



TURJAF

12(12): 2024
ENGLISH ISSUE



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Turkish Journal of Agriculture - Food Science and Technology
International Peer-Reviewed Journal | ISSN: 2148-127X
www.agrifoodscience.com



Vol. 12 No. 12 (2024)

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- [WorldCat libraries\(WorldCat\)](#),
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ISSN: 2148-127X



Turkish JAF Sci.Tech.



TR – DİZİN Bilgileri

Türk Tarım - Gıda Bilim ve Teknoloji dergisi

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Atıf Ortalaması

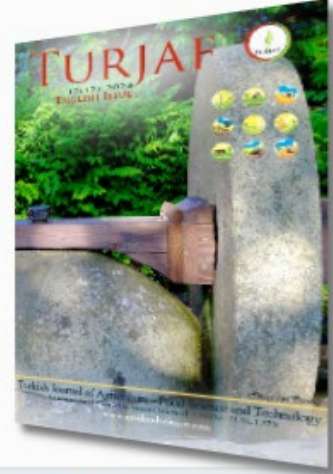
0,99

Kendine Atıf Oranı

%29,14

Konu Kategorisi: Fen > Ziraat Fen > Mühendislik

Konu Alanları: Ziraat Mühendisliği Gıda Bilimi ve Teknolojisi



EISSN: 2148-127X

İlk Yayın Yılı: 2013

Diziniendiği Yıllar: 2014-2024 (Fen)

Yayın Periyodu: Ocak, Şubat, Mart, Nisan, Mayıs, Haziran,

Temmuz, Ağustos, Eylül, Ekim, Kasım, Aralık

Yayın Formatı: Elektronik

Yayın Dili: Türkçe | İngilizce

Editör: Hasan Eleroğlu

Yayıncı: Turkish Science and Technology Publishing (TURSTEP)

Web Adresi: <http://www.agrifoodscience.com>

Makale & Atıf Sayısı



Makale Türleri

Araştırma Makalesi Deleme Diğer Ölü Süreli Düzeltme



Adaptation and Growth Performance of F1 Progeny of Crossbred Sheep in Bangladesh

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ARTICLE INFO

ABSTRACT

Research Article

Received : 24.07.2023

Accepted : 07.10.2024

Keywords:

Crossbred sheep
Growth performance
Perendale crossbred
Dorper crossbred
Damara crossbred

The research was conducted to assess the growth performance of different crossbred sheep at Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. In the crossbreeding program, Perendale, Dorper and Damara breed were considered as sire and BLRI improved native sheep (BNS) was used as dam. The production performance of assorted F₁ progeny were evaluated and compared with BLRI improved native sheep. Data analysis was carried out using Generalized Linear Model (GLM) procedure of Statistical Package for the Social Sciences (SPSS) version 25.0. Each crossbred genotype outperformed native sheep in terms of live weights and Average Daily gain (ADG). The live weights ($p < 0.001$ and $p < 0.01$) and ADG ($p < 0.001$) significantly influenced by genotype except the 6 months live weight. Seasonal effects were found non-significant on live weights and ADG except birth weight ($p < 0.05$). Among the crossbreds, the highest birth weight was found in Dorper crossbred (2.37 ± 0.13 kg) while 12 months live weight was found higher in Perendale crossbred (22.33 ± 0.99 kg), respectively. In case of cumulative growth performance of male, the highest value was found in Damara crossbred while, Perendale crossbred female was found better compared to other crossbred. Major disease frequently occurred in crossbred sheep was diarrhea. The survivability rate (%) of crossbred sheep at lamb (0-3 months of age) and grower (3-8 months of age) stage were 92.55 and 90.8, respectively indicates positive influence on the crossbreeding program. Though, this is very first work regarding crossbred sheep, further research is needed in corporation with other economic trait associated with growth and reproduction to evaluate all the crossbred genotype as well as select a suitable crossbred for the production of commercial market lamb in Bangladesh.

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Introduction

Sheep is one of the promising livestock species after cattle and goat which have great potentiality for profitable lamb production in the revolution in livestock industry of Bangladesh (Sun et al., 2020; Hashem et al., 2020; Hossain et al., 2021). Present sheep population of the country is 3.90 million, which has increased 6 times during the last ten years with average annual growth rate of 1.99% (DLS, 2024). Sheep farming might be a sustainable tool to produce animal protein, eradicate poverty, women empowerment and socio-economic development of Bangladesh (Kendall and Telfer, 2000; Haque et al., 2020; Amin et al., 2020).

There is no established sheep breed in Bangladesh, but only the native sheep which have slow growth rate. In addition to conventional sheep, crossbred sheep are a potential resource to accomplish the increasing demand of animal protein in Bangladesh. Crossbreeding is generally considered as an effective tool to improve the production potentiality of native livestock species in the developing countries. Genetic improvement of the productive and reproductive traits of small ruminant is the results of selective breeding within breeds, development of new breeds or genotypes and crossbreeding. Heterosis is the primary benefit of an organized crossbreeding program that rapidly improves the productivity of crossbred animals

compare to the average performance of a pure breed (Fathala et al., 2014).

Different research work incorporating crossbreeding or synthetic breed development program revealed that crossbred performance using exotic sires were superior as compared to the native sheep stock (Sanna et al., 2001; Boujenane, 2002; Tibbo, 2006; Tsegay et al., 2013; Lakew et al., 2014; Tilahun et al., 2014; Lalit et al., 2016).

On the other hand, no research has been conducted on the development of synthetic sheep breeds in Bangladesh. Numbers of crossbreeding program were undertaken in our country to improve the indigenous sheep with high performing exotic sheep viz. Lohi, Romney Marsh, Suffolk and Perendale but the attempts did not succeed (Bhuiyan, 2013; Hamid, 2019). Latterly, Bangladesh Livestock Research Institute (BLRI) had started research work with some exotic sheep breed originated from Australia named Suffolk, Perendale, Dorper (Giasuddin et al., 2018) and Damara which is originated from South Africa with the aim to develop a suitable crossbred sheep for meat production. The program has begun using Perendale, Dorper and Damara as sire line and BLRI improved native sheep as dam line for crossbreeding. To augment the genetic superiority as well as diminish the degenerating performance of crossbred animals in subsequent generation, it is cardinal to integrate crossbreeding program with selective breeding (Gizaw et al., 2012). Therefore, to utilize the breeding program effectively, the current work was conducted to evaluate the relative performance of various crossbred sheep genotypes.

Materials and Method

The breeding program was carried out at Sheep Research Farm, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. The data were collected from January, 2018 to January, 2020.

Animal Selection and Breeding

The crossbreeding program was conducted with exotic sheep viz. Dorper, Perendale and Damara as sire line while BLRI improved native sheep (BNS) was used as dam line. The average birth weight, live weight at 3 and 6 months of age and mature live weight of BNS were 1.46, 6.96, 11.16 and 25.83 kg, respectively (Pervage et al., 2009; Mowsume et al., 2023). BNS that attained a birth weight and mature weight of at least 1.5 and 25 kg and achieved at least one parity was selected as dam. Natural service was performed for mating the animals. The breeding plan is given in Table 1.

Three crossbred sheep (Dorper, Perendale and Damara crossbred) were considered as treatment group and BNS was regarded as the control group to compare the production performance of their offspring. A total number of 40 native sheep was considered as control group.

Feeding and Overall Management

All the research animal were housed in a permanent slatted floor raised above the ground level with enough space for giving them more comfort. Crossbred sheep were given 8 hours to graze (from 8 AM to 4 PM) with one hour as resting period (12:00 PM to 01:00 PM). Animals were given a concentrate combination comprising 17% CP, 11 MJ ME/kg DM supplied for ewes, rams, and lambs twice

daily in the morning and evening at rates of 200, 250, and 100 g/d/head, respectively. Adequate fresh water was supplied for all the time. The ingredients of concentrate mixture is given in Table 2.

The PPR (Peste Des Petits Ruminants) vaccine was administered to each animal regularly. Deworming and dipping with 0.5% melatheaon solution was practiced at a regular interval to control the internal and external parasites. Animals with severe skin disorders, stunted growth, unthriftiness, repeat breeding and unhealthy conditions were removed from the flock.

Record Keeping

Data on birth weight, live weight at 3, 6, 9 and 12 months, average daily weight gain at 0-3, 3-6, 6-9 and 9-12 months of age, disease incidence and lamb mortality were observed and recorded. Genotype and season were considered as fixed effects. The year round seasonal effect were recorded as summer season (March-June), rainy season (July-October) and winter season (November-February).

Statistical Analysis

Sample size of the experiment was unbalanced due to the result of natural service. All the recorded data were compiled in Microsoft Excel Worksheet, organized and analyzed by General Linear Model (GLM) procedure of Statistical Package for the Social Sciences (SPSS) version 25.0. For mean comparisons, the Duncan's Multiple Range Test (DMRT) was applied.

Table 1. The breeding plan

	Perendale (♂)	Dorper (♂)	Damara (♂)
BNS (♀)	Perendale (♂)		
	× BNS (♀)		
BNS (♀)		Dorper (♂) ×	
		BNS (♀)	
BNS (♀)			Damara (♂) ×
			BNS (♀)

¹BNS= BLRI Improved Native Sheep

Table 2. The ingredients of concentrate mixture

Feed ingredients	Quantity in the concentrate mixture (%)
Crushed maize	21
Wheat bran	50
Soybean meal	14
Grass pea meal	12
Protein concentrate	1
Di-calcium phosphate (DCP)	0.5
Vitamin-mineral premix	0.5
Salt	1
Total	100

Results and discussion:

Live weight gain

All crossbred genotypes outperformed native sheep in terms of live weight gain at various ages. (Table 3). The genotype has significant effects ($p < 0.001$ and $p < 0.01$) on the live weights except the 6 months live weight. Dorper crossbred had the highest birth weight (2.37 ± 0.13 kg) among the crossbreds followed by Perendale (2.33 ± 0.12

kg) and Damara (2.12 ± 0.09 kg), respectively. Damara crossbred performed better in case of 3 and 9 months live weight (10.30 ± 0.39 and 18.39 ± 0.66 kg), on the other hand, Perendale crossbred was found superior at the age of 6 and 12 months live weight (14.37 ± 0.69 and 22.33 ± 0.99 kg). Season did not significantly affect live weight except birth weight ($p < 0.05$). In case of genotype- season interaction, there was no significant difference in live weight at different ages except birth weight ($p < 0.05$). Zaffer et al. (2015) found the birth weight, weaning weight, 6 and 12 months live weight of crossbred sheep as 2.86 ± 0.06 kg, 15.27 ± 0.29 kg, 17.03 ± 0.26 kg, 22.32 ± 0.29 kg, respectively, which is nearly identical to the current findings but lambing season significantly affected only weaning weight and 12 months live weight which contradicts the findings. Higher live weight compare to the present findings were found by Belete et al. (2015) which were 2.20 ± 0.47 , 19.00 ± 0.27 and 31.85 ± 0.89 kg, respectively for birth weight, weaning weight, and 12 months live weight, respectively for Dorper crossbred sheep. Tarekegn et al. (2014) also found higher performance compare to the present findings as 3.24 kg, 20.51 kg and 64.69 g/d for the birth weight, 6 months body weight and 3 months growth rate, respectively. Similarly, relatively higher live weight than this result was also found

by Tesema et al. (2022) for Dorper crossbred sheep and the values were and 3.03 ± 0.02 , 14.5 ± 0.18 , 20.4 ± 0.26 , 24.8 ± 0.31 , and 28.3 ± 0.40 kg, respectively for birth weight, weaning weight, 6, 9 and 12 months live weight, respectively. In another study conducted in Ethiopia, the average birth weight, 3, 6, 9 and 12 months live weight of Dorper crossbred were found as 2.55, 13.78, 20.78, 19.45 and 26.16 kg, respectively which are much higher than the current findings (Gebreyowhens et al., 2017). Giorgis et al., (2017) also found higher birth weight of Dorper F₁ crossbred (2.54 kg) compare to the present findings. However, the variations might be originated from the differences in management, environment and genotype-environment interactions.

Among the male of different genotypes, cumulative growth performance was better in Damara crossbred followed by Dorper, Perendale and native sheep genotype and the average live weight at 12 months of age were 24.19, 22.52, 21.77 and 19.14 kg, respectively (Figure 1). But in case of female, Perendale crossbred was superior in cumulative growth performance followed by Dorper, Damara and native sheep genotype and the values were 22.23, 22.16, 19.66 and 16.9 kg, respectively at 12 months age (Figure 2).

Table 3. Effect of genotype and season on live weights of crossbred sheep at different ages-

Factors	N	Least Squares Means (LSM)±SE for different Live weight (kg)				
		Birth weight	3 months	6 months	9 months	12 months
Overall	144	2.12±0.05	9.63±0.23	13.57±0.32	17.35±0.39	20.93±0.45
Minimum		1.00	5.45	8.30	10.10	11.65
Maximum		3.80	18.10	26.80	35.00	42.20
Genotype						
Perendale crossbred	31	2.33 ^a ±0.12	10.14 ^a ±0.51	14.37±0.69	18.28 ^a ±0.85	22.33 ^a ±0.99
Dorper crossbred	26	2.37 ^a ±0.13	10.24 ^a ±0.56	13.63±0.78	17.45 ^a ±0.93	21.36 ^a ±1.08
Damara crossbred	47	2.12 ^a ±0.09	10.30 ^a ±0.39	13.76±0.54	18.39 ^a ±0.66	22.12 ^a ±0.77
BNS	40	1.68 ^b ±0.84	7.83 ^b ±0.37	12.51±0.50	15.25 ^b ±0.62	17.92 ^b ±0.72
Significance level		***	***	NS	**	***
Season						
Winter (November-February)	63	2.18 ^a ±0.09	10.15±0.39	14.40±0.53	18.01±0.66	21.66±0.76
Summer (March- June)	43	2.29 ^a ±0.08	9.89±0.35	13.50±0.47	17.52±0.58	20.98±0.68
Rainy (July-October)	38	1.90 ^b ±0.10	8.85±0.46	12.80±0.63	16.50±0.77	20.16±0.89
Significance level		*	NS	NS	NS	NS
Genotype×Season		*	NS	NS	NS	NS
Significance level						

BNS: BLRI improved native sheep; ***: Highly significant ($p < 0.001$); **: moderately significant ($p < 0.01$); *: significant ($p < 0.05$); NS: non-significant ($p > 0.05$); SE: standard error mean; N: number of observations.

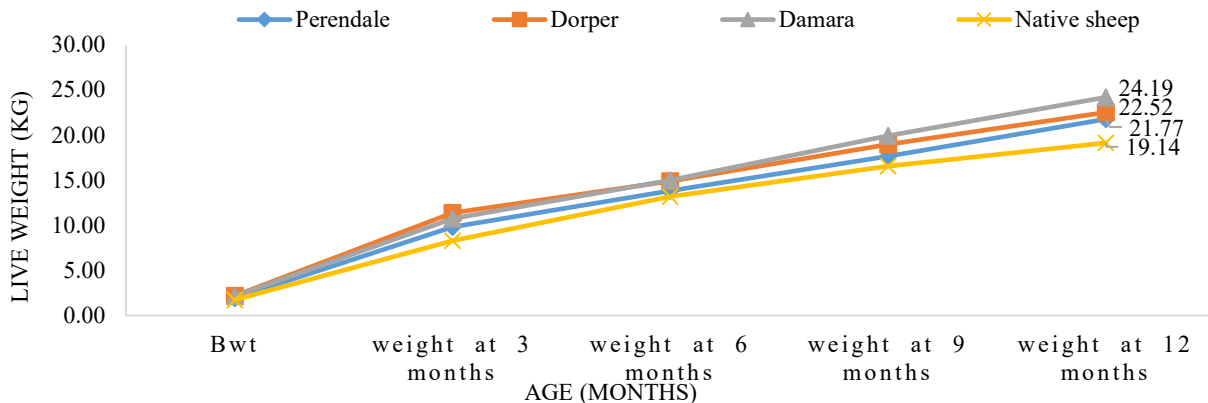


Figure 1. Cumulative growth performance of different crossbred male

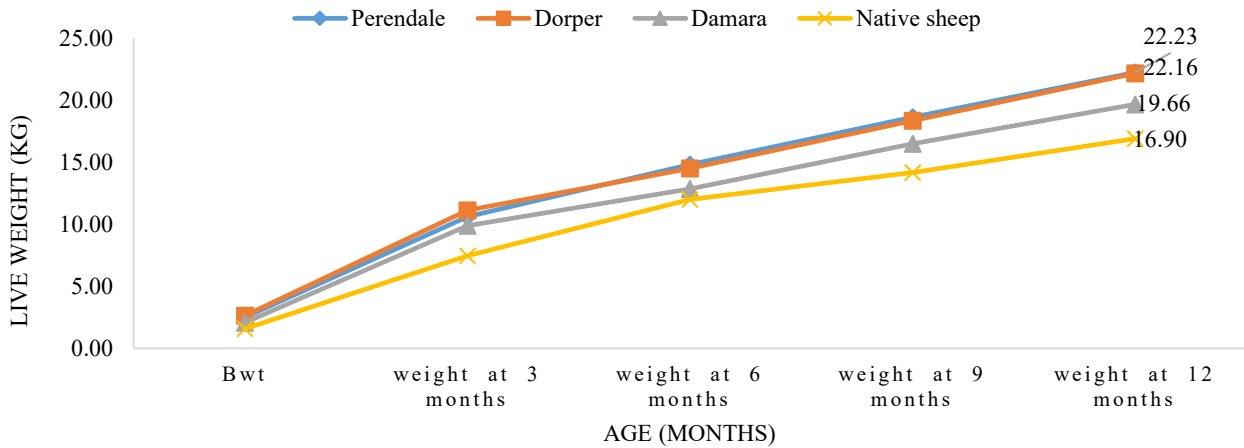


Figure 2. Cumulative growth performance of different crossbred female

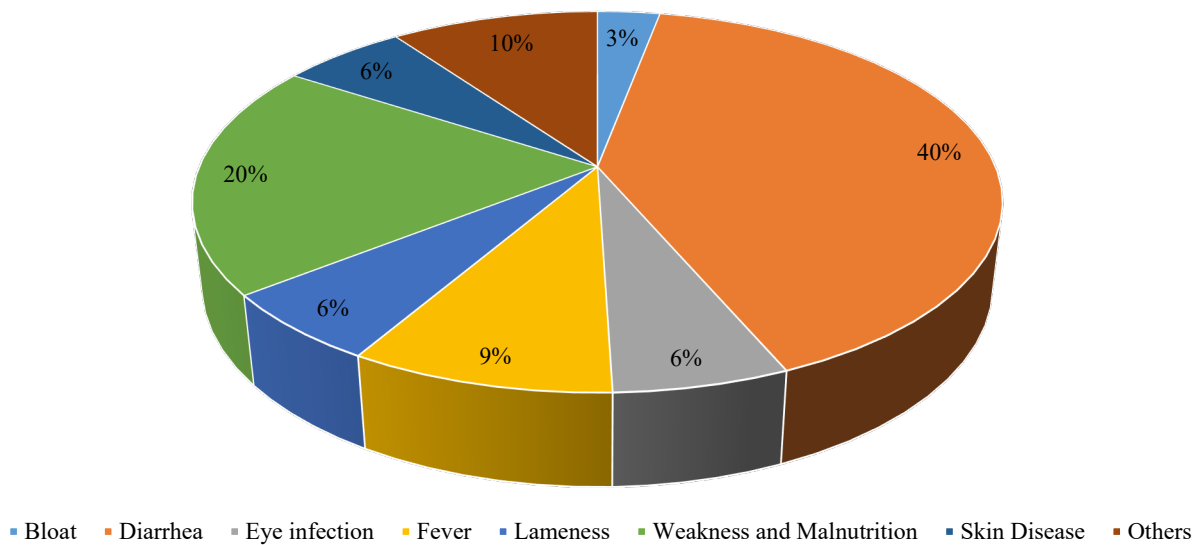


Figure 3. Disease incidence of crossbred sheep
Figures in the parenthesis represent the number of observations.

Average Daily Weight gain (ADG)

There was significant impact of genotype on ADG ($p < 0.001$) at different ages of sheep (Table 4). All the crossbred genotype were superior in ADG than BNS except in case of daily weight gain at 3-6 months of age. Highest ADG at 0-3 months and 6-9 months were found in Damara crossbred (89.89 ± 3.92 and 50.94 ± 2.54 g/d), respectively. In case of 3-6 months ADG, native sheep were found to be superior (51.39 ± 2.54 g/d) than all the crossbred genotypes. Perendale crossbred performed better in case of 9-12 months ADG (45.04 ± 3.21 g/d). However, season had no significant effect on ADG. In case of genotype- season interaction, no significant effect was found in ADG at different ages except at birth to 3 months ADG ($p < 0.05$). Relatively lower ADG of Dorper crossbred sheep at 0-3 months of age (69.4 g/day) than the present findings was found by Tsegay et al. (2013). In another study, ADG of and 50% Dorper crossbred at 3-6 months of age were found as 132 and 126 g/d, respectively (Tilahun et al., 2014) which are much higher than the current findings. Ayichew (2019) reported 125.84 g/day ADG of Dorper F₁ crossbred at 3-6 months of age in Ethiopia,

which is also much higher than the present findings. Relatively much higher result was found for Dorper crossbred (199.6 g/d ADG at 0-3 months of age) than the current findings reported by Gavojdian et al. (2013). This could be due to the differences in management practices or environment of the study area.

Disease Incidence and Lamb Survivability

Major disease occurred in crossbred sheep was diarrhea (40%) followed by weakness and malnutrition (20%), fever (9%), eye infection (6%), lameness (6%), skin disease (6%) and bloat (3%). Other health problem was 10% including mechanical injury and fighting etc.

Most of the crossbred sheep affected with fever as a result of exposure with sunlight during grazing. It might be due to the consequences of heat stress. Tesema et al. (2020) found GI (gastrointestinal) parasites, pneumonia and septicemia as important diseases of crossbred animals. Internal parasites and pneumonia could have occurred as a result of grazing habit and a lack of suitable post-lambing management of crossbreds.

There was higher mortality found in crossbred sheep as compared to the native sheep (Table 5). In all the crossbred sheep, higher mortality was found in growing stage (9.2%) rather than lamb (7.45%). It might be due to the result of weaning stress. But in case of native sheep, lamb mortality was higher (5.00%) rather than growing stage (4.21%). Relatively much higher mortality were found by Tesema et al. (2020) than the present findings, which were 86.0, 76.6, and 67.9% for Dorper x native crossbred lambs at 3, 6 and 12 months of age, respectively. Getachew et al. (2015) reported the mortality rate of Menz sheep at weaning and

one year of age as 16.7% and 36.3%, both of which are greater than the current finding. Gavojdian et al. (2013) reported 90.6% survivability rate of Dorper crossbred sheep at 0-3 months of age which is lower than the current result (92.55%). Relatively lower survival percentage of Dorper x Menz crossbred sheep (81%) up to 3 months of age was noted by Abebe et al. (2015) than the present findings. The current crossbreeding program found positive influence on the survivability rate of lamb compared to the reviewed research. This might be due to the good mothering ability of BNS to raise their lamb.

Table 4. Effect of genotype and season on Average Daily Gain of crossbred sheep at different ages-

Effect	N	Least Squares Means (LSM)±SE for different Average Daily Gain (g/d)			
		0-3 months	3-6 months	6-9 months	9-12 months
Overall	144	82.45±2.3	43.30±1.59	41.4±1.49	39.84±1.47
Minimum		44.51	11.54	10.99	12.22
Maximum		175.82	126.37	102.97	114.56
Genotype					
Perendale crossbred	31	85.80 ^a ±5.04	46.54 ^{ab} ±3.49	42.86 ^a ±3.26	45.04 ^a ±3.21
Dorper crossbred	26	86.43 ^a ±5.51	37.27 ^b ±3.83	42.03 ^a ±3.57	43.35 ^a ±3.51
Damara crossbred	47	89.89 ^a ±3.92	38.02 ^b ±2.72	50.94 ^a ±2.54	41.28 ^a ±2.50
BNS	40	67.67 ^b ±3.66	51.39 ^a ±2.54	30.08 ^b ±2.37	29.70 ^b ±2.33
Significance level		***	***	***	***
Season					
Winter (November-February)	63	87.60±3.88	46.75±2.69	39.64±2.52	40.52±2.48
Summer (March- June)	43	83.47±3.43	39.70±2.38	44.10±2.22	38.38±2.19
Rainy (July-October)	38	76.29±4.55	43.46±3.16	40.69±2.95	40.63±2.90
Significance level		NS	NS	NS	NS
Genotype×Season		*	NS	NS	NS
Significance level					

BNS: BLRI improved native sheep; ***: Highly significant ($p < 0.001$); **: moderately significant ($p < 0.01$); *: significant ($p < 0.05$); NS: non-significant ($p > 0.05$); SE: standard error mean; N: number of observations.

Table 5. Survivability (%) of different crossbred genotype

Genotype	Lamb (0-3 months of age)		Growing (3-8 months of age)	
	Mortality (%)	Survivability (%)	Mortality (%)	Survivability (%)
Perendale	6.67 (3)	93.33 (42)	7.14 (3)	92.86 (39)
Dorper	5.26 (2)	94.74 (36)	8.33 (3)	91.67 (33)
Damara	8.57 (9)	91.43 (96)	10.42 (10)	89.58 (86)
Total crossbred	7.45 (14)	92.55 (174)	9.2 (16)	90.8 (158)
Native sheep	5.00 (10)	95 (190)	4.21 (8)	95.79 (192)

Conclusion

It may be concluded that, the growth performance of Damara and Perendale crossbred were better. Crossbred sheep showed higher growth compare to native sheep. Though mortality rate was higher in crossbred sheep, it may decrease with each successive generation. Schematic and scientific breeding plan with proper nutrition and other managemental facilities may contribute to upgrade or improvement of native sheep by exotic sheep. Thus, the crossbreeding with exotic sheep should continue to develop a sustainable meat type sheep in Bangladesh.

Declarations

Acknowledgement

The authors are grateful to the authority of Bangladesh Livestock Research Institute for funding as well as facilitating to conduct the research project entitled "Exotic sheep adaptation and their crossbreds production in Bangladesh".

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Spatial Evaluation of Carp Production by Using Geography Information Systems (GIS) in the Anatolian Region of Türkiye

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ARTICLE INFO

Research Article

Received : 17.04.2024
Accepted : 31.10.2024

Keywords:

Carp Production
GIS Mapping
Spatial Analysis
Anatolian Region
Türkiye

ABSTRACT

Fish industry has significant importance all over the world because red meat is not enough to complete the protein requirements of growing population. Therefore, focus is now on those fish species production rate which are more suitable. In this study we focused on common carp which has important commercial value due to its size and tasty meat. Common carp is a fresh water fish and mostly found in rivers, ponds, dams and lakes. It is special due to its ability of adjustment in any aquatic habitat and sometimes beneficial also for other aquatic animals by releasing nutrients in habitat. Türkiye is a rich country in terms of rivers, dams and lakes. In this research, we described famous water reservoirs in Türkiye and in which regions higher amount of carp fish produced. It was focused on Central Anatolian Region of Türkiye which has large amount of water reservoirs. Geography Information System (GIS) based mapping and spatial analysis was used in this study to check the production rate of carp in Central Anatolian Region of Türkiye for long period from year 2000 to 2019. It was concluded from this study that highest average production rate of carp is obtained in Konya province (total: 11919,2 tons/2000-2019; average: 596 tons/20 year) which is west part of Anatolian region and lowest average production rate is found in the Niğde province (total: 163,5 tons/2000-2019; average: 8,2 tons/20 year) which is south part of the Central Anatolian Region.

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Introduction

Fishing remains a considerable source of income for humans since ancient times. Countries concerned of balanced nutrition have focused on optimum utilization of water reservoirs and to expand their animal protein bases. For this purpose many new projects have been developed. Major production is through fishing from natural habitats. Although, the participation of aquaculture in fisheries is increasing gradually. Türkiye has also affected by this global revolution because aquaculture sector has considerably increased also in Türkiye (Akova, 2015).

Even though there are many modifications in countries, 16.7% of animal protein sources are provided by fishery foods. This includes as 59% in Taiwan, 3% in Austria, 55% in Japan and 7.1% in Germany. Fifty percent population in developing countries fulfill 40% of their animal protein requirement from fish. All over the world, in 1960 average amount of fish consumed by people was 9.9 kg per capita, while in 2009 it increased to 18.4 kg and in 2012 increased to 19.2 kg. Average amounts of fish consumed in Europe,

North America, Oceania, Asia, and Africa was 22 kg, 24.1 kg, 24.6 kg, 20.7 kg, and 9.1 kg per capita respectively (Akova, 2015). Fish yields have been decreasing over the year due to quickly increase in world population. Fish reservoirs also in threat due to many adversarial environmental aspects inpite of using many advance techniques in fishing. Earth population is more than seven billion. Fish farming make an important contribution to fulfill the nutritional requirements of this growing population, providing raw material for the fish industry and increasing facilitation for employment (Yılmaz et al., 2008). In general, the worldwide yield of fishery captured reached from 13.13 million tones in 1990 to 66.6 million tons in 2012. The produced income was 144.4 billion US dollars (Akova, 2015).

Fish farming through aquaculture is divided into two ways as freshwater aquaculture and marine aquaculture. Freshwater aquaculture globally has great contribution in provision of fish protein (Belton et al., 2018; Belton and

Bush, 2014; Bayhan et al., 2017). Freshwater aquaculture comprise a large variety of systems throughout economic and physical scales, species, value chains, ownership and infrastructure configurations. It mostly contains household organized ponds and commercial businesses at medium and small levels which yields many species of carp fish and some other fish in polyculture schemes for consumption of local people (Hernandez et al., 2018). Freshwater aquaculture is mostly known for the production of striped catfish and tilapia that are mainly produced in mud ponds for usage at national and international level. A key factor of freshwater aquaculture production throughout the past twenty years has been the propagation of value food chains inside and outside of country situated in South and Southeast Asia (Belton et al., 2017; Hernandez, et al., 2018; Belton and Filipinski, 2019; Belton and Little, 2008; Loc et al., 2010). China is the largest provider of freshwater fish at domestic level and also at international level (Fluet-Chouinard et al., 2018). In 2003, approximate 400.000 tons of common carp, grass carp, chub and crucian carp were produced in China for commercial use in the native market. Asia is the major producer of aquaculture, as in 2017 comprising 92% of the aquatic animals and seaweeds (FAO, 2019). Türkiye gained the 36th position in the global fishing yield in 2005, and 23rd position in the global aquaculture fish farming yield. Freshwater fishery production was the greatest in East Anatolian region from 1998 to 2008. The mostly farmed species in Türkiye are trout, sea bass and sea bream. Fishery products in Türkiye produced from freshwater is 52.7%, while production from marine water is 47.3%. Rainbow trout which is most dominant farmed specie, accounts 56% of the whole production. In previous nine years trout farming increased three times (from 2004 to 2013). According to location Türkiye has opulent water reservoirs, and due to rich reservoirs of water and suitable climate, it is actually best state for aquaculture (TUIK, 2014).

Inland waters of Türkiye comprised 236 fish species and 26 subspecies of fish families. The Cyprinidae fish family has 166 species which is 49% of the whole fish yield. Common carp fish, is the most common specie of cyprinid family that makes a significant portion of inlandwater (stream, lake and dam lake,) fish production in many areas of Türkiye (Çetinkaya, 2006; Balık et al., 2006). Inland waters always had a great potential of fish production in terms of variety due to sufficient freshwater reservoirs that comprised about 175 km of rivers, 1 million hactar of natural lakes, 170.000 hactar of reservoirs, 70.000 hactar of lagoons and 700 small reservoirs. Many species including carp, mullet, wells and eel are major produced species in these freshwater reservoirs (Rad, 2000). Additionally, as marine fish storage in the marine adjoining Türkiye are slowly decreasing, inland water reservoirs gives a major choice for establishment of freshwater fish harvest and fish farming (Celebi, 2010; Karabas et al., 2018).

Common carp linked to the Cypriniformes order and the Cyprinidae family, which is known the biggest family of freshwater fish. It mostly produced and lived in freshwater habitates specifically in rivers, lakes and ponds and very rarely found in salty water (Barus et al., 2001). It is generally found in almost whole countries of the world though is very famous in Asia and in European countries

(Weber and Brown, 2011; Kloskowski, 2011a; Parkos and Wahl, 2014). Globally Common carp is the third major cultured and commercially significant freshwater fish specie (FAO, 2013). It is known as commercially important fish specie because of its excellent adaptive ability to both food and ecosystem (Soltani et al., 2010; Manjappa et al., 2011; Rahman, 2015; Erguven and Aydin, 2019). Over than 80% of whole fish yield comes from common carp in few European countries (Woyanovich et al., 2010; Anton-Pardo et al., 2014). Common carp is mostly known as 'ecological engineer' due to its ability of create modification in properties of aquatic ecosystems (Matsuzaki et al., 2009; Bajer and Sorensen 2015; Rahman, 2015).

Globally the production of common carp is about 4 million tons. According to global production of finfish aquaculture in 2010, grass carp categorized as first, silver carp categorized as second and common carp categorized as third. Only China produced 77% of the worldwide aquaculture production of common carp fish in 2009 (FAO, 2012). The most produced six finfish species in china including grass carp, silver carp, common carp, bighead carp, crucian carp and tilapia (FAO, 2013). Common carp (*Cyprinus carpio*) has an important place in Türkiye's fishing industry and it is mostly found in our freshwater reservoirs such as rivers, streams, ponds, lakes and dam lakes. It is commonly found in calm-flowing bottom areas of big rivers in reigon of Anatolian but not in very cold mountain lakes (Geldiay and Balık, 1988). In 2006 Carp fish contained 27.5% of whole freshwater captured fish production in Türkiye (Anonymous, 2007).

In Türkiye carp specie was initially examined by Numann (1958). He observed many ecological and biological properties of carp fish population living in Beyşehir, Akşehir, İznik, Eğirdir, Süleymaniye, Manyas and Apolyont Lakes. Actual biological properties of Common carp (*Cyprinus carpio*) were identified in Mogan Lake, in Hirfanlı Dam Lake, in Eymir Lake, in Hafik Lake (Sivas), Akşehir Lake (Konya), Bafra Balık Lakes (Samsun), in Çıldır Lake, Gölhisar Lake (Burdur), in Kaz Lake (Tokat), İznik Lake (Bursa) (Tanyolaç and Karabatak, 1974; Karabatak, 1977; Tanyolaç, 1979; Cengizler, 1987; Çetinkaya, 1992; Demirkalp, 1992; Yerli, 1997; Alp and Balık, 2000; Karataş, 2000; Özeren, 2008). The commercial value of common carp is increasing day by day by increasing the growth rate, higher weight and length, high yield of meat, adjustable in any habitat and good taste (Demirkalp, 1992). Common carp fish is a very famous benthivorous fish that has bottom-up effects. The bottom-up effects of common carp msostly rely on the integration of nutrients which are derived from benthos and nutrients which are excrete from bottom sediment through feeding on benthos.

Common carp increases productivity of phytoplankton by excreting nutrients such as soluble phosphorus from the sediment. These all characteristics of common carp are important elements for resuspension of sediment. (Rahman et al., 2008a; Rahman, 2015). This influences positively on those fish production, which directly or indirectly rely on natural food. For example, rohu fish is a planktivorous fish, which produced better in those ponds where common carp also exist nither in a monoculture (Rahman et al., 2006). Common carp also increases the oxygen accessibility in the

bottom soil through sediment resuspension. However, resuspension of the bottom soil through common carp increases the depth and limit of oxygen penetration into the bottom soil. Enough oxygen availability enhances the aerobic breakdown of organic matter which also increases the amount of minerals in the bottom soil (Rahman et al., 2009; Yathavamoorthi et al. 2010). Common carp also excrete enough amount of phosphorus and nitrogen which transfers from the bottom sediment to the water column (Morgan and Hicks, 2013).

The most important benefit of GIS analysis is that it gives the desired map as the end result by considering the distribution maps formed by including many factors within a specific database (Xu et al., 2001; Akdeniz et al., 2011; Arslan, 2008; Erguven and Sener, 2019). GIS consists of four major parameters: collection of data and installation of data, second is data base of area geography, third is analysis of data and modeling of data, then fourth is data imagining and presentation. The purpose of spatial analysis is creating the relationship between parameters of water quality by making an estimation from the point values of sample.

This data which is obtained through spatial analysis is used to check the involvement of each parameter of water quality throughout the lake on a GIS spatial distribution map. GIS has been used for sectorial, regional and national studies of aquaculture (Nath et al., 2000; Salam et al., 2003).

In this study we focused on evaluation of carp production with GIS-based mapping and spatial analysis in Central Anatolian Region of Türkiye for long periods from 2000 to 2019. Geography Information System (GIS) analysis is a broadly used technique specifically for management of water quality in previous years.

Material And Method

This study was conducted in Anatolian Reigon of Türkiye. According to geography, total area of Central Anatolian Region is 163.057 km² while its total population is 12.896.55. Central Anatolian region is located in the middle of Türkiye. There are 13 provinces in total. The location of research area is presented in Figure 1.

In this research, carp production included from the year of 2000-2019 years. All this production rate information data got from TÜİK reports (TÜİK, 2019). In order to determine the distribution of carp production by provinces and the relationship between the carp production, the linear regression method was analyzed. In addition to the standard deviations of the total 20 years production amounts of the provinces of the Central Anatolian Region between the years 2000-2019 were determined (Schneider et al., 2010). In the study, the carp production amount of the provinces of the Central Anatolian Region was analyzed spatially by using Arc GIS 10.3.1 one of the geography information system software (Anonymous, 2010). All this data was analysed by using GIS-based mapping and spatial analysis. In the research, distribution maps were created by using spline interpolation method for spatial evaluation of carp production amounts in Anatolian region's provinces (Hou and Andrews, 1978; Hummel, 1983; Lee, 1983).

Results And Discussion

According to GIS-based mapping and spatial analysis we found the following maps and graphs in which the carp production amounts are given according to the cities in the map, and a comparison of these production amounts is given in the graphs. Each graphs shows the detail of five year data of carp production.

In Figure 2 it can be seen in 2000 Aksaray carp production is 80 tons, Ankara production is 422 tons, Eskişehir production is 296 tons, Karaman production is 72 tons, Kayseri production is 421 tons, Kirikkale production is 70 tons, Kırşehir production is 432 tons, Yozgat production is 170 tons, Çankırı production is 305 tons, Sivas production is 186 tons and Niğde carp production is 20 tons. The Nevşehir production is 18 tons with the lowest value and highest carp production was reported in Konya with a highest value of 683 tons. In maps we see the blue places where the carp production is mostly conducted, which means that carp production is highly conducted around the west part of the research area as spatial, while the production is getting lower near the south part of the research area as spatial.



Figure 1. The location of research area

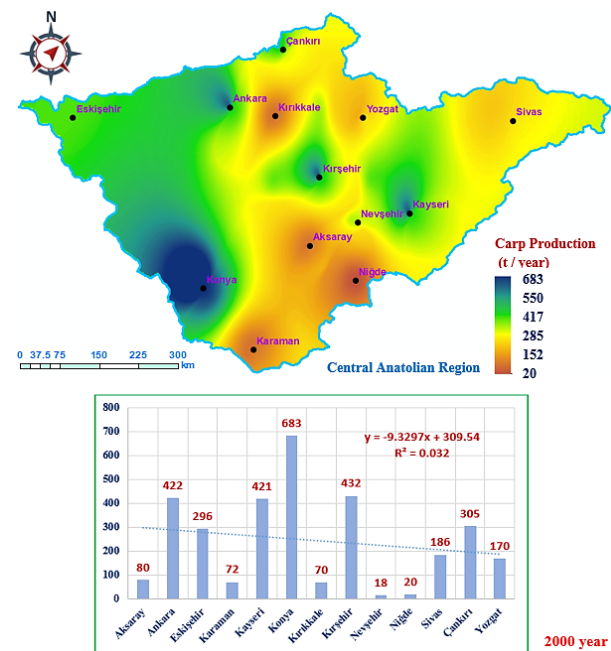


Figure 2. Spatial analysis of carp production in 2000

As seen in the Figure 3, in 2005 Aksaray carp production is 91 tons, Ankara production is 574 tons, Eskişehir production is 351 tons, Karaman production is 86 tons, Kayseri production is 274 tons, Kırıkkale production is 74 tons, Kırşehir production is 433 tons, Yozgat production is 135 tons, Çankırı production is 283 tons, Sivas production is 185 tons and Nevşehir carp production is 36 tons. The Niğde production is 7 tons with the lowest value and highest carp production was reported in Konya with a highest value of 869 tons. In spatial maps we see the blue places where the carp production is mostly conducted, which means that carp production is highly conducted around the west part of the research area as spatial, while the production is getting lower near the south part of the research area as spatial.

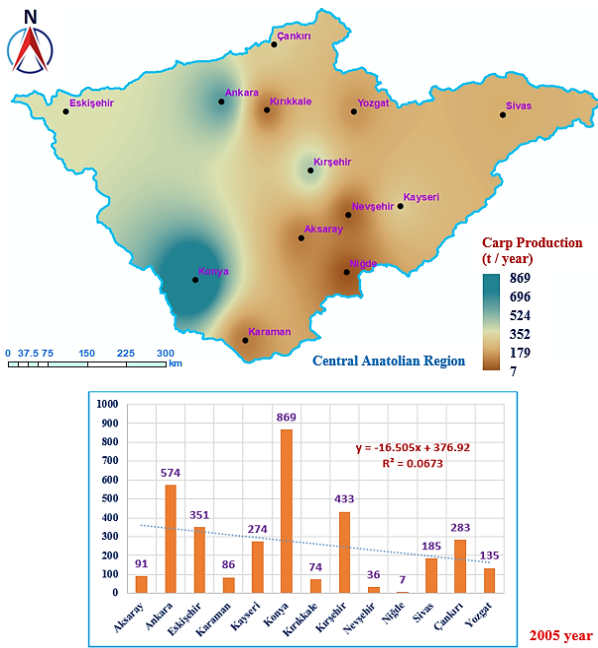


Figure 3. Spatial analysis of carp production in 2005

Figure 4 shows that in 2010 Aksaray carp production is 79 tons, Ankara production is 557 tons, Eskişehir production is 336 tons, Karaman production is 82 tons, Kayseri production is 237 tons, Kırıkkale production is 87 tons, Kırşehir production is 241 tons, Yozgat production is 123 tons, Çankırı production is 131 tons, Sivas production is 184 tons, Nevşehir carp production is 17 tons. The Niğde production is 2 tons with the lowest value and highest carp production was reported in Konya with a highest value of 897 tons. In spatial maps it can be seen that the green places where the carp production is mostly conducted, which means that carp production is highly conducted around the west part of the research area as spatial, while the production is getting lower near the south part of the research area as spatial.

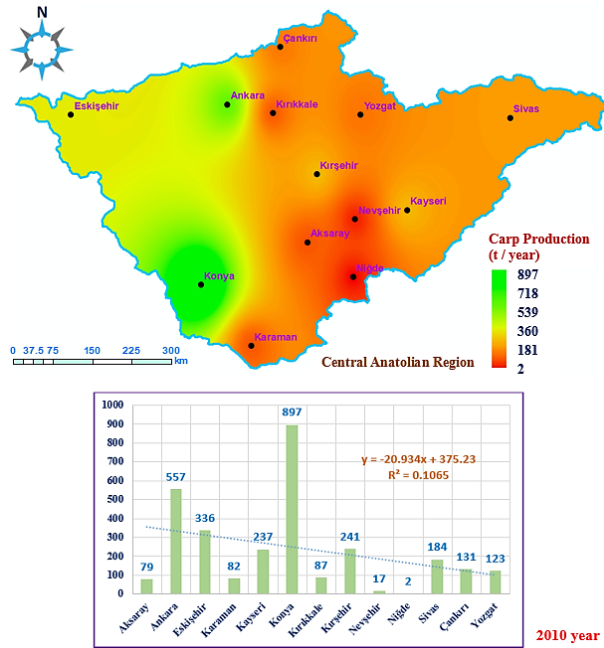


Figure 4. Spatial analysis of carp production in 2010

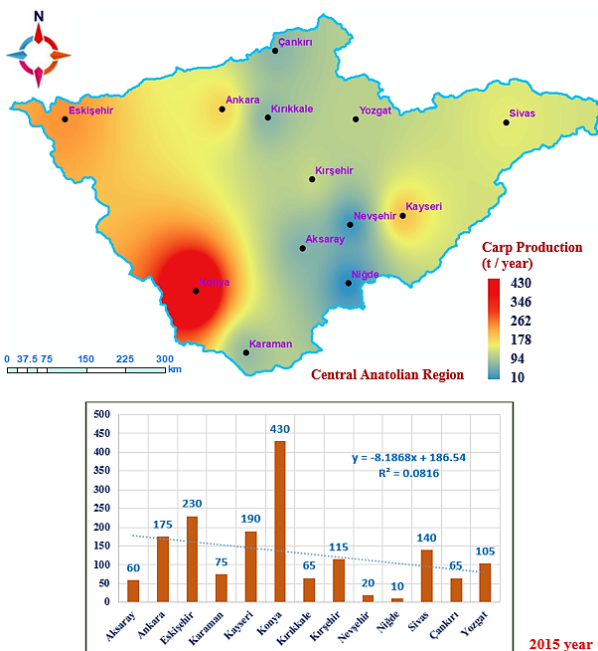


Figure 5. Spatial analysis of carp production in 2015

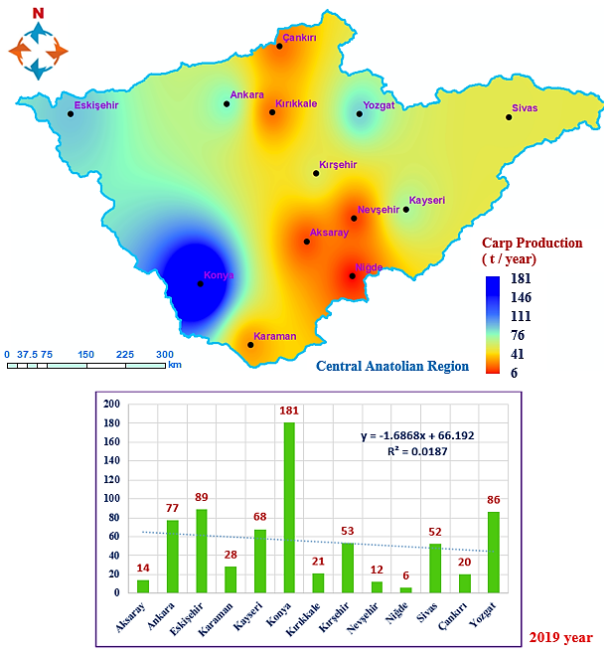


Figure 6. Spatial analysis of carp production in 2019

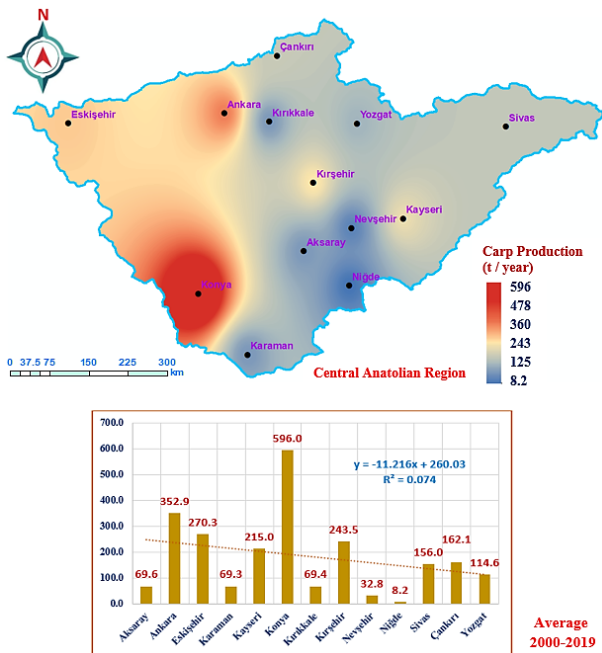


Figure 7. Spatial analysis of carp production in average of long periods (2000-2019)

In 2015 Aksaray, Ankara, Eskişehir, Karaman, Kayseri, Kırıkkale, Kırşehir, Yozgat, Çankırı, Sivas, Nevşehir carp production is 60 tons, 175 tons, 230 tons, 75 tons, 190 tons, 65 tons, 115 tons, 105 tons, 65 tons, 140 tons and 20 tons, respectively. The Niğde production is 10 tons with the lowest value and highest carp production was reported in Konya with a highest value of 430 tons. In maps we see the red places where the carp production is mostly conducted, which means that carp production is highly conducted around the west part of the research area as spatial, while the production is getting lower near the south part of the research area as spatial.

Aksaray carp production is 14 tons, Ankara production is 77 tons, Eskişehir production is 89 tons, Karaman production is 28 tons, Kayseri production is 68 tons, Kırıkkale production is 21 tons, Kırşehir production is 53 tons, Yozgat production is 86 tons, Çankırı production is 20 tons, Sivas production is 52 tons, Nevşehir carp production is 12 tons in 2019 (Figure 6).

The Niğde production is 6 tonnes with the lowest value, and the highest carp production was reported in Konya with a highest value of 181 tonnes. In maps we see the blue places where the carp production is mostly conducted, which means that carp production is highly conducted around the west part of the research area as spatial, while the production is getting lower near the south part of the research area as spatial.

In Figure 7 we can see graph shows the average carp production amount of each provinces for 20 years from 2000 to 2019 by doing spatial analysis. It shows Aksaray carp production is 69.6 tons, Ankara production is 352.9 tons, Eskişehir production is 270.3 tons, Karaman production is 69.3 tons, Kayseri production is 215 tons, Kırıkkale production is 69.4 tons, Kırşehir production is 243.5 tons, Yozgat production is 114.6 tons, Çankırı production is 162.1 tons, Sivas production is 156 tons, Nevşehir carp production is 32.8 tons. The average

production of all these years Niğde is 8.2 tons with the lowest value and highest average carp production was reported in Konya with a highest value of 596 tons. In maps we see the red places where the carp production is mostly conducted, which means that carp production is highly conducted around the west part of the research area as spatial, while the production is getting lower near the south part of the research area as spatial. Table 1 shows the total and average values of carp production in 2000-2019 of each city.

It was reported that the carp (*Cyprinus carpio*) has an important place in fishing activities in Türkiye and it is widely distributed in freshwater ecosystems including lake, river, pond and dam lake. There is suitable water conditions and carp mostly captured from fresh waters, however carp farming is quickly decreasing in Türkiye. According to previous literature carp production decreased from year 2003 to 2013, while there was only 146 tons carp cultured in 2013 (TÜİK, 2013). The production of aquaculture carp in Türkiye in 2004, 2008 and 2013 was reported as 683, 629 and 146 tons, respectively (TÜİK, 2013).

On other hand the total amount of captured and aquaculture production of common carp in Türkiye in 2003, 2007 and 2012 was 14363 tons, 12886 tons and 10195 tons/year, respectively. The world total carp population was 3.023.675 (2003), 2.887.591 (2007), 3.877.118 (2012) tons/year, respectively in these years. (FAO-FIGIS, 2014). According to previous study total carp production in the Eastern Anatolian Region is 28.790,8 tons and the highest production is conducted in Elazığ. It records for half of the total production in the East Anatolian Region. After Elazığ, production rate is going to declined in Malatya, Tunceli, Van, Erzincan, Erzurum, Muş, and Bitlis, while Iğdır, Ardahan, Kars, respectively and Bingöl have the lower most production rate (Akova, 2015). Overall production in the Central Anatolian Region is 19.015 tons with trout being the dominant species. Mirror carp is farmed only in Kırşehir and Yozgat as 11.2 tons and 27.2 tons, respectively. Kayseri production comprise more than half of the overall production in the Central Anatolian Region. After Kayseri carp production rate is going to declined in Sivas, Karaman, Kırşehir, Konya, Yozgat, Eskişehir, Ankara, Niğde and Çankırı, respectively. Overall production in the Southeastern Anatolian Region is 10.331,8 tons. Approximately half is cultured in Şanlıurfa. After Şanlıurfa production rate is going to declined in Diyarbakır, Gaziantep, Batman, and Adıyaman. Lower Production values were found in Hakkari, Mardin, Şırnak and Siirt (Akova, 2015).

Conclusions

It is concluded from this article that fisheries have remained an important industry from ancient times to date. As the global population increased captured fish is not enough to complete the requirements of protein in human. Therefore fish farming sector is established all over the world which is called aquaculture. Aquaculture include both farming in fresh water and farming in marine water. In this article we were focused on carp production in Central Anatolian Reigon of Türkiye.

Table 1. The average or total values and standard deviation of carp production in Central Anatolian Region

Provinces	Carp Production (tons/year) in 2000-2019			
	Total	Average	Standard Deviation	R ²
Aksaray	1391.0	69.6	23.661	0.6587
Ankara	7058.9	352.9	192.860	0.6409
Eskişehir	5406.4	270.3	91.697	0.6125
Karaman	1386.0	69.3	21.721	0.3053
Kayseri	4300.9	215.0	84.224	0.7866
Konya	11919.2	596.0	231.013	0.666
Kırıkkale	1387.0	69.4	25.516	0.3421
Kırşehir	4869.0	243.5	148.293	0.9138
Nevşehir	344.7	32.8	7.506	0.1826
Niğde	163.5	8.2	4.705	0.0834
Sivas	3119.1	156.0	48.606	0.584
Çankırı	3241.0	162.1	111.057	0.9463
Yozgat	2291.0	114.6	27.695	0.8116

GIS based-mapping and spatial analysis were used in this study to check the production rate of carp in Central Anatolian Region from year 2000 to 2019. It was concluded from this study that the highest production rate of carp is obtained in Konya province which is the west part of Anatolian region and the lowest production rate is found in the Niğde province which is the south part of the Anatolian region.

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Response Surface Methodology for Modelling and Optimizing Efficiency in Deep Well Pumping Systems

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ARTICLE INFO

ABSTRACT

Research Article

Received : 21.04.2024

Accepted : 28.06.2024

Keywords:

Deep Well Pumps
Response Surface Methodology
Pump System Efficiency
Optimization
Irrigation

This study presents research on modelling the efficiency and flow rate of deep well pumping facilities using the response surface method, evaluating the models, and assessing optimization based on target flow rate. Regression and variance analysis techniques have been successfully employed to evaluate the relationship between input factors (input pressure and power drawn from the grid) and responses (system efficiency and flow rate). ANOVA analysis has been used to examine the effects of linear and quadratic terms, and the results have shown that pressure and power drawn from the grid have a significant effect on pump system efficiency. Additionally, the performance of the regression models has been evaluated using error metrics such as R^2 value, RMSE, and MAPE. These values for the pumping facility system efficiency model were found to be 0.9993, 0.292%, and 0.71%, respectively, and for the flow rate model, they were 0.9997, 0.69 m^3h^{-1} , and 1.07%. The results obtained demonstrate that the model operates with high accuracy and explains a large part of the variance in the response variables. An optimization study was conducted to maximize pump system efficiency by maintaining the flow rate at a certain target value. According to the experimental results obtained, the target flow rate was predicted with an error rate of 1.49%, and the pump system efficiency was predicted with an error rate of 2.14%. This study highlights the effective use of various analytical and experimental methods to improve the efficiency of pump systems. Future researchers are encouraged to conduct similar analyses on a larger scale and under different operating conditions. Furthermore, evaluating different optimization strategies to improve the energy efficiency of pump systems, which can lead to significant energy savings in industrial applications, is recommended.

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Introduction

Deep well pumps are indispensable devices employed for the extraction of water and other fluids from significant depths, playing a crucial role in various industrial, agricultural, and environmental applications. These pumps are meticulously designed to function efficiently under challenging conditions, where the depth of the water source necessitates specialized extraction equipment. Deep well pumps are characterized by their ability to reach depths that exceed those achievable by standard pumps. They are suitable for applications where the water sources are deep underground (Michael & Voss, 2008). The design and performance of deep well pumps are critical in ensuring a reliable water supply and fluid extraction. Frequently exposed to high pressures and demanding operating conditions, these pumps require robust construction and efficient hydraulic performance. Optimizing the design of deep well pumps is essential to enhance their efficiency, durability, and overall performance (Gao et al., 2023).

The optimization of pump designs is of paramount importance in improving the efficiency and performance of various pump types. One effective method for achieving such optimization is the application of the Response Surface Method (RSM). RSM, a statistical technique, is utilized to model and analyze the relationship between input factors and output responses, thereby facilitating the identification of optimal operating conditions (Alawadhi et al., 2021). By integrating RSM with advanced simulation techniques such as 3D-RANS simulations, researchers can compute objective functions that are used to optimize pump geometries. This approach has proven particularly beneficial in designing centrifugal pumps for slurry transport and multistage pump impellers, with an emphasis on analyzing the relationship between structural parameters and performance (Alawadhi et al., 2021; Peng et al., 2021).

Moreover, the application of RSM in pump design has extended to the cooperative optimization of components such as impellers in multiphase pumps (Peng et al., 2021). By creating development of surrogate models based on numerical results using RSM, researchers have enhanced the hydraulic performance of high-speed magnetic drive pumps and achieved performance improvements in centrifugal slurry pumps (Abdolahnejad et al., 2022; Xu et al., 2022). Additionally, RSM has facilitated the optimization of sweep and blade lean for diffusers to suppress hub corner vortex in multistage pumps, demonstrating its versatility in addressing complex flow dynamics (Ning et al., 2021).

In conclusion, the integration of RSM in pump applications has proven to be a valuable tool for optimizing pump designs, improving performance, and reducing computational costs. By leveraging RSM alongside advanced simulation techniques, researchers have made significant advancements in pump technology, resulting in more efficient and reliable pump systems. However, while previous studies have predominantly focused on optimizing pump design parameters, there is a notable gap in research regarding the modeling and optimization of operational parameters in pumping plants (Alawadhi et al., 2021; Peng et al., 2021; Cheng et al., 2024). This study aims to fill this gap by modeling the efficiency of the pumping system and flow rate based on the pump outlet pressure and power drawn from the network using RSM. This approach is unique as it seeks to determine the most suitable operating conditions through regression analysis and optimization techniques, thereby enhancing the overall efficiency of pumping systems. This study distinguishes itself from existing literature by focusing not only on the design parameters but also on the operational parameters, providing a more comprehensive optimization strategy for pumping systems.

Materials and Methods

This study was carried out in the Deep Well Pump Test Tower (Figure 1) established within the scope of the

project numbered TUBITAK 213O140 at Selçuk University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering. The deep well was fed from the tank with 4" and 6" pipes from the top.

Gravel with a bulk density of 1.54 kg/m³ and a geometric diameter of 7-15 mm was placed around the 10cm wide well screen. In this way it has formed the environmental work of a deep well. Table 1 shows the technical specifications of the deep well submersible pump and submersible motor used in the present experiments. The technical specifications of the measuring instruments used in the present experiments are given in Table 2.

Software and an automated system were developed to record the measured values. A wireless communication card transmits the sensor data to a computer. The information stored in the central unit was entered into the software; under the operator's name, in the desired intervals. The recording process was designed to obtain data every second. Once 50 data points had been received from a sensor, recording was initiated after the pumping regime.

Flow rate (Q), outlet pressure (m), power drawn from the network (kW) and well drawdown (Δ) values were measured at 10 different valve openings at the optimum operating speed of the pump. Pump manometric head (H_m) value and pumping plant system efficiency (η) were calculated. All parameters were measured in triplicate at each valve opening. The EN ISO 9906 standard was used for the measurements and calculations of the pump operating characteristics.

Results and Discussion

General characteristics of the pumping plant

The average values of the general characteristic curves of the deep well pumping plant are presented in Figure 2. As the Q increased, the H_m value decreased, and the well drawdown level increased.

Table 1. Technical specifications for submersible pump and motor

Pump technical specifications	Pump	Submersible motor technical specifications	Submersible motor
Pump outside diameter (mm)	203.2	Brand	Watermot
Pump material (TSE EN 1591)	Cast iron	Volt (V)	380
Pump shaft material	Stainless steel	Amper (A)	13.6
Pump shaft diameter (mm)	30	Hz	50
Pump number of stages	1	rpm (l/min)	2780
Number of blades	6	Power (kW)	5.5
Blade thickness (mm)	5	Shaft diameter (mm)	25
Impeller outlet diameter (mm)	150	Cooling type	Water-cooler
Impeller outlet width (mm)	20	Cable cross-section	3x2.5 mm ²

Table 2. Technical specifications for measurement devices

Device	Technical specifications
Flow meter	S MAG 100 TİP, DN 125 flange connection electromagnetic flow meter, 220 V supplied digital indicator, instant flow, percent flow, total flow indicators. Adjustable 4-20 mA plus and frequency output. Measurement Range: 15-440 m ³ /h. Measurement error: $\pm 0.5\%$.
Manometer	WİKA, 0-10 bar, Bottom installed, 4-20 mA output. Accuracy $< \pm 1\%$
Water level meter	Hydrotechnik brand, 010 type/1,5 V, 150 m scaled cable, voice and light indicator type.
Temperature sensors	Turck brand, 10-24 VDC, -50...100 °C, 4-20mA output.
Computer	Asus intel core i7.

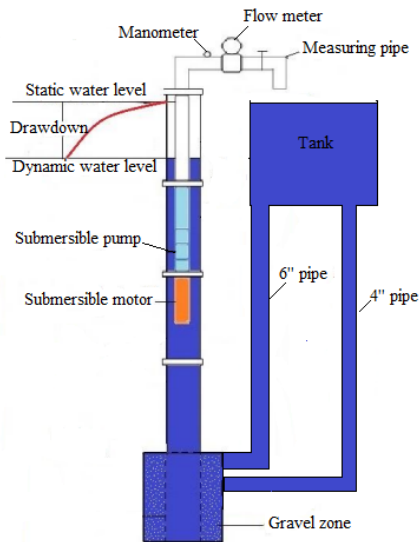


Figure 1. Deep Well Pump Test Tower

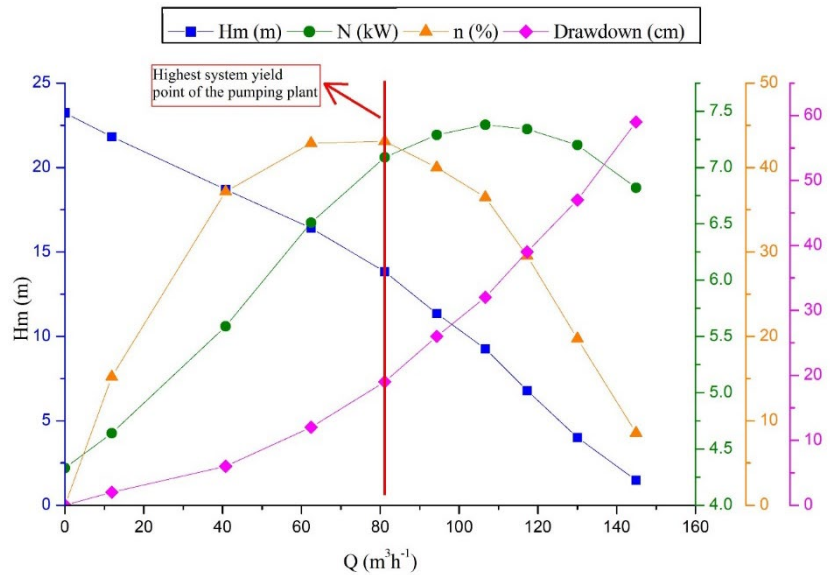


Figure 2. General characteristic curves of the pumping plant

Table 3. Experimental results related design table

Std Order	Run Order	Pt Type	Blocks	Pressure (bar)	Power (kW)	Pump system efficiently (%)	Flow (m ³ h ⁻¹)
11	1	0	1	2.05	4.66	15.21	11.88
9	2	0	1	2.02	4.62	15.29	11.88
2	3	1	1	1.51	6.51	42.87	62.45
8	4	-1	1	1.53	6.49	43.24	62.03
12	5	0	1	1.27	7.07	43.64	80.73
4	6	1	1	1.25	7.07	43.23	81.15
13	7	0	1	1.02	7.29	40.71	94.33
10	8	0	1	1.03	7.29	41.25	94.75
6	9	-1	1	0.79	7.38	36.47	106.65
3	10	1	1	0.77	7.38	35.24	105.38
1	11	1	1	0.77	7.38	35.38	105.80
5	12	-1	1	0.53	7.34	29.19	117.70
7	13	-1	1	0.53	7.34	29.19	117.70

Consequently, the power drawn from the network increased up to a flow rate of 108 m³ h⁻¹. At the maximum efficiency point of the pumping plant, which is 43%, the Hm is 13.83 m (1.27 bar), the power drawn from the network is 7.09 kW, and the well drawdown level is 19 cm. These results are consistent with the general characteristics of pumping plants. Çalışır (2010); Doğan and Yağmur (2023); Orhan et al. (2012) reported similar results in their studies. These results show that Hm decreases with increasing Q, which is a general characteristic of the pump, and the pumping system efficiency increases up to a certain flow rate level and then decreases.

RSM Application in Pumping Plant

As a computational application for modelling and optimization, RSM is becoming increasingly popular within engineering (Uslu and Celik, 2020). The basic aim of this method is to determine the effects of various input factors on response parameters and to optimize them. RSM evaluate the relationship between input and output parameters and, optimize responses based on the input parameters. Specifically, RSM use the least squares method to evaluate significant factors, to achieving the desired results and optimize the interaction between the

variables (Inayat et al., 2019; Kumar and Dinesha, 2018). This approach employs the least squares method to establish a relationship between input and output parameters, thereby optimizing the response based on the input factors.

In this study, RSM was employed to model pump system efficiency and flow as functions of outlet pressure and grid consumption across different pump system orifices. RSM begins by identifying an appropriate correlation between output and input parameters, utilizing a quadratic equation model for this correlation. The model is illustrated below (Uslu and Celik, 2020):

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \sum_{j \geq 1}^k \beta_{ij} x_i x_j + \sum_{i=1}^k \beta_{ii} x_i^2 + \varepsilon \quad (1)$$

Where β is regression constant, k is the number of input parameters, i is the linear measurable quantity, j is the quadratic factor and ε is the random error, X_1, X_2, \dots, X_k are the input parameters and Y is the response.

Table 3 presents the design matrix along with the experimental data. In the RSM model, pressure and power were selected as input variables, while pump system efficiency and flow rate were chosen as output variables.

Table 4. Variance analysis of pump system efficiently values

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1191.85	238.370	2231.2	0
Linear	2	225.27	112.637	1054.31	0
P	1	0.03	0.032	0.3	0.6
N	1	0.59	0.588	5.51	0.051
Square	2	1.97	0.985	9.22	0.011
P*P	1	1.85	1.848	17.29	0.004
N*N	1	1.01	1.014	9.49	0.018
2-Way Interaction	1	0.02	0.025	0.23	0.646
P*N	1	0.02	0.025	0.23	0.646
Error	7	0.75	0.107		
Lack-of-Fit	5	0.74	0.148	30.12	0.032
Pure Error	2	0.01	0.005		
Total	12	1192.6			

P: Pressure (bar), N: Power drawn from the mains (kW)

Table 5. Analysis of the variance of the flow rate values

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	15182.8	3036.57	5042.5	0
Linear	2	12288.3	6144.17	10203	0
P	1	6	5.95	9.89	0.016
N	1	3.6	3.61	5.99	0.044
Square	2	1.1	0.54	0.9	0.45
P*P	1	0.9	0.92	1.53	0.257
N*N	1	0	0.02	0.04	0.848
2-Way Interaction	1	0.2	0.15	0.26	0.628
P*N	1	0.2	0.15	0.26	0.628
Error	7	4.2	0.6		
Lack-of-Fit	5	4.1	0.83	18.72	0.051
Pure Error	2	0.1	0.04		
Total	12	15187.1			

P: Pressure (bar), N: Power drawn from the mains (kW)

ANOVA was utilized to identify significant relationships between input factors and responses. A higher F-value and a lower P-value indicate the greater importance of each term in the proposed model for the response, with a P-value accepted as significant at 0.05 (Dana et al., 2020). As shown in Table 4, for the linear coefficients, the P-value for all variables is greater than 0.05. However, regarding the quadratic coefficients, the P-values for pressure and power drawn from the mains are less than 0.05. This indicates that pressure and power drawn from the mains have a significant impact on the efficiency of the pumping system.

In Table 5, the p-values for the linear coefficients of the pressure variable and power drawn from the mains are less than 0.05. Conversely, for the quadratic coefficients, all variables have p-values greater than 0.05. This indicates that the squared coefficients of the pressure variable and the power drawn from the grid have a significant effect on the flow rate.

RSM produces squares to estimate pump system efficiency and flow parameters based on input parameters as shown in Eqs. (2) and (3).

$$\eta_{\text{system}} = -147.2 + 36.3P + 47.3N - 17.31P \times P - 3.57N \times N + 1.88P \times N \quad (2)$$

$$Q = 87 - 99.4P + 4.3N + 12.21P \times P + 0.55N \times N + 4.7P \times N \quad (3)$$

Where η_{system} is the system efficiency of the pumping plant, P is the pump outlet pressure, N is the power drawn from the grid and Q is the pump flow rate.

The model was assessed using the model the coefficient of determination R^2 value, root mean square error (RMSE), and mean absolute percentage error (MAPE). The R^2 value was employed to measure the performance of the model, as it indicates the percentage of variation in the response variable that can be explained by the independent variables (Shahhosseini et al., 2019). A stronger predictive relationship is indicated by a higher R^2 value. Conversely, RMSE and MAPE are used as error measures, with lower values indicating better model performance (Gültepe, 2019; Wang and Xu, 2004). The R^2 values of the pump system efficiency and flow rate models were calculated to be 0.9993 and 0.9997, respectively (Figure 3). The RMSE for the pump system efficiency (Figure 3a) and flow rate (Figure 3b) models were 0.29 (%) and 0.69 ($\text{m}^3 \text{h}^{-1}$), and the MAPE were 0.71 (%) and 1.07 (%), respectively. Considering these low error metrics, it can be concluded that the application of RSM to pump system efficiencies was highly successful.

Figure 4(a) presents the surface plot of pumping system efficiency as a function of pump pressure and power drawn from the grid. The efficiency of the pumping system increased up to a certain point with rising pump outlet pressure and power.

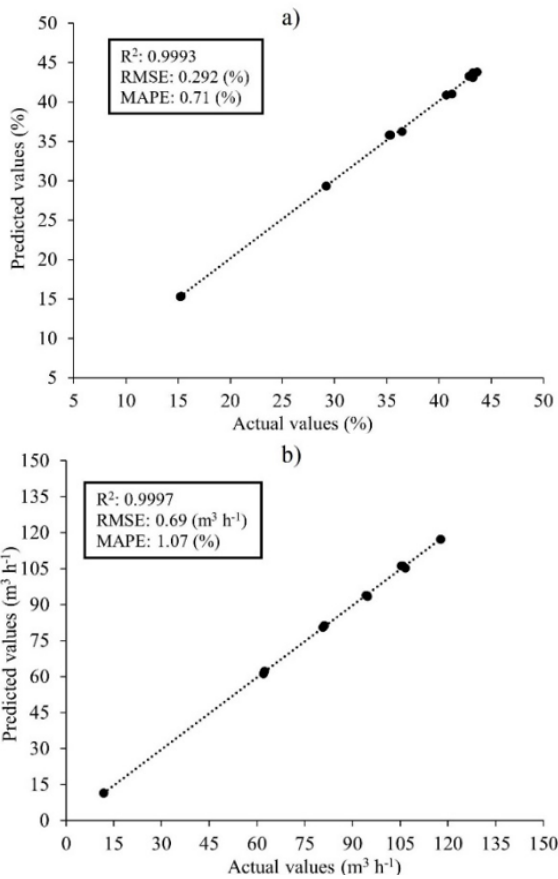


Figure 3. Comparison of actual and predicted values of pump system efficiency (a) and flow rate (b)

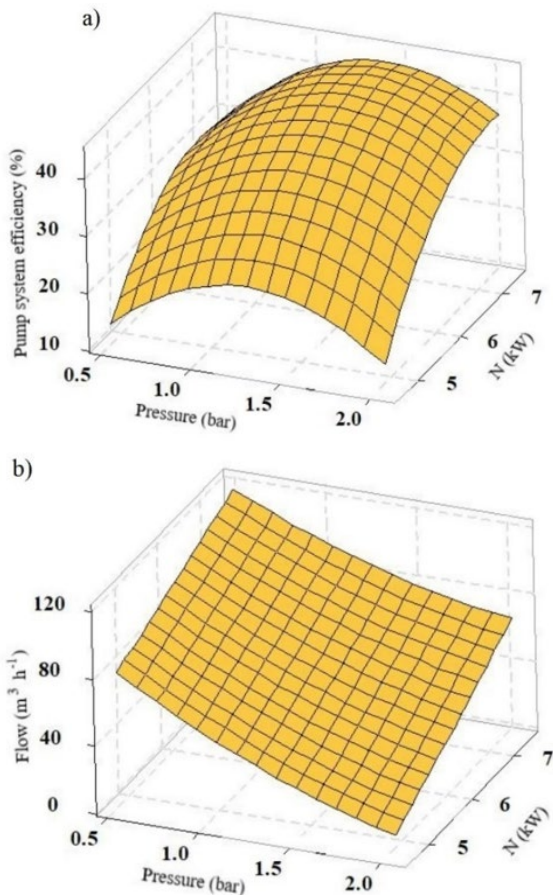


Figure 4. Effect of outlet pressure and power drawn from the mains on pump system efficiency (a) and flow (b)

Beyond this point, further increases resulted in a decrease in pump system efficiency. These trends, depicted in Figure 4(a) and Figure 4(b), are consistent with the general characteristics of pumps (Çalışır, 2010; Doğan and Yağmur, 2023; Korkmaz, 2015; Orhan et al., 2012).

The optimization graph is shown in Figure 5. In these graphs, a target value of $100 m^3 h^{-1}$ was set for the flow rate while maximizing the pump system efficiency. The desirability (D) value represents the effectiveness of the input factors in achieving the desired objectives in the optimization process. As the combined desirability approaches 1, it indicates a strong alignment between the input factors and the overall goals, thereby highlighting the success of the optimization process (Aydın et al., 2020). In this study, the combined desirability value was found to be 0.92. The optimal RSM setting, where the target flow rate is $100 m^3 h^{-1}$ and the pump system efficiency is maximized, corresponds to a pump outlet pressure of 1.09 bar and a power draw of 7.38 kW from the grid. In the experimental setup, the pump outlet pressure was set to 1.09 bar. The system was operated under these conditions, and the results are presented in Table 6. The average error values of the input parameters (P, N) and output parameters (Q, η), estimated by RSM and compared with the experimental results, were calculated as 0%, 1.89%, 1.49%, and 2.14%, respectively.

Conclusion

This study employed a variety of experimental and analytical methods to enhance the efficiency of a pumping system. Regression and analysis of variance (ANOVA) techniques were successfully utilized to assess the relationship between input variables and responses. ANOVA analysis examined the impacts of linear and quadratic terms, revealing significant effects of pressure and power drawn from the grid on pumping system efficiency. Furthermore, the performance of regression models was evaluated using R-squared (R^2), root mean square error (RMSE), and mean absolute percentage error (MAPE). The results demonstrated high accuracy of the models, with the independent variables explaining a substantial portion of the variance in response variables.

Optimization efforts aimed to maintain the flow rate at a specified target value to maximize pump system efficiency. According to experimental results, the estimated error rate for the target flow rate was 1.49%, and for pumping system efficiency, it was 2.14%.

This study demonstrates that a range of analytical and experimental methods can be used effectively to improve the efficiency of pumping systems. Future researchers recommend conducting similar analyzes on a larger scale and under different operating conditions. In addition, the evaluation of different optimization strategies to improve the energy efficiency of pumping systems can lead to significant energy savings in industrial applications. It is also recommended that the methods and findings used in this study be tested in a wider scope by applying them to studies on similar systems. In this way, more effective and optimized solutions for improving the efficiency of pumping systems can potentially be developed.

Table 6. RMS predicted and experimental results and error in both results

	Output variables		Input variables	
	Q (m ³ h ⁻¹)	η (%)	P (bar)	N (kW)
RSM-predicted results	92.74	41.47	1.09	7.38
Experimental results	94.15	40.58	1.09	7.18
Error (%)	1.49	2.14	0	1.89

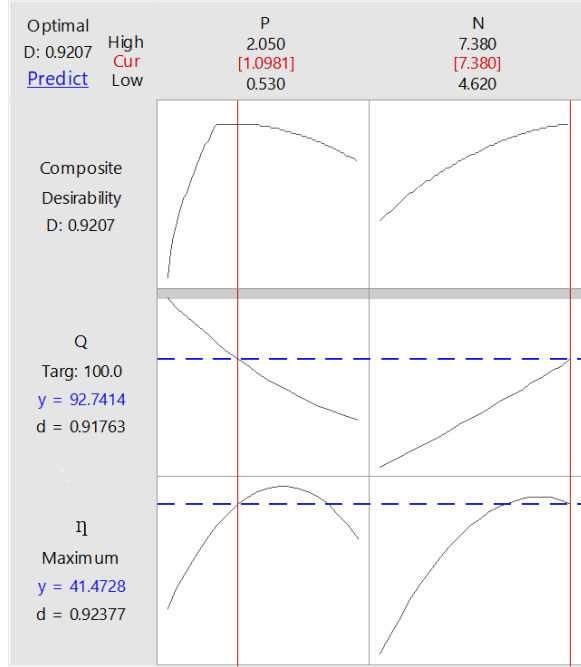


Figure 5. Optimization plot

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Haematological Indices and Fertility Potential of Rabbits Receiving Camels Foot (*Piliostigma thonningii*) Essential Oil Supplemented Diet

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ARTICLE INFO

ABSTRACT

Research Article

Received : 18.05.2024

Accepted : 05.08.2024

Keywords:

Bucks
Tropical phytogetic
Feed supplement
Blood
Semen

The current study aimed to evaluate *Piliostigma thonningii* seeds-derived essential oil (PTO) effect on hematological and reproductive parameters in rabbits. Three groups consisting of 15 animals each were randomly assigned and with an average initial body weight (BW) of 262.89 ± 22.36 g in a fully randomised experimental design. Group 1 received the control diet, while for groups 2 and 3 the basal control diet was supplemented with 2 mL PTO/kg diet and 4 mL PTO/kg diet, respectively. At the end of the experiment, blood samples were collected and the blood was analysed using the ABACUS ROSS haematology analyser. The results indicated significant differences in rabbits receiving PTO supplemented feed, namely; Packed cell volume, red blood cells, white blood cells, mean corpuscular haemoglobin concentration, neutrophil increased ($P < 0.05$) with increasing level of PEO supplementation. Mean corpuscular volume and haemoglobin, lymphocyte, monocyte and platelet were higher ($P < 0.05$) in T2 and T3 than in T1. Neutrophil/lymphocyte ratio was higher ($P < 0.05$) in T3 than T1 with T2 being intermediate ($P > 0.05$) between T1 and T3. Semen volume, concentration and motility were higher ($P < 0.05$) in T2 and T3 than in T1. While semen abnormalities and bucks' reaction time to does (libido) were greater ($P < 0.05$) in T1 than in T2 and T3, Live dead ratio was higher ($P < 0.05$) in T3 relative to T1 while T2 was intermediate between T1 and T3 ($P > 0.05$). semen color and pH were not affected ($P > 0.05$) by treatments. It was therefore concluded that *P. thonningii* essential oil supplementation enhanced both haematological and fertility potential of the experimental rabbits.

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Introduction

Rabbit farming in Africa, mainly Nigeria have been tremendously challenging due to several multitudinous problems, which have resulted to a gross insufficiency of animal products to meet up the increasing population challenge in the country (Anaso, 2023a).

In West Africa, precisely in Nigeria, the acute scarcity of meat due to farmer-herder crises, the past global pandemic effects and poor economic policies caused by corruption have adherently compelled livestock farmers to improve feed resources utilisation, health status and meat production from their cattle, goat and sheep (Anaso et al., 2021), however there still exist a shortage, which can be bridged by the farming of highly prolific monogastrates with short production cycles like rabbits.

Rabbits generally possess high fertility and rapid growth rates, thereby characterizing them an excellent source of meat and protein of animal origin (Anaso et al., 2024). Rabbit meat is characterized as high quality due to adequate animal protein and polyunsaturated fatty acids, and low calories (El-gogary et al., 2018).

Anaso (2023a and 2023b), described have essential oils to have potentially important aromatic characteristic of plant when harvested, extracted and isolated. *Piliostigma thonningii* Schum., or camel's foot Milne-Rech (Caesalpiniaceae) is a fairly little tree that grows crookedly and has a characteristic dark brown to black fissured bark.

It is commonly found in savannah areas. *Piliostigma thonningii* is widely accessible and has been used for many years by African and Nigerian traditional healers as a treatment for dermatosis and later malaria. Recent research has revealed that it also possesses normal flavouring, antioxidant, insecticidal, and antibacterial qualities Anaso (2023a). Ogbiko et al. (2021) reported no mortality at 2 g/kg p.o after 24 hours and showed no sign of delayed toxicity or mortality after 14 days of observation. Ajiboye et al. (2017) concluded from their findings that the seed oils of *P. thonningii* may be of significant importance medicinally and industrially, and could, therefore, also be used as a substitute for other oils or blended with other vegetable oils. Jimoh and Oladiji (2005) reported Seeds of *P. thonningii* were also found to be rich in protein (crude protein), carbohydrate, and several essential mineral elements. Mineral analysis of *P. thonningii* showed the seed to be an exceptionally excellent source of antioxidant micronutrients such as iron, calcium, selenium, zinc and manganese. The phytochemical investigation (screening) of the seed showed the presence of saponins, flavonoids, phenolics, glycosides, anthraquinones as well as cardiac glycosides while tannins, steroids, phylobatannins and triterpenes were absent (Anaso, 2023a). Elghalid et al. (2020) reported that administration of lower (LA) and higher doses (HA) of a newly developed mixture of herbal plants and spices enriched with special extracts and essential oils decreased blood cholesterol, triglycerides and

low-density lipoproteins; however, the LA treatment increased high-density lipoproteins and total antioxidant capacity but decreased malondialdehyde relative to the control treatment. The rabbits were fed a basal diet without additives (Control rabbits) or supplemented with 0.5 mL (LA) or 1mL (HA) of the additives mixture per litre of drinking water.

Anaso (2023b) presented in Table 1 showing the bioactive ingredients.

Anaso (2023b) explains that Beta pinene was the most abundant and are well-known representatives of the monoterpenes group, and are found in many plants' essential oils. A wide range of pharmacological activities of β -pinene have been reported to include antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-*Leishmania* and analgesic effects (Anaso 2023b; Bahare et al., 2019). Pandi et al. (2021) reported limonene as monoterpenes which possesses antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune modulatory, anti-fibrotic and anti-genotoxic properties. Gamma terpineol, hexenyl acetate, fenchol and camhene have functions similar to the flavouring and aroma characteristic of the β -pinene (Anaso, 2023b). Similarly, n-nonanol has relative flavouring characteristic but can be harmful to internal body organs when large doses are administered and ingested.

Table 1. Bioactive compounds of *Piliostigma thonningii* essential oil detected by the gas chromatography-mass spectrometry

Compounds	Percentage area	Mass peak
Azulene	0.42	25, 40, 42, 57
Beta myrcene	6.05	25, 72, 102, 106, 110
Beta limonene	17.85	25, 41, 43, 57
Cis- linaloxide	0.21	25, 39, 47, 49, 55
Alpha cymene	0.15	25, 44, 48
Beta ocimene	0.06	25, 88, 106
Trans beta ocimene	0.30	25, 39, 66, 70
4-methylpropiohenone	0.12	25, 88, 106, 109
1,3 dimethyl-4-Isopropylbenzene	3.11	25, 48, 51, 58, 69
Dodecane	0.19	25, 41, 43
Tridecane	1.20	60, 68, 77, 103
2,3-dimethylnepthalene	0.71	25, 44, 68, 92
1,8-dimethylnepthalene	0.22	25, 81, 87, 102, 117
Alpha thujene	0.19	25, 29, 38, 44
Beta pinene	25.02	25, 62, 68, 100
Terpinen-4-ol	2.60	25, 42, 47, 58
Alpha terpineol	3.70	25, 88, 93, 106
Gamma terpineol	5.11	25, 71, 79, 100
Alpha cubebene	2.09	25, 63, 69
Alpha yiangene	1.02	25, 41, 55, 108, 127
Alpha selinene	1.10	25, 40, 48, 51
Chrysathenine	0.08	25, 47, 57, 63
Alpha allyltoluene	0.29	25, 39, 47, 58
Borneol	1.44	25, 33, 39
Hexenyl acetate	2.83	25, 28, 37, 108
Camhene	1.50	25, 63, 68, 101
n-Nonanol	0.04	25, 69, 104
Trans-pinocarveol	0.62	25, 27, 29, 41, 55, 67
Cis pinocarveol	1.77	25, 41, 55, 67, 81, 104
Fenchol	0.96	25, 41, 58, 138
Alpha murolene	9.63	25, 39, 51, 105, 109

Trans-pinocarveol and Cis-pinocarveol are harmful when ingested in large quantity (Anaso, 2023b). Similarly, Alpha cubebene, alpha yiangene, alpha selinene, chrysanthenine, alpha allytoluene and alpha muurolene are generally sesquiterpene hydrocarbons exhibiting antifungal, antioxidant and antiinsecticidal properties and may be harmful in large quantity (SCLabs, 2016).

The current epidemic of antimicrobial resistance challenge and subsequent ban on antibiotic growth promoters by several countries have compelled the search for alternatives of improving animal productivity and minimizing adverse effects on human consumers. Due to this ban, a great deal of study has been done to explore the use of phytonics as alternate feed additives in animal nutrition Anaso (2023a).

There is no information on the hematological and fertility potential of rabbits supplemented PEO. The objectives of the present research were, therefore, to: 1) Evaluate the haematological parameters and 2) Determine the fertility potentials of rabbits fed PEO supplemented diet

Materials And Methods

Ethical Approval

Ethical approval was granted by the Animal Ethics and Conduct Board of the Department of Animal Science University of Abuja, Nigeria following the presentation of the research proposal on the 17th Day of November 2022, with approval registration number 19/501/ANSJ/002. The approval granted before the thesis proceeded and was strictly adhered to and scrutinized to conform with international standards for conducting research on rabbits, acknowledging the current international recommendations on the rational use of antibiotics.

Assemblage of *P. thonningii* Seeds and Subsequent Essential Oil Extraction

Piliostigma thonningii seeds were carefully obtained from the University of Abuja's surroundings and they were later verified by a licensed taxonomist at the Department of Biological Science at the Forestry Research Institute of Nigeria.

The Clevenger apparatus was used to extract the essential oil in accordance with Anaso's (2023b) and Mohamed et al. (2006) technique. The *P. thonningii* seeds were carefully ground, dried in enough shade, and kept at room temperature until they were needed again (extraction). Laboratory procedure involved placing the ground seed sample in a steel apparatus and allowing it to soften and produce the essential oil forms by intermittently heating up after connecting the condenser to a water inlet and outlet, precisely 100 g of dried ground sample was suspended in precisely 700 ml of distilled water using distillation process at about 100°C for three hours. The resultant essential oil droplets were congregated in a cooling system after prior mixing with steam and passage through the carrier.

Experimental Site

The study was conducted at the University of Abuja Teaching and Research Farm's Monogastric Unit, which is situated in Giri inside the Gwagwalada Area Council in the Federal Capital Territory of Abuja, Nigeria. Rabbits were housed in individual open sided metabolic hutches which can separate faeces from urine.

Experimental Animals, Management and Treatment

For the experiment, 45 clinically certified healthy weaned male Dutch rabbits weighing an average of 262.89 ± 22.36 g were used, they were about five weeks old. A reliable source (The National Animal Production Research Institute at Ahmadu Bello University in Zaria, Nigeria), is where the rabbits were bought. Two weeks prior to the arrival of the rabbits, the hutches and Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and antiseptic (Morigad) were used to sterilize and disinfect the area around them. The animals received preventative medication and were placed in quarantine for exactly two weeks. The prophylactic measures comprised injecting a subcutaneous dose of an anti-parasitic medication (Avomec®) at 0.5 mg/kg of the animal body weight (BW) to control endo and ecto parasites, administering an anti-stress drink (Vitalyte®), and administering a parenterally administered intramuscular injection of the broad-spectrum antibiotic oxytetracycline HCl at 1.0 mL/10 kg BW. Additionally, at the beginning of the trial, rabbits received a single subcutaneous treatment with coccidiostat (Sulphadimidine Sodium BP solution) at a dose of 1 mL/rabbit per manufacturer's advice.

Daily sanitation of the intermittent hutches was carried out with a strong disinfectant. With precisely fifteen rabbits in each group, the rabbits were systematically divided into three groups. After balancing for body weight (BW), the rabbits in each group were assigned to one of three treatments in a completely randomised manner, with their beginning BWs being numerically equal.

Based on the guidelines provided by the NRC (1984), a base control diet was formulated for growing rabbits. For a duration of 12 weeks, water and feed were given freely, with feeding occurring twice a day at 8:00 and 16:00. In the initial treatment, a baseline control food was given to the rabbits. In the other treatments, two and four milliliters of PEO per kilogram of the control food were added.

Blood Collection and Analyses

Blood samples were taken from each treatment's individual rabbits on the last day of the trial. Before the rabbits in each treatment group had access to food and water in the morning, blood samples were drawn from the marginal veins in their ears. After being collected into multiple 5 ml vacuum tubes and chilled using ice packs, the blood samples were promptly transported to the laboratory for examination. Four hours after collection, a haematological analysis was conducted utilizing an ABACUS ROSS haematology analyzer (Model 212, Indian). The packed cell volume (PCV), hemoglobin concentration, red blood cell (RBC), white blood cell (WBC), and their differentials were all determined using the entire count. The following values were obtained: mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) using the following formulas;

$$\text{MCV} = \text{PCV} \times 10/\text{RBC},$$

$$\text{MCH} = \text{Hb} \times 10/\text{RBC}, \text{ and}$$

$$\text{MCHC} = \text{Hb} \times 100/\text{PCV} \text{ as described by Jain (1986).}$$

Semen Collection and Characteristics, and Libido Test

An improvised artificial vagina (AV) filled with a warm water at about 45°C was used in collecting semen from bucks, as the doe was fitted with the AV and presented to bucks. Samples that were collected were examined immediately for semen volume via of millimetre direct reading, expressed in millilitre (mL). The samples were then kept in water bath at 37°C, and evaluations were made in sequence according to Colegio Brasileiro de Reprodução (CBRA, 1998) animal manual. The appearance of the semen (color) was determined by visualization of consistence of ejaculates and classified as: creamy marble, creamy, thick milky, milky and watered. Smear of each sample of semen was prepared, allowed to air dry, labeled, and stored for additional analysis.

As soon as the sample was taken, live and dead sperm were identified by making a smear of the corresponding sample from each replicate using the eosin-nigrosin stain. A drop of individual semen sample was placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosin solution was then placed alongside the drop of semen on the slide. A gentle circular mixing (turning) of the slide was done to allow a uniform mixture of the two samples. One-quarter of the part of another clean slide was held in place, on top of the first sample on the slide at a 45-degree angle, to make contact with the semen sample slide and carefully drawn apart to prepare and produce a thin smear. This was then left and allowed to dry and thereafter respectively labelled. This process was carried out on separate samples. After that, the slides were finally mounted unto the microscope to count the live and dead sperm cells. The principle of the stain and procedure was that the dead sperm cells accepted the stain and appeared stained while the live sperm cells rejected the stain and remain unstained. The procedure above was developed by Hancock (1951) and modified by Anaso et al. (2024).

Libido/male reaction time to female was determined by exposing bucks to estrogenized doe in enclosed area. A stop watch was adopted to take the time for mounting without intromission and ejaculation, and duly recorded as described by Angel-Gracia et al. (2015).

Statistical Analyses

Data on haematological parameters and semen characteristics were subjected to analysis of variance in a completely randomized design using the SPSS (23.0). The

same software's Duncan multiple range test was performed to assess the significance of the mean difference at the $P \leq 0.05$ level.

The statistical model is shown below

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where:

Y_{ij} = the overall response to the specific parameter under investigation,

μ = the general mean peculiar to each observation,

t_{ij} = the fixed effect of the dietary treatments ($i = 3$) on the observed parameters and

e_{ij} = the random error term for each estimate

Results

Haematological Parameters of Rabbits Fed Piliostigma Thonningii Essential Oil Supplemented Diet

Table 2 shows the haematological parameters of the rabbits fed PEO supplemented diet. PCV varied from 38.00 to 49.95%, Hb from 13.13 to 18.50 g/dL, RBC from 11.87 to 17.52 $\times 10^6/L$, WBC from 5.60 to 8.77 $\times 10^9/L$, MCHC from 29.80 to 35.91% and neutrophil from 22.45 to 32.78% among treatment groups in the order: T3 > T2 > T1 ($P < 0.05$). MCV, MCH, lymphocytes, monocytes and platelets were 58.02, 64.02 and 65.12 fl; 17.46, 22.45 and 23.67 pg, 49.80, 70.40 and 73.07%; 1.28, 2.40 and 2.72% and 340.16, 530.74 and 580.11 $\times 10^3/uL$ for T1, T2 and T3 respectively with T1 having lower values ($P < 0.05$) than T2 and T3, which had similar values ($P > 0.05$). Eosinophil varied from 1.36 to 2.22% and was higher ($P < 0.05$) in T3 than in T1 and T2, which were similar ($P > 0.05$). Neutrophil/lymphocyte ratio (0.41 - 0.45) was higher ($P < 0.05$) in T3 than and T1 but was similar ($P > 0.05$) between T1 and T2, and T2 and T3.

Semen Characteristics of Rabbits Fed Piliostigma thonningii Essential Oil Supplemented Diet

Table 3 shows the semen parameters of rabbits fed PEO supplemented diet. The semen colour was similar (creamy) across treatments (T1, T2 and T3). Semen pH was 6.00 for each of the treatments and was not affected by treatments. Ejaculatory volume varied from 0.53 to 1.08 ml, sperm motility from 68.33 to 86.00 % and semen concentration varied from 222.20 to 321.80 ($\times 10^6$), presenting lower ($P < 0.05$) values in T1 compared to T2 and T3 which were similar ($P > 0.05$).

Table 2. Haematological parameters of rabbits fed Piliostigma thonningii essential oil supplemented diet

Parameter	T1	T2	T3	SEM	RV
Packed cell volume (%)	38.00 ^c	45.25 ^b	49.95 ^a	0.96	33-50
Haemoglobin concentration (g/dL)	13.13 ^c	16.57 ^b	18.50 ^a	0.48	13-18
Red blood cell count ($10^6/L$)	11.87 ^c	14.72 ^b	17.52 ^a	0.53	11-18
White blood cell count ($10^9/L$)	5.60 ^c	7.55 ^b	8.77 ^a	0.23	5-12.5
Mean corpuscular volume (fl)	58.02 ^b	64.02 ^a	65.12 ^a	2.03	58-67
Mean corpuscular haemoglobin (pg)	17.47 ^b	22.45 ^a	23.67 ^a	0.58	17-24
MCHC (%)	29.80 ^c	33.57 ^b	35.91 ^a	0.63	29-37
Lymphocyte (%)	49.80 ^b	70.40 ^a	73.07 ^a	1.34	30-85
Monocyte (%)	1.28 ^b	2.40 ^a	2.72 ^a	0.27	1-4
Neutrophil (%)	22.45 ^c	29.16 ^b	32.78 ^a	0.90	20-75
Eosinophil (%)	1.36 ^b	1.60 ^b	2.22 ^a	0.17	1-4
Platelet ($10^3/uL$)	340.16 ^b	530.74 ^a	580.11 ^a	26.1	250-650
Neutrophil:lymphocyte ratio	0.44 ^b	0.41 ^{ab}	0.45 ^a	0.01	0.34-2.64

^{abc} means along the row with the various superscripts are significantly ($P < 0.05$) different; T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet. RV: reference values as stated Beers (2006).

Table 3. Semen characteristics of rabbits fed *Piliostigma thonningii* essential oil supplemented diet

Parameter	T1	T2	T3	SEM
Semen colour	Cream	Cream	Cream	
pH	6.00	6.00	6.00	0.01
Semen volume (ml)	0.53 ^b	0.83 ^a	1.08 ^a	0.12
Motility (%)	58.33 ^b	70.00 ^a	76.00 ^a	6.11
Semen concentration (x10 ⁶)	222.20 ^b	290.60 ^a	321.80 ^a	27.26
Abnormalities (%)	19.52 ^a	11.83 ^b	9.95 ^b	1.62
Live dead ratio	1.50 ^b	2.53 ^{ab}	3.29 ^a	0.58
Libido/reaction time (secs)	20.33 ^a	12.33 ^b	11.67 ^b	2.31

^{abc} Means with the different superscripts along the row are significantly ($P < 0.05$) different; T1, 0 ml *P. thonningii* essential oil; T2, 2 ml *P. thonningii* essential oil; T3, 4 ml *P. thonningii* essential oil/kg diet.

Sperm live cell (live spermatozoa) varied between 60.00 to 76.67% and live dead ratio (1.50 to 3.29) with T3 higher and T1 lower ($P < 0.05$) but was similar ($P > 0.05$) between T1 and T2 and T2 and T3. Contrarily, dead sperm cell (23.33 to 40.00%) varied with T3 lower and T1 higher ($P < 0.05$) but was similar ($P > 0.05$) between T1 and T2 and T2 and T3. Reaction time from 11.67 to 20.33 secs and sperm abnormalities 9.95 to 19.52% presented higher ($P < 0.05$) values in T1 compared to T2 and T3 which were similar ($P > 0.05$).

Discussions

Haematological Parameters of Rabbits Fed Piliostigma thonningii Essential Oil Supplemented Diet

Haematological parameters are an index and are a reflection of the dietary effects on an animal with respect to the quality of the diet consumed and the nutrient supplied to satisfy the physiological requirements of the animal. It is important to note that the study's experimental animals, particularly those that were fed *P. thonningii* EO based diet, did not show apparent clinical signs of ill health. The absence of signs and symptoms of ill health, morbidity and mortality in the animals suggests the used doses of *P. thonningii* EO in the current study were not toxic. Mahmoud et al. (2016) also illustrated and concluded that thyme extract and other dietary essential oils were not toxic to experimental animals. Ogbiko et al. (2021) reports *Piliostigma thonningii* leaf extract to be orally safe up to a dose of 2 g/kg body weight.

In general results obtained for PCV, HB, RBC, MCHC, WBC, neutrophils and eosinophils were highest in treatment groups supplemented with 4 ml PEO. Highest WBC, RBC, Hb, and lymphocytes were similar to work of Bassiony et al. (2015) who reported cinnamaldehyde and thymol to significantly improve these parameters.

One significant indicator of anemia is PCV. The values obtained for PCV were within the stipulated range 33-50% for clinically healthy rabbits (Beers, 2006). The higher PCV values for the T2 and T3 indicate that PEO supplementation at 2 and 4 ml/kg diet enabled provision of quality protein to the rabbits, as PCV is useful in assessing protein status of a fed diet (Ayoade et al., 2015). PEO enhanced the PCV suggesting improved protein utilization, possibly due to enhanced protein intake and digestibility. The normal PCV values for all the treatments which were stipulated range, is suggestive of the absence of toxic factors such haemagglutinin which adversely affects blood formation (Ayoade et al., 2015). The higher value of T3 than T2 implies that PEO at 4ml/kg diet enhanced the

rabbit's PCV compared to T2. This further justifies the non-toxicity of the high PEO.

Haemoglobin concentration and PCV are also indicative of adaptation to adverse situations in form of oxidative stress conditions. Low hemoglobin levels are a sign that not enough red blood cells are supplying oxygen to the body's peripheral tissues. The significantly higher levels of Hb in T2 and T3, although within the stipulated range of 13-18 g/dL for healthy rabbits is, therefore, indicative of antioxidative property of PEO ensuring reduced oxidative stress. The result also indicates better iron utilisation for the formation of haemoglobin by the PEO supplemented diets. However, T3 enhanced iron utilisation than T2, indicating superiority of PEO at 4ml/kg diet. There is a direct correlation between PCV and Hb, as seen by the comparable trends that both test results showed (Olafadehan, 2011).

The MCHC, MCH, and MCV values are comparable to those disclosed by Bassiony et al. (2015) and were within the physiological ranges stated in Beers (2006) for healthy growing rabbits. The normal values in the three treatments further confirm the absence of anaemia, particularly a hypochromic microcytic type (Olafadehan et al., 2014). The MCV values within stipulated reference range implied that the animals did not stand the risk of haemoconcentration and anaemia (Brian, 2009). The higher MCH values in the animals fed PEO supplemented diet show enhanced oxygen carrying capacity of the RBC because MCH is an indicator of the oxygen carrying capacity of the RBC (Brain, 2009). Changes in total RBC count and its indices (MCV and MCHC) are of value in determining the physiological effect of PEO to the experimental animals' health and well-being.

The higher WBC, lymphocytes and neutrophils values in treatments (2 and 4ml PEO) are indicative of improved activation of the body defense and immunity to infections or toxic substances than in the control. The absence of overwhelming disease in the rabbits throughout the feeding period showed improved gut associated lymphoid tissue development, this tissue development may have been possibly due to the decrease in the pathogenic bacteria as a result of bioactive components leading to a change in the microbial ecology, which favours, beneficial microbial species in the gut (Anaso et al., 2023; Ismail and Bealish, 2014). This could be linked to the increased count of beneficial Lactobacilli in the caecum. WBC counts are in agreement with the values reported by Bassiony et al. (2015). The WBC counts also fell within the stipulated range of 5-12.5 $10^3/\mu\text{l}$ for healthy rabbits as stipulated by Beers (2006) Merck's Veterinary Manual.

The high lymphocytes and platelets values in T2 and T3 imply that the rabbits had a better potential to ward off invading disease-causing organisms. According to the Beers (2006) Merck's Veterinary Manual, lymphocytes are a variety of WBC that directly attack disease-causing bacteria, viruses and toxins and regulate the other part of the immune system as well as produce antibodies, which neutralize invaders or mark them for destruction by other agents of the immune system. According to Mahgoub et al. (2009), lymphocytes are necessary for humoral and cell-mediated immune responses. Jelalu (2014) explained that differential leukocytes are responsible for the defence of an organism and depressed levels of lymphocytes indicate a depleted immune system while an elevated level indicates an active infection.

The eosinophil content was within reference values in the Beers Merck's Veterinary Manual (2006) for clinically healthy rabbits. The higher eosinophil count in rabbits supplemented 4 ml of the EO suggests a more induced immunological and cytotoxic processes (Chattopadhyay et al., 2007).

Since monocytes are the progenitors of macrophages, they are vital to the animal immune system. The values obtained were in line with 1 to 4% reported by Merck's Veterinary Manual (2006) for rabbits. The normal monocyte value indicates the critical role played in tissue development and homeostasis is performed at the cellular system. However, the higher monocytes values in T2 and T3 imply that they were equally equipped to identify harmful bacteria, viruses and toxins, attack foreign substances and provided all that were needed for rabbit's health, survival and enhanced performance.

It can be concluded that *P. thoningii* EO can be added to grower rabbits' diets as a supplement to improve their haematological indices without endangering their health or causing intoxication, as all the studied haematological parameters were within the normal ranges for healthy rabbits and they did not exhibit any clinical signs or symptoms of illness.

Semen Characteristics of Rabbits Fed Piliostigma thoningii Essential Oil Supplemented Diet

According to studies by Anaso et al. (2024), Anaso et al. (2023) and Ososanyo et al. (2013), who noted a creamy colour characteristic for domestic animals, the experimental rabbits' semen had a similar colour. High-quality semen has a creamy white appearance. Low concentration is indicated by translucent semen, while contamination or poor quality is indicated by blood stains and strange colour. Thus, the similar semen colour of all the rabbits irrespective of treatment indicates good quality semen. This further indicates that PEO could be supplemented up to 4ml/kg diet without compromising the semen quality.

Semen pH is crucial factor affecting sperm motility and viability in term of its ability to fertilize an egg (Zhou et al., 2015). The pH difference in the semen was not statistically significant, which was in line with the findings of Abdel-Wareth and Metwally (2021) who found no significant variation in the pH of the semen from animals fed thyme essential oil (TEO) in comparison to the control group. The pH value in rabbit is between 6.00-6.33 which is slightly acidic (Abdel-Wareth and Metwally, 2020). According to Zhou et al. (2015), abnormal pH values above

the range may indicate an underlying infection It might therefore have an impact on its viability.

El-Gindy et al. (2020) observed improved semen characteristics, including higher volume, motility, concentration, and fewer abnormalities, after feeding potato peel extract, a strong antioxidant, to growing rabbits. The study conducted by Abdel-Wareth and Metwally (2020) revealed a noteworthy enhancement in the semen characteristics of the treatment groups of growing rabbits that were administered dietary thyme essential oil and phytogetic supplement. Reduced ejaculate volume cases may result from season, collectors' factor, pooled volume, collecting frequency, or management (Anaso et al., 2023). They may also result from poor nutrition. Therefore, the oxidative and antibacterial activities of the bioactive chemicals in PEO may be the cause of a slight change in semen volume.

Sperm motility is connected to sperm viability, which is a measure of sperm count that may be high or low, claims Osinowo (2016). In general, motile cells are always viable, and determining whether non-motile cells are alive or dead depends on their viability. According to Osinowo (2016), sperm motility above 65% is deemed good. Strong, progressive motility, which is frequently observed as swirling, wave-like motions in highly concentrated ejaculates, is an important indicator of the survivability of the sperm.

Elevated feed intake is directly correlated with high semen concentration, which can also be caused by variations in temperature and frequency of ejaculation (Anaso et al., 2024). PEO supplementation's strong antioxidant activities of beta-pinene and caryophyllene may therefore be responsible for the enhanced delivery of nutrients for the spermatogenic process and higher sperm concentration in T2 and T3. Comparable to the present results, El-Ratel et al. (2021) observed an improvement in the progressive motility, vitality, sperm cell concentration, sperm outputs, and fertility in developing rabbits fed extra virgin olive oil (EVOO), betaine (BET), and ginger (GIN) as natural antioxidants.

Higher libido score in animals supplemented PEO proves the resultant effect of the bioactive constituent (components) of the essential oil, mainly in terms of its antioxidative property. The libido score is a function of the reaction time, higher libido is inversely proportional to lower reaction time. Libido test is becoming a common practice in breeding soundness examination for domestic animals, compared to other livestock species, increasing rabbits' sexual performance is currently the subject of more research and interest. Generally, libido (sex drive) is an important component of male fertility. It is independent on gonadal and extragonadal sperm reserves, semen quality, BW, growth rate or masculinity.

Conclusion

P. thoningii essential oil supplementation improved the haematological parameters and reproductive potentials under the circumstances of the current findings, suggesting a beneficial influence and thus encouraging its usage in other domestic animals. The study established 4 ml *P. thoningii* essential oil supplementation per kg of basal diet as the optimum dose for the rabbits due to their improved physiological response and reproductive potentials.

Declarations

The author declares no conflict of interest in the design, collection, writing of manuscript and decision to publish this work

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Efficacy of The Essential Oil of *Coriandrum sativum* against *Sitophilus oryzae* (Coleoptera:Curculionidae)

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ARTICLE INFO

ABSTRACT

Research Article

Received : 28.05.2024

Accepted : 17.07.2024

Keywords:

Fumigant activity

Storage pests

Biodegradable

Coriander

Türkiye

Recently, there has been a great interest in the use of natural products of plant origin due to the side effects of synthetic substances. Since synthetic chemicals used in the agricultural field a great threat to the environment and public health, many studies are carried out on the use of natural products from production to storage. Given the environmental and public health risks associated with synthetic chemicals commonly used in agriculture, extensive research efforts are focused on exploring the utilization of natural products throughout the entire agricultural process, from production to storage. The efficacy of *Coriandrum sativum* L. seed essential oil (Cs-EO) was evaluated in the laboratory conditions against the rice weevil-*Sitophilus oryzae* L. adults. The Cs-EO essential oils were applied at four different dose rates (3%, 6%, 9% and 12%) on wheat and fumigant toxicity assay was recorded dead adults after 3, 5, 7, 9 and 11th days. In the 12%, the highest mortality 87.86 % and the lowest mortality at concentration of 3%, 23.28% and of the eleven days. In this study, GC-MS analysis of *C. sativum* was also determined at the same time. Cs essential oil was generated by steam distillation and compounds were identified by GC-MS analysis. GC-MS analysis of EOs from Cs seeds showed it to be reach linalool 79.12%. This study suggest that essential oil of Cs (Cs- EOs) can be used as alternatives to pesticides for management of stored pest control.

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Introduction

Pest and postharvest insect damage and residues cause losses to grain in storage for farmers. The huge amount of agricultural production is provided by destroying harmful grasses and insects. Storage pests degrade the quality and quantity of grains such as corn, rice, and wheat, and are a global issue (Jayakumar et al., 2017; Stejskal, et. al. 2010). To keep the grains from being harmed during storage, the right temperatures and preservatives must be utilized. The synthetic chemicals-called pesticides are used for this purpose, but pesticides have harmful effects on the environment and public health as well as increasing crop production (Jayakumar et al., 2017). From this point of view, there is an urgent need for effective, environmentally friendly, biodegradable, non-toxic control tools for storage pest. Many natural pesticides (from plants) are used as an alternative to synthetic pesticides, since they do not cause environmental pollution. Different plant derived compounds including plant extract, and essential oils (EOs) have been suggested for stored-grain pests in recent studies (Nenaah, 2014; Al-Harbi, 2021).

Because of their wide range of bioactivity, contact and fumigant toxicity, repellent, oviposition, and feeding deterrent qualities, plant origin pesticides have been investigated as potential pest management agents. Fumigation is a process, and the most frequent ingredients are methyl bromide and phosphine gas (Nenaah, 2014). Fumigants work by disrupting both the neurological and respiratory systems of insects, causing them to die (de Araújo, 2019). Therefore study into the use of plant-derived molecules, has become more important. Hence, investigations into the utilization of plant-derived molecules have assumed heightened significance.

Rice weevil (*L.*) is one of the most destructive pests of stored grain. *Sitophilus oryzae*, the adults of which are 2mm long and brown/black in color, pierce the stored grain and damage it by feeding from the inside (Koutsaviti; 2018). Several researchers have described in vivo/vitro application of EOs as repellent, ovicidal, insecticidal and fumigant activity in the control of *S. oryzae*. Plant essential oils have been documented to exhibit fumigant and repellent activities against *S. oryzae* (Al-Harbi, 2021; de

Araújo et al., 2019; Koutsaviti et al., 2018; Sriti Eljazi, 2018). Evaluated repellent activity of essential oil from *Mentha piperita* Linn. leaves (Jesser et al., 2020). Cs-EOs (*C. sativum* essential oil) is a common product used in perfume, cosmetics, and food. It has been reported that coriander EO has antibacterial, antifungal, anti-cancer, antidiabetic, analgesic and antioxidant effects (Rajeshwari et al, 2012; Prachayasittikul et al., 2017; Sriti Eljazi et al., 2018). In this study was evaluated to the efficacy of Cs-EOs against *S.oryzae* in terms of its mortality rate. In this investigation, we evaluated the insecticidal efficacy of coriander essential oils (Cs-EOs) against *S. oryzae*, focusing on their impact on mortality rates.

Material and Methods

Chemicals and Reagents

All the chemicals and standards were of analytical grade. Milli-Q water was used for preparing all solutions and cleaning.

Test Insects

Adult rice weevils were obtained from a colony maintained by the department of Plant Protection of Agricultural Faculty of Tokat Gaziosmanpaşa University. *S. oryzae* was reared on sterilized whole wheat. We sourced adult rice weevils from a colony maintained by the Department of Plant Protection at the Faculty of Agriculture, Tokat Gaziosmanpaşa University. *S. oryzae* was reared on sterilized whole wheat. All test procedures were carried out at 23±2°C, 65% RH and L/D regime of 12:8 hours. Test insect used in fumigant toxicity studies were two week post emergence.

Plant materials, Essential Oil and GC-MS analysis

Coriander seeds were supplied from cultivar plant areas in Tokat Gaziosmanpaşa University, Faculty of Agriculture. The seeds were dried at shade and stored in a room temperature. Coriander seeds were grinded, washed with distilled water. They were subjected to hydro distillation (4 h) in a Clevenger's apparatus and obtained essential oil.

The chemical profile of Cs-EOs was analyzed by Gas chromatography coupled with mass spectrometry (GC-

MS). GC-MS analysis carried out using a Trace 1310 gas chromatograph equipped with an ISQ single quadrupole mass spectrometer (Thermo Fischer Scientific, Austin, TX). The GC oven temperature was adjusted an initial temperature 70°C for six minutes then heated up to 235°C at 3°C/min and finally 10 min 235°C. The ion source and detector temperature were set at 250°C. A thermos TG-WAXMS GC column (60m×0.25mm×0.25µm) was used for identification.

The carrier gas was helium with a flow rate 1.2mL/min. The chemical contents of the obtained oil were determined according to their retention times in mass spectroscopy and corrected by comparison of the known compounds using mass spectral library search against the National Institute of Standards and Technology (NIST).

Fumigant Assay

In order to fumigant toxicity test Cs-EOs was tested against adults of *S. oryzae*. Ten adults with of mixed male/female were placed with two gram of wheat grains in a polystyrene vial (10 cm height and 3 cm diameter). A filter paper strip (2 cm diameter) treated with essential oil solution prepared in acetone (8%, 14%, 20%, 26%, and 32%) was fastly put to the vial. All the vials were closed and stored in dark. Each set of concentration was six replicates. A positive control without essential oil was kept for comparison. After 3,5,7,9 and 11th day of fumigation, mortality of adults was recorded.

Statistical Analysis

All statistical analyzes were carried out with the help of MINITAB (Release 14) package program. Dose-death trial results were analyzed with the help of Polo-PC probit package program.

Results AND Discussion

Chemical contents of Cs-EOs were analyzed by GC-MS. The identification of the chemical contents was based on retention time and percentage comparison with authentic standards. Major compound was linalool (79.12%) which is in consent with previous reports (Eikani et al., 2006; İzgi & Telci, 2017), (Table 1) (Figure 1).

Table 1. Chemical composition of Cs-Eos

Rt	Compound name	%
7.79	α-Pinene	2.67
9.20	Camphene	0.49
10.89	2- α-Pinen	0.25
11.56	Sabinene	0.15
14.07	α-Myrcene	0.43
16.50	l-Limonene	1.28
20.66	çTerpinene	2.82
23.25	Benzene, methyl(1-methylethyl)	1.12
24.46	α-Terpinolene	0.30
58.95	Camphor	6.16
66.41	L-Linalool	79.12
69.71	3-Cyclohexen-1-ol,4-methyl-1-(1methylethyl)	0.33
77.42	(+)-α-Terpineol	0.88
81.71	Geranyl acetate	2.10
87.34	Geraniol	1.88

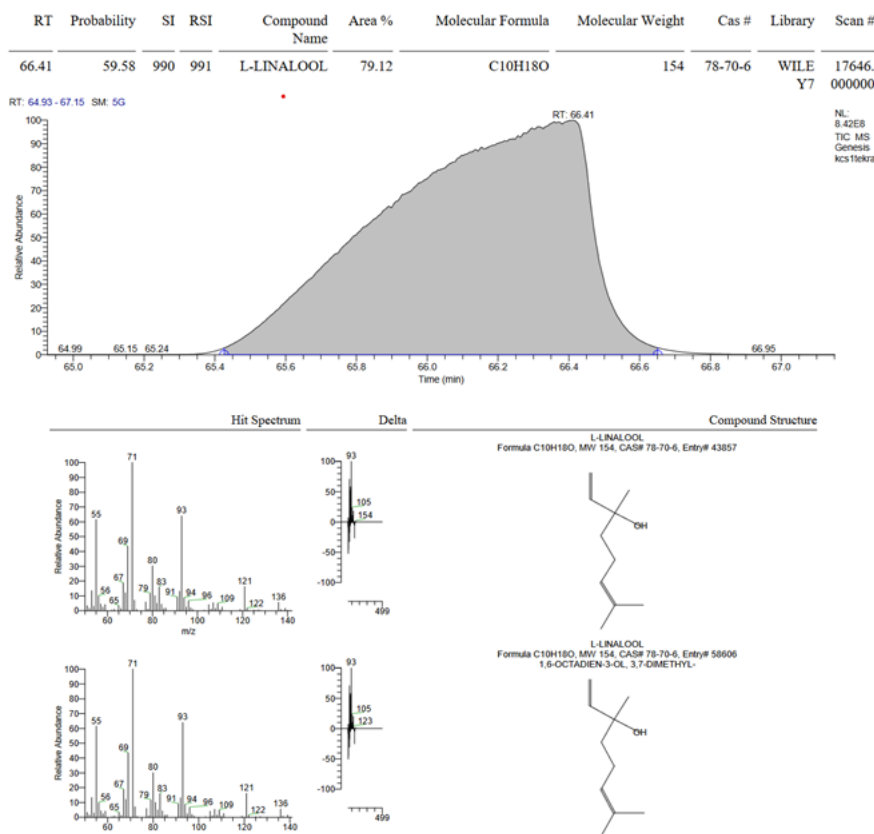


Figure 1. GC-MS spectra of essential oil

Table 2. The main compounds of coriander essential oil from diferent countries

Samples	Essential oil	References
Iran	Linalool (73.05%), a-Pinene (9.18%), Gama-Terpinene (7.65%), hydro-distillation with a clevenger apparatus	[Zamindar et al., 2016]
Tunisia	Linalool (26.12–66.08%), extracted with OMEGA 20 extruder a-Pinene (11.65–43.80%), p-Cymene (3.34–5.20%),	[Hossein et al., 2014]
Canada	Linalool (78.96%), Cymene (6.38%), Ocimene (4.46%), Camphor (3.62%), hydro-distillation with a clevenger apparatus	[Galata et al., 2014]
Iran	Linalool (66.29–63.27%), hydro-distillation with a clevenger apparatus	[Hossein et al., 2014]
Türkiye	Linalool (78.96%), hydro-distillation with a clevenger apparatus	[Zhang et al., 2015]
Tunisia	Linalool (79.22%), hydro-distillation with a clevenger apparatus	[Siriti et al., 2018]
Serbia	Linalool (64.04%), Isolated EOs in n-hexane	[D. Micić et al., 2019]
Türkiye	Linalool (79.12%), hydro-distillation with a clevenger apparatus Camphor (6.16%),α-Pinene (2.67%)	Present Study

The contents in the essential oil of the plant may differ depending on the geographical location, climate and seasonal conditions. As can be seen in Table 2 the coriander seeds contain more than 60% linalool, oxygenated monoterpen as major components.

Similar to our study, it was observed that more than 70% of the content of coriander seeds collected from different places such as Maraghe, Khoramabad, Estahbanat, Tabriz, Hamedan, Bajestan, Amol, and Yazd was linalool in 2010 (Ebrahimi, et al., 2010). Existing literatures has established that ecological variations exert a substantial impact on seed yields, oil content, and compositional traits across various Coriandrum varieties were reported. Previous research has demonstrated that the essential oil content and composition of *C. sativum* can be influenced by cultivation practices, ontogenetic factors, and genetic influences (Telci et al., 2006; Msaada et al., 2007).

The fumigant toxicity of Cs-EOs samples of *S. oryzae* adults is shown in Table 3. The fumigant toxicity tests indicated that Cs-EOs had a significant fumigant activity on *S. oryzae* as compared to control sets. Mortality of insect was paralleled with increase dose and exposure time of essential oil. While there are many studies on the toxic effects of essential oils on storage pests, there are few data on their mechanism of action.

High level of fumigant activity against *S. oryzae* especially on the 9th and 11th days and killed more than 50% (Table 3). Increasing mortality was showed for the rice weevil when the concentrations and exposure times increased. The highest concentration 12% that caused 87.86% mortality for test insects after trial. It was determined that the all concentration was effective according to the control at all doses and statistically significant as well. The LC₅₀ values of the essential oils tested are summarized in Table 4.

Table 3. Fumigant toxicity of coriander seeds essential oils against *Sitophilus oryzae*

Concentration %	3 d	5d	7 d	9 d	11 d
3	0.00±0.48 CC	11.10±0.99 AA	20.89±0.24B B	21.25±0.47 BB	23.78±0.51 DD
6	1.27±0.51 BC	14.41±0.33 AA	26.18±0.06 BB	28.71±1.01 BB	58.23±0.34 CC
9	4.05±0.68 AB	18.91±0.99 AA	31.73±0.37 BB	45.53±0.12 AA	73.24±0.10 BB
12	9.81±0.42 AA	19.26±0.45 AA	39.02±0.08 AA	51.25±0.12 AA	87.86±0.19 AA
Control	0.00±0.00 CC	0.00±0.00 BB	0.00±0.00 CC	0.00±0.00 CC	3.69±0.56 EE

Table 4. LC₅₀ values of coriander seeds EOs against the adults of *Sitophilus oryzae*

Essential oil	LC ₅₀ (95%CI)	Slope±SE	χ ²	Heterogeneity
5 day	125.72 (37.342-0.19)	0.74±0.26	15.43	0.73
7 day	24.89 (14.78-145.28)	0.94±0.23	36.81	1.23
9 day	11.32 (9.34-15.47)	1.39±0.23	17.68	0.58
11 day	5.16 (4.69-5.11)	3.040 ±0.25	19.88	0.66

LC₅₀ values of coriander against *S. oryzae* were 125.72, 24.89, 11.32 and 5.16 respectively. In 11. day Cs-EOs vapour caused high mortality and toxic effect compared to other days. The mortality in *S. oryzae* was increased with increasing exposure time and concentration (Table 4). Toxic effect of using treatment methods showed that increasing mortality with increasing concentration (Kraikrathok et al., 2013; Karan et al., 2018). The different results of the insect species to the EOs has previously been reported for stored product insects (Negahban et al., 2007).

In conclusion, chemical composition analysis of Cs-EOs was performed by library searching and retention time with GC-MS method. This study revealed the fumigant activity of coriander essential oil rich in linalool. The use of Cs-EOs especially linalool can be considered as an excellent alternative to synthetic insecticides for control *S. oryzae* without negative health effects (Ismann & Grienesen, 2014). De Clerck et al., 2020 pointed out that biological activities of plant terpenoids include repellency and insecticidal effect, growth inhibition through altered protein availability, and direct toxicity. Plant-derived essential oils (EOs) represent promising alternatives for pest management due to their diverse biological activities, biodegradability, and minimal impact on non-target organisms and the environment (Boyer et al., 2012; Peschiutta et al., 2019; Patiño-Bayona et al., 2021).

Essential oils are volatile, which could be of great interest for the reduction of the residues, as well as agricultural applications (Zanellato et al., 2009). Particular emphasis has been placed on the relative efficacy against pathogens and insects, multifaceted mechanisms of action, and the comparatively low toxicity to mammals and humans associated with essential oils. Overall our works and results showed that essential oils from coriander seeds present important perspectives for useful application in Rice weevil. Also this study adds knowledge to the development of newer and safer bio insecticides based on essential oils for *S. oryzae* control in rice. The results indicated that Cs EOs have potential to be candidate as natural insecticides for the control of stored grain.

Declarations

Acknowledgement

We would like to thank Prof. Dr İsa Telci (Isparta University of Applied Sciences) for providig the plant material (coriander). Also the authors wish to thank Master

student Sinem Dilibal (Tokat Gaziosmanpaşa University, Agricultural Faculty, Plant Protection Department of Tokat_Türkiye) for the assistance in maintaining the culture of insects and Mesut Gok for the GC-MS measurements.

Author's Contribution

Kadriye Ozlem Saygı: Conceptualization, Methodology, Formal analysis and investigation, Writing - original draft preparation, Writing - review and editing
Ayşe Yeşilayer: Conceptualization, Methodology, Formal analysis and investigation, Writing - review and editing

Conflict Interest

Authors declare that they no conflict interest.

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Effect of Thermal Manipulation During Embryogenesis on Pre and Post-Hatch Performance of Stored Hatching Eggs of Japanese Quails

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ARTICLE INFO

Research Article

Received : 09.06.2024
Accepted : 20.08.2024

Keywords:

Hatching traits
Quails
Incubation
Storage
Post hatch performance

ABSTRACT

This research investigated the influence of high incubation temperature on hatching, and post-hatch characteristics of stored hatching eggs of Japanese quails. Hatching eggs of Japanese quails were stored for 7 days and incubated under two temperature conditions. The T1 group (control, 75 eggs) was subjected to a standard incubation temperature (37.5°C) while the T2 group (75 eggs) was exposed to a thermal manipulation protocol (of 38.5°C for 5 hours daily between embryonic days 5-15). The egg weight classification, chick weight, chick length, wing length, weekly body traits, body weight, total feed intake, and stress responses, weight of internal organs, whole carcass, breast, neck, wing, thigh, and neck did not significantly differ ($P < 0.05$) between the incubation treatments. Hatchability was higher and early embryonic mortality was lower in T2 than in T1. Late embryonic mortality was lower in T1. Significantly ($P \leq 0.05$) higher pectoral muscle width at hatch and carcass yield/dressing percentage were observed in quails exposed to thermal manipulation protocol during embryogenesis. It was concluded that exposure of stored eggs to thermal manipulation protocol (of 38.5°C for 5 hours between embryonic days, ED 5-15, T2) during embryogenesis could enhance embryonic and growth traits, as well as carcass traits without any negative effect on stress indicators.

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Introduction

The rapidly growing world's population has increased the demand for cheap and affordable chicken meat which has in turn increased the search for the production of other alternative poultry species. The commercial production of Japanese quails (*Coturnix japonica*) as an alternative poultry specie is gaining a significant interest compared to the production other alternative poultry species. The unique flavour of their meat and eggs (Kayang et al., 2004) has been reported to be the cause for the rise in the interest for commercial production of Japanese quails. According to Oguz and Minvielle (2001), Japanese quails have small body sizes with short generation interval, high resistance to diseases and egg production, and low maintenance cost, making these birds suitable for laboratory purposes. Furthermore, Bagh et al. (2016) reported that in India, the Japanese quail is a promising poultry species for rural farmers due to their lower management requirements.

An egg is an important source of nutrients and it was reported that the eggs of Japanese quails are rich in minerals, vitamins, and antioxidants with a 3-4 times

nutritional value compare to the eggs of chickens (Lalwani, 2011; Tunsaringkarn et al., 2013). A comparative analysis by Loniță et al. (2008) revealed that compared to meat of broiler chickens and ducks, quail meat has the highest level of protein and the lowest calorie. A different author (Vali, 2008) also reported that quail meat has become a superior economic source of animal protein due to its lower cholesterol and lean nature.

In underdeveloped and developing countries, poultry meat and eggs are the most affordable and cheapest source of protein. This has increased the regional and global demand for poultry meat and eggs leading to the expansion of commercial hatcheries and the number of daily hatched chicks. However, in many countries especially underdeveloped and developing countries, commercial hatcheries have very limited capacities leading to the regular storage of hatching eggs and chicks being hatched in batches. Indeed, Romao et al. (2008) reported that the eggs of quails are collected and normally stored between 1-3 weeks prior to incubation.

For efficient pre and post-hatch performance, it is recommended that hatching eggs should not be stored for more than 7 days or that the optimum egg storage period should be between 5-7 days. According to Whitehead et al. (2002) the optimum storage period for chicken fertilized eggs is 7 days and each extension of this period increases embryonic mortality and decreases hatchability. Fassenko (2007) also reported that the storing eggs before incubation does not adversely affect hatchability when the storage period does not exceed 7 days. In some cases, hatching eggs may be stored beyond the recommended time, which has several negative effects on both pre and post-hatch performance. Eggs stored for longer duration are known to have poor embryonic development, fertility, hatchability, higher embryonic mortality, lower chick weight at hatch and poor-quality hatched chicks. For instance, Garip and Dere (2011) observed an hatchability of 35.4% and 78.4% and for quail eggs stored for 15 d and 5 d at 21°C. Other authors have also reported negative correlation between hatchability and duration of egg storage (Narahari et al., 2002; Fassenko et al., 2001). Naraharim et al. (1988) also observed the highest fertility and hatchability of fertile eggs in hatching egg stored for 1-3 days compared to those stored for longer durations. An increase in the number of storage days' increases embryonic mortality and failed hatch with a reduction in the internal and external egg quality traits of hatching eggs (Ayeni et al., 2020; González-Redondo et al., 2023; Nasri et al., 2020; Carvalho et al., 2023). It has been reported that chicks that hatched from eggs stored for 14 days had the lowest weight at hatch and at 5 weeks during the rearing cycle (El-kazaz and Abo-Samaha, 2018). Furthermore, Mayes and Takeballi (1984) observed a 5% reduction in hatchability per day after 7 days of egg.

Thermal manipulation during the incubation of non-stored hatching eggs has been reported to enhance embryonic development, and hatchability, decrease incubation time and improve chick quality, post-hatch growth, and welfare performance (Yahav et al., 2004; Piestun et al., 2008; Collin, et al., 2005; Piestun et al., 2009; Al-Zhgoul et al., 2013; Al-Rukibat et al., 2017; Piestun et al., 2011; Piestun et al., 2013; Yalcin et al., 2012).

Therefore, in this research, it was hypothesized thermal manipulation of stored hatching eggs would improve pre and post-hatch performance. To our understanding, this study is one of the few preliminary researches testing the effect of incubation temperature on stored hatching eggs of Japanese quails.

Material and Methods

Experimental Groups and Storage Conditions

In this study, a total of 150 hatching eggs of Japanese quails were used. The eggs were first weighed using an electronic balance with a precision of 0.01g and divided into 2 separate groups (75 eggs per group); control-T1 and the thermal manipulated group-T2. The eggs were then stored with an average temperature and humidity of 18°C and 55% respectively for a week in the storage room. After storage, the eggs were again weighed with an electronic balance and then subjected to a prewarming protocol (26°C, RH 55%) for 5 hours. Moisture (%ML) loss during storage was evaluated using the formulae below.

$$\%ML = \frac{\text{Weight before storage} - \text{Weight after storage}}{\text{Weight before storage}} \times 100$$

Incubation Treatments

The T1-control were subjected to an incubation temperature of 37.5°C throughout the incubation period while the T2 eggs were subjected to a high temperature of 38.5°C for 5 hours between embryonic days (ED) 5-15. On incubation day 15, eggs were transferred to the hatcher.

Evaluation of Hatching Traits

Hatching traits such as embryonic mortality and hatchability were recorded and evaluated. To determine the stage of embryonic mortality, unhatched eggs at the end of the hatching period were cracked. The dead embryos were assessed and the stage of embryonic development was used to determine the stage of embryonic mortality by specialist with vast understanding of embryonic mortality.

$$H = \frac{\text{(The total number of chicks that hatched)}}{\text{(The total number of eggs incubated)}} \times 100$$

$$E = \frac{\text{(Number of early embryonic deaths)}}{\text{(Total number of embryonic deaths in that group)}} \times 100$$

$$M = \frac{\text{(Number of mid-embryonic deaths)}}{\text{(Total number of embryonic deaths in that group)}} \times 100$$

$$L = \frac{\text{(Number of late embryonic deaths)}}{\text{(Total number of embryonic deaths in that group)}} \times 100$$

H: Hatchability

E: Early embryonic mortality

M: Middle embryonic mortality

L: Late embryonic mortality

Evaluation of Post Hatch Traits and Animals Selected for the Growing Period

At hatch, chick quality traits (chick length, chick weight, and chick pectoral muscle length) in all the chicks that hatched from the respective incubation groups were assessed. The chick weight was measured using scale with a precision of 0.01 g and the chick length was measured using a rule/line gauge attached to a table. The pectoral muscle length was measured using a digital vernier caliper. After that, only chicks of high grade quality with no leg problem or having better locomotion abilities were selected for the growing or rearing cycle. Using this criteria, 25 healthy chicks per incubation treatment were reared for 5 weeks.

Housing Facility during the Rearing Cycle

The poultry housing facility at Çukurova University Farm was used in this study. The quails were reared in cages with dimensions 28cm×92cm×44cm (height, length, and width respectively). There were 3 replicates per experimental group. The number of birds and replicates used in the current experiment are given in Table 1.

Table 1. The number of experimental birds and replicates used in the study

Treatments	T1R1	T1R2	T1R3	Total
T1	8	8	9	25
T2	T2R1	T2R2	T2R3	25
	8	8	9	

T1; Treatment 1, T2; Treatment 2, R1; Replicate 1, R2; Replicate 2, R3; Replicate 3

Experimental Diet

A broiler chick diet containing 3000 kcal/kg ME and 24% crude protein were given to chicks between 0-2 weeks of the experiment. From the 3-5 weeks of the experiment, a diet containing 3000 kcal/kg ME and 22% crude protein were provided for the grower quails.

Measurements/Evaluation of Feed Intake and Body Weight

Using an electronic balance with a precision of 0.01 g, feed intake and body weight were recorded weekly. The formulae below were used in evaluating the weekly feed intake.

$$(FG - FL)$$

FG: Feed given at the beginning of a particular week
 FL: feed left at the end of that week

Measurement of Body Traits During the Rearing Period

All the quails (25 quails per incubation treatment) were assessed for body traits such as chick length, wing length, and length and width of the pectoral muscle on weekly basis throughout the production period using a digital Venier calliper.

Measurement of Stress

The rectal temperature and leg composite asymmetry were used as the measure of stress in this study.

- **Rectal Temperature Measurements**

The rectal temperature of the 25 quails per experimental treatment was recorded by inserting a digital thermometer 3cm inside the cloaca (Kursun et al., 2024).

- **Measurements of Fluctuating Asymmetry of Leg**

The leg fluctuating asymmetry (FA) was recorded using a digital calliper (Archer et al., 2009) after slaughter in all the slaughtered quails (25 quails per incubation treatment). FA was evaluated using the formula below.

- $$FA = \frac{ML(L-R) + MW(L-R) + MTL(L-R)}{3}$$

R (Right); L (Left); ML (Metatarsal length); MTL (Middle Toe length); MW (Metatarsal width)

Evaluation of Carcass Traits

All the experimental birds were slaughtered (25 quails per incubation treatment), the whole carcass was measured and each part of the carcass (neck, wings, breast, and thigh) were measured separately. The measurements were done using an electronic balance with a precision of 0.01 g. The formula below was used to evaluate the carcass yield or dressing percentage;

$$DP = \frac{(\text{Carcass weight})}{(\text{Live weight})} \times 100$$

DP: Dressing percentage/Carcass yield

Measurement of Immune and Other Internal Organs

The immune organ (spleen) weight, weight of proventriculus, heart, gizzard (ventriculus), liver, and intestines were all measured using an electronic balance with a precision of 0.01 g.

Statistical Analysis

Using SPSS version 26, test of normality was conducted using the Shapiro-Wilk. After confirming the normality of the data, independent sample t-test analysis was then used to evaluate the statistical mean difference of the various incubation treatments for all the measured data except for hatching traits. The hatching traits were analysed by dividing the occurrence of a particular trait by the total number of all the traits and expressed in percentages.

Results

The weight of the eggs before and after storage as well as during transfer to the hatcher is presented in Table 2. The weight of the egg before and after storage as well as during transfer to the hatcher was not statistically ($P \geq 0.05$) different between the control and the thermal manipulated group.

The results of incubation treatments on the hatching traits of stored eggs are given in Table 3. The T2 group had higher hatchability than the T1 group. The percentage of early embryonic mortality was higher in T1, however, the T2 group had the higher percentage of late embryonic mortality.

Table 2. The weight (g) of the eggs before and after storage and during transfer to the hatcher

Experimental Treatment	Classifications of Egg Weight		
	Weight Before Storage	Weight After Storage	Weight Before Transfer to Hatcher
T1 (Control)	12.67 ± 0.11	12.55 ± 0.11	11.30 ± 0.15
T2	12.89 ± 0.10	12.72 ± 0.10	11.24 ± 0.16
P Value*	0.139	0.262	0.263

*T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

Table 3. Influence of incubation treatments on embryonic mortality (%) and hatchability (%)

Experimental Treatment	Number of Hatched Chicks (N)	Hatchability (%)	Embryonic Mortality (%)		
			Early Embryonic Mortality (%)	Middle Embryonic Mortality (%)	Late Embryonic Mortality (%)
T1	29	38.67	85.71	-	14.29
T2	33	44.00	66.67	-	33.33

T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

Table 4. Influence of incubation treatments on chick body traits at hatch

Experimental Treatment	Chick Body Traits at Hatch			
	Chick Weight (g)	Chick Length (cm)	Pectoral Muscle Width (mm)	Wing Length (mm)
T1	9.22 ± 0.15	11.08 ± 0.10	13.68 ± 0.27	21.83 ± 0.31
T2	8.94 ± 0.12	10.98 ± 0.14	14.74 ± 0.26	22.08 ± 0.46
P values	0.148	0.547	0.006	0.642

T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

Table 5. Effect of incubation treatments on chick body traits during the rearing period

Body Traits	Treatments	Weeks of Production			
		1	2	4	5
Chick Length (cm)	T1	15.84 ± 0.14	20.88 ± 0.24	27.22 ± 0.28	27.92 ± 0.84
	T2	16.20 ± 0.17	21.12 ± 0.23	26.73 ± 0.35	37.89 ± 9.65
P value		0.113	0.478	0.281	0.309
Pectoral Muscle Width (mm)	T1	21.38 ± 0.49	26.21 ± 0.42	41.30 ± 1.40	43.36 ± 0.85
	T2	21.37 ± 0.45	26.32 ± 0.41	41.08 ± 0.88	42.56 ± 0.72
P value		0.986	0.850	0.899	0.483
Pectoral Muscle Length (mm)	T1	46.83 ± 0.66	53.14 ± 1.01	60.18 ± 1.32	67.14 ± 1.06
	T2	48.03 ± 0.83	52.83 ± 1.10	59.71 ± 1.15	66.68 ± 1.28
P value		0.267	0.841	0.789	0.787
Wing Length (mm)	T1	43.75 ± 0.72	66.00 ± 1.79	87.93 ± 1.92	96.83 ± 0.96
	T2	45.61 ± 1.27	67.47 ± 1.45	89.70 ± 1.40	96.89 ± 1.26
P value		0.220	0.525	0.458	0.972

T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

Table 6. Effect of incubation treatments on total feed intake and average weekly body weight

Experimental Treatments	Body Weight (g) /Week					Total Feed Intake (g)
	1	2	3	4	5	
T1	33.16 ± 0.85	84.96 ± 1.89	155.63 ± 3.82	212.46 ± 5.57	253.45 ± 7.80	4229.28 ± 750.47
T2	33.44 ± 1.02	81.87 ± 2.66	149.55 ± 5.56	210.92 ± 7.41	257.38 ± 9.93	4302.32 ± 791.73
P Values	0.832	0.355	0.380	0.869	0.757	0.948

T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

Table 7. Influence of incubation treatments on cloacal/rectal temperature and fluctuating asymmetry of the leg.

Experimental Treatments	Stress Responses	
	Rectal/Cloacal Temperature (°C)	Fluctuating Asymmetry
T1	41.94 ± 0.10	0.79 ± 0.12
T2	42.04 ± 0.11	1.00 ± 0.19
P Values	0.492	0.357

T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

The results of incubation managements on chick body traits at hatch is presented in Table 4. The incubation treatments had no statistical effect ($P \geq 0.05$) on wing length, chick length and chick weight at hatch between the two experimental treatments however, the T2 group had significantly ($P \leq 0.05$) higher pectoral muscle width at hatch than the T1 group.

The effect of incubation treatments on weekly body traits during the production/rearing phase is presented in Table 5. The weekly body traits were not statistically ($P \geq 0.05$) different between the two incubation treatments. The experimenters (students) that measured the body traits data on week 3 were inexperienced thereby recording wrong values for that week. Therefore, the body trait data for week 3 was excluded/ discarded from the research.

The influence of the incubation treatments on total feed intake and weekly body weight is given in Table 6. The

incubation treatments did not have any statistical influence ($P \geq 0.05$) on the total feed intake and weekly body weight evaluated in this study.

The influence of incubation treatments on responses to stress is presented in Table 7. No significant ($P \geq 0.05$) effect of the incubation treatment was observed on the measured stress parameters (fluctuating asymmetry of the leg rectal/cloacal temperature and).

The influence of the incubation treatments on carcass trait is given in Table 8. The weight of the whole carcass, breast, wings, thigh, and neck did not differ significantly between the incubation treatments ($P \geq 0.05$). However, the T2 group had statistically ($P \leq 0.05$) higher dressing percentage compared to the compared to T1 group. Furthermore, the weight of the heart, liver, gizzard, proventriculus, and intestines did not statistically ($P \geq 0.05$) differ between the two incubation treatments.

Table 8. Influence of incubation treatments on carcass parameters

Weight of Carcass and Internal Organs (g)	Treatments		P Values
	T1	T2	
Whole carcass	173.35 ± 6.29	185.02 ± 6.92	0.218
Breast	87.16 ± 17.94	89.33 ± 19.23	0.682
Wing	12.32 ± 0.41	12.67 ± 0.52	0.60
Thigh	37.54 ± 1.35	40.19 ± 1.64	0.220
Neck	6.87 ± 0.26	6.89 ± 0.27	0.958
Dressing Percentage (%)	68.22 ± 0.82	72.12 ± 1.02	0.004
Heart	2.36 ± 0.16	2.17 ± 0.10	0.311
Liver	6.21 ± 0.33	6.93 ± 0.37	0.150
Gizzard	5.06 ± 0.24	4.87 ± 0.24	0.588
Proventriculus	0.92 ± 0.06	1.03 ± 0.06	0.214
Intestines	9.77 ± 2.93	9.44 ± 2.35	0.668

T1: Treatment 1 (control, stored eggs exposed to normal incubation temperature). T2: Treatment 2 (Stored eggs exposed to thermal manipulation).

Discussion

In terms of egg weight before and after storage, no statistical difference between the experimental treatments were observed. The eggs from the two experimental treatments were from the same breeder parents, similar average egg weight, and similar storage conditions and this reason may have accounted for the lack of significant difference in terms of egg weight before and after storage between the experimental groups. Furthermore, egg weight loss before transfer to the hatcher was not significantly different between the incubation treatments however, it was numerically higher in T2 compared to T1. In line with our observations, other authors (Abdelfattah, 2019; Kamanli et al., 2021; Lin et al., 2017; Farghly et al., 2022) have reported higher egg weight loss in non-stored eggs exposed to high incubation temperature (38.1°C, 38.5°C, 39±1°C or 41°C) compared to those exposed to low or standard incubation temperature. Another author also reported that the exposure of non-stored eggs to a temperature of 39°C decreased egg weight (Sgavioli et al., 2016). The exchange of gas between the egg and its surrounding is regulated by the eggshell conductance and this has been reported to regulate metabolic heat and water loss (Campos and Santos, 2003; Hamidu et al., 2007). The greater loss of egg weight in the T2 group observed in the present experiment could probably be due to the high evaporation of water from the developing embryos (Shafey, 2002). Contrary to our results higher egg weight loss in stored eggs exposed to normal incubation temperature than in stored eggs exposed to thermal manipulation during embryogenesis was observed (Alkis, 2021).

The T2 group had higher hatchability than the T1 group in the current study. The findings of this experiment are in agreement with the results of other studies (Farghly et al., 2022; Abdelfattah, 2019; Lin et al., 2017) which also observed that non-stored eggs subjected to higher temperature during incubation had higher hatchability than those subjected to standard incubation temperature. The higher hatchability in the group subjected to high temperature during incubation could be related to an excessive reduction in the number of early embryo mortality in the present study. Contrary to our findings, others (Shah and Özkan, 2022; El-Shater et al., 2020; El-

Shater et al., 2021; Abuoghaba et al., 2021) observed higher hatchability in embryos exposed to standard incubation temperature (37.7°C or 37.5°C) than those subjected to thermal manipulation protocols (41°C or 39.5°C). The diversity in the various scientific reports might be related to the differences in the thermal manipulation protocol used by the different authors.

Early embryonic mortality was higher in the T1 than in T2. Eggs exposed to storage have been reported to have a lower number of embryonic cells due to apoptotic death of cells which has a huge influence on embryonic mortality after standard incubation conditions (temperature and humidity) are restored. It could be possible that thermal manipulation of the T2 group increased the proliferation of embryonic cells resulting in an adequate number of cells for initial embryonic development. Our observation is supported by the findings of other researches (Shah and Özkan 2022) who also reported a lower percentage of early embryonic mortality in eggs exposed to thermal manipulation. The higher number of late mortality among the T2 group could be due to excessive dehydration of the embryo due to evaporation caused by higher heat exposure. Contrary to our observations several authors (Abdelfattah, 2019; Vitorino Carvalho et al., 2020; Abuoghaba et al., 2021) have observed a higher number of late and early embryonic mortality in non-stored eggs exposed to higher incubation temperature. The differences between the findings of the present experiment and other experiments in terms of early embryonic mortality might be related to the storage effect which was part of the current study or the thermal manipulation protocol used.

In the present study, in terms of chick body traits, significantly ($P \leq 0.05$) higher pectoral muscle width/diameter at hatch was observed in the T2 group compared to the T1 group. Other authors also reported that the exposure of non-stored eggs to thermal manipulation increased breast muscle hypertrophy (El-Shater et al., 2021). In non-stored eggs, thermal manipulation has been reported to enhance proliferative activity, increase the number of muscle cells in embryonic and post-hatch chicks as well as IGF-I which is known to stimulate the proliferation and differentiation of satellite cells and increase myofiber hypertrophy (Paul and Rosenthal, 2002;

Adams and McCue 1998; Piestun, et al., 2009; Adams et al., 2000). Again, it was reported that exposure of embryos to thermal manipulation protocols increased muscle development and the diameter of myofiber (Piestun et al., 2009). This reason may account for the higher pectoral muscle width in T2 than in T1 observed in the current study. Other chick body traits (wing length, chick weight, and chick length) at hatch were not statistically different from one another between the experimental groups. In line with our reports, other authors have also reported that thermal manipulation had no statistical effect of on chick weight and chick length at hatch (Farghly, et al. 2022; Shah and Özkan, 2022). Although not statistically different, numerically higher chick weight and length in T1 compared to T2 were observed at hatch. Higher chick weight and chick length at hatch or day-old in non-stored and stored eggs exposed to normal incubation temperature than those exposed to high incubation temperature have been reported by several authors (Abdelfattah, 2019; Alkıs, 2021; Sgavioli et al., 2016; Abuoghaba et al., 2021; El-Shater et al., 2021; Amjadian and Shahir, 2020; Kamanli et al., 2021). The lower chick weight and chick length traits at hatch or day old reported in the group subjected to thermal manipulation might be related to the reduction in the utilization of yolk causing a subsequent reduction in the availability of nutrients for embryonic development (Willemsen et al., 2010). However, Alkıs (2021) observed higher chick weight in stored eggs subjected to high temperature during embryogenesis.

The current experiment is one of the first to evaluate the influence of thermal manipulation on chick body traits from hatch till slaughter age. Although several authors have reported the long-lasting and immediate effect of thermal manipulation from hatch till slaughter age (Piestun et al., 2009; Vitorino Carvalho, et al., 2020), The measured body traits from the first week of age till slaughter were not significantly different between the experimental groups.

The experimental groups in the present study did not differ statistically in terms of weekly body weight gain. Vitorino Carvalho et al. (2020) also observed no statistical influence of high incubation temperature on the body weight of quails at 35 days of age. Contrary to our findings other authors reported higher post-hatch (5 weeks) body weight in quails that hatched from eggs exposed to thermal manipulation during incubation (41°C for 3h/day at ED 6-8) (Alkan, et al., 2013; El-Shater et al., 2021). It was reported that at 8 weeks of age, chickens that hatched from eggs subjected to thermal manipulation during incubation (39 ± 1°C for 2 h during ED 4–14) had higher live weight (Farghly et al., 2022). Other authors also reported lower body weight in quails exposed to higher incubation temperature than those incubated with standard incubation temperature at 5 weeks and 25 days of age (Vitorino Carvalho et al., 2020; Abuoghaba et al., 2021). The differences in results could be related to the thermal manipulation protocol used or the quail breeder genotype from which eggs were obtained.

The total feed intake throughout the production period was not statistically different between the incubation treatments in the current study. In agreement with our results, several authors have reported no statistical effect of thermal manipulation during embryogenesis on total feed intake (Farghly et al., 2022; Amjadian, and Shahir, 2020).

Contrary to our results, significantly lower total feed intake in quails exposed to thermal manipulation during incubation have been reported (Abuoghaba et al., 2021).

Furthermore, the physical stress parameters (rectal temperature and fluctuation asymmetry of the leg) measured in the present study did not significantly between the experimental groups. In line with the current study, several authors (Amjadian, and Shahir, 2020; Yalçın, et al., 2006; Sgavioli et al., 2016) have reported no significant effect of thermal manipulation during embryogenesis on cloacal temperature. It is possible that the thermal manipulation protocol employed during embryogenesis did not have any significant effect on the mechanism that influences the acquisition of thermoregulation or thermotolerance leading to the subsequent lack of significant effect on the cloacal temperature of the birds. However, some authors (Abdelfattah, 2019; Abuoghaba, 2016) observed higher rectal temperature at hatch in quails that hatched from eggs subjected to high incubation temperature and others (Al-Zghoul et al., 2015; Shah, 2021; Al- Al-Zghoul, 2018; Al-Rukibat, et al., 2017; Zghoul, et al., 2019) also reported that thermal manipulation during embryogenesis decreased post hatch rectal/cloacal temperature at normal rearing temperature or during post-hatch heat challenge. The variations in results are probably due to the timing of the thermal manipulation, incubation temperature, and the duration of thermal manipulation.

The T2 group had statistically higher carcass yield/dressing percentage than the T1 group in the present study. This is a reflection of the higher carcass weight and live body that was observed in the T2 group weight at the end of the experiment. Contrary to our findings, no significant effect of thermal manipulation on dressing percentage has been reported (Farghly et al., 2022). No significant effect of the incubation treatments on the weight of the full carcass, breast, thigh, wing, neck, heart, liver, gizzard, proventriculus, and intestines was observed in the present study. In line with our reports, other researchers (Abuoghaba, et al., 2021; Lin, et al., 2017; Farghly et al., 2022) also reported no statistical influence of thermal manipulation on carcass percentage, liver heart, gizzard, and intestines. Contrary to our results, higher percentages of breast, heart, and gizzard at hatch and slaughter age in chicks that hatched from non-stored eggs exposed to normal incubation temperature than those exposed to thermal manipulation have been reported (Abdelfattah, 2019; Lin, et al., 2017; Abuoghaba, et al., 2021).

Conclusion

Our preliminary research revealed that thermal manipulation of stored eggs could decrease early embryonic mortality as well as improve live body weight, feed intake, and carcass traits without any negative effect on production and welfare performance.

Declarations

Ethical of approval

The ethics committee of Cukurova University granted the approval/ethic report (approval number: 28.03.2024/2) for this study.

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Cost-Benefit Analysis of the Fattening of Morkaraman Lambs with Different Dietary Plant Protein Sources

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ARTICLE INFO

Research Article

Received : 10.06.2024

Accepted : 05.08.2024

Keywords:

Corn

Cost

Gluten

Profitability

Protein

ABSTRACT

This study investigated the effects of different plant protein sources incorporated into feed concentrates on the live weight gain and feed conversion ratio of Morkaraman lambs by days 28, 42 and 56 of fattening, and presents a cost-benefit analysis. The study animals included 24 male Morkaraman lambs with a mean age of 9 months, which were assigned to 3 study groups. The dietary plant protein sources provided to the animals were soybean meal and safflower meal in Group I, wheat gluten in Group II, and corn gluten in Group III. The total feed intake values (kg) of Group I, Group II and Group III were 40.75±1.08, 39.18±0.88, and 37.67±0.62, respectively, during the period between days 0-28 of fattening; 62.77±1.67, 60.14±0.96, and 57.54±1.28, respectively, during the period between days 0-42 of fattening; and 83.31±1.89, 77.79±1.43, and 75.97±1.67, respectively, during the period between days 0-56 of fattening ($p<0.05$). The live weight gain values (kg) of Group I, Group II and Group III during the period between days 0-56 of fattening were 14.82±0.84, 11.97±0.51, and 13.71±0.91, respectively ($p<0.05$). The feed conversion ratio was observed not to have a statistically significant effect on production yields ($p>0.05$). In conclusion, while the highest income from live weight gain during the period between days 0-56 of fattening was achieved with the use of soybean meal and safflower meal as dietary plant protein sources, the lowest fattening cost was achieved with the use of corn gluten.

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Introduction

Proteins, as the second major building block of the human body after water, constitute almost 50% of the cell's dry weight. Proteins are known to have various functions, including, among others, the repair, growth, and maintenance of the body (Wyness, 2016; Yetim & Tekiner, 2020; Yıldırım et al., 2021). Based on assessments of their biological value, proteins found in foods of animal origin are more potent than those found in foods of plant origin. Thus, food products of animal origin are essential to adequate and balanced nutrition (Yıldırım et al., 2021). Animal proteins can be obtained through the consumption of red meat, fish, eggs, and milk and milk products. Owing to its high content of protein, which on average is 24%, red meat is considered a major dietary protein source (Wyness, 2016). According to data pertaining to the year 2019, meat consumption per capita is 100.6 kg in the United States of America (USA), and almost half of it (50.1 kg) is from poultry meat consumption, while meat consumption per capita is 65 kg in the European Union (EU-28), and 24.9 kg of it is from poultry meat consumption. When compared

to the USA and EU, meat consumption per capita is reported as 36.3 kg in Türkiye, and 60% of it is from poultry meat consumption (ESK, 2019). Thus, these data show that red meat is less consumed in Türkiye. This comparison also points out a positive correlation between the level of development of countries and the animal protein consumption of their populations (Sarıözkan et al., 2007). The large difference observed in the meat and red meat consumption of Türkiye stems from the various problems of the livestock sector, including, among others, the underemphasis of small ruminant breeding. Another setback is the poor organization of the animal holdings and the limited number of producer/farmer cooperatives (Günlü & Mat, 2021).

Until the 1980s, Türkiye had a significantly large population of sheep. The sharp decline observed as of 1980 was, to a great extent, linked to the transition to a market economy and the implementation of the stability program (dated January 24, 1980) that had been initiated in the livestock sector. The reduced state aid provided to sheep

production and the increase in feed costs during this transition period eventually led to a sharp decline in the sheep population of the country. During this period, cattle production was placed at the forefront and benefited from state aid, incentives, and safeguard measures, such that farmers lost interest in sheep breeding. This trend continued until 2010. Although the sheep population displayed a gradual increase thereafter, this did not suffice to compensate for the preceding loss (Günlü & Mat, 2021). Despite the highly satisfactory fattening performance of sheep against increasing feed costs, the sustainability of sheep production can only be ensured through the increase in production yields. This would also enable the production of an adequate volume of red meat and contribute to the closing of the supply deficit. In this respect, the sheep breeding sector is highly important for red meat production (Günlü et al., 2002).

The decline in the total area and quality of grassland in Türkiye, together with the deficit in the production of roughage and the limited implementation of genetic animal improvement programs, have all contributed to the critical importance of nutrition in the sheep and goat sectors (Aksu Elmalı et al., 2010). The targeted performance values of high-producing breeds can only be achieved with the provision of high-quality feed to animals (Can et al., 2024). Thus, the protein sources incorporated into the feed ration are of particular importance. In Türkiye, the primary dietary protein sources used in animal nutrition are soybean meal, sunflower meal, cotton seed oil meal and corn gluten. The gluten protein is obtained from cereals, including barley, wheat, corn and oats, by means of the separation of starch and other components. While gluten-containing food products are widely consumed across the world, the use of corn gluten is also very common in animal nutrition. In Türkiye, corn is the third most produced cereal after wheat and barley. Not only is gluten used as a protein source in animal nutrition, but it is also used as an energy source in the livestock sector. On the other hand, the advantage of wheat is its high adaptation capacity to

different climates and geographical conditions. Wheat is not only a staple food crop for humans, but it is also commonly used in animal nutrition. Wheat grains contain on average 5.4% of gluten (Can, 2023).

This study was aimed at determining the fattening performance of Morkaraman lambs fed on different dietary plant protein sources by days 28, 42 and 56 of fattening, and at identifying the most profitable feed ration in terms of sheep production economics.

Material and Method

Animal Material, Study Groups and Nutrition

The animal material of the study comprised of 24 male Morkaraman lambs of a mean age of 9 months. The body condition scores and mean live weights of the lambs were similar. The lambs were assigned to three study groups, including a control group (Group I), a group fed on wheat gluten (Group II), and a group fed on corn gluten (Group III). The animals were housed in a closed farm system, in individual stalls. The animals were housed in individual stalls in a closed farm system of a private enterprise in Bayburt province. The feed rations provided to the animals were formulated to be isonitrogenous (crude protein (CP): 17%) and isocaloric (metabolic energy (ME): 2700 kcal/kg). The protein sources incorporated into the feed rations were soybean meal and safflower meal in Group I, wheat gluten in Group II and corn gluten in Group III. After a 21-day acclimatization period, the lambs were fattened for a period of 56 days. After being transferred to the farm, the lambs were firstly treated for internal and external parasites, and then vaccinated twice against enterotoxemia with the commercial vaccine Coglavax®. The lambs were fed twice a day, at 8.00 a.m. and 4.00 p.m., with preweighed amounts of feed. Two feed troughs were placed in each stall to separately calculate the intake of concentrated feed and roughage. Table 1 presents the composition of the feed concentrates provided to the study groups.

Table 1. The composition of the feed concentrates containing different dietary protein sources, %.

Ingredients, %	Groups		
	Group I	Group II	Group III
Wheat gluten (%75 HP)		10.3	
Corn gluten (%61 HP)			14.78
Soybean meal (%45 HP)	15.93		
Safflower meal (%22 HP)	7.47		
Rice bran	10		
Barley	60	52.5	60
Wheat		30	
Corn			18.22
Molasses	3	3	3
Marble dust	2.4	1.65	2.35
Dicalcium phosphate		1.51	0.96
Soy oil	0.6	0.33	
Salt	0.3	0.31	0.3
Ammonium chloride	0.2	0.3	0.28
Vitamin-Mineral premix	0.1	0.1	0.1
Total	100	100	100
Nutrient composition			
Crude protein, %	17	17	17
Metabolisable energy, (kcal/kg)	2.700	2.700	2.700

Determination of Performance Parameters

The body weight of each lamb was measured at the beginning of the study and on days 28, 42 and 56 of fattening, in the morning, before the provision of feed to the animals. Live weight measurement was performed with the aid of a special cage equipped with a scale. Daily feed intake was calculated based on the amounts of concentrated feed and roughage remaining in the feed troughs before the morning feed replenishment. Daily live weight gain was calculated by subtracting the initial weight from the final weight for each animal and dividing by the total number of days between the two consecutive weighings. The feed conversion ratio was calculated on the basis of the amount of feed consumed for the gain of 1 kg of live weight.

Calculation of Cost and Income

The feed cost per animal was calculated by multiplying the amounts of concentrated feed and roughage consumed during the periods between days 0-28, 0-42 and 0-56 of fattening with the ruling feed prices of the year 2021. Operating costs were calculated on the basis of the costs of the feed concentrates and roughage. The costs of vaccination, medication and labor, and other costs were not taken into consideration as they did not differ between the study groups.

The income generated per animal was calculated by multiplying the total live weight gain achieved during the periods between days 0-28, 0-42 and 0-56 of fattening with the ruling live weight sales prices per kg in December 2021. The cost/income ratio was determined by dividing the sales income of the farm by the total production cost (Güneş et al., 2001).

Statistical Analyses

The study data was statistically analyzed using the SPSS 20 software package. While feed intake, feed conversion ratio and live weight gain data were subjected to one-way analysis of variance (ANOVA), the significance of the differences between the study groups was determined with Duncan's test. P values smaller than 0.05 were considered statistically significant (Tekin, 2003; Tekin, 2010).

Findings

The feed intake, live weight gain and feed conversion ratio of the animals for the different periods of fattening are presented in Table 2.

As shown in Table 2, while no statistically significant difference was determined for roughage intake, live weight gain and the feed conversion ratio during the period between days 0-28 of fattening ($p>0.05$), it was observed that both the feed concentrate intake and total feed intake of Group III were significantly lower than those of the control group ($p<0.05$). Likewise, during the period between days 0-42 of fattening, no statistically significant difference was detected for roughage intake, live weight gain and the feed conversion ratio ($p>0.05$). During this period, Groups II and III displayed similar feed concentrate intake levels, which were significantly lower those that of Group I ($p<0.05$). Furthermore, total feed intake was significantly higher in Group I in comparison to Group III ($p<0.05$). Finally, the assessment of the period between days 0-56 of fattening also revealed no statistically significant difference for roughage intake and the feed conversion ratio ($p>0.05$). During this period, Groups II and III presented with similar feed concentrate and total feed intake levels, which were significantly lower than those of Group I ($p<0.05$). On the other hand, for the entire fattening period, it was ascertained that live weight gain was significantly higher in Group I, when compared to Group II ($p<0.05$).

The results of the cost and income analysis made for the lambs fed on the different dietary protein sources for the periods between days 0-28, 0-42 and 0-56 of fattening are presented in Table 3.

As shown in Table 3, during all three fattening periods, the highest income from live weight gain was achieved in Group I. The lowest incomes from live weight gain were detected in Group III during the period between days 0-28 of fattening and in Group II during the periods between days 0-42 and 0-56 of fattening. In the present study, the mean cost/income ratio was 1.50. Based on this result, it was determined that, in view of the costs of the supply of feed concentrate and roughage alone, and by calculating the income generated through the live weight gain of the lambs, an input of 1 Turkish Lira (TL) produced an output of 1.5 TL. The cost/income ratio was lowest during the period between days 0-28 of fattening in Group II. Periodic assessment demonstrated that the mean cost/income ratios during the periods between days 0-28, 0-42 and 0-56 of fattening were 1.46, 1.51, and 1.54, respectively.

Table 2. Live weight gain, feed intake and feed conversion ratio data for the different periods of fattening.

Period (Days)	Groups	Feed Concentrate Intake (kg)	Roughage Intake (kg)	Total Feed Intake (kg)	Live Weight Gain (kg)	Feed Conversion Ratio
0-28	Group I	36.45±1.11 ^a	4.29±0.48	40.75±1.08 ^a	7.63±1.01	5.36±1.13
	Group II	34.1±1.05 ^{ab}	5.08±0.28	39.18±0.88 ^{ab}	5.62±0.55	6.98±0.78
	Group III	32.89±0.67 ^b	4.78±0.21	37.67±0.62 ^b	5.5±0.52	6.84±0.56
0-42	Group I	56.96±1.66 ^a	5.81±0.77	62.77±1.67 ^a	11.05±1.07	5.69±0.68
	Group II	52.51±1.31 ^b	7.63±0.43	60.14±0.96 ^{ab}	9.24±0.71	6.51±0.47
	Group III	50.22±1.11 ^b	7.32±0.40	57.54±1.28 ^b	9.65±0.85	5.99±0.48
0-56	Group I	76.85±1.89 ^a	6.46±0.86	83.31±1.89 ^a	14.82±0.84 ^a	5.62±0.33
	Group II	69.19±1.83 ^b	8.59±0.53	77.79±1.43 ^b	11.97±0.51 ^b	6.49±0.18
	Group III	67.75±1.35 ^b	8.23±0.53	75.97±1.67 ^b	13.71±0.91 ^{ab}	5.54±0.32

Table 3. The total cost of production and the income (TL) from live weight gain for the different periods of fattening and the different dietary plant protein sources used.

Expenditure/Income	0-28 Days			0-42 Days			0-56 Days		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
Total Cost of Feed Concentrate (TL)	1049.87	1636.86	973.64	1640.52	2520.6	1486.41	2213.28	3321.3	2005.4
Total Cost of Roughage (TL)	31.27	37.02	34.82	42.29	55.56	53.34	47.07	62.57	59.89
Total Costs (TL)	1081.14	1673.88	1008.46	1682.81	2576.16	1539.75	2260.35	3383.87	2065.29
Total Live Weight Gain (Kg)	61.04	45.01	44.03	88.43	73.95	77.26	118.6	95.8	109.73
Income From Live Weight Gain (TL)	2105.88	1552.85	1519.04	3050.84	2551.28	2665.47	4091.7	3305.1	3785.69
Cost/Income Ratio	1.95	0.93	1.51	1.81	0.99	1.73	1.81	0.98	1.83

The prices of feed concentrate used for cost calculations were 3.60 TL/Kg for Group I, 6.00 TL/Kg for Group II and 3.70 TL/Kg for Group III. The price of wheat straw used as roughage source for all groups is 7.30 TL/Kg.

Discussion and Conclusion

The rapid increase in the global population has led to an increased demand for animal products. The consumption of sheep meat (mutton) and lamb meat has a traditional place in Turkish culture. In Türkiye, the majority of the sheep population is comprised of local (indigenous) breeds, which have not been genetically improved due to their current adaptation traits. Although these local breeds are well adapted to extensive breeding, they do not produce satisfactory performance results under intensive production conditions. In recent years, the reduced size of grassland, resulting from management plans/projects and rehabilitation/improvement programs implemented for pastures has increased the importance of intensive production systems for low-producing local breeds. The priority of animal holdings is the profitability of fattening rather than the length of the fattening period. This is because high profitability enables sheep production to be performed more sustainably. In return, sustainable production enables an increase in both the small ruminant population and the production of red meat. It is projected that an increase in red meat production would reduce sales prices and increase red meat consumption per capita in Türkiye, similar to the case in developed countries.

In the present study, the differences observed in the performance parameters of Morkaraman lambs, which were fed on different dietary plant protein sources during the period between days 0-56 of fattening, were found to be statistically significant ($p < 0.05$).

In a previous study on Tuj (Tushin) lambs, the mean live weight gain achieved during the period between days 0-60 of fattening was determined as 11.66 kg, and the feed conversion ratio was reported as 6.16 kg for the same period (Aksu Elmalı et al., 2010). In another study on Awassi lambs, the mean live weight gain achieved in a fattening period of 56 days was determined as 12.73 kg, and the mean feed concentrate conversion ratios ascertained for the periods between days 0-14, 14-28, 28-42 and 42-56 of fattening were 5.36 kg, 5.22 kg, 5.59 kg, and 6.53 kg, respectively. The mean roughage conversion ratios determined for the same periods in this particular study were 2.41 kg, 2.86 kg, 4.16 kg, and 4.52 kg, respectively (Kul & Akcan, 2002). In a study on Akkaraman lambs, the mean live weight gains achieved in a fattening period of 56 days in the control group and

Groups 1, 2, 3 and 4 were reported as 7.95 kg, 11.14 kg, 15.42 kg, 15.30 kg, and 14.69 kg, respectively (İmik et al., 2003). Another fattening study on Morkaraman lambs reported the mean live weight achieved by day 56 as 40.86 kg and indicated the mean live weight gain achieved from day 0 up to day 56 of fattening as 15.21 kg. This study demonstrated the amount of feed required for the gain of 1 kg of live weight, in other words, the feed conversion ratio as 5.38 (Küçük et al., 2002). Another research conducted by Demir (2019) on Awassi lambs revealed that the live weight gain achieved by the end of a 56-day fattening period was 13.92 kg and the feed conversion ratio was calculated as 6.29. In a more recent study by Erensoy (2022), the mean live weight gains achieved in lambs included in three different groups by the end of 56 days of fattening were reported as 11.60 kg, 13.9 kg and 12.7 kg, and the feed conversion ratios were determined as 4.97, 4.53 and 5.18. A different study on Morkaraman lambs reported live weight gains of 15.61 kg, 14.79 kg, 19.48 kg, 15.99 kg and 18.68 kg to have been achieved in different study groups by the end of a 49-day fattening period (İmik and Günlü, 2011). Furthermore, in research investigating the impact of creep feeding and conventional feeding on growth performance in male Akkaraman lambs, the difference between the live weights measured on days 20 and 62 was reported as 5.03 kg in the animals subjected to creep feeding and 4.69 kg in those subjected to conventional feeding (Yildirim et al., 2023). The comparison of the data obtained in the present study, as shown in Table 2, with the data of previous studies demonstrated a similarity in the mean live weight gains and feed conversion ratios, which showed only numeric differences. These differences may have arisen from several factors that affect the fattening performance. Breed, sex, age, the length of the fattening period, body condition, health status and fattening season are listed among the many factors known to affect the fattening performance (Pala & Gülşen, 2021).

As presented in Table 3, according to a periodic assessment, the mean cost/income ratios determined for the periods between days 0-28, 0-42 and 0-56 of fattening were 1.46, 1.51, and 1.54, respectively. In a previous study, the mean cost/income ratios for fattening periods of 0-30, 0-60 and 0-90 days were reported as 3.48, 3.07 and 2.79,

respectively (Aksu Elmalı et al., 2010). In the present study, an increase in the length of the fattening period was observed to be associated with the irregular increase or decrease of the cost/income ratio. On the other hand, in a study conducted by Aksu Elmalı et al. (2010), an increase in the length of the fattening period was reported to be associated with a decrease in the cost/income ratio. Furthermore, the cost/income ratios reported by Aksu Elmalı et al. (2010) were higher than those reported in the present study. Thus, the results of the two studies differ from each other. This may be attributed to differences arising from the years in which the studies were conducted and various factors (i.e., breed, age, etc.) that affect fattening performance. In previous studies on lambs, the mean cost/income ratio was reported as 1.49 for the Karaman province (Cevger, 1997) and as 1.41 for the Konya province (Günlü et al., 2002). The results of the previous studies conducted in the Karaman and Konya provinces agree with the results of the present study.

The present study investigated the impact of the incorporation of alternative protein sources into the feed ration on the cost of fattening for the periods between days 0-28, 0-42 and 0-56. Based on the assessment of the study results, it was concluded that the use of corn gluten in Group III had reduced the cost of fattening. Moreover, to maximize production profitability it is suggested to employ a fattening period length of 56 days.

Apart from being used as an energy source in animal nutrition, owing to its non-degradability in the rumen, richness in bypass methionine and high level of digestibility, corn is also commonly used for the nutrition of high-producing animals to close the protein deficit. In fact, Darabighane et al. (2020) reported that the incorporation of dry corn gluten into the feed ration of lactating cows, instead of clover hay increased both dry matter intake and digestibility. Furthermore, Maklad Eman et al. (2017) reported that the replacement of corn grains and oilseed meal in the feed concentrate provided to lactating cows by 15% corn gluten increased both the volume of milk produced and the economic efficiency of the dairy farm. Moreover, in the present study, the best feed conversion ratios were achieved in the animals fed corn gluten as a dietary protein source. However, different from the previous studies referred to above, in research conducted by Taylor et al. (2017), an increased rate of corn gluten in the feed ration of cows was reported to have decreased both dry matter intake and the feed conversion ratio. In a study on broiler chickens, Giannenas et al. (2017) reported that the use of corn gluten instead of soybean meal decreased feed intake, live weight gain and the feed conversion ratio, and increased the cost of feeding. Wheat is not only a staple food product for humans but is also critical to animal nutrition. However, due to the characteristics of its gluten form, wheat has been used primarily in the bakery, confectionary and industrial food sectors. The low protein solubility of wheat gluten, despite its high protein content, limits its use in animal nutrition (Can, 2023; Fang et al., 2017). However, similar live weight gain levels have been achieved by İmlik (2019) in different groups of rats fed soybean meal and wheat gluten as dietary protein sources, which is interesting.

In this study, based on cost analysis, the lowest feed concentrate costs were determined in the group fed with

corn gluten at 0-28, 0-42 and 0-56 days of fattening periods. However, it was noted that, throughout the entire fattening period, feeding the lambs a dietary protein combination of soybean meal and safflower meal increased profitability, compared to the other groups, although it increased the feed cost.

Owing to its high ruminal degradability, high and balanced composition of exogenous amino acids and high protein content, soybeans meal has found common use in the nutrition of young animals and poultry (Ergin & Aydemir, 2018). However, the cultivation of soybean is rather limited in Türkiye and its supply heavily depends on imports, which leads to continuously changing prices in relation to the foreign exchange rate. On the other hand, despite being the third most produced crop in Türkiye, after wheat and barley, corn (gluten) is still imported at much lower prices than soybean meal. Wheat gluten is not preferred by animal breeders, given its adverse effects on the performance parameters of lambs and high import prices.

Given that soybeans are part of human, ruminant and poultry diets, they are an important commodity in terms of international trade, which could easily increase the production costs of the livestock sector. In this context, the availability of alternative feedstuffs for incorporation into ruminant rations is critical to reducing the feed costs of animal production. In conclusion, based on our cost-benefit analysis, while the combined use of soybean meal + safflower meal is most profitable, corn gluten could be used as an alternative protein source, depending on the foreign exchange rate and its import price.

Declarations

Ethics Committee Approval

This study was approved by the 17.12.2020 dated and 181 numbered decision of the Local Ethics Board for Animal Experiments of Atatürk University.

Author Contributions

The authors contributed equally to the development and writing of the manuscript.

Declaration of Interests

The authors have no conflict of interest to declare.

Funding

The authors thank the Scientific Research Projects Coordination Unit of Atatürk University for their financial support (Number: TDK-2021-9222). This manuscript is based on the analysis of the data presented in the doctorate thesis of the corresponding author by a different method.

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Growth Performance of Broiler Chicken (*Gallus gallus domesticus*) in Response to Cinnamon Powder as Feed Additives

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ARTICLE INFO

ABSTRACT

Research Article

Received : 19.06.2024

Accepted : 11.09.2024

Keywords:

Cinnamon Powder

Feed additives

Broiler chickens

Growth performance

Broiler feeds

One hundred-twenty (120) heads of day-old Cornish-cross broiler chicks were used in the study, which employed a Completely Randomized Design (CRD). The purpose of the study was to evaluate the growth performance of broiler chickens in response to cinnamon powder supplementation. There were four treatments which were replicated three times having 10 broiler chicks per replication. Treatment 1 (T₁) had 50g cinnamon powder as feed additives, Treatment (T₂) with 75g cinnamon powder, Treatment 3 (T₃) with 100g cinnamon powder and the Control group (T₀) had a basal diet of commercial feeds with no feed additives. The study investigated the growth parameters of broiler chickens for 30 days, after the termination of the study the chickens were slaughtered and weighed to determine the carcass performance of the chickens. After the period of experimentation broiler chickens from Treatment 3 (T₃) with 100 grams of cinnamon powder obtained the highest live weight compared to other treatments. Also, it was observed in the study that broiler chickens from Treatment 3 (T₃) had the highest return on investment with 64%.

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Introduction

The poultry industry is one of the most profitable businesses of agriculture that provides nutritious meats and eggs for human consumption within the shortest possible time. The chicken is the most exploited species of poultry, which is utilized for food production in the whole world. On the other hand, Philippine poultry industry, chickens are ranked one, followed by ducks in economic importance because their products are the main source of meat and eggs. Broiler production and egg production are one of the sunrise industries in the Philippines today. It started as a backyard enterprise but later it shifted to the more commercialized and integrated farming operations. Chickens are one of the most common and widespread domestic animals, with a total population of 23.7 billion as of 2018 from more than 19 billion in 2011 (PSA 2011). Recently, the price of the chicken jump up to 180 to 230 pesos per kilo due to its demand and availability. It is highly nutritious and a good source of protein. Providing commercial poultry feeds all around the period is just not financially feasible, hence there is a need to reduce the cost of poultry production by replacing the costly commercial feed with some comparatively cheaper feed resources. However, this fast-growing industry has been faced with

many backlogs especially in backyard farming because of the prohibition of antibiotic-based feed additives, and the higher cost of feeds. Hence, cinnamon has been seen as an alternative feed additive. Cinnamon powder is renowned for its anti-inflammatory properties. (Adedeje et al., 2021) highlighted the anti-inflammatory effects of cinnamon, suggesting its potential in managing conditions related to inflammation. This includes diseases such as arthritis and certain cardiovascular conditions. In terms of nutritional content, cinnamon is notably low in calories and provides essential nutrients. While it may not be a significant source of macronutrients, such as proteins and fats, it does contain small amounts of minerals like manganese and calcium. Manganese, in particular, plays a crucial role in bone health and metabolism of chickens. Cinnamon's role in modulating immune responses in broiler chickens has also been investigated in a study by (Al-Abdullatif et al., 2023), cinnamon powder supplementation was associated with enhanced immune parameters in broilers. The researchers observed increased antibody titers and improved cellular immune responses, suggesting that cinnamon may contribute to the immunological well-being of broiler chickens. Moreover, the impact of cinnamon on oxidative

stress and antioxidant status in broiler chickens has been a subject of interest. (Kanani et.al., 2016), in a study that reported that cinnamon supplementation led to a reduction in oxidative stress markers and an improvement in antioxidant enzyme activities. These findings indicate that cinnamon may play a role in mitigating oxidative damage in broiler chickens. Thus, this study aimed to determine the growth performance of broiler chicken fed with commercial ration supplemented with varying levels of cinnamon powder.

Materials and Methods

Experimental House and Cages

The one hundred-twenty (120) day-old broiler chicks were housed in a 160 m² stress-free housing within environmentally safe vicinity to ensure high ethical standards. The environment wherein the chicken cages were placed is a bit humid during day time and much colder during night time, hence the used of light and cooler materials were needed. The poultry house was constructed using light materials like bamboo, nipa, binder, and wood to protect the broilers from predators. Anent to this, the living condition of chickens was observed to be in minimum standard requirements, the cages temperature was maintained at 33-32°C on the first week and gradually decreasing it to 18-24°C as the age of the chicken progress. Moreover, the feeder used was a manual feeding of chickens since the experiment is done on smaller population, water troughs or drinkers was also provided still in manual water drinkers, while insuring the availability of water so that the living condition of chicken is maintained. The poultry cages were cleaned and disinfected once every seven days to maintain good hygiene and avoid the entry of pathogens.

Study Design

The study was purely experimental and used a Completely Randomized Design (CRD). The study use cinnamon powder mixed in commercial feed. The following were the amount of cinnamon powder used along with commercial feeds; T₁ = 50 grams cinnamon powder with 950 grams of commercial feeds, T₂ = 75 grams of cinnamon with 925 grams commercial feeds and T₃ = 100 grams cinnamon powder with 900 grams commercial feeds respectively, while T₀ (Controlled group) were fed with pure commercial fee. It was conducted at Sibugay

Technical Institute Incorporated (STII) Eco Agro-Tourism Farm Animal Science Experimental Area at Upper Pangil, Ipil, Zamboanga Sibugay from July 17, 2023 to August 17, 2023 covering 30 days period of experimentation.

Data Collection

The data obtained from the study were, the weekly weights of broiler chickens starting from the 7th day of experimentation until the 30th day. Also, data like the dressed weight and Average Daily Gained Weight (ADG) were collected to further evaluate the effects of cinnamon powder.

Brooding of Chicks

Brooding of chicks happened when the day-old chicks were brood for at least to 14 days. Using incandescent light bulbs during the day and especially during nighttime, this was done to ensure that the chickens are able to insulate themselves and it enhances their eating habit. Cage doors were closed every night to maximize insulation and avoid cold stress. Other bedding materials such as used newspapers and rubbish cartons were used in order to ensure clean pens. Also, chicks during the brooding period were provided with waterers to keep them hydrated during day time.

Broiler chickens were equally fed with readily available commercial feeds during the first seven days of the study. On the first week of the study experimental treatments were introduced based on the randomization techniques. To ensure the physical and dietary well-being of the experimental animals, a gradual change of feeding was employed. The cinnamon powder used in the study was mixed to commercial feeds as mentioned above, hence the cinnamon was just a supplementation not as part of the formulation.

Statistical Analyses

To calculate for significantly different statistical analysis in the growth performance of broiler chickens in response to cinnamon as feed additives, the study used analysis of variance (ANOVA) and using JAMOVI a free and open-source program for data encoding and analysis. When ANOVA results were significant Tukey Honest Significant Difference as post hoc test was done to assess the significance of differences between pairs of the treatment means.

Table 1. Nutritional composition of commercial feeds used in the study

Nutritional Composition of Commercial Feeds used in the Study*					
Chick Booster (1-15 days)		Broiler Starter (16-26 days)		Broiler Finisher (27 – harvest day)	
Crude Protein	Min. 21.50%	Crude Protein	Min. 19.50%	Crude Protein	Min. 18.00%
Crude Fat	Min. 4.00%	Crude Fat	Min. 5.00%	Crude Fat	Min. 6.00%
Crude Fiber	Max. 3.50%	Crude Fiber	Max. 4.50%	Crude Fiber	Max. 5.50%
Moisture	Max. 12.00%	Moisture	Max. 12.00%	Moisture	Max. 12.00%
Calcium	0.85-1.15%	Calcium	0.80 – 1.10%	Calcium	0.80-1.10%
Phosphorus	Min. 0.70%	Phosphorus	Min. 0.70%	Phosphorus	Min. 0.70%

*One brand was used but remained unnamed in order not to promote the brand

Table 2. Summary of the growth performance of broiler chickens in response to cinnamon as feed additives

	Weight of Broiler Chickens (g)							
	1 st week	F	2 nd week	F	3 rd week	F	30 th day	F
T ₀	145.1		368		734.1		1284.7	
T ₁	146.7	12.4*	409.6	34.3*	748.1	33.7*	1292.1	421*
T ₂	152.5		420.9		767		1377.8	
T ₃	159.8		429.9		792		1461.2	

*Very significant at $\alpha=0.05$

Table 3. Carcass traits of broiler chicken in response to cinnamon powder as feed additives.

Carcass traits	Control (T ₀)	Treatment 1 (T ₁)	Treatment 2 (T ₂)	Treatment 3 (T ₃)
Dressing percentage	53.93	56.32	57.86	62.59*
Dressed weight	26724	26671	28659	30022
SEM	2.16	1.81	5.24	4.85

*very significant at $\alpha = 0.05$

Results and Discussions

Weekly weights of chickens

As presented in Table 2, during the first week after introducing the cinnamon powder as feed additives, chickens from Treatment 3 (T₃) with 100g cinnamon powder had the highest average weight of 159.8g, followed by Treatment 2 (T₂) with 152.5g, Treatment 1 (T₁) with 146.7, and the control group (T₀) with 145.1g. In the second week of the study, it was observed that still Treatment 3 (T₃) had the highest average weight of 429.9g, followed by Treatment 2 (T₂) with 420.9g, Treatment 1 (T₁) with 409.6g, and the control group (T₀) with 368g. During the third week of the study, it was found that Treatment (T₃) had the highest accumulated gained weight of 792g, followed by Treatment 2 with 767g, Treatment 3 had an average weight of 748.1g and the control group had 734.1g, while on the twenty-eighth day of the study; broiler chickens from Treatment 3 (T₃) had the highest accumulated weight of 1235g, it was followed by Treatment 2 with 1196g, while Treatment 1 had 1190.1g and the control group had an average weight of 1184.5g. On the 30th day before the termination of the study, broiler chickens from Treatment 3 (T₃) had the highest gained weight of 1461.2g, while Treatment 2 had 1377.8g, and Treatment 1 had an average weight of 1292.1g, and the control group had 1284.1g. These results had been subjected to statistical analyses employing analysis of variance (ANOVA), it was found that these averages had a very significant difference among each other suggesting a very efficient response of broiler from cinnamon powder as feed additives since $p < 0.05$. Furthermore, using Tukey HSD test it was found that among all treatments, Treatment 3 (T₃) with 100g cinnamon powder had a significant difference among all other treatments with $p < 0.01$.

Moreover, these significant results during the 1st week, 2nd week, 3rd and 30th day of the study can be associated with cinnamon powder as a feed additive aligns with the study of (Ali et.al., 2021), which states that cinnamons' bioactive components and microbial effects may enhance nutrient absorption and utilization of animals, contributing to sustained and accelerated growth and health enhancement of the broiler chickens. On the 28th day of the study, chickens were found to be responding rapidly to weather-induced stressors, although (Oduyayo et.al., 2021) exclaimed that cinnamon has stress-mitigating effects, it can never be denied that broilers are sensitive types of

chickens (Krauze et.al., 2021). Weather-related stressors such as temperature fluctuations or humidity levels (Apalowo et.al., 2024), may have introduced variability, masking the potential effects of cinnamon powder supplementation. The non-significant outcome during the 28th day may underscore the importance of optimizing environmental conditions for supplement efficacy.

Dressed Weight

The dressed weight of broiler chickens was found to be high in Treatment (T₃) with 100g cinnamon powder gaining 1203g, it was followed by Treatment 2 with 1146.4g, Treatment 1 had 1067g, while the control group with 1069g average weight. It was found that Treatment 3 with 100g cinnamon powder had a very significant difference compared to other treatments (Table 3). Furthermore, the post hoc analysis revealed that comparing all the mean differences of each treatment Treatment 3 was still very significant with $p < 0.01$.

This significant result in dressed weight is in contrast to (Behera et.al., 2020; El-Hack et.al., 2020), wherein their studies they have found that the inclusion of varying levels of cinnamon powder had no significant effect on the carcass traits. Additionally (Khan and Ahmad, 2023) also reported that cinnamon powder supplementation had improved the BWG and FCR of the chicken but had no significant effect on other carcass parameters.

As presented in Table 4, the average daily gained (ADG) weight of broiler chicks was found to be highest at Treatment 3 (T₃) gaining 47g, followed by Treatment 2 with 45g, while Treatment 1 had 42g and control group had an average of 41.5g respectively. Statistical analysis revealed that there was a very significant difference in the average daily gained weight broiler chickens when varying levels of cinnamon powder was used as feed additives. To further evaluate this significant difference Tukey HSD was employed and it revealed that Treatment 3 with 100g cinnamon powder had a very significant effect on the average daily gained weight performance of broiler chickens.

The results from this study regarding the average daily gained weight of broiler chickens in response to cinnamon powder as a feed additive is in agreement with (Sethy et.al., 2020), in their study they found that broiler chickens' supplemented cinnamon powder had significantly affected

the body weight of the chickens compared to the control group. They also added that broiler chickens supplemented with cinnamon powder had the highest feed conversion ratio (FCR), which led to higher average gained weight. This result also concurs to (Adedeji et.al., 2021) findings, which reflected that the use of cinnamon powder as natural feed additive has a promising effect on the feed intake efficiency, growth performance, and carcass quality of broiler chickens.

Table 4. Average Daily Gained (ADG) weight of broiler chickens in response to cinnamon powder.

Treatments	ADG	F	p-value
Control	41.5		0.633
Treatment 1	42	396**	<0.001
Treatment 2	45		<0.001
Treatment 3	47		<0.001

**Very significant at 0.05

Conclusions

It has been proven by the study and other relevant studies that cinnamon powder as a feed additive can enhance the weight performance of broiler chicken. The findings suggest that cinnamon's bioactive components contribute to sustained growth promotion, cumulative health benefits, prolonged immunomodulation, stress mitigation, and sustained antioxidant protection. These conclusions are consistent with prior research, highlighting the potential of cinnamon powder as a valuable and effective feed supplement for optimizing the growth, health, and overall well-being of broiler chickens over an extended supplementation period.

Declarations

Acknowledgment

The researchers would like to acknowledge the entire faculty of the College of Agriculture of Sibugay Technical Institute Incorporated, for their support and for allowing the researchers to conduct the study in their experimental area.

Ethical Approval Certificate

This study was cordially approved by Sibugay Technical Institute Incorporated – Animal Science Research Committee, and agreed on the decision and permission was obtained from STII Research Ethics Committee to conduct the study on broiler chickens with cinnamon powder as feed additives on the Animal Science Research Area with approval certificate number 342-STII-0015-2023 dated July 17, 2023 to August 16, 2023.

Author Contribution Statement

Ramil B. Entana Jr.: Investigation, formal analysis, manuscript revision, interpretation of data gathered

Mary Ann C. Alejandro: Data collection, investigation, minor manuscript editing

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

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Farmland Challenges in the Haor Basin of Bangladesh: Nature and Solutions

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ARTICLE INFO

Research Article

Received : 26.06.2024

Accepted : 05.08.2024

Keywords:

Haor Basin
Bangladesh
Agriculture
Farmland problems
Netrokona

ABSTRACT

Haor regions are inhabited by one of the most economically disadvantaged communities, which rely on agriculture and endure numerous challenges due to its vulnerability. This article analyzes the data obtained from Key Informant Interviews (KIIs) and Focus Group Discussions (FGDs), which were subsequently connected to prior publications to identify the nature of the agricultural land-related problems in the Netrokona Haor basin. Multiple concerns and their nature have been uncovered through the examination. Among those, difficulties in irrigation systems pose the greatest challenge for regional producers. Additional challenges encompass land fragmentation, pollution, erosion, fishing-related concerns, drainage infrastructure, and flood. This study discusses probable solutions with the directive to new research that claims collaborative venture through government and private agencies. Carefully designed research-based policy framework prioritizing strict implementation of existing laws is crucial to effectively mitigate the problem.

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Introduction

A Haor is a unique body of water distinguished by its bowl-shaped topography and distinct ecological system. The Haor region of Bangladesh covers an area of 1.99 million hectares across seven districts, with a population of 19.37 million (Haor & Wetland Development Board, 2012). The livelihoods of the inhabitants primarily depend on agriculture and related activities, such as crop cultivation, fishing, and livestock rearing (Barkat et al., 2019; Haor & Wetland Development Board, 2012). Additionally, the region contributes 6% to the overall Gross Domestic Product (GDP) (Ministry of Environment, Forest, and Climate Change, 2021).

Netrakona is the only district in the Mymensingh division known for its Haor ecosystem, consisting of 52 Haors and Beels, covering 79,345 hectares. According to the Haor & Wetland Development Board (2012), it was the fourth largest area in the Haor region, accounting for 28.92% of the total land area in Haor. The district has numerous prominent haors, including Dingi Pota Haor, Medar Beel, and Talar Haor (netrokona.gov.bd, 2023). Agriculture in these low-lying areas is the primary means of sustenance for the local population. Agriculture is known to be vulnerable to natural disaster and with the geography of the region exacerbated the susceptibility. Therefore, climate change-related and human-induced challenges cause enduring hardships. Over the years, flash

floods, drought, and changed participation patterns caused serious issues that posed a great toll on agriculture and, thus, on the inhabitants of this low land people. Besides those issues, there are compelling issues related solely to the farmland of this region, which need to be reflected in existing haor management policy. To enhance the overall standard of living and eradicate the said hardship, it is essential to identify and tackle the issues facing agriculture in the Haor basin. This can be done by investigating the daily hardships experienced by the community in Netrokona, identifying the underlying reasons for these issues, and proposing viable solutions that may enhance the region's quality of life.

Identifying farmland-related concerns is vital due to the region's status as the most disadvantaged in the country, with agriculture functioning as the mainstay of income and being directly linked to land usage and management. This study seeks to examine the challenges faced by local farmers in relation to agricultural land. It will analyze the underlying causes and potential solutions, focusing on whether there is/are any significant challenges farmer faces particularly regarding land, if there is any gap in government policy and laws, if there are any flaws in policymaking or implementation, and what can be the way out of addressing those issues.

Existing Policies Regarding Haor Basin

The national water policy (1999) encompasses provisions for allocating water resources towards the conservation of Haor, Baors, and Beels, aiming to preserve aquatic ecosystems and facilitate effective drainage systems. The Ministry of Agriculture (MoA) places significant emphasis on surface water irrigation in its National Agriculture Policy (1999). The Ministry of Fisheries and Livestock introduced the National Fisheries Policy in 1998, outlining the prohibition of untreated industrial effluent disposal and the conservation of fish habitats and complete removal of water (dewatering) from lakes, beels, ditches-canals, and other open water bodies, including Haor. The National Environmental Policy (1992) was designed to maintain ecological integrity by preventing pollution, adopting a land management approach that considers the ecosystem, and preserving wetlands and migratory bird habitats. The National Land Use Policy (2001) is viewed to prevent landfilling and establish a strict prohibition on the encroachment of pre-existing wetland areas. 2009, the government implemented the Jalmohal Management Policy to enhance fisheries' resource production and biodiversity.

Literature Review

Global climate change is causing an ongoing decline in the ecosystem and increasing its susceptibility to damage. The Haor and wetland regions are particularly susceptible to these issues, with the vulnerability of wetlands being influenced by the geographical landscape and dependence on water resources. Specifically, the reliance on rainwater makes wetlands highly vulnerable to climate change effects such as changes in precipitation and temperature (Winter, 2000). These changes can lead to a transformation in the characteristics of wetlands, causing them to shift from being carbon reservoirs to carbon emitters (Salimi et al., 2021). In contrast, Desta et al. (2012) highlight the anthropogenic activities that significantly threaten wetland ecosystems, further exacerbated by climate change. One of their findings suggests that the effect could be a modification in land utilization in the mentioned area.

The haor and wetlands significantly impact the socio-economic well-being of the local population (Kangalawe & Liwenga, 2004). Excessive utilization of resources, coupled with extensive deforestation, can lead to environmental problems. Verhoeven and Setter (2009) stated that the most sustainable use of wetland rice production is to consider its sustainability. However, this study demonstrates that this particular approach can lead to issues such as eutrophication, fish mortality, and a decline in biodiversity due to the toxic effects of pesticides and the lack of wetland preservation. The water scarcity, as observed by King et al. (2021), may be attributed to the excessive subsidization of water usage and the inefficiency of the agricultural sector. Mondal (2010) identified land loss, population increase, and climate change as the primary obstacles facing agricultural agriculture in Bangladesh. The haor and wetland, which have a dynamic and circular function in the economy (Byomkesh et al., 2008), are currently facing a major issue in the form of changes in land use for human habitation. This problem was identified in research conducted in the Tahirpur Upazila in the Sunamganj district (Uddin et al., 2015).

Cited, the literature focuses on the agricultural utilization and climate change-led challenges associated with wetlands. Previous literature on the context of Bangladesh revolves around floods and the significant issue posed by floods in the haor area. Although those studies loosely focus on land-related difficulties, emphasis is not enough to explain those specific issues. In that sense, there is a dearth of literature focusing specifically on farm-land-related issues of wetlands and haor. This study aims to fill this research gap by focusing on land-related issues, the form of those factors, and how they impact farmers

Methodology

The study is extensively qualitative and utilizes field-level data using Focus Group Discussions (FGD). Subsequently, the data are analyzed and evaluated, considering existing scholarly works, and matched with Key Informant Interviews (KII) information.

To collect field-level data, three (03) Focus Group Discussions (FGDs) with farmers of 8-12 members were conducted in Gobindasree, Uchitpur, and Dokkhin Para in the Madan Upazila. To match the data from those FGDs and for a better understanding, two (02) separate FGDs with students and prominent civil citizens were conducted to better understand the information from previous FGDs. The selected students were directly connected with Haor because of their family dependency on agriculture or because they reside in one of the Haor regions. Civil citizens include teachers from renowned institutions, NGO workers, and journalists. Subsequently, the provided information has been qualitatively analyzed to identify challenges and matched with existing literatures. Findings then were further discussed for critical explanation and evaluation through semi-structured interviews with two fishers, one local representative, one NGO worker, and three government officials working in the Department of Agriculture, Department of Fisheries, and Bangladesh Agricultural Development Corporation. Expert opinion has been sought to rank the issues regarding their intensity.

Challenges of Farmland in Netrokona

Land Fragmentation

Land fragmentation in the Haor region is caused by the rapid rise of the population and the rising need for land for residential uses. The main factors contributing to the issue are the diverse land composition and the lack of a well-established land market in South Asia (Niroula & Thapa, 2005). The increase in family members leads to the division of their property and decreased accessibility of cultivable land. The division of inherited property among descendants diminishes extensive land areas into smaller plots, posing difficulties for the inheritor in establishing their household, who opted to use their fertile land to build houses and necessary infrastructure.

Land fragmentation results in a lack of cultivable land for families with reduced size, leading to a decline in crop output (Rahman & Rahman, 2009). According to The Business Standard (2020), the amount of land suitable for farming in Bangladesh has decreased by 69,000 hectares per year.

Table 1. Description of Data Source

No.	Data source	Number	Mode of Data collection
01	FGDs with farmer	03	Field
02	FGDs with civil citizens	02	At the researcher's institute
03	KII	07	In-person and online

Soil Erosion

The Haor area has two primary forms of soil erosion: riverbank erosion and residential premises erosion due to waves. Both lead to the loss of living spaces and changes in arable land's physical features and fertility (Wolfgramm et al., 2007; Novara et al., 2018). Heavy rainfall creates strong currents that lead to soil and river erosion (Zhao et al., 2022).

Pollution and Reduction of Soil Fertility

Soil pollution and reduced fertility are due to various factors, such as the practice of monoculture (Ewel et al., 1991; Shen et al., 2004), the excessive use of chemical fertilizers and pesticides (Havugimana et al., 2015; Savci, 2015; Scholz & McIntyre, 2018), the use of chemicals in fisheries, the iron infestation through irrigation from shallow pumps (Fageria et al., 2008), and pollution from human settlements and tourism activities.

The region is characterized by monoculture, where farmers are adept at repeatedly cultivating the same variety of crops, making soil infertile and eventually decreasing crop production (Shen et al., 2004). Chemical fertilizers and pesticides were introduced to optimize crop yield and lessen pest infestation, often without adequate knowledge of the application procedures and the optimum amounts. Hence, reckless consumption of chemical products leads to increased expenses, decreased soil quality, and environmental contamination, thus creating havoc on Haor's natural ecology. That could also have harmful effects on human health. The same thing applies to fisheries.

Shallow pump water, a prominent irrigation mode, leads to iron proliferation on agricultural land. Typically, the sedimentation process can minimize the intensity of iron. Nevertheless, farmers are driven to irrigate the land without undertaking such necessary treatment due to the lack of reservoir and the urgency of irrigation. This leads to an elevation in iron absorption, resulting in a decline in oxidation, degradation of soil pH, soil fertility, and rice genotypes (Fageria et al., 2008). Farmers typically seek advice from local fertilizer vendors to mitigate the negative effects on crop health, who often recommend synthetic fertilizers only to make money. This results in another episode of rising costs and elevated soil pollution, with no effect on iron infestation.

The production of significant amounts of both perishable and non-perishable waste is widespread in our everyday lives, with insufficient waste management systems in rural/suburban areas leading to exposure of garbage to open space. Furthermore, modernization and the rising trend of tourism in specific regions have led to a rise in the generation of non-biodegradable garbage, such as plastic bottles, bags, and other packaging materials. This garbage contributes to the prevalence of microplastics in the environment, harming aquatic species and perhaps degrading soil quality (Karbalaeei et al., 2018).

Irrigation Problem

The irrigation issue in this region is complex and multifaceted, with natural and anthropogenic catalysts.

Among natural factors, climate change badly impacted Haor and wetland ecosystems. The region is experiencing harsh effects of climate change, including heat waves, erratic precipitation patterns, and prolonged summers without rain. Though June to September is the prime time for precipitation, precipitation patterns exhibit notable fluctuations with the absence or minimal occurrence of rainfall during anticipated times, followed by abrupt episodes of intense rainfall due to the recurrent prevalence of low atmospheric pressure. Monir et al. (2023) showed an up to 82% declining trend in rainfall during the monsoon period, and it will worsen in the near future.

The altered precipitation patterns and the absence of a water reservoir in the Haor region, coupled with the negligent actions of the leaseholder, pose significant challenges to the reliance on surface and precipitation water, which were the main source of irrigation. As a result, reliance on groundwater is increasing. However, the prevalence of declining groundwater caused by excessive exploitation stirs the trouble. Though there are some rules and prescriptions regarding optimal distances between irrigation pumps (Bangladesh Gazette, 2019), no tendency is observed to follow them, resulting in excessive groundwater exploitation. Based on farmers' responses, shallow-water pumps with a depth range of 60-120 feet and deep-water pumps with a depth of 500 feet or more generally access enough water in any season. However, many pumps with depths ranging from 250-350 feet face challenges accessing water due to declining water levels, specifically in summer. The water from the deep pump is devoid of arsenic and iron and is safe for drinking or irrigation. In contrast, water from shallow to moderate depths typically contains high iron intensity, making it unsuitable.

The leasers (leaseholders) engaging in the practice of artificially inundating water bodies to facilitate fish growth impedes the cultivation of paddy during planting season and dewatering the Jalmahal (local name of waterbody) by shallow pumps (a practice that is strictly prohibited by National Fisheries Policy, 1998) for fishing in the months of Falgun and Chaitra causes significant irrigation problem as it is considered the peak time for irrigation. Minimizing cost rationale for their illegal actions, as the rainfall is minimal, and water is at a minimum point.

Flood

This region faces enormous challenges because of flash floods during the monsoon and pre-monsoon seasons, which are triggered by severe rainfall and water inflow from upstream India, particularly due to intense precipitation in Meghalaya. It is one of the consequences of climate change and the insufficiency of appropriate preventative actions (Dey et al., 2021; MoWR, 2012). Every year, a great loss happens due to floods. For

instance, the Netrokona region experienced around 10.5% loss of yields in 2017 (Barkat et al., 2019). The catastrophic floods of 2022 resulted in three consecutive flood occurrences that severely impacted crops, causing a total loss of \$547.6 million (MDMR, 2022). Besides, these floods have many ramifications on agricultural land. Sediment deposition usually enriches the soil but reduces the depth of Haor wetlands and water reservoirs. This raises concerns over navigation during the rainy season and the availability of water resources during the dry season.

Problems Regarding Fisheries and Fishing

The local government manages Haor and Jalmahals leasing to generate revenue. However, this system causes an inefficient and inequitable allocation of wealth with a potential ecological hazard through the endangerment of native species, disruption to irrigation systems, and resource misallocation through unethical practice of encroachment.

The well-known slogan "Jal Jar Jola Tar" (whomever the fisher should have the right to the water reservoir) is the foundation of the 'Jalmahal Management Policy, 2009'. According to this policy, Haor areas should only be leased to registered Jele (fisherman) approved by the Ministry of Local Government, Rural Development & Cooperatives. However, it has come to light that powerful and influential groups are exploiting the loop of the law. Using their physical, monetary, and political strength, these communities utilize the id of fishers to participate in auctions or engage in bidding processes and, upon obtaining the authority, exercise control over and exploit water resources solely to maximize their financial gains. Though the lease is bestowed upon the fisher in white and black, the occupancy belongs to this group. Evidence shows that these groups control a much larger area than what they are entitled to (Barkat et al., 2019). No one is permitted in fishing activities within this area, even within one's farm or private pond, if it is somehow linked with the Jalmahal (the link happens when the whole area is submerged under water in the rainy season, then it looks like a piece of large water reservoir). Consequently, the local populace has no access to fishing, which is supposed to be freely available, forcing them to purchase alternatives from the local market. These influential individuals disrupt the allocation of natural resources, causing the common people's deprivation and financial and nutritional losses.

The exploitation of water resources extends beyond felony trespassing. As mentioned earlier, waterlogging to cultivate fish prevents crops from growing, and dewatering impedes irrigation at the end of the winter season. An important concern arises from using rotenone, which is practiced capturing fish submerged in mud. The primary purpose of rotenone is to manage unwanted and hazardous fish species (Ling, 2002, p. 9) turns into extensive utilization, posing a significant risk to public health (Ling, 2002, p. 25) and issues to amphibians and vertebrates (Dalu et al., 2015; Liu et al., 2015; Sherer et al., 2003). It also endangered indigenous fish species by risking their eggs and larvae.

Lack of Sideway to Carry Crops

Another regional issue is the absence of suitable pathways for transporting crops, livestock, and other agricultural goods to and from their farmland. In the local context, this thoroughfare is commonly called 'Gopat'.

Due to intense precipitation and subsequent flooding, the condition of this sideway deteriorates, which poses a challenge in transporting agricultural produce and livestock from the field to the residence.

Poorly Managed Embankment and Drainage

Establishing embankments and roads often neglects ecological considerations and, even if considered, fails to address environmental needs due to insufficient planning and inadequate maintenance, manifests undesired waterlogging. On the other hand, because of the widespread corruption prevalent in embankment construction, the overall quality of the work is not up to standard. This renders the susceptibility to breakage or erosion during flash floods, resulting in significant hardships for the affected inhabitants.

Sluice gates on the embankments aim to enable the efficient management of irrigation and drainage systems due to poor management and maintenance and lack of utility for agricultural use. Due to the lack of officials of the Water Development Board in the Haor region, maintenance or reconstruction of a malfunctioned or damaged gate takes a long route via local administration to local representatives and often has minimal remedial measures. Moreover, fishery owners utilize the negligence of maintenance to serve their purpose by regulating water flow according to their requirements, disregarding the needs of farmers.

The following table shows the order of the identified issues by their intensity of impact on agricultural land and their relative importance in resolving them. The rank is assigned by the preference of farmers who face it with the consideration of expert opinion.

Discussion

While flooding is commonly regarded as the most significant problem in the Haor area, it positively impacts the fertility of the ground and the biodiversity of the wetland. Moreover, it has been discovered that the community does not acknowledge it as harmful to the land, specifically regarding irrigation. The irrigation situation has worsened lately due to the altered precipitation pattern and decreased subsurface water levels. The Jalmahal Management Policy (2009) is considered the most objectionable and troublesome policy regulation implemented by the government. It leads to an inefficient distribution of resources among stakeholders and obstructs water management for irrigation. Consequently, most of the participants in the FGDs strongly support the elimination of the open water leasing scheme. The issues of soil erosion, flooding, and irrigation problems are interconnected with the inadequate management of the region's infrastructure. Inadequate management systems can exacerbate flooding and erosion, leading to mismanagement of water resources. Pollution and land fragmentation are associated with land management and the implementation of sustainable agricultural methods. The table below displays the ranking of identified concerns based on the severity of the impact on agricultural land and their relative importance in fixing them. The rank is determined based on the feedback from Focus Group Discussions (FGDs) and the insights from experts who participated in Key Informant Interviews (KIIs).

Table 2. Rank of the Problems by the Intensity of Their Impact

No.	Problems	Relative Rank of the Problems
01	Irrigation Problem	1
02	Problems Regarding Fisheries and Fishing	2
03	Land Fragmentation	3
04	Soil Erosion	4
05	Poorly Managed Embankment and Drainage	5
06	Flood	6
07	Pollution and Reduction of Soil Fertility	7
08	Lack of Sideway to Carry Crops	8

Policy Recommendations

Efficient Land Use

Strategies to optimize land utilization can mitigate the negative impacts of land loss and fragmentation. Given the low land-to-population ratio, diligence and careful consideration are needed. Maximum emphasis should be given to the efficient use of land such that no land should be left uncultivated. Promoting human settlement in agricultural land should be discouraged, along with promoting vertical accommodation for a large group of people. Land consolidation can assess and address agricultural land fragmentation (Muchová & Jusková, 2017; Nithinyurwa et al., 2020). Among various approaches, a comprehensive and focused study is crucial to determine the most suitable land use approach and cultivation method for the haor region. Encouraging the cultivation of native crops and the practice of pearl culture during the flood season could potentially enhance the economy.

Minimizing Side Effects of Tourism

Tourism is primarily advantageous for certain groups of people (Barkat et al., 2019), but the resulting pollution surpasses the possible advantages. Therefore, tourism promotions should consider ecological well-being. Failure to address ecological sustainability might lead to adverse outcomes.

Using Eco-friendly and Biodegradable Products

Environmentally friendly products should be adopted to protect the environment and ensure sustainability. The widespread production of synthetic items has been discovered to be a major cause of substantial environmental damage. Therefore, they must be prohibited by enforcing legal measures and public consciousness.

Sideway to Farmland to Carry the Crops

To optimize the transportation of agricultural commodities and livestock, it is imperative to regularly maintain the region's routes. The feasibility of constructing concrete roadways will guarantee sustainability.

Crop Diversity and Controlled Use of Chemicals

Diversification in crop cultivation enhances crop productivity and soil fertility and promotes the sustainability of agricultural practices (Di Falco & Zoupanidou, 2017; Li et al; 2021). In this region, the geographical features of the farmland make it difficult to diversify crops. Therefore, research from agricultural scientists is needed to determine the most suitable combination of crops according to land type. At the same

time, biofertilizer and pesticide use practices should be promoted through public awareness programs and extensive campaigns. Setting a lab in every union for better management of fertilizer use can act as an immersive task for optimal fertilizer use. The concerned department should ensure the availability of information. Implementing the Internet of Things (IoT) in agriculture can solve all of these problems just by a snap of the finger that will lead to an initiative for smart farming (Farooq et al., 2019; Stočes et al., 2016).

Afforestation and Construction of Pavements to Stop Soil Erosion

Ensuring the upkeep of rivers, canals, and reservoirs, as well as constructing embankments and concrete walls, are crucial steps in protecting residential homes from erosion. Implementing extensive programs like CARE, Bangladesh, to install concrete pavement projects in their residential area can reduce erosion. Alternatively, implementing extensive reforestation efforts and constructing living fences around the area can be environmentally friendly to combat wave erosion.

Facilitating Irrigation and Drainage Through Proper Management of Embankment

Ensuring the protection of water reservoirs, carrying out planned construction of embankments and gates, and proper maintenance of them can enhance the efficiency of irrigation systems. A quick response team from the concerned department can effectively reduce the dillydally of maintenance. Regular dredging actions are necessary to ensure sufficient depth in the Haor and Beel for effective drainage, irrigation, and navigation.

The National Water Policy (1999) strictly requires the protection of natural water bodies and the conservation of aquatic ecology. The National Agricultural Policy (1999) also strictly forbids water removal from natural water reservoirs. Hence, controlling the self-interested behaviors of those renting out these resources is imperative to maximize the efficient use of surface water in agricultural fields.

Probable Solution for Fisher

Thorough monitoring can guarantee the right to lease Jalmahal to the fishing community. To eliminate financial constraints on fishing communities that may result in their unwillingness to participate in auctions, a transparent leasing and financial aid system is needed.

Local farmer associations' participation in taking over the lease has been noted as an efficient way to address the

water use problem from the reservoir. This method grants farmers a certain degree of regulatory authority. Under these circumstances, farmers can effectively prevent landlords from drying up the Haor when it is necessary for them to do so. Leasing the Jalmahal to genuine fisher folk or associations could decrease self-interest-driven activity.

Creating More Natural Breeding Zones and Reserved Areas

Policy implementation to prevent extinction could protect native fish species. Immediate action is required to establish further protected areas for fish reproduction to ensure the survival of endangered and vulnerable species. This will simultaneously contribute to the water supply for irrigation. This may be aided by applying synthetic fertilizers and pesticides to agricultural areas as efficiently as possible.

Conclusion

Since Haor is socioeconomically disadvantaged and agriculture makes up most of the Haor economy, adopting sustainable agriculture and agro-based enterprises is essential to raising the standard of living for Haor citizens by increasing income and ensuring food access and extenuating climate change-related issues (Raihan et al., 2024) This study focuses on the district's agricultural land problem and found that the primary concern regarding agricultural land pertains to crop field irrigation. Other issues include land fragmentation (which is more or less a national problem), increasing contamination and declining soil fertility, fishing-related worries, infrastructure constraints, etc.

Diverse organizations are working to eliminate these issues through state-led efforts such as the Hilip by the Government of Bangladesh (GoB) and the International Fund for Agricultural Development (IFAD). However, the scope of the initiatives must be increased to promote inclusivity. Target-led initiatives must also be implemented to lessen the region's agriculture industry's difficulties. To prioritize ecological aspects in policymaking, raising farmers' awareness and literacy levels regarding the best ways to use land, chemicals, and biodiversity in wetland ecosystems is imperative. The concerned government agencies must exercise greater vigilance to safeguard the rights of ordinary citizens. As was shown in the previous section, more research is required to collaborate between public and private institutions.

Limitations Of the Study and Future Research Window

This study aimed to examine the current issues related to agricultural land in Netrokona, which are contributing factors in food production, considering the limitations of time and expense. Therefore, not out of limitations. These include collecting first-hand interview data from a few participants and incorporating geological and agri-scientific knowledge to address the problems posed in various research areas. However, these factors delineate a trajectory for potential avenues of research. Potential research areas include studying the most efficient land use system for Haor, identifying optimal crop combinations

and yields to maximize farmland utilization and productivity, conducting geological surveys to determine water levels and potential solutions, exploring the optimal use of synthetic and chemical fertilizers, and developing a rural waste production and management system.

Declarations

Acknowledgement

Special thanks to BARCIK, Bangladesh, for their initiative and technical support in this project.

Ethics Committee Approval Statement

As the article does not contain any experimental or sensitive data on humans or animals it does not need any ERB approval.

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The Effects of DGAT1-K232A Gene Polymorphisms on Milk Performance Traits in Simmental Cattle

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ARTICLE INFO

ABSTRACT

Research Article

Received : 27.06.2024

Accepted : 13.08.2024

Keywords:

K232A

Polymorphism

RFLP

Simmental

Animal breeding

Growing world population, scientists aim to achieve high-yielding products by using new techniques and methods in the fields of food, agriculture, and livestock. The aim of this study is to identify the DGAT/K232A gene polymorphism for Enhancing Performance Characteristics in Simmental cattle and to use it in breeding programs. DGAT/K232A gene polymorphism was analyzed by RFLP method in 70 Simmental cattle using CfrI restriction enzyme. The frequency of the K allele was found 0.77, while A allele was 0.23. The distribution of DGAT1-K232A genotype frequencies in the breed was not balanced ($p < 0.05$). No significant relationship was found between DGAT1/K232A gene polymorphisms and milk yield due to the small number of samples.

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Introduction

The presence of multiple genes controlling economically significant traits could pose limitations to contemporary breeding methodologies in animal breeding. Therefore, new molecular techniques and statistical methods need to be used to understand the effects of specific genes and non-genetic factors that influence the incidence of a trait in populations. (Akbaş & Bilgiç, 2023).

DGAT1 is an enzyme found in the microsome and is closely related to cholesterol acyltransferases 1 and 2, which are involved in the metabolism of fatty acids and acyl-CoA. Its primary role is to facilitate the synthesis of triglycerides by catalyzing the acylation of diacylglycerol at the sn-3 position (Farese et al. 2000). DGAT1 may impact various physiological processes, including the creation of adipose tissue, lipoprotein assembly, and intestinal fat absorption, and the regulation of lactation plasma Triacylglycerol concentrations, as well as influence lactation and production (Mohammed et al. 2015; Anggraeni 2019). The DGAT1 gene is responsible for encoding the DGAT1 enzyme, which is essential in the final step of triglyceride synthesis in the mammary gland (Schennink et al. 2007; Leskova et al. 2013).

The variation in the Bos taurus cattle breeds, found at positions 10433 and 10434 on exon 8 of the DGAT1 gene (rs AJ318490.1), leads to the substitution of alanine for lysine amino acid at position 232. This variation may have an impact on milk production, growth, and reproductive traits in cattle breeds (Winter et al. 2002; Grisart et al. 2002; Gautier et al. 2007). This genetic variation has been found to have a significant impact on both milk yield and milk composition in dairy cattle. (Gautier et al. 2007; Naslund et al. 2008). The K232 Lysine variant, has been found to be linked to higher protein and fat contents, as well as fat yield, whereas the A232 Alanine variant, is linked to higher milk and protein yields (Winter et al. 2002). This genetic variation is responsible for a significant quantitative trait locus (QTL) that affects milk production traits not only in Holstein dairy cattle but also in other breeds of cattle (Gautier et al. 2007). The frequency of these two alleles varies in different breeds of cattle, and it is possible that there may be additional mutations that contribute to the observed QTL effect on milk production yields (Bijl et al. 2014; Boichard et al. 2014; Vanbergue et al. 2016).

The DGAT K232A gene polymorphism is known to have an effect on milk and meat yield in cattle, and it is considered to have high potential as a marker for improving performance traits of Simmental cattle and for use in breeding programs. Therefore, knowledge of this gene polymorphism is an important source of information for breeders aiming to increase cattle productivity. The purpose of this research was to investigate the DGATI/K232A gene variation as a potential marker in Simmental cattle and to assess its impact on milk production characteristics.

Material and Methods

Animal material and DNA Extraction

Blood samples taken from 66 Simmental cattle breed raised in semi-instantive environment in Erzurum were used as DNA material. All of the cattle were subjected to the same feeding system and had low levels of inbreeding. Blood samples were collected from the tail vein of the animals using vacutainers that contained K3EDTA as an anticoagulant. DNA was isolated from the gathered blood samples with the QIAGEN Genomic DNA Purification kit (Gentra Puregene, USA) following the provided guidelines. Primers were designed based on the DGAT1 sequence (AA/GC) at positions 10433 and 10434 (rs AJ318490.1) GenBank acc. ARS-UCD1.2 (GCF_002263795.1) and used Primer3web version 4.1.0 (<https://bioinfo.ut.ee/primer3-0.4.0>). The primer sequences used and the relevant PCR programs are presented in Table 1.

PCR Conditions and RFLP Analysis

The PCR reaction mixture (20 mL) contained; 2,5 µL genomic DNA, 0,5 µL F –Primer, 0,5µL R-Primer, 0,5 µL of dNTPmix (D7595: Sigma, St. Louis, MO, USA, 0.25mM), 0,5 units of Taq DNA polymerase (D1806: Sigma), 10 µL of 10x PCR Buffer, 2,5 µL of 0.25 mM MgCl2 and ddH2O making the total volume of 20 µL were used for PCR amplification (Table1).

The PCR amplified DGAT/K232A gene region was digested using CfrI(EaeI) restriction enzyme and incubated at 37 °C for 2 hours. The digested products were then separated on a 3.0% agarose gel for 3.0 hours at 45 Volts and viewed under UV light. The allele frequency of the base mutation of each gene was determined using the PopGen 32 software developed by Yeh et al. (1999) to verify whether they were in Hardy-Weinberg (HW) equilibrium.

Statistical Analysis

The effects of DGATI/K232A gene polymorphisms on lactation and some other milk yield traits were investigated in Simmental cattle raised in a private enterprise in Erzurum. A correlation analysis was performed between the milk yield records of animals in different lactations whose yield records were systematically kept between 2017-2020 and the relevant polymorphic regions. The statistical analysis used real milk yield, 305-day milk yield, lactation period, and daily milk yield as parameters to evaluate the association between genotype and milk yield traits. The general linear model in the SPSS 25.0 software program was used to examine the data (IBM SPSS 25.0 Corp. Inc.). Environmental factors such as lactation order, genotype, calving seasons and were considered to have an impact on the relevant yield trait and were taken into account in the analysis. According to the yield traits in the research, the following statistical model was employed (Equation 1).

$$y_{ijk} = \mu + a_i + b_j + c_k + e_{ijk} \tag{1}$$

Yijk is any of the milk yield traits (305-day milk yield, lactation milk yield and daily milk yield); μ is the population average; ai is the ith genotype effect; bj is the effect of the jth lactation order (j: 3; 1st Lactation: 1, 2nd Lactation: 2, 3rd Lactation: 3... 7 Lactation: 7th); ck is the effect of the kth calving season (k: 2; 1: winter-spring, 2: summer-autumn); eijk is the margin of error.

Results and Discussion

PCR and RFLP results and band sizes of 66 samples, excluding 70 samples with no PCR bands observed after PCR amplification, are shown in Figure 1. The DGAT1/K232A polymorphisms were analyzed using PCR-RFLP, and the expected band sizes for each genotype were determined. Following digestion with the restriction enzyme, DGAT1/K232A PCR products revealed KK genotype as bands of 429 bp and 209 bp, KA genotype as bands of 429 bp, 219 bp, and 209 bp, and AA genotype as bands of 219 bp and 209 bp (Figure 1).

The DGATI gene in Simmental cattle was found to have genotype frequencies of 0.64, 0.26, and 0.10 for KK, KA, and AA, respectively. The corresponding allele frequencies for KK and AA were determined to be 0.77 and 0.23, respectively. The Hardy-Weinberg genetic equilibrium test showed that the distributions of DGATI genotype distributions not in balance (P<0.05) in the studied breeds (Table 2).

Table 1. Table 1. DGATI gene primers and PCR conditions

PCR Primers				
Gene region	Reference	Primer sequences	PCR product	Softwares
DGATI/ CfrI	ARS-UCD1.2 (GCF_002263795.1)	F: 5-TGGCCCTGATGGTCTACA-3 R: 5-AGGAAGCGCTTTCGGATG-3	429	https://www.ncbi.nlm.nih.gov/tools/primer-blast/
PCR Conditions				
Initial denaturation	Denaturation	Extension	Number of cycles	Final extension
95 C/45 sec	60 C /50 sec	72 C/2 min	35	72 C/5 min

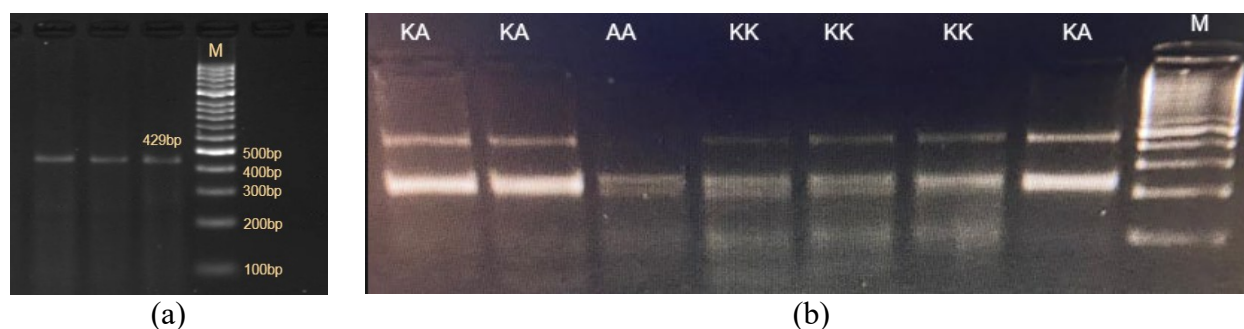


Figure 1. a.PCR results b. DGAT1/K232ARFLP results (KK, KA, and AA genotypes of the DGAT1 gene with Standard 100 bp DNA marker images, respectively)

Table 2. The genotype and allele frequencies and H-W equilibrium DGATI/K232A genes

Genotype	N	allele K	allele A	Observed Frequency	%	H-W Expected Frequency	H-Wa (p value)
KK	42	84	0	42	63.64	38.64	
KA	17	17	17	17	25.76	23.72	
AA	7	0	14	7	10.61	3.64	0.021*
Total	66	101	31	66	100.00	66.00	
Allel Frequencies	132	0.77	0.23	132			

* P<0.05 indicates that the sampled population is imbalanced Hardy-Weinberg equilibriums, a. p-value for the test for Hardy-Weinberg equilibrium.

Table 3. Effects of DGATI/K232A genotypes on milk yields in Simmental cattle

Variation Sources		N	Lactation milk yield X ± Sx	305-day milk yield X ± Sx	Daily milk yield X ± Sx	Lactation period X ± Sx
DGATI/CfrI	AA	9	5439.632±611.955	5385.193±472.945	17.660±1.551	312.760±29.857
	KA	23	5533.781±373.474	5743.017±288.636	18.868±.947	289.876±18.222
	KK	69	5142.732±249.400	5340.576±192.747	17.516±.632	297.560±12.168
	P		0.449	0.432	0.789	0.589
Calving season	1	62	5267.697±308.177	5387.134±238.172	17.672±.781	294.581±15.036
	2	39	5476.400±371.123	5592.056±286.820	18.357±.941	305.551±18.107
	P		0.589	0.493	0.484	0.561
Lactation order	1	3	5138.145±1021.702	5352.121±789.615	17.554±2.589	299.218±49.849
	2	8	4899.464±654.154	4946.878±505.558	16.258±1.658	305.915±31.916
	3	18	5670.094±440.776	5769.680±340.650	18.943±1.117	301.813±21.506
	4	22	5509.839±410.575	5573.682±317.310	18.309±1.041	308.043±20.032
	5	23	5230.101±387.050	5641.915±299.128	18.506±.981	268.256±18.884
	6	18	5141.044±430.314	5169.697±332.565	16.951±1.091	304.638±20.995
	7	9	6015.651±611.740	5973.192±472.778	19.580±1.550	312.575±29.847
P		0.810	0.580	0.582	0.731	
Total	101	5372.048±281.461	5489.595±217.525	18.015±.713	300.066±13.733	

* X: General Means, Sx: Standard deviation.

The DGATI gene in Simmental cattle was found to have genotype frequencies of 0.64, 0.26, and 0.10 for KK, KA, and AA, respectively. The corresponding allele frequencies for KK and AA were determined to be 0.77 and 0.23, respectively. The Hardy-Weinberg genetic equilibrium test showed that the distributions of DGATI genotype distributions not in balance (P<0.05) in the studied breeds.

Based on the analysis of the DGATI/K232A fragment differences in 66 Simmental cattle over 7 lactations, there were no significant differences in milk yield averages based on genotype, calving season, or lactation order factors (p>0.05). Table 3 shows the values for daily milk yields, corrected milk yields, and lactation periods for the breed (Table 3).

In the study, the general averages for milk yield, 305-day milk yield, daily milk yield and lactation period of 66

Simmental cattle in different lactations were 5372.048 kg, 5489.595 kg, 18.015 kg and 300.066 days, respectively. The KA genotype had the highest average milk yield, while the KK genotype had the lowest average. The second season range in the DGATI genotype had the highest overall averages for all yield values, while the first season range had the lowest averages. The highest average daily milk yield was 19,580 kg in the seventh highest lactation, and the second lowest was 16,258 kg. The highest average real milk yield was 6015.651 kg in the seventh highest lactation, and the second lowest was 5093.744 kg. The highest average 305-day milk yield was 5973.192 kg in individuals in the seventh lactation, and the lowest average was 4946.878 kg in animals in the second lactation. The seventh highest lactation period was 312.575 days, while the fifth lowest was 268.256 days.

Table 4. Allele frequency distributions of DGAT1 K232A gene obtained from the present study and other literatures

DGAT1/CfrI Breeds	Allele frequencies			
	N	K	A	References
Borgou	83	0.77*	0.23	Houaga et al.2018
White Fulani	96	0.92*	0.08	Houaga et al.2018
Hardhenu (Holstein Friesian cross breed)	181	0.60*	0.40	Gothwal et al.2022
Sahiwal	83	0.96 *	0.04	Gothwal et al.2022
Polish Holstein- Fresian	144	0.28	0.72	(Kęsek-Woźniak et al. 2020)
Holstein	53	0.43	0.57	(Anggraeni, 2019)
Italian Simmental	95	0.01	0.99	(Scotti et al. 2016)
Holstein Friesian	53	0.57*	0.43	Bhat et al.2017
Jersey	200	0.60*	0.40	Bhat et al.2017
Jersey cross (Local Kashmiri)	200	0.58*	0.42	Özkan Ünal et al. 2015
Anatolian Black (AB)	42	0.58 *	0.42	Özkan Ünal et al. 2015
South Anatolian Red (SAR)	47	0.80*	0.20	Özkan Ünal et al. 2015
Holstein Friesian (HF) bulls	281	0.59*	0.41	(Patel et al. 2009)
Angus, Charolais, Simmental	243	0.09	0.91	(Li et al. 2013)
Croatian beef cattle	175	0.70*	0.30	(Kelava et al. 2013)
Iran Holstein	398	0.37	0.63	(Koopaei et al. 2012)
Holstein cows	1061	0.14	0.86	(Mao et al. 2012)
Simmental beef cattle	26	0.17	0.83	Karolyi et al. 2012
Iranian Holstein Bulls	103	0.80*	0.20	(Mashhadi et al. 2012)

We conducted a study on Simmental cattle, focusing on the allele frequencies for the DGAT1/K232A genes. For the DGAT1/K232A gene polymorphism, the dominant K allele frequency was 0.77 and the A allele frequency was 0.23. The allele frequencies of previous studies on DGAT1/K232A gene polymorphism in different cattle breeds are presented in Table 4. Those similar to the allele frequencies we determined in the Simmental breed are indicated with * symbol.

Hardy-Weinberg equilibrium test was conducted to evaluate the balance of genotype frequencies in the breed for the DGAT1/K232A gene. The test results revealed that the genotype frequencies were not balanced and did not meet the criteria for Hardy-Weinberg equilibrium, which could be attributed to a high rate of inbreeding and the limited sample size of the cattle analyzed ($P < 0.05$). In studies conducted on different cattle breeds, it is observed that genetic distribution is not in HW equilibrium in Czech Simmental and Holstein breeds, Hardhenu and Sahiwal, and Simmental beef cattle breeds due to the low number of cattles (Karolyi et al. 2012; Citek et al. 2021; Gothwal et al. 2022). On the other hand, in Holstein, purebred Holstein cows, Fleckvieh, and Croatian (Simmental, Hereford, Charolais) cattle breeds, it has been observed that DGAT1/K232A genotype frequencies are balanced in distribution (Kelava et al. 2013; Bartoň et al. 2016; Ardicli et al. 2018; Anggraeni, 2019), and in different cattle breeds such as 351 Italian Brown cows and 1061 cows sampled from 2 Chinese Holstein cattle, allele distributions are in Hardy Weinberg equilibrium equilibrium, which consisted of a large number of breeds (Conte et al. 2010; Mao et al. 2012a).

Due to the limited size of the Simmental breed sample and the available milk yield records, In the study did not find any significant correlation between the DGAT1/K232A genotype polymorphisms and milk yield ($P < 0.05$). A parallel study conducted on Holstein cattle using microarray analysis on mammary tissue also found that the gene expression of DGAT1 had negligible effects

on milk yield (Mach et al. 2012). Similarly, studies investigating the association between the DGAT1 K232A gene polymorphism and milk yield have reported no significant associations with milk yield or milk components in Holstein cattle and daily milk yield in Dutch Holstein Friesian cattle (Mach et al. 2012).

There are studies available on different breeds with large population sizes investigating the K232A gene polymorphisms and their associations with 305-day milk yield, total milk yield, milk protein and fat yield, milk composition, daily milk yield, and milk quality parameters (pH, milk fat, milk density, and milk acidity) including Holstein ($n=1236$), Holstein Friesian ($n=415$), Jersey ($n=340$) and Hungarian Simmental ($n=481$) (Smaragdov, 2011; Anton et al. 2012; Mao et al. 2012; Cerit et al.2014; Ardicli et al. 2018; Citek et al. 2021).

In the study we conducted, milk yield was found to be higher in individuals with the KA and lowest KK genotype of DGAT1 K232A, but the differences in milk yield averages were not statistically significant ($P > 0.05$). The DGAT1 polymorphism, especially the allele of Lysine (K), showed the strongest association with milk fatty acid, milk fat yield and fat content, protein content, and saturated fatty acid composition, which confirms the crucial role of DGAT1 in the lipid metabolism of the mammary gland (Conte et al. 2010; Vanbergue et al. 2016; Bhat et al. 2017; Houaga et al. 2018; Palombo et al. 2018; Kęsek-Woźniak et al. 2020). Additionally, membrane arrangement or cell structure of mammary gland epithelial cells is impacted by the DGAT1 KK and AA gene polymorphism (Lu et al. 2015).

The DGAT1 K232A gene polymorphism is widely used as a marker in beef cattle breeds, as it affects not only milk production in dairy breeds but also various performance traits in beef cattle. In beef cattle breeds, it has been shown to affect backfat thickness, backfat retention rate, meat yield, intramuscular fat content, fatty acid composition, and types (Anton et al. 2011; Curi et al. 2011; Karolyi et al. 2012b; Barton et al. 2016). It also has

significant effects on important meat quality traits such as marbling score, backfat thickness, fat color, Warner-Bratzler shear force and longissimus muscle area, as well as on meat tenderness and marbling in beef cattle and saturated fatty acid content in milk (Wu et al. 2012; Karolyi et al. 2012b; Li et al. 2013; Babii et al. 2018).

Conclusions

Several association analysis studies have been published that investigate the potential of DGAT1 genes polymorphisms as markers in cattle breeding, with significant implications found for body weight, meat yield, and reproductive performance in general. However, there is still much to learn about the specific effects of DGAT1 gene polymorphisms on performance traits in Simmental cattle. Future research should aim to expand sample sizes to improve the statistical power of the study, as well as to examine other genetic factors that may interact with the gene to influence performance traits. The DGAT1 K232A polymorphic region has a high potential to be used as a marker in Simmental cattle breeding.

Declarations

Acknowledgment

We would like to express gratitude to the genetics laboratory teams of Atatürk University Faculty of Agriculture, Department of Animal Science, for their invaluable assistance during of the study. We are grateful for the support and assistance of everyone who contributed to this study.

Authors' Contributions

The project idea, design, and study implementation were aided by ZS, HU, and SK. The lab analyses were under the supervision of ZS and HM. The finalization of the paper and scientific editing fell to ZS and SK.

Conflict of Interest

The authors declare no conflict of interest

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Analysis of Specialization in Agricultural Products After WTO Membership: A Review for Türkiye and Mercosur Countries Agricultural Trade Relation

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ARTICLE INFO

Research Article

Received : 04.07.2024
Accepted : 17.09.2024

Keywords:
Agriculture
Livestock
Specialization
Türkiye
MERCOSUR

ABSTRACT

The increase in the volume of foreign trade between Türkiye and MERCOSUR countries in recent years has an important potential. The fact that these countries are economically similar further increases the mutual foreign trade potential. These trade relations are important in terms of economic cooperation and are expected to strengthen the economic interests of countries over time. Both Türkiye and MERCOSUR countries have a high potential in terms of production and foreign trade in agricultural and livestock products. Of course, the fact that countries are members of the World Trade Organization has increased free foreign trade in these sectors. Therefore, in this study, the level of foreign trade specialization in the agriculture and livestock sector after Türkiye and MERCOSUR countries became a member of the World Trade Organization was analysed. In this study covering the years 1995-2022; Net Trade, Export-Import Ratio, Lafay and Michaely indices were used. The findings show that the highest specialization is in Argentina and the lowest in Paraguay. In addition, Türkiye and Brazil have an advantage in numerically similar product groups. Türkiye needs to increase productivity in agriculture to increase the level of specialization in foreign trade.

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Introduction

Established in 1991, the South American Common Market (MERCOSUR) is an economic cooperation organization established by countries in South America to specialize and increase competitiveness on a global basis. MERCOSUR covers an area of approximately 15 million km² and has a population of nearly 300 million. In addition, it is ranked 5th in the world economy. For this reason, it is predicted that the organization, which has been developing since the day it was founded, will increase its international competitiveness (MERCOSUR, 2023).

Foreign trade relations between Türkiye and MERCOSUR countries are generally important and maintain their potential. MERCOSUR, as an economic union of South American countries, consists of Brazil, Argentina, Uruguay, Paraguay and Venezuela (Venezuela is currently suspended). Türkiye, on the other hand, has taken various steps to improve its trade relations with these countries. Although the trade volume between Türkiye and Mercosur countries varies from time to time, it generally tends to increase. Türkiye's exports to major economies such as Brazil and Argentina are noteworthy. Both Türkiye and MERCOSUR countries offer each other significant

economic opportunities with their large populations and diverse industrial sectors. This potential enables the increase in trade volume and the deepening of economic cooperation. Foreign trade relations between Türkiye and Mercosur continue to develop with the awareness of mutual trade potential and are expected to be further strengthened in the future.

In 2021, approximately 44% of Türkiye's trade with South American countries was made to 4 countries that are full members of MERCOSUR. Türkiye's exports to MERCOSUR countries reached USD 1.751 billion in 2021. Türkiye's imports amounted to USD 4.9 billion in 2021 (Ministry of Foreign Affairs, 2023). Negotiations for the signing of the Türkiye-MERCOSUR Free Trade Agreement (FTA) were first held in Buenos Aires on April 22, 2008, and the negotiation process is still ongoing (Ministry of Trade, 2023). Türkiye-MERCOSUR Political Consultation and Cooperation Mechanism Memorandum of Understanding was signed on December 16, 2010. In this context, it is envisaged that political consultation meetings will be held between Türkiye and MERCOSUR.

To accelerate growth and development, emphasis is placed on the industry and services sector rather than the agricultural sector. So, how did the agriculture and livestock sector take shape with the membership of Türkiye and MERCOSUR countries to the World Trade Organization, which takes the economic growth and development of countries as its mission? In this context, in the study, the level of specialization in the agriculture and livestock sector of MERCOSUR and Türkiye after becoming a member of the WTO was analysed and the answer to this question was tried to be sought.

Literature Review

In the literature, there are many publications in which the competitiveness and specialization level of the agricultural sector are analysed with various indexes. For instance; Ceylan (2019) aimed to measure the competitiveness of the wheat sectors in Hungary and Türkiye. The study examines the competitiveness level of both countries using the Revealed Comparative Advantages (RCA) and the Michaely Indexes (MI). According to the results of the analysis, Hungary has a higher degree of expertise in the wheat sector than Türkiye. Matkovski et al. (2019) analysed the European Union (EU) competitiveness of agri-food crops in Southeastern European countries. RCA index was used in the study. In addition, a model was created to determine the determinants of the level of specialization of agricultural products. The results demonstrate that there is a comparative advantage in the agri-food goods in all Southeast European countries except Albania.

Maqbool et al. (2020) used the Symmetric RCA (RSCA), Vollrath (RXA) index, Relative Trade Advantage (RTA) and Net Trade Index (NTI) along with the RCA index to measure Pakistan’s competitiveness in the grain sector. The results of analysis occur the existence of competitiveness in Pakistan’s grain sector. Sukkmaya et al. (2021) measure the specialization performance of agricultural products between Indonesia and Australia using the RCA index, Lafay index (LFI) and Trade Balance index (TBI). According to the results of both RCA and LFI, Indonesia has a high negative TBI rate in total foodstuffs both globally and in Australia. In other words, the country has not been able to specialize in agricultural products to a large extent. Cimpoeş and Sarbu (2020) analysed Moldova’s agricultural product competitiveness using the RCA, RSCA and TBI indices. According to the results of the analysis, it was found that more than half of the other product groups, where specialization and competitiveness were high in four product groups, were disadvantaged.

Karaman et al. (2023) investigate the competitive advantage of Türkiye’s agri-food exports to the EU and Shanghai Cooperation Organization (SCO) markets. While the LFI and trade rates are used, the results show that Türkiye has a competitive advantage in fruit and vegetable exports to the EU and SCO countries. Bahta and Mbai (2023) analysed the competitiveness of Namibia’s agri-food crops not only with the RCA, LFI, Export Diversification (EDI), Hirschman (HI) and Major Export Category (MEC) indices, but also with regression analysis, the Household Food Insecurity Access Scale, and various indices. It was revealed that Namibia is dependent on the

agri-food commodities included in this study and there is no specialization.

In the literature, there are studies examining the competitiveness of many countries and country groups both in various agricultural products and in certain agricultural product groups. However, we have not come across any study examining the agriculture and livestock sector of MERCOSUR countries and Türkiye. Therefore, we think that the study will contribute to the literature and fill an important gap.

Material and Method

The analysis was conducted using data from the COMTRADE database of the World Integrated Trade Solution (WITS) site. Venezuela, one of the MERCOSUR countries, has been excluded from the scope of the analysis due to the lack of foreign trade data. Four indices including both export and import data were used to analyze the level of specialization. Explanations of these indices will be made in this section.

Net Trade Index

The Net Trade Index (NTI) is the ratio of the net export value in a sector to the value of foreign trade volume and is calculated in this way. In this context, the index is used to measure intra-industrial trade and the competitiveness of the country (Kösekahyaoglu & Sarıçoban, 2017). The NTI is formulated as follows:

$$NTI_{jkt} = \frac{X_{kt}^j - M_{kt}^j}{X_{kt}^j + M_{kt}^j} \tag{1}$$

X_{kt}^j denotes the sum of country j’s exports in sector k at time t, and M_{kt}^j denotes the sum of country j’s imports in sector k at time t. The index values between -1 and +1. A positive index value indicates that the country specializes in that sector and has a competitive power vice versa.

Export-Import Ratio Index

The Export-Import Ratio Index (EIRI) demonstrates an industry’s competitiveness and expertise in foreign trade. The index shows the ratio of a country’s share of exports in one sector to its share of imports in the same sector (Balassa, 1977).

The EIRI is formulated as follows:

$$EIRI_{jkt} \log = \left[\frac{X_{kt}^j / X_t^j}{M_{kt}^j / M_t^j} \right] \tag{2}$$

X_{kt}^j denotes the sum of country j’s exports in sector k at time t and X_t^j denotes country j’s total exports at time t. M_{kt}^j denotes the sum of country j’s imports in sector k at time t and M_t^j denotes country j’s total imports at time t.

The logarithmic form of the index gives information about the competitiveness of the country. If the EIRI is > 0.50, the competitiveness of that country is high. If the EIRI is < -0.50, the country's competitiveness is low.

Lafay Index

The Lafay Index (LI) uses not only export data, but also import data, just like other indices that measure the level of specialization. Like NTI, the LI allows the measurement of intra-industry trade and re-export flows. (Desai, 2012).

The LI is formulated as:

$$LI_{ikt} = 100 \left[\frac{X-M}{X+M} - \frac{\sum X-M}{\sum X+M} \right] \frac{X+M}{\sum X+M} \quad (3)$$

X and M refer to the export and import values of the analysed industry. If the index value is zero, the comparative advantage is neutral. If the index value is positive, there is a specialization in that sector. If it is negative, there is no specialization of that sector. The higher the index value, the higher the level of specialize (Sukmaya, Saptana, & Perwita, 2021).

Michaely Index

The Michaely index is an index created by Michael Michaely in 1962. It has been put forward as an alternative to other specialization indices. The Michaely index is formulated as follows (Michaely, 1962):

$$MI_{ij} = \frac{X_{ij}^j}{\sum X_i^j} - \frac{M_i^j}{\sum M_i^j} \quad (4)$$

X_{ij} in the formula; denotes the exports of country j in sector I and M_{ij} denotes the imports of country j in sector i. $\sum X_{ij}$ represents the country's total exports, $\sum M_{ij}$ represents the country's total imports. (Laursen, 2015). The index takes values between -1 and +1. If the value of the index is positive, the country specializes in that sector, and if it is negative, it does not. (Sujova, Hlavackova, & Marcinekova, 2015).

Results

The empirical analysis was conducted on 44 product groups that included Standard International Trade Classification (SITC) Rev. 3,3-digit agricultural goods.

The study covers the years 1995-2022 to understand the specialization of MERCOSUR countries in the agriculture and livestock sector after joining the WTO in 1995. In the analysis comments, each country is classified within itself, and it is aimed to see the country-based analyzes more clearly.

Results of Türkiye's Analysis

The results of empirical analysis of Türkiye (NTI, EIRI, LFI and MI) are shown in Table 1, Table 2, Table 3, and Table 4. In the tables, only those products in which specialization is available are shown. Since the analysis scores cover a period of 28 years (1995-2022), the arithmetic average of the product groups in question was taken and interpreted accordingly.

Türkiye's NTI results (Table 1) show that there is specialization in 25 products, while 19 do not. It can be said that intra-industrial trade is also high in product groups with high NTI values (012, 046, 056, 058, 062 coded products) as both export and import values are high.

Türkiye's EIRI results (Table 2) show that there is a specialization in 32 products (according to logarithmic forms) and not in 12 products. The 046 flour and wheat, has the highest EIRI value. In addition, product groups 058 and 091 are the product groups with the highest value after 046.

Türkiye's LFI results (Table 3) show that there is specialization in 21 products, while 23 do not. The product group with the highest LFI index value is the product group with the code 057 (fruit, nuts, fresh or dried).

Türkiye's MI results (Table 4) show that there is specialization in 32 products, while 12 do not. The highest level of expertise is in the group of fruits, nuts, fresh or fried products with the code 057.

Results of Argentina's Analysis

Results of the empirical analysis of Argentina (NTI, EIRI, LFI and MI) are shown in Table 5, Table 6, Table 7, and Table 8. In the tables, only those products in which specialization is available are shown. Since the analysis scores cover a period of 28 years (1995-2022), the arithmetic average of the product groups in question was taken and interpreted accordingly.

Table 1. Türkiye's NTI Results

Code	Name	NTI	Code	Name	NTI
012	Meat & offal	0.900	054	Veg., fresh, chilled, frozen	0.569
016	Meat, salted, dried, smoked	0.282	056	Veg., preserved	0.909
017	Meat, prep., preserved	0.798	057	Fruit & nuts, fresh, dried	0.805
022	Milk, cream, milk products	0.156	058	Fruit, prep.,preserved	0.935
024	Cheese & curd	0.454	059	Fruit & veg. juices	0.740
025	Eggs	0.529	061	Sugars, molasses, honey	0.237
034	Fish, fresh, chilled,frozen	0.366	062	Sugar confectionary	0.916
035	Fish, dried, salted,smoked	0.700	098	Edible goods & prep. n.e.s.	0.121
036	Crustaceans & molluscs	0.536	111	Non-alcoholic beverages n.e.s.	0.556
037	Marine prod., prep. preserved	0.845	112	Alcoholic bevarages	0.205
046	Flour of wheat	0.992	121	Tobacco, unmanuf.; refuse	0.167
047	Other cereal & flours	0.882	122	Tobacco, manufactured	0.288
048	Cereal, flour & starch	0.825			

Source: Calculated by the author based on COMTRADE (2023)

Table 2. Türkiye's EIRI Results

Code	Name	EIRI	Code	Name	EIRI
011	Meat of bovine animals	2.123	056	Veg., preserved	3.896
012	Meat & offal	4.364	057	Fruit & nuts, fresh, dried	2.994
016	Meat, salted dried, smoked	1.199	058	Fruit, prep., preserved	4.501
017	Meat, prep., preserved	3.352	059	Fruit & veg. juices	2.396
022	Milk, cream & milk products	1.596	061	Sugars, molasses & honey	2.080
024	Cheese & curd	1.317	062	Sugar confectionary	3.655
025	Eggs	2.187	073	Chocolate and cocoa prep.	1.848
034	Fish, fresh, chilled, frozen	1.123	074	Tea	0.658
035	Fish, dried, salted, smoked	2.883	075	Spices	2.225
036	Crustaceans & molluscs	2.281	091	Margarine & shortening	4.564
037	Marine prod., prep. preserved	3.600	098	Edible goods & prep. n.e.s.	0.622
043	Barley, unmilled	1.686	111	Non-alcoholic beverages n.e.s.	2.515
046	Flour of wheat	9.086	112	Alcoholic bevarages	1.264
047	Cereal meals & flours	4.603	121	Tobacco, unmanuf.; refuse	0.912
048	Cereal, flour & starch prep.	2.822	122	Tobacco, manufactured	1.078
054	Veg., fresh, chilled, frozen	2.212	431	Animal/veg. fats/oils, processed	0.935

Source: Calculated by the author based on COMTRADE (2023)

Table 3. Türkiye's LFI Results

Code	Name	LFI	Code	Name	LFI
012	Meat & offal	0.705	054	Veg. fresh, chilled or frozen	1.730
017	Meat, prep., preserved	0.067	056	Veg. preserved	2.359
024	Cheese & curd	0.102	057	Fruit & nuts, fresh, dried	9.362
025	Eggs	0.311	058	Fruit, prep., preserved	2.789
034	Fish, fresh, frozen	0.389	059	Fruit & veg. juices	0.433
035	Fish, dried, salted, smoked	0.055	062	Sugar confectionary	1.131
036	Crustaceans and molluscs	0.089	073	Chocolate & cocoa prep.	0.642
037	Marine prod., prep, preserved	0.183	075	Spices	0.250
046	Flour of wheat	1.948	091	Margarine & shortening	0.496
047	Cereal meals & flours	0.065	111	Non-alcoholic beverages n.e.s.	0.162
048	Cereal, flour & starch prep.	2.370			

Source: Calculated by the author based on COMTRADE (2023).

Table 4. Türkiye's MI Results

Code	Name	MI	Code	Name	MI
001	Live animals	0.0002	057	Fruit & nuts, fresh or dried	0.0309
012	Meat & offal	0.0018	058	Fruit, prep. preserved	0.0082
017	Meat, prep., preserved	0.0002	059	Fruit & veg. juices	0.0015
022	Milk, cream, milk products	0.0003	061	Sugars, molasses & honey	0.0017
024	Cheese & curd	0.0005	062	Sugar confectionary	0.0035
025	Eggs	0.0010	073	Chocolate & cocoa prep.	0.0024
034	Fish, fresh, chilled, frozen	0.0019	074	Tea	0.0001
035	Fish, dried, salted, smoked	0.0001	075	Spices	0.0011
036	Crustaceans & molluscs	0.0004	091	Margarine & shortening	0.0019
037	Marine prod., prep., preserved	0.0007	098	Edible goods & prep. n.e.s.	0.0013
043	Barley, unmilled	0.0003	111	Non-alcoholic beverages n.e.s.	0.0008
046	Meal & flour of wheat	0.0051	112	Alcoholic bevarages	0.0004
047	Other cereal meals & flours	0.0002	121	Tobacco, unmanuf.; refuse	0.0054
048	Cereal, flour & starch prep.	0.0071	122	Tobacco, manufactured	0.0016
054	Veg. fresh, chilled, frozen	0.0082	421	Fixed soft veg. fats/oils	0.0002
056	Veg. preserved	0.0074	431	Animal/veg. fats/oils, processed	0.0009

Source: Calculated by the author based on COMTRADE (2023).

Argentina's NTI results (Table 5) demonstrate that there is specialization in 39 products, but it cannot specialize in only 5 product groups. Among the 39 product groups, the highest specialization is in the 011, 036, 041, 042, 044, 045, 046, 074 and 081 product groups.

Argentina's EIRI results (Table 6), 37 products have specialization and 7 do not. While unmilled wheat with

code 041 has the highest index value, this product group is followed by meal and flour, or wheat coded 046 and milled barley with code 043.

Argentina's LFI results (Table 7), there is specialization in 15 products, while 29 do not. The products with the highest index value are animal feed with code 081 and fixed soft vegetable oils with code 421.

Table 5. Argentina's NTI Results

Code	Name	NTI	Code	Name	NTI
001	Live animals	0.231	054	Veg. fresh, chilled, frozen	0.898
011	Meat of bovine animals	0.968			
012	Meat & offal	0.522	056	Veg. preserved	0.507
017	Meat, prep., preserved	0.864	057	Fruit & nuts, fresh, dried	0.582
022	Milk, cream & milk products	0.880	058	Fruit, prep., preserved	0.482
023	Butter	0.763	059	Fruit & veg. juices	0.905
024	Cheese & curd	0.751	061	Sugars, molasses, honey	0.853
025	Eggs	0.444	062	Sugar confectionary	0.411
034	Fish, fresh, chilled, frozen	0.888	073	Chocolate, cocoa prep.	0.376
035	Fish, dried, salted, smoked	0.780	074	Tea	0.909
036	Crustaceans & molluscs	0.975	081	Animal feeds, excl. unmilled cereals	0.981
041	Wheat, unmilled	0.999	091	Margarine & shortening	0.711
042	Rice	0.946	098	Edible goods & prep. n.e.s.	0.248
043	Barley, unmilled	0.864	111	Non-alcoholic beverages n.e.s.	0.324
044	Maize, unmilled	0.984	112	Alcoholic bevarages	0.674
045	Other cereals, unmilled	0.952	121	Tobacco, unmanuf.; refuse	0.862
046	Meal & flour of wheat	0.977	411	Animal oils & fats	0.310
047	Other cereal meals & flours	0.589	421	Fixed soft veg. fats/oils	0.992
048	Cereal, flour & starch prep.	0.703	431	Animal/veg. fats/oils, processed	0.557

Source: Calculated by the author based on COMTRADE (2023).

Table 6. Argentina's EIRI Results

Code	Name	EIRI	Code	Name	EIRI
001	Live animals	0.629	054	Veg. fresh, chilled, frozen	3.143
011	Meat of bovine animals	6.242		Veg. preserved	1.181
012	Meat & offal	1.612	056	Fruit & nuts, fresh, dried	1.399
017	Meat, prep., preserved	2.887	057	Fruit, prep., preserved	1.675
022	Milk, cream & milk products	4.042	058	Fruit & veg. juices	3.225
023	Butter	6.821	059	Sugars, molasses & honey	3.033
024	Cheese & curd	2.437	061	Sugar confectionary	0.860
025	Eggs	7.212	062	Chocolate & cocoa prep.	0.887
034	Fish, fresh, chilled, frozen	3.254	073	Tea	3.497
035	Fish, dried, salted, smoked	2.359	074	Animal feeds, excl. unmilled cereals	4.670
036	Crustaceans & molluscs	4.535	081	Margarine & shortening	2.186
041	Wheat, unmilled	10.251	091	Edible goods & prep. n.e.s.	0.714
042	Rice	3.769	111	Non-alcoholic beverages n.e.s.	2.134
043	Barley, unmilled	9.211	112	Alcoholic beverages	2.780
044	Maize, unmilled	5.136	121	Tobacco, unmanuf.; refuse	0.756
045	Other cereals, unmilled	4.810	122	Animal oils & fats	1.219
046	Meal & flour of wheat	9.572	411	Fixed soft veg. fats/oils	6.257
047	Other cereal meals & flours	2.838	421	Animal/veg. fats/oils, processed	1.486
048	Cereal, flour & starch prep.	2.266	431		

Source: Calculated by the author based on COMTRADE (2023).

Table 7. Argentina's LFI Results

Code	Name	LFI	Code	Name	LFI
011	Meat of bovine animals	0.560	045	Other cereals, unmilled	0.062
017	Meat, prep.,preserved	0.103	046	Meal & flour of wheat	0.072
022	Milk, cream & milk products	0.015	054	Veg. fresh, chilled, frozen	0.053
036	Crustaceans & molluscs	0.355	059	Fruit & veg. juices	0.044
041	Wheat, unmilled	0.880	074	Tea	0.028
042	Rice	0.043	081	Animal feeds, excl. unmilled cereals	2.476
043	Barley, unmilled	0.064	421	Fixed soft veg.fats/oils	1.738
044	Maize, unmilled	0.961			

Source: Calculated by the author based on COMTRADE (2023).

Table 8. Argentina's MI Results

Code	Name	MI	Code	Name	MI
001	Live animals	0.0004	054	Veg. fresh, chilled or frozen	0.0089
011	Meat of bovine animals	0.0233	056	Veg. preserved	0.0026
012	Meat & offal	0.0052	057	Fruit & nuts, fresh, dried	0.0122
017	Meat, prep., preserved	0.0052	058	Fruit, prep., preserved	0.0034
022	Milk, cream & milk products	0.0090	059	Fruit & veg. juices	0.0057
023	Butter	0.0006	061	Sugars, molasses & honey	0.0052
024	Cheese & curd	0.0025	062	Sugar confectionary	0.0010
025	Eggs	0.0002	073	Chocolate & cocoa prep.	0.0011
034	Fish, fresh, chilled, frozen	0.0113	074	Tea	0.0024
035	Fish, dried, salted, smoked	0.0004	081	Animal feeds, excl. unmilled cereals	0.1227
036	Crustaceans & molluscs	0.0152	091	Margarine & shortening	0.0015
041	Wheat, unmilled	0.0358	098	Edible goods & prep. n.e.s.	0.0013
042	Rice	0.0038	111	Edible goods & prep. n.e.s.	0.0002
043	Barley, unmilled	0.0043	112	Non-alcoholic beverages n.e.s.	0.0080
044	Maize, unmilled	0.0538	121	Alcoholic beverages	0.0046
045	Other cereals, unmilled	0.0031	411	Animal oils & fats	0.0002
046	Meal & flour of wheat	0.0030	421	Fixed soft veg. fats/oils	0.0779
047	Other cereal meals & flours	0.0001	431	Animal/veg. fats/oils, processed	0.0015
048	Cereal, flour & starch prep.	0.0049			

Source: Calculated by the author based on COMTRADE (2023)

Table 9. Brazil's NTI Results

Code	Name	NTI	Code	Name	NTI
001	Live animals	0.304	071	Coffee & coffee substitutes	0.989
011	Meat of bovine animals	0.762	072	Cocoa	0.322
012	Meat & offal	0.975	073	Chocolate & cocoa prep.	0.156
016	Meat, salted dried, smoked	0.728	074	Tea	0.732
017	Meat, prep., preserved	0.990	075	Spices	0.681
025	Eggs	0.383	081	Animal feeds, excl. unmilled cereals	0.922
036	Crustaceans & molluscs	0.504	091	Margarine & shortening	0.142
044	Maize, unmilled	0.408	098	Edible goods & prep. n.e.s.	0.113
057	Fruit & nuts, fresh, dried	0.175	121	Tobacco, unmanuf.; refuse	0.955
058	Fruit, prep., preserved	0.093	122	Tobacco, manufactured	0.814
059	Fruit & veg. juices	0.983	421	Fixed soft veg. fats/oils	0.578
061	Sugars, molasses & honey	0.984	431	Animal/veg. fats/oils, processed	0.296
062	Sugar confectionary	0.621			

Source: Calculated by the author based on COMTRADE (2023).

Argentina's MI results (Table 8), just like the EIRI results, 37 products are specialized, and 7 are not. The product group with the highest index value is animal feeds with the code 081.

Results of Brazil's Analysis

The results of empirical analysis of Brazil (NTI, EIRI, LFI and MI) are shown in Table 9, Table 10, Table 11, and Table 12. In the tables, only those products in which specialization is available are shown. Since the analysis scores cover a period of 28 years (1995-2022), the arithmetic average of the product groups in question was taken and interpreted accordingly.

Brazil's NTI results (Table 9), demonstrate that specialized in 25 products and failed to specialize in 19 products. The product groups with the highest index values are 012, 017, 059, 061, 071 and 081.

Brazil's EIRI results (Table 10) demonstrate that it specializes in 28 products and 16 do not. The product groups with the highest index values are the products with codes 071, 0,17 and 059.

Brazil's LFI results (Table 11) demonstrate that specialization in 17 products and non-specialization in 27 products. According to the LFI results, Brazil is specialized in fewer product groups than other indices. The products with the highest index value are the products with the codes 071, 061, 012 and 081.

Brazil's MI results (Table 12) demonstrate that 22 specialize in 22 products, while 22 do not specialize. The product groups with the highest index values are the products with the codes 071, 061, 012 and 081, like the results of the Lafay index.

Results of Uruguay's Analysis

The results of empirical analysis of Uruguay (NTI, EIRI, LFI and MI) are shown in Table 13, Table 14, Table 15, and Table 16. In the tables, only those products in which specialization is available are shown. Since the analysis scores cover a period of 28 years (1995-2022), the arithmetic average of the product groups in question was taken and interpreted accordingly.

Table 10 Brazil's EIRI Results

Code	Name	EIRI	Code	Name	EIRI
001	Live animals	2.558	071	Coffee & coffee substitutes	7.004
011	Meat of bovine animals	2.512	072	Cocoa	2.824
012	Meat & offal	4.676	073	Chocolate & cocoa prep.	0.603
016	Meat, salted dried, smoked	3.382	074	Tea	2.077
017	Meat, prep., preserved	6.058	075	Spices	3.534
025	Eggs	0.850	081	Animal feeds, excl. unmilled cereals	3.033
036	Crustaceans & molluscs	3.498	091	Margarine & shortening	3.171
043	Barley, unmilled	1.397	111	Non-alcoholic beverages n.e.s.	0.851
044	Maize, unmilled	2.276	112	Alcoholic beverages	0.394
047	Other cereal meals & flours	4.292	121	Tobacco, unmanuf.; refuse	3.447
058	Fruit, prep., preserved	2.082	122	Tobacco, manufactured	2.609
059	Fruit & veg. juices	5.169	411	Animal oils & fats	2.740
061	Sugars, molasses & honey	4.544	421	Fixed soft veg. fats/oils	1.039
062	Sugar confectionary	3.485	431	Animal/veg. fats/oils, processed	0.566

Source: Calculated by the author based on COMTRADE (2023)

Table 11: Brazil's LFI Results

Code	Name	LFI	Code	Name	LFI
011	Meat of bovine animals	1.419	062	Sugar confectionary	0.015
012	Meat & offal	3.680	071	Coffee & coffee substitutes	4.042
016	Meat, salted dried, smoked	0.112	074	Tea	0.030
017	Meat, prep., preserved	0.819	075	Spices	0.026
036	Crustaceans & molluscs	0.158	081	Animal feeds, excl. unmilled cereals	3.764
044	Maize, unmilled	0.493	121	Tobacco, unmanuf.; refuse	1.526
047	Other cereal meals & flours	0.012	122	Tobacco, manufactured	0.199
059	Fruit & veg. juices	1.811	421	Fixed soft veg. fats/oils	0.062
061	Sugars, molasses & honey	4.341			

Source: Calculated by the author based on COMTRADE (2023).

Table 12. Brazil's MI Results

Code	Name	MI	Code	Name	MI
001	Live animals	0.0009	062	Sugar confectionary	0.0010
011	Meat of bovine animals	0.0168	071	Coffee & coffee substitutes	0.0316
012	Meat & offal	0.0320	072	Cocoa	0.0008
016	Meat, salted dried, smoked	0.0012	073	Chocolate & cocoa prep.	0.0003
017	Meat, prep., preserved	0.0067	074	Tea	0.0004
025	Eggs	0.0001	075	Spices	0.0010
036	Crustaceans & molluscs	0.0013	081	Animal feeds, excl. unmilled cereals	0.0322
044	Maize, unmilled	0.0099	098	Edible goods & prep. n.e.s.	0.0004
057	Fruit & nuts, fresh or dried	0.0012	121	Tobacco, unmanuf.; refuse	0.0134
059	Fruit & veg. juices	0.0145	122	Tobacco, manufactured	0.0021
061	Sugars, molasses & honey	0.0386	421	Fixed soft veg. fats/oils	0.0078

Source: Calculated by the author based on COMTRADE (2023)

Table 13: Uruguay's NTI Results

Code	Name	NTI	Code	Name	NTI
001	Live animals	0.902	035	Fish, dried, salted, smoked	0.062
011	Meat of bovine animals	0.975	036	Crustaceans & molluscs	0.446
012	Meat & offal	0.579	041	Wheat, unmilled	0.480
016	Meat, salted dried, smoked	0.328	042	Rice	0.995
017	Meat, prep. preserved	0.738	046	Meal & flour of wheat	0.514
022	Milk, cream & milk goods	0.944	048	Cereal, flour & starch prep.	0.531
023	Butter	0.962	059	Fruit & veg. juices	0.417
024	Cheese & curd	0.918	122	Tobacco, manufactured	0.619
034	Fish, fresh, chilled, frozen	0.759	411	Animal oils, fats	0.641

Source: Calculated by the author based on COMTRADE (2023).

Table 14. Uruguay's EIRI Results

Code	Name	EIRI	Code	Name	EIRI
001	Live animals	4.029	035	Fish, dried, salted, smoked	1.349
011	Meat of bovine animals	11.644	036	Crustaceans & molluscs	1.847
012	Meat & offal	1.613	046	Meal & flour of wheat	2.198
016	Meat, salted dried, smoked	2.700	048	Cereal, flour & starch prep.	1.484
017	Meat, prep., preserved	2.666	057	Fruit & nuts, fresh, dried	1.341
022	Milk, cream, milk products	3.766	059	Fruit & veg. juices	1.429
023	Butter	10.411	091	Margarine & shortening	1.117
024	Cheese & curd	3.917	122	Tobacco, manufactured	2.050
034	Fish, fresh, chilled, frozen	3.324	411	Animal oils & fats	1.727

Source: Calculated by the author based on COMTRADE (2023).

Table 15. Uruguay's LFI Results

Code	Name	LFI	Code	Name	LFI
001	Live animals	0.989	034	Cheese & curd	1.081
011	Meat of bovine animals	10.671	036	Fish, fresh, chilled & frozen	0.050
012	Meat & offal	0.035	041	Wheat, unmilled	0.250
017	Meat, salted dried, smoked	0.305	042	Rice	4.612
022	Meat, prep., preserved	2.643	122	Tobacco, manufactured	0.186
023	Milk, cream & milk products	0.488	411	Animal oils & fats	0.092
024	Butter	1.216			

Source: Calculated by the author based on COMTRADE (2023).

Table 16. Uruguay's MI Results

Code	Name	MI	Code	Name	MI
001	Live animals	0.0150	037	Marine prod., prep., preserved	0.0003
011	Meat of bovine animals	0.1677	041	Wheat, unmilled	0.0119
012	Meat & offal	0.0173	042	Rice	0.0680
016	Meat, salted dried, smoked	0.0003	043	Barley, unmilled	0.0005
017	Meat, prep., preserved	0.0069	046	Meal & flour of wheat	0.0011
022	Milk, cream & milk products	0.0424	048	Cereal, flour & starch prep.	0.0188
023	Butter	0.0074	057	Fruit & nuts, fresh or dried	0.0124
024	Cheese & curd	0.0196	059	Fruit & veg. juices	0.0009
034	Fish, fresh, chilled, frozen	0.0230	091	Margarine & shortening	0.0036
035	Fish, dried, salted, smoked	0.0001	122	Tobacco, manufactured	0.0065
036	Crustaceans & molluscs	0.0026	411	Animal oils & fats	0.0049

Source: Calculated by the author based on COMTRADE (2023).

Table 17. Paraguay's NTI Results

Code	Name	NTI	Code	Name	NTI
011	Meat of bovine animals	0.983	059	Fruit & veg. juices	0.355
012	Meat & offal	0.832	061	Sugars, molasses & honey	0.713
023	Butter	0.044	074	Tea	0.246
041	Wheat, unmilled	0.633	081	Animal feeds, excl. unmilled cereals	0.863
042	Rice	0.714	411	Animal oils & fats	0.544
044	Maize, unmilled	0.673	421	Fixed soft veg. fats/oils	0.918
046	Meal & flour of wheat	0.386	422	Other fixed veg. fats/oils	0.888

Source: Calculated by the author based on COMTRADE (2023).

Uruguay's NTI results (Table 13) show that there is specialization in 18 products and not in 26. The products with the highest index value are the products with the codes 001, 011, 022, 023, 024, 042. The fact that the index value of these products is close to 1 demonstrates that intra-industrial trade is very high in these sectors. Uruguay's EIRI results (Table 14) show that, just like NTI, it specializes in 18 products and fails to specialize in 26 products. Again, like the NTI results, the products with the highest index value are the products with the codes 001, 011, 023.

Uruguay's LFI Results (Table 15) show that it specialized in 13 groups and not in 31 groups. The product groups with the highest LFI value are the products with codes 011, 022, 024 and 042.

Uruguay's MI results (Table 16) show specialization in 22 products and no specialization in 22 products. The product groups with the highest index values are the products with the codes 011, 022, 034, 042.

Results of Paraguay's Analysis

The results of empirical analysis of Paraguay (NTI, EIRI, LFI and MI) are shown in Table 17, Table 18, Table 19, and Table 20. In the tables, only those products in which specialization is available are shown. Since the analysis scores cover a period of 28 years (1995-2022), the arithmetic average of the product groups in question was taken and interpreted accordingly.

Paraguay's NTI results (Table 17) show that it was able to specialize in only 14 products, while it could not specialize in 30 products. The product groups with the highest index value are the products with codes 011, 012, 421 and 422.

Paraguay's EIRI results (Table 18) show that it was able to specialize in 15 products, while it did not specialize in 29 products. The products with the highest index value are the products with codes 011, 041, 042 and 422.

Paraguay's LFI results (Table 19) show that there is specialization in 13 products and not in 31 products. The products with the highest index value are the products with codes 011, 044, 081 and 421.

Paraguay's MI results (Table 20) show that there is specialization in only 10 products and not in 34 products. The products with the highest index values are the products with codes 011, 041, 042 and 421.

Conclusion

In the study, we examined the specialization analyses of Türkiye and MERCOSUR countries by various indexes and reached certain conclusions. Although these results are different in each country and in each index, we can generally say the following. Türkiye specializes in 25 product groups according to NTI, 32 according to EIRI and MI, and 21 according to LFI. Argentina specializes in 39 product groups according to NTI, 37 according to EIRI and MI, and 15 according to LFI. Brazil specializes in 25 product groups according to NTI, 28 according to EIRI, 17

according to LFI and 22 according to MI. Uruguay specializes in 18 product groups according to NTI and EIRI, 13 according to LFI, and 22 according to MI. Paraguay specializes in 14 product groups according to NTI, 15 according to EIRI, 13 according to LFI and 10 according to MI.

Based on these results, Argentina has the highest specialization in each index among Türkiye and MERCOSUR countries (except Venezuela). Argentina is followed by Türkiye and Brazil. According to Argentina's NTI results, products with codes 011, 041, 044, 045, 046, 081, 421 have a very high index value. This shows that intra-industry trade in these product groups is quite high. According to the EIRI results, there is a higher level of specialization in products coded 041, 043 and 046, and according to LFI and MI, there is a higher level of specialization in products coded 081 and 421 compared to other product groups.

The index values of all countries reveal that the countries specialize in almost the same products. Likewise, the results of these indexes should be compatible with each other. Otherwise, the reliability of the index results disappears. While the index results positively differentiate Argentina in the agricultural sector, the competitive advantage of Türkiye and Brazil is like each other. Considering the agricultural and livestock potential of Türkiye and South American countries, the results should not surprise us. The climate and the suitability of agricultural land are very important in the agriculture and livestock sector. Türkiye needs to make better use of both agricultural and pasture areas. Effective use of agricultural lands, development of irrigation systems, improvement of nutrition and health conditions of animals will increase the agricultural and livestock standards and specialization level of these countries. This situation will reveal the profiles of countries that can compete with the world in the agriculture and livestock sector.

Table 18. Paraguay's EIRI Results

Code	Name	EIRI	Code	Name	EIRI
011	Meat of bovine animals	8.091	059	Fruit & veg. juices	1.777
012	Meat & offal	4.095	061	Sugars, molasses & honey	3.238
017	Meat, prep., preserved	0.887	074	Tea	1.462
041	Wheat, unmilled	8.659	081	Animal feeds, excl. unmilled cereals	3.263
042	Rice	5.171	411	Animal oils & fats	3.994
044	Maize, unmilled	2.407	421	Fixed soft veg. fats/oils	3.971
046	Meal & flour of wheat	3.033	422	Other fixed veg. fats/oils	6.177
047	Other cereal meals & flours	0.850			

Source: Calculated by the author based on COMTRADE (2023).

Table 19. Paraguay's LFI Results

Code	Name	LFI	Code	Name	LFI
011	Meat of bovine animals	10.480	061	Sugars, molasses & honey	0.652
012	Meat & offal	0.460			
041	Wheat, unmilled	1.486	074	Tea	0.012
042	Rice	0.944	081	Animal feeds, excl. unmilled cereals	8.126
044	Maize, unmilled	2.469	411	Animal oils & fats	0.105
046	Meal & flour of wheat	0.009	421	Fixed soft veg. fats/oils	6.028
059	Fruit & veg. juices	0.032	422	Other fixed veg. fats/oils	0.392

Source: Calculated by the author based on COMTRADE (2023).

Table 20. Paraguay's MI Results

Code	Name	MI	Code	Name	MI
001	Live animals	0.0018	044	Maize, unmilled	0.0284
011	Meat of bovine animals	0.0888	061	Sugars, molasses & honey	0.0060
012	Meat & offal	0.0045	411	Animal oils & fats	0.0014
041	Wheat, unmilled	0.0135	421	Fixed soft veg. fats/oils	0.0474
042	Rice	0.0103	422	Other fixed veg. fats/oils	0.0024

Source: Calculated by the author based on COMTRADE (2023)

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Evaluation of University Students' Chicken Meat Consumption Preferences and Knowledge of Chicken Production

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ARTICLE INFO

ABSTRACT

Research Article

Received : 04.07.2024
Accepted : 23.10.2024

Keywords:

Chicken meat
Consumption
Preference
Student
Türkiye

This study was conducted to evaluate university students' chicken meat consumption preferences and their level of knowledge about chicken production. The survey was conducted with 61 structured online questionnaires using the Kobo-collect Tool Box. The questionnaires were administered online via student WhatsApp groups. Chicken breast meat was the most consumed with cubed meat being the most preferred. The expiration date was the main factor affecting chicken meat purchasing decisions, and the majority of the respondents did not have any preferred cooking method. Heavier meat (>1 kg) and fresh chicken meat were the most preferred with the highest chicken meat consumption of 1-3 times per week. Chicken meat consumption was highest at dinner and in the winter season with viral diseases being perceived as the most dangerous chicken diseases. The Coronavirus outbreak affected the chicken meat consumption of the majority of the respondents and most of them bought meat from reliable sale points. Most of the respondents could differentiate between slow and fast-growing broiler hybrids with Ross 308 being the most recognized commercial broiler hybrid by the respondents. It was concluded that chicken meat consumption was higher among the respondents however, agriculture students should be motivated to improve their knowledge about chicken production.

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Introduction

In underdeveloped and developing countries, chicken eggs (Abdallah et al., 2022) and meat serve as a cheaper source of protein compared to other protein sources, increasing global production, consumption, exports and imports of poultry meat, eggs and products. Pomaah et al. (2023) reported that the rise in income and standard of living has influenced the demand for poultry products. According to Uzundumlu and Dilli (2022), chicken meat accounted for 57% of total meat consumed and 118 million tons of chicken meat were produced worldwide, of which 17% was supplied by the US, 12.3% by Brazil, 11.5% by China, 4% by Russia and 3.5% by India. In the same period, 16.9 million tons of chicken meat were exported, of which 14.7 million tons were unprocessed and 2.2 million tons were processed, and 24% of this amount came from Brazil, 20.5% from the USA, 8.4% from the Netherlands, 6.2% from Poland, 3.5% from China, 3.1% from Belgium and 3% from Türkiye (Uzundumlu and Dilli, 2022). Furthermore, due to the lack of any religious restrictions or condemnation on poultry meat, it is an acceptable meat source all over the world (Barbut and Leishman, 2022; Uçar and Türkoğlu, 2018).

The global economic crisis and the rise in inflation have also changed the protein or food consumption pattern in the world and in the last decade, the increase in the prices of poultry meat, fish and red meat due to the economic crisis has completely changed the animal protein consumption pattern in the Republic of Türkiye (Kursun et al., 2024). Dierks (2024) reported that the total amount of poultry meat produced in Türkiye in 2023 was approximately 2.4 million tons, with the majority being obtained from chicken meat (2.33 million tons) and approximately 1.2 billion chickens were slaughtered in 2023. Furthermore, according to TUIK (2023), chicken meat production in Türkiye in 2023 was 202,143 tons, while chicken egg production was reported to be 1.80 billion eggs. According to Türkiye Nutrition and Health Survey (TDHS) 2019 data, the average total meat consumption per capita was 86.25 g, while poultry meat consumption per capita was 28.24 g (Turkey Nutrition and Health Survey, 2020). FAOSTAT (2023) also reported that poultry meat consumption per capita in Türkiye reached 20.1 kg in 2021, an increase of 0.05% compared to the previous year however, the all-time high per capita consumption of poultry meat in Türkiye

reached 21.4 kg in 2017 and the all-time low of 2.23 kg in 1964. This explains the significance of the poultry industry in contributing to sustainable protein consumption in Türkiye.

Therefore, this study was conducted to evaluate university students' chicken meat consumption preferences and their level of knowledge about chicken production.

Materials and Method

Survey Region and Target Group

This study was conducted in Çukurova University which is located in city of Adana in the Republic of Türkiye. Adana is located in the southern part of Türkiye and it is situated on the Seyhan River, 35 km (22 mi) inland from the Mediterranean Sea. The latitude and longitude of this city are 36.9914° N, 35.3308° E. Adana has a hot summer Mediterranean climate (Csa) according to Köppen classification and a dry summer subtropical climate (Cs) according to Trewartha classification. Winters are mild and rainy with frost at night, but snow is very rare. Summer months are hot, humid, dry and long, and temperatures often reach or exceed 40 °C (104.0 °F) during heat waves. The targeted group for this survey were the students (undergraduate and post-graduate) in the Faculty of Agriculture in Çukurova University.

For foreign trade, Adana's exports have increased by 38% in the last 5 years and reached 2.5 billion dollars. The 2021 import figure is 3.6 billion dollars and the total foreign trade volume reached 6.1 billion dollars. 35% of Adana's exports are made to European countries and 30% to Middle Eastern countries. Iraq, Germany, Spain, USA and Italy are the top 5 countries in Adana exports. Chemical, food and textile sectors are in the top 3 in exports. On product basis, minibuses, and buses, polyethylene terephthalate, polyester, wheat flour, chemically pure fructose, uncut and frozen meat, cotton trousers, shorts, milling machines, corn starch, synthetic non-continuous fibres from polyesters, steel wires, mandarins, sunflower seeds and oil stand out.

However, job opportunities and income levels are extremely low among the inhabitants of Adana, with some of the parents, elderly or the youth either struggling between jobs or being unemployed. As a result, the majority of the inhabitants are not able to afford some animal proteins such as fish, and red meat. This has created a huge dependence on chicken meat, with an exponential rise in its consumption due to its cheaper price.

Questionnaire Design

The questionnaires for this study were prepared online using Kobo-Toolbox software and consisted of 3 main sections. The first section of the questionnaire included questions about the demographic characteristics of the participants (age, gender, beliefs, etc.), the second section included questions about chicken meat consumption preferences (do you consume chicken meat? preferred parts of chicken meat, cooking methods of chicken meat, etc.) and the third section included questions about broiler chicken production (chicken diseases, breeding systems, broiler hybrids, etc.).

Sample Size

Based on the 2023 data, the total number of students in Çukurova University and in the Faculty of Agriculture, of Çukurova University were 50455 and 2078 respectively. The total population of Çukurova University was subjected to a software called Raosoft program with 99% confidence level and 5% margin of error and the sample size for the entire Çukurova University was generated as 655. The sample size for only Faculty of Agriculture of Çukurova University was calculated using the formula below (Abive-Bortsi et al. (2022):

$$\frac{PS}{PE} \times 655 = \frac{2078}{50455} \times 655 = 27$$

PS: Population of students in the faculty of agriculture in Çukurova University

PE: Population of the entire students in Çukurova University

According to the formula above, the sample size for only the Faculty of Agriculture is 27 for this study. A sample of 61 participants was used instead of the 27 generated using the above formula. This is because larger sample sizes can provide greater precision in estimation, greater statistical power, generalizability of results and confidence in the results. It also reduces the effect of random variability and increases the chances of obtaining statistically significant results.

Data Collection

Online questionnaire designed using the Kobo-collect Toolbox website were administered to the students of the Faculty of Agriculture of Çukurova University (all departments) using the questionnaire's link. The questionnaires were shared in the student WhatsApp groups of the various departments in the Faculty of Agriculture for one week in May 2024. After the data collection period, incomplete or partially completed data were deleted and only the data with all the responses completed by the participants were used in this study.

Statistical Analysis

The data in the current study were sorted using the Kobo-collect toolbox. Uncompleted responses were deleted from the study and only responses that were fully completed by the participants were used for statistical analyses. Both Microsoft Excell 2013 and the Kobo-collect Toolbox were used for descriptive analyses of the results. The results of the statistical analyses were presented as either tables, bar or pie charts.

Results and Discussion

The respondents' gender, age, religion, marital and employment status and monthly salary are given in Table 1. It was revealed that the majority (60.66%) of the respondents were male and 39.34% were female. The higher percentage of males than females could be attributed to the fact that agriculture as a major demands more physical work from students such as cleaning and managing livestock and poultry, making the Faculty of Agriculture more suitable for men than women. Similar to

our study, several survey studies conducted at the university or in the agricultural department have also confirmed a higher number of male students than female students (Kursun et al., 2024; Kara et al., 2020; Avcılar et al., 2023; Akin et al., 2019).

The majority (77.05%) of the respondents were single and 22.95% were married. The reason why the proportion of single respondents was higher than the proportion of married respondents is that students are not considered to be of marriageable age in modern Turkish ideology, or they are simply continuing their education and may prefer to get married when they graduate or start to have a stable source of income for married life. Similar to our findings, other authors (Avcılar et al., 2023; Kursun et al., 2024) reported lower rates of marriage status in survey studies with university students as the target population.

In the current study, the vast majority (91.8%) of the respondents were Muslims which is due to the fact that the current survey was conducted in a Muslim-majority country. According to USDSOIRF (2022) data, the Muslim population in Türkiye is reported to be around 99%, which explains the high proportion of Muslims observed in the current survey, while Dyvik (2023) also reported that the proportion of Muslims living in Türkiye is 83.23%. This may explain the gradual increase in the number of people with no religious affiliation observed in the current study.

The majority (44.26%) of the participants were ≥ 26 , while 42.62% and 13.11% were between 21-25 and 17-20 age groups, respectively. Similar to our findings, some survey studies conducted at universities revealed that the majority of participants were over the age of 25 (Kursun et al., 2024). In contrast to the findings of the current study, several authors (Kara et al., 2020; Avcılar et al., 2023) reported that the average age of the participants in their university-based survey was between 20-22 years. It is likely that the majority of the students participating in the current survey are master's and doctoral students whose age are generally 26 and above.

The proportion of non-working participants was 62.3% and the proportion of working participants was 37.7%. Similar to the findings of the current study, Kursun et al. (2024) also reported that the proportion of non-working participants was 57.31% in their university-based survey study. In the current study, the majority of the participants were not working and this may be attributed to the continuity of the educational studies, which may have prevented most of the students from having additional time for either part-time or full-time jobs.

Among the working respondents, 86.96% had an income of >17000 TL, while 8.69% and 4.35% had an income of 11000-17000 TL and <10000 TL respectively. The majority of participants earned more than 17000 TL per month and it is speculated that these participants may be master's or doctoral students working full-time or part-time in addition to being students. Kursun et al. (2024) also found that most working students in their survey study earned the highest income value set for the survey (>10000 TL).

The answers of the participants regarding chicken meat consumption, chicken carcass and chicken meat form preferences, factors affecting chicken meat purchasing decisions, cooking method preferences and chicken meat sources are given in Table 2.

Table 1. Demographic characteristics of respondents

	Outcomes	Percentage %
Gender	Male	60.66
	Female	39.34
Religion	Muslim	91.8
	Christian	6.56
	No affiliation	1.64
Employment history	Employed	37.7
	Unemployed	62.3
Income (TL)	<10000	4.35
	11000-17000	8.69
	>17000	86.96
Age	17-20	13.11
	21-25	42.62
	≥ 26	44.26
Marital status	Single	77.05
	Married	22.95

Most (98.36%) of the participants consumed chicken meat, while (1.64%) did not consume chicken meat. Durmuş et al. (2012) also reported that in their study, 98.26% of the participants consumed chicken meat, similar to the results of the current study. In recent years, the economic crisis and the increase in unemployment in Türkiye has led to an increase in the purchase and consumption of chicken meat due to its cheaper prices compared to other animal protein sources. Furthermore, the target group in this study are students, the majority of whom are not working and therefore have limited ability to purchase expensive animal proteins such as mutton or beef. This may explain the increase in the number of participants consuming chicken meat observed in the current study. In some studies, the majority of participants reported that chicken meat was the most affordable compared to mutton and beef, and some studies have reported that the affordability of poultry meat is one of the reasons for increased global poultry meat consumption (Roeningk 1999; Aral et al., 2013; Wadud, 2006).

While 40% of the participants preferred breast meat, 25% did not have any chicken meat part preference. 18.33%, 8.33% and 8.33% of the participants had preferences for the thigh, wing and whole chicken meat, respectively. Similar to the findings of the present study, several studies (İskender et al., 2015; Kara et al., 2020; Adamski et al., 2017) have also reported that most of the participants in their study had a higher preference for chicken breast meat. However, in some studies, most of the participants had a higher preference for whole chicken meat or chicken thighs (Durmuş et al., 2012; Dokuzlu et al., 2013; Jayaraman et al., 2012; Memon et al., 2009). Due to the higher number of young male participants in the present study, it was speculated that the higher preference for chicken breast meat may be related to the fact that young men in Türkiye participate more in sports and fitness activities and have the perception that chicken meat, especially chicken breast meat, has a higher protein content than other parts of chicken meat and therefore this may have accounted for the higher preference for chicken breast meat than other parts observed in this current study.

While 51.67% of the participants in the current study preferred cubed chicken meat, 1.67% preferred minced meat. However, the form of chicken meat did not affect the

purchasing decision of 46.67% of the respondents. It can be explained that the reason for the preference for cubed chicken meat may be due to the fact that the meat is cooked faster and is more delicious. Also, in Türkiye, there is a higher interest in barbecues among friends and family during vacations and other family gatherings. Because of this many people may prefer to buy cubed meat which is readily available and suitable for barbecue and this may explain the higher preference for cubed meat than minced meat observed in this study.

The expiration date affected the purchasing decisions of the majority of the participants (66.67%), while 11.67% paid attention to the nutritional content when purchasing chicken meat. Moreover, 11.67% of the respondents paid attention to the production company and 10% stated that the weight of meat was the most important factor affecting purchasing decisions. In line with the findings of the current study, several studies (Durmuş et al., 2012; İskender et al., 2015; Kara et al., 2020) have also identified that the purchasing decision of the majority of the participants was influenced by the expiration date. However, in a study conducted by Adamski et al (2017), it was revealed that while the purchasing decision of the majority of the participants was influenced by the freshness of the product, the expiration date was the third most influential factor affecting the chicken meat purchasing decision. It is evaluated that university students who participated in this survey are aware of food poisoning due to expiration date related problems and may prefer fresh products even if food items with a close expiration date are cheaper. In addition, students are also aware of the lower nutritional value of food items with closer expiration dates, and this may be the reason why the majority of the respondents preferred the expiration date compared to other factors.

While 38.33% of the participants did not have any preferred cooking method, 35% preferred the frying method, and 13.33% preferred the oven-roasting method,

and 13.33% preferred cooking the oven roasting method. The preference for barbecue and boiling methods were 8.33% and 5% respectively. In contrast to the findings of the present study, Memon et al. (2009) reported that 47% of the participants preferred the frying method. In addition, in other studies (Durmuş et al., 2012; Dokuzlu et al., 2013; İskender et al., 2015), most of the participants had a higher preference for the boiling or oven method.

In the current study, 65% of the participants purchased chicken meat from the market and 35% from the butcher. In line with the findings of the present study, other studies (Kara et al., 2020; Vukasovič, 2014; Büyüknisan, 2008) have also confirmed that the majority of their participants purchased chicken meat from markets. In contrast to our findings, in several studies (Neima et al., 2021; Parlakay et al., 2022; Adamski et al., 2017; Ahmed and Mustapha, 2020), most of the participants purchased chicken meat from trusted chicken outlets, chicken product dealers, street vendors and butchers.

Respondents' preferences for different chicken meat weights are given in Fig. 1. Most (58.33%) of the respondents preferred chicken carcasses weighing >1 kg, while the preference for carcasses weight of 0.900-1kg, 0.400-0.800kg and <0.400kg was 21.67%, 16.67% and 3.33%, respectively. Similar to the present study, in a study by Neima et al. (2021), most of the participants had a higher preference for chicken meat that weighs more than 1 kg. In contrast to our results, in a study by Memon et al. (2009), the majority of the participants preferred 1 kg of chicken meat. In other studies, the majority of the participants also reported that they consumed 1 kg or 3-5 kg of chicken meat (Parlakay et al., 2022; Gurram et al., 2018). The preference for a carcass weight of more than 1 kg may be attributed to the recent economic crisis in Türkiye, which caused many people to increase their consumption of chicken meat due to its lower price compared to other types of meat (red meat and fish).

Table 2. Respondents' answers to chicken meat consumption, chicken carcass and meat form preferences, factors affecting chicken meat purchasing decision, cooking method preferences and sources of chicken meat

	Outcomes	Percentage (%)
Do you consume chicken meat?	Yes	98.36
	No	1.64
Chicken carcass part preferences	Breast	40
	Indifferent	25
	Thigh	18.33
	Wing	8.33
	Whole chicken	8.33
Preferences for different forms of chicken meat	Cubed	51.67
	Indifferent	46.67
	Minced	1.67
What do you pay attention to when buying chicken meat?	Expiration date	66.67
	Nutritional content	11.67
	Meat processing company	11.67
	Meat packaged weight	10
Respondents cooking method preferences	Indifferent	38.33
	Frying	35
	Roasting	13.33
	Barbecue	8.33
	Boiling	5
Sources of chicken meat	Butcher shop	35
	Market	65

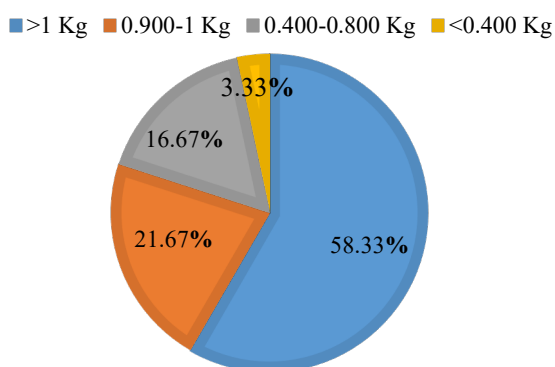


Figure 1. Respondents preference for different chicken carcass weight

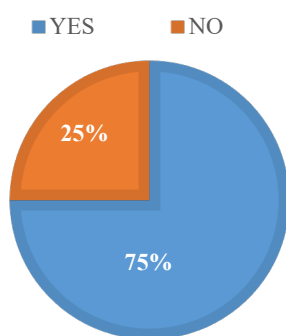


Figure 2. Do you participants pay attention to TSE when purchasing chicken meat?

It is also speculated that the survey target group of young men and women (students) engage in more sports activities and are aware of the higher protein content of chicken meat for muscle production and therefore prefer to buy heavier chicken meat compared to lighter ones.

The answers of the participants on whether they pay attention to the Turkish Standards Institute (TSE) label when purchasing chicken meat are shown in Figure 2. 75% of the participants paid attention to the (TSE) label, while 25% did not. The reasons why the respondents pay attention to the TSE label can be explained by the fact that it proves that a quality and healthy service is provided by facilitating the circulation of quality products in accordance with the standards of the Turkish Government and this brings some form of confidence and trust to consumers as well as ensuring customer satisfaction.

Participants' chicken meat preferences, weekly chicken meat consumption, chicken meat consumption at different meals and seasonal effect on chicken meat consumption are given in Table 3. In the present study, 56.67% and 25% of the participants preferred fresh and frozen chicken meat, respectively, while 18.33% of the participants did not have any chicken meat purchasing preference. Similar to the findings of the current study, other studies (Yıldız and Duru, 2019; Adamski et al., 2017; Büyüknisan, 2008; Parlakay et al., 2022) also revealed that most of the participants preferred fresh chicken meat. The participants' preference for fresh meat may be associated with the better taste or nutritional content of fresh meat.

While 63.33% of the participants consumed chicken meat 1-3 times a week, 31.67% and 5% consumed chicken meat more than 4-6 times and >6 times per week,

respectively. Similar to the findings of the current study, Adamski et al. (2017) also found that most of the participants consumed chicken meat 2-3 times a week in their study. In addition, in a study conducted by Yıldız and Duru (2019), the total weekly chicken meat consumption of the group consuming chicken meat once a week was observed to be higher. Memon et al. (2009) also observed that weekly chicken meat consumption was higher in their study. However, Gurram et al. (2018) reported that the proportion of participants who consumed chicken meat monthly was higher. In addition, Asante-Addo and Weible (2020) also reported that the proportion of participants consuming chicken meat 2-3 times a month was higher. The target audience for this survey group is students, most of whom are unemployed and mostly live in dormitories or at home with their families and are therefore mainly dependent on the food provided to them by their parents or dormitories. Although chicken meat is the cheapest meat compared to red meat or fish, the economic crisis in Türkiye has increased the prices of foodstuffs, making it difficult for the average Turkish family to afford regular chicken meat or for dormitories to provide regular chicken meat to students. This has forced many families or organizations (dormitories) to reduce their budgets or cut their regular supply of animal protein, or rather replace animal protein with vegetable protein. This may explain why the majority of respondents consumed chicken meat 1-3 times a week.

While 58.33% of the participants prefer chicken meat at dinner, 8.33% prefer chicken meat at lunch. However, the meal time or period did not affect the consumption pattern of 33.33% of the participants in the current study. In Turkish food culture, chicken meat is generally consumed as a main diet at dinner. Since the target survey group are students and the majority of them are unemployed, it is speculated that most of them live in dormitories or with families who provide them with food, and in these places chicken meat is usually consumed as the main diet at dinner.

Seasons had no effect on the chicken consumption of 35% of the respondents, while 65% of the respondents' chicken meat consumption was affected by the seasons. In general, differences in the various seasons of the year (from moderate cold to severe cold and from moderate heat to high heat) lead to various physical and hormonal imbalances, which can have a positive or negative impact on food consumption habits. This may explain why the consumption of chicken meat by the majority of participants in the present study was affected by the seasons.

While the majority of participants (79.49%) consumed chicken meat in winter, the consumption of chicken meat in summer and spring were 12.82% and 7.69% respectively. The fact that the representation of the autumn season is zero (0%) means that this season has no effect on the participants' chicken meat consumption habits, which is the main reason why the Excel spreadsheet application excluded the autumn season and its percentage representation from the graphic representation.

It was therefore speculated that higher consumption of chicken meat in winter may be related to higher energy requirements to maintain thermoregulation (body heat/temperature) during the cold months.

Table 3. Respondents' chicken meat preference, chicken meat consumption per week, chicken meat consumption at different meals and seasonal effect on chicken meat consumption

	Outcomes (%)	
What type of chicken meat do you prefer?	Fresh meat	56.67
	Frozen meat	25
	Indifferent	18.33
Chicken meat consumption per week	1-3	63.33
	4-6	31.67
	>6	5
Chicken meat consumption at different meals	Dinner	58.33
	Indifferent	33.33
	Lunch	8.33
Does season affect your chicken meat consumption pattern?	Yes	65
	No	35
Chicken meat consumption per season	Winter	79.49
	Summer	12.82
	Spring	7.69
	Autumn	0

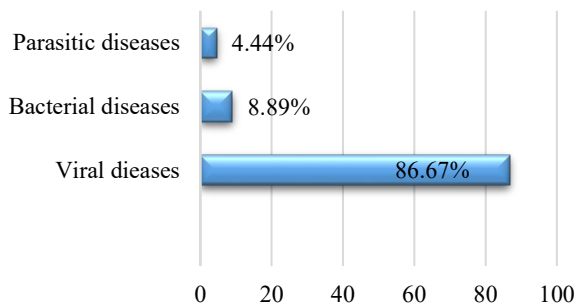


Figure 3. Respondents answers on dangerous chicken diseases

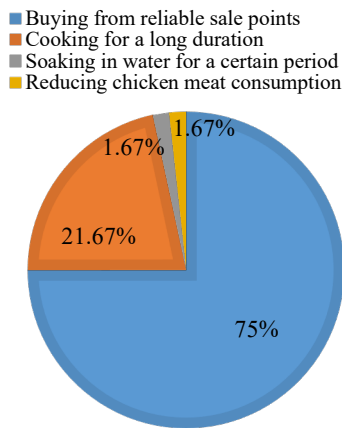


Figure 4. Safety measures taking by respondents to prevent disease spread from chicken meat

Consistent with the findings of the current study, other authors (Kara et al., 2020; Memon et al., 2009) also reported higher consumption of chicken meat during winter months among survey participants. Contrary to our findings, Lee et al. (2017) reported that in their study, 57.3% of the participants consumed chicken meat in the summer.

Participants' evaluation of chicken diseases based on the degree of mortality is given in Figure 3. While the majority of participants (86.67%) evaluated viral diseases as the most dangerous chicken disease, 8.89% and 4.44%

evaluated bacterial and parasitic diseases as the most dangerous diseases respectively. Rahman and Samad (2005) revealed that Newcastle disease is the most common chicken disease and viral diseases such as Newcastle and Avian Influenza are considered the most dangerous poultry diseases with high mortality rates. Since the target audience of the survey are agricultural students taking courses related to livestock and poultry diseases, it is expected that most of these students will be knowledgeable about the effective nature of viral diseases and their impact on poultry production worldwide. This explains the reasons for a higher number of participants who evaluated viral diseases as the most dangerous poultry disease however, Talukdar et al. (2017) reported bacterial diseases as the most common chicken diseases in their study.

Different methods used by the participants to protect themselves from poultry diseases are given in Figure 4. While 75% of the participants buy chicken meat from reliable sales points, 21.67% cook it (chicken meat) for a long time before consumption. Additionally, 1.67% of the participants keep chicken meat in water for a certain period of time before cooking, and 1.67% do not consume chicken meat regularly. Durmuş et al. (2012) also reported that 41.01% of the respondents in their survey did not consume chicken meat to avoid contracting the disease, while 5.63% purchased poultry meat and products from well-known producers.

Participants' answers regarding whether the Coronavirus outbreak affected their chicken meat consumption habits are given in Figure 5. While 61.67% of the participants stated that the coronavirus affected their consumption of chicken meat, the chicken meat consumption pattern of 38.33% of the respondents was not affected by the coronavirus outbreak. Ganesh et al. (2021) reported a 5% decrease in poultry meat consumption in the Tamil Nadu region of India due to the coronavirus outbreak. Additionally, Tzimitra-Kalogianni (2022) reported that a 14.2% decrease in poultry meat imports in Greece between 2019 and 2020 indicates the possible effects of the COVID-19 pandemic on poultry meat consumption and possibly chicken meat consumption. Similarly, Yılmaz et al. (2020) reported that the food consumption and purchasing habits of students at Gümüşhane University were affected by the COVID-19 pandemic. In a study conducted by Jia et al. (2021) at different education levels (high school, university, and graduate) in China, the authors reported that the Coronavirus pandemic caused a decrease in the consumption of poultry meat among female participants compared to male participants. However, Mikail and Kaplan (2021) reported that the coronavirus epidemic in Türkiye did not affect the meat consumption of 64.4% of the respondents.

Participants' information about commercial broiler hybrids is given in Table 4. In the current study, 65.57% of the participants had knowledge of commercial broiler hybrids, while 34.43% of the participants had no information about broiler hybrids. The higher proportion of participants with high knowledge of commercial broiler hybrids may be directly proportional to the fact that agriculture students, who take poultry and animal husbandry courses, and topics related to poultry species and hybrids are an integral part of the poultry breeding courses they take.

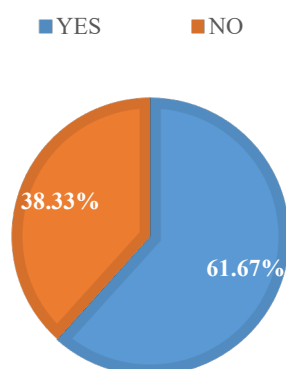


Figure 5. Respondents answers to whether Corona virus affected their chicken meat consumption patterns

Table 4. Respondents knowledge about commercial broiler hybrids

	Outcomes	(%)
Are you familiar with commercial broiler hybrids?	Yes	65.57
	No	34.43
If yes which hybrids are you very familiar with?	Ross	75
	Anadolu T	25
	Cobb	0
Are you familiar with fast and slow-growing commercial broiler hybrids?	Yes	57.38
	No	42.62
If yes which of the following is a fast-growing hybrid?	Ross	80
	Sussex	11.43
	Hubbard	8.57
If yes which of the following is a slow-growing hybrid?	Hubbard	60
	Sussex	31.43
	Ross	8.57

In the current study, 75% of the participants were familiar with the Ross commercial hybrid, while the rate of participants who were familiar with Anadolu T was 25%. It was observed that none of the participants (0%) were familiar with Cobb commercial broiler hybrids. Most of the commercial broiler farms and companies in Türkiye use Ross 308 for production because those hybrids are more resistant to the local environmental conditions and perform better than other commercial broiler hybrids. This reason has increased the rate of Ross 308 in the Turkish market compared to other hybrids, and the fact that the target survey group is more exposed to broiler chickens in school farms, that uses Ross 308 may be the reason why most of the students are familiar Ross 308 better than other hybrids.

In this study, 57.38% of the participants had knowledge of slow and fast-growing commercial broiler hybrids, while the percentage of participants who did not have any knowledge of slow and fast-growing hybrids was 42.62%. The majority of the participants (80%) chose the correct answer by choosing Ross 308 as fast-growing hybrid. However, some of the participants chose either Sussex (11.43%) or Hubbard (8.57%) as fast-growing broiler strains, which was a wrong answer. Additionally, while 60% of the participants chose the right response by choosing Hubbard as the slow-growing hybrid, 31.43% of the participants chose Sussex and 8.57% chose Ross as slow-growing boiler strain, which was a wrong answer. The higher number of participants who were knowledgeable about commercial broiler hybrids, as well as participants who chose correct answers for fast- and slow-growing hybrids, may be

related to the courses they took as agricultural students because an integral part of these courses are theoretical and applied poultry science courses.

Conclusion

It was concluded that the rise in the economic crisis has increased chicken meat consumption preferences and chicken breast meat as well as the expiration date are some of the most influential factors affecting purchasing decisions. Chicken meat consumption was lower among the respondents triggering an emergent situation to ensure sustainable poultry production in Türkiye. It is encouraged that the government should help subsidize poultry farmers in terms of provision of or resources such as chicks, feeds and other production equipment to ensure lower production costs which could also lead to lower market prices of chicken meat. This could help increase the average chicken meat consumption per week/ per capita.

Declarations

Funding

This research did not receive any external fund source

Ethic Report

The ethic report for this survey with the ethic report number E-74009925-604.01-1004777 was granted by the ethic committee of Çukurova University, Adana, Türkiye.

Conflict of Interest

The authors declare no conflict of interest

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Acceleration of Breaking Buds Dormancy on Apricot Trees by Using Alternatives of Hydrogen Cyanamide (Dormex) and Assessment of Resulting Fruits Quality

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ARTICLE INFO

ABSTRACT

Research Article

Received : 10.07.2024

Accepted : 20.08.2024

Keywords:

Dormancy

Dormex alternatives

Apricot

Breaking buds

Fruit quality

The purpose of this research was to determine the effectiveness of alternatives of dormex on apricot budbreak and their effect on apricot fruit quality. Our study was carried out on five-years old "Canino" apricot at Badr district, El Behira Governorate, Egypt, during the two seasons 2022 and 2023, respectively. Twenty-four uniform apricot trees were selected and sprayed to the runoff once on 20 and 25 January during 2022 and 2023, respectively, by following treatments: control, mineral oil at 2%, low pyrite urea at 2%, ammonium nitrate at 1.5%, potassium nitrate at 2%, mineral oil combined with low pyrite urea at the same concentration of 2%, mineral oil at 2% combined with ammonium nitrate at 1.5%, mineral oil combined with potassium nitrate at the same concentration of 2%. For each of the treatments that were used, 0.05% (v/v) of the non-ionic surfactant tween 80 was applied. In both seasons, the use alternatives dormex chemicals resulted in better budbreak than control trees especially the formulation of mineral oil at (2%) plus potassium nitrate (2%).

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Introduction

Cultivation of low chill fruit trees has been widely adopted in the last few years especially in arid regions. However, late bud opening and the need to break uniformly at the end of-dormancy have encouraged many growers to use chemicals that stimulate bud opening. Many growers lately have been using Dormex (hydrogen cyanamide) to break bud dormancy. However, there has been a reduction in using Dormex since 2008. Countries within the European Union have prohibited the use of hydrogen cyanamide due to its high toxicity (EFSA, 2010). In searching for alternatives to Dormex, one must consider efficiency, low environmental and plant toxicity, as well as low cost of use these three primary desirable qualities that were reported by (Erez, 2000) that must be available in the new bud breaking agents. Thus, the use of Dormex has been ceased because of its extreme toxicity even though its use is still going on in many countries as a mean to break bud dormancy, not only in grapevine, but also in other deciduous trees such as peach, plum, nectarine and apple trees.

This research focuses on the use of some relatively new agents especially combinations that could have synergistic effects on Canino apricot trees to break their bud dormancy especially following a relatively warm winter to be able to harvest early which increases the potential of gaining higher marketing value since this crop has the potential to marketing in Egypt and in many Arab countries in addition the export. The use of potassium, low concentration of ammonium and low pyrite urea in combination with summer-mineral oil in a synergistic formulation could raise the buds respiration and provide the growers with an application in their hands and provide an economic treatment.

Therefore, the aims of this research were to provide the apricot growers with an effective alternative to application of Dormex that is applicable on a field scale with one spray. It is also desirable to have a synergistic influence of certain combination which means lower cost with high efficacy. It is also a plus to have higher quality fruits as compared with the control.

Materials and Method

This study was completed in both of the seasons 2022 and 2023 respectively, using five years old “Canino” apricot trees cultivar (*Prunus armeniaca* L) spaced at 4×5 m and grown under drip irrigation system in a private orchard at Badr district, El Behira Governorate, Egypt. Trees were subjected to conventional agricultural methods throughout the season. The soil had a sandy texture and a drip irrigation system was implemented. The treatments were organized using a fully randomized block design. Each treatment was replicated three times, with one apricot tree representing each replication. A total of twenty-four trees, including the control group, were used in this study for each season. A total of twenty-four “Canino” apricot trees were randomly allocated to one of eight treatments. The treatments were administered by spraying the trees once on January 20th and January 25th in both 2022 and 2023. Twenty four apricot uniform trees were selected and sprayed by one of following treatments:

- Control (sprayed with water).
- Mineral oil at 2% (v/v) (K.Z (oil in miscible type) developed by Kafer El-Zayat pesticides & Chemicals Co.,)
- low pyrite urea at 2% (w/v)
- Ammonium nitrate at 1.5% (w/v)
- Potassium nitrate at 2% (w/v)
- Mineral oil combined with low pyrite urea at the same concentration 2%.
- Mineral oil at 2 % combined with ammonium nitrate at 1.5 %.
- Mineral oil at 2% combined with potassium nitrate at 2%.

To each of the treatments that were used, 0.05% (v/v) of the non-ionic surfactant tween 80 was applied. The trees received the usual standard horticulture practices of fertilization, irrigation and pruning. Trees had labeled ten shoots all around the canopy where five at the northern half of tree canopy and other five shoots at the southern half of canopy. Randomized complete block designs (RCBDs) were used to organize these treatments.

The following parameters were determined to evaluate the tested treatments:

- *Buds manner*: Start of bud burst was recorded for each treatment. The numbers of total buds and opened buds were counted and the percentage of opened and closed buds were calculated.
- *Chemical characteristics*: A Carl-Zeiss hand refractometer was used to measure the percentage of total soluble solids (TSS) in fruit juice (AOAC, 1994).

According to (AOAC, 1994), total titratable acidity (%) is expressed as g of malic acid per 100 milliliters of juice. Soluble solids content (SSC) / acid ratio: This ratio was determined using the titratable acidity and TSS data for fruit juice.

Identification of total carotenoids by using the technique described by Ranganna (1995). Five grammes of fresh apricot fruit, or a known weight of the sample, were weighed and then coarsely pulverised in acetone using a mortar and pestle until the residue was colourless. A conical flask was used to collect the acetone extract. The carotenoid pigments were separated using a separating funnel. After moving the carotenoid extract into a

separating funnel, petroleum was added along with 10% Na₂SO₄. To separate the carotenoid layer, swirl the funnel. Volumetric flasks held the collected separated carotenoids. Until no colour was left in the extract, the procedure was repeated. The absorbance was measured spectrophotometrically at 452 nm. Total carotenoids were estimated using following formula:

$$TC \text{ (mg/100g F.W basis)} = \frac{3.87 \times A \text{ (452 nm)} \times VM \times DF \times 100}{VS(g) \times 1000}$$

TC : Total carotenoids

A : absorbance

VM : Volume make up

DF : Dilution factor

VS : weight of sample

Determination of vitamin C content was determined using the spectrophotometer (APEL, PD-303S Japan). Desai and Desai (2019) state that, in technique, upon the addition of bromine water, ascorbic acid undergoes oxidation and transforms into dehydroascorbic acid. A coupling reaction takes place when 2, 4 dinitrophenyl hydrazine is subjected to heating at a temperature of 37°C for a duration of three hours. Following the addition of 85% H₂SO₄ to the solution, a colored complex formed after three hours. The absorbance at 491 nm was then measured.

Statistical Analysis

It was done according to Gomez and Gomez, 1984, using CoStat (Version 6.303, CoHort, USA, 1998–2004).

Results

The data in (Table 1) and (Figure 1 and 2) showed that variations in opened buds in the southern and northern directions of the tree due to applied treatments. The data indicated that the greatest open buds in the southern half of the tree were obtained with the use of mineral oil plus potassium nitrate ranging from 88 to 95 % followed by the same components (mineral oil at 2% plus ammonium nitrate at 1.5%) which resulted in the percentage of open buds between 60 and 70 % in two seasons. That was the case at the northern direction of the tree. Since the greatest percentage of opened buds was obtained also by application of mineral oil 2% plus potassium nitrate at 2 % followed by mineral oil at 2 % plus ammonium nitrate at 1.5 %. The greater magnitude was found with the buds at the southern half of the tree when compared with the northern half. The second magnitude of increase in opened buds was obtained by mineral oil at 2 % plus low pyrite urea at 2 %. Meanwhile, that was the case with the southern half but with lower magnitude ranging between 27 % to 38 %. Mineral oil at 2 % alone resulted in 10 to 16 % and 6 to 7 % in the southern and northern parts of the tree respectively.

The data in (Table 2) show the changes in total soluble solids (TSS) in response to the various studied applications as an alternative to Dormex. The data provided evidence that the tree that had a progress in the development also reflected greater advancement in the development of the TSS.

Table 1. Percentage of opened (southern and northern) buds of “Canino” apricot as affected by pre-harvest applying during the two seasons 2022 and 2023.

Treatments	percentage of the southern opened buds		percentage of the northern opened buds	
	2022	2023	2022	2023
Control	3.14 ^{g*}	9.48 ^g	0.80 ^g	1.43 ^h
Mineral oil 2%	10.67 ^f	16.38 ^f	6.57 ^f	7.17 ^g
low pyrite urea 2%	25.50 ^e	20.05 ^{ef}	7.66 ^f	12.03 ^f
Ammonium nitrate 1.5 %	30.64 ^e	23.75 ^e	12.80 ^e	15.11 ^e
potassium nitrate 2%	40.28 ^d	28.60 ^d	18.12 ^d	20.87 ^d
Mineral oil 2% + low pyrite urea 2%	55.00 ^c	44.34 ^c	27.56 ^c	38.20 ^c
Mineral oil 2% + ammonium nitrate 1.5 %	70.57 ^b	61.99 ^b	48.72 ^b	54.57 ^b
Mineral oil 2% + potassium nitrate 2%	94.69 ^a	88.44 ^a	76.33 ^a	67.27 ^a
LSD _{at 5%}	7.09	3.92	2.48	2.87

* Values in each column that were followed by similar letters did not differ substantially at 5%, the Least Significant Difference was used to compare the means.

Table 2. TSS, acidity and TSS/acidity of “Canino” apricot as affected by pre-harvest applying during the two seasons 2022 and 2023.

Treatments	TSS (%)		Acidity (%)		TSS/Acidity (ratio)	
	2022	2023	2022	2023	2022	2023
Control	11.39 ^{ft*}	11.85 ^{ff}	1.56 ^a	1.62 ^a	7.29 ^f	7.32 ^g
Mineral oil 2%	11.71 ^{ef}	12.24 ^{ef}	1.49 ^{ab}	1.54 ^a	7.86 ^{ef}	7.95 ^g
low pyrite urea 2%	12.12 ^{de}	12.64 ^{de}	1.40 ^b	1.42 ^b	8.68 ^e	8.91 ^f
Ammonium nitrate 1.5 %	12.43 ^{cd}	12.93 ^{cd}	1.28 ^c	1.33 ^{bc}	9.69 ^d	9.76 ^e
potassium nitrate 2%	12.73 ^{bcd}	13.23 ^{bc}	1.21 ^{cd}	1.25 ^{cd}	10.55 ^{cd}	10.55 ^d
Mineral oil 2% + low pyrite urea 2%	13.13 ^{abc}	13.75 ^{ab}	1.16 ^{de}	1.20 ^{de}	11.29 ^c	11.48 ^c
Mineral oil 2% + ammonium nitrate 1.5 %	13.37 ^{ab}	13.92 ^a	1.07 ^e	1.10 ^e	12.50 ^b	12.69 ^b
Mineral oil 2% + potassium nitrate 2%	13.72 ^a	14.27 ^a	0.97 ^f	0.97 ^f	14.21 ^a	14.71 ^a
LSD _{at 5%}	0.71	0.58	0.10	0.10	0.89	0.68

* Values in each column that were followed by similar letters did not differ substantially at 5%, the Least Significant Difference was used to compare the means.

For example, the trees that had received mineral oil at (2%) plus potassium nitrate at (2%) achieved progress in their TSS that was significantly higher compared to the control or mineral oil 2% alone, as well as potassium nitrate at 2% alone. In parallel progress there was a significant increase in the TSS with the application of mineral oil at (2 %) plus ammonium nitrate at (1.5 %) as compared with the control or with the individual treatment of ammonium nitrate alone or with mineral oil at 2 % alone. Therefore, the progress in opening the flower buds led to a significant increase in TSS in the trees, as shown in (Table 2). Hence, there was an increase in the TSS values reflecting the progress that occurred in breaking their bud dormancy. With regard to the response of fruit acidity to used treatments, the data in (Table 2) showed that mineral oil at (2 %) resulted in the highest juice acidity that was similar to that of the control in both seasons followed by the sole addition of urea (low pyrite) then ammonium nitrate as an individual treatment in a same pattern in both seasons. Meanwhile, applying potassium nitrate individually resulted in a juice acidity that was similar to that of ammonium nitrate in both seasons. Meanwhile, the combination treatments whether the mineral oil was mixed with the low pyrite urea or with ammonium nitrate had similarly juice acidity without any significant difference in both seasons. Meanwhile, the lowest juice acidity was obtained with the combination of mineral oil plus potassium nitrate at (2 %) and even significantly lower than the control.

With regard to the ratio between TSS and acidity in response to different treatments, the data in (Table 2) revealed that the highest value was found with the combination of mineral oil plus potassium nitrate related to the control and all other treatments followed by mineral oil plus ammonium nitrate. In the combination treatments, the combination of mineral oil plus low pyrite urea was effective but in a lower magnitude than others. It is noticeable that the use of individual application had significantly lower efficacy than the control and the combination treatments maintained a constant pattern across the two seasons. The only sole application that did not vary from the control in TSS/ acidity was the addition of mineral oil at (2%).

Changes in the percentage of total sugars in “Canino” apricot fruit during the two seasons 2022 and 2023 at the harvest time presented in table 93). The data indicated that the highest content of total sugar was obtained with the application of mineral oil at (2 %) plus ammonium nitrate at (1.5 %) followed by the application of mineral oil at (2 %) plus potassium nitrate at (2 %) when comparing with the control group during both seasons. While there was no notable distinction between the two formulations. Moreover, many other treatments did not significantly vary from the control such as potassium nitrate alone, ammonium nitrate alone and low pyrite urea especially in the first season, as well as mineral oil alone. Thus, the delay in buds opening was reflected on the content of total sugars at the harvest time.

Table 3. Total sugar, vitamin C and total carotenoid of “Canino” apricot as affected by pre-harvest applying during the two seasons 2022 and 2023.

Treatments	Total sugar (%)		Vitamin C (mg/100g)		Total carotenoid (mg/100g)	
	2022	2023	2022	2023	2022	2023
Control	7.51 ^{fr}	7.93 ^g	11.35 ^h	11.83 ^h	1.31 ^f	1.36 ^g
Mineral oil 2%	7.87 ^{ef}	8.29 ^{fg}	11.95 ^g	12.43 ^g	1.58 ^f	1.70 ^{fg}
low pyrite urea 2%	8.14 ^{de}	8.55 ^{ef}	12.68 ^f	13.19 ^f	1.89 ^e	2.02 ^{ef}
Ammonium nitrate 1.5 %	8.49 ^{cd}	8.95 ^{de}	13.35 ^e	13.95 ^e	2.23 ^d	2.33 ^{de}
potassium nitrate 2%	8.74 ^c	9.23 ^{cd}	13.97 ^d	14.54 ^d	2.53 ^c	2.67 ^{cd}
Mineral oil 2% + low pyrite urea 2%	9.18 ^b	9.61 ^{bc}	14.70 ^c	15.22 ^c	2.74 ^{bc}	2.85 ^{bc}
Mineral oil 2% + ammonium nitrate 1.5 %	9.82 ^a	10.32 ^a	15.22 ^b	15.81 ^b	3.17 ^a	3.36 ^a
Mineral oil 2% + potassium nitrate 2%	9.49 ^{ab}	10.02 ^{ab}	15.82 ^a	16.41 ^a	2.98 ^{ab}	3.14 ^{ab}
LSD at 5%	0.40	0.49	0.36	0.46	0.28	0.38

* Values in each column that were followed by similar letters did not differ substantially at 5%, the Least Significant Difference was used to compare the means.

Percentage of The Northern Opened Buds

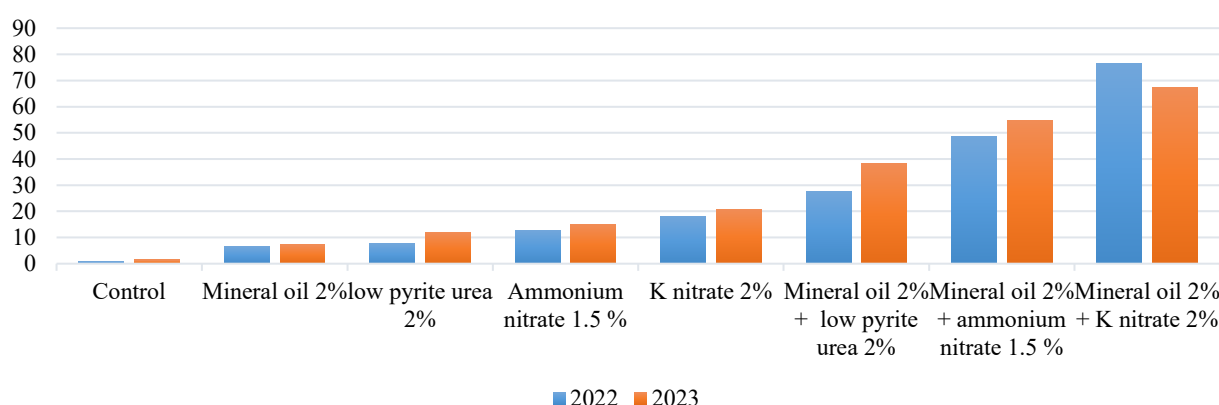


Figure 1. Effect of treatments on percentage of the northern opened buds in both seasons 2022 and 2023

Percentage of The Southern Opened Buds

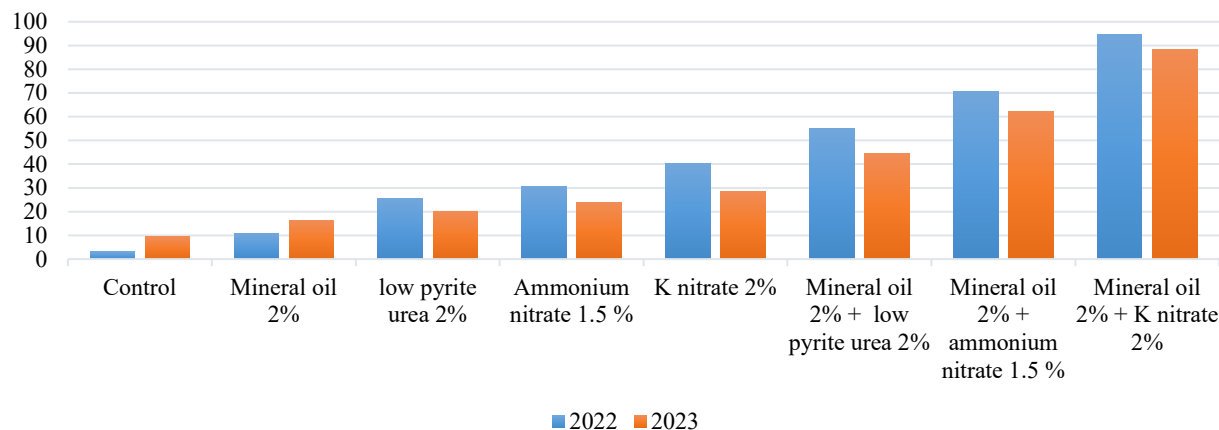


Figure 2. Effect of treatments on percentage of the southern opened buds in both seasons 2022 and 2023

The response of vitamin C in apricot fruits at harvest time to various early applications to break bud dormancy (one application in January in 20 or 25 in the two seasons respectively), was published in (Table 3). The statistics indicated that mineral oil plus potassium nitrate resulted in the greatest content of vitamin C in the fruit at harvest followed by mineral oil combined with ammonium nitrate. Such variations were consistent during the two seasons. The third magnitude of vitamin C content was achieved with the application of mineral oil but combined with low

pyrite urea as compared with the control, the same pattern was true in two seasons. In addition, when either potassium nitrate or ammonium was applied alone, there was a significant reduction in vitamin C content as compared with the combination treatments during both seasons. Thus, it was better to apply the used concentration of the mineral oil than to use the individual treatment in terms of gaining higher content of vitamin C in the fruit at the harvest time.

The content of carotenes in the fruit at the harvest time in response to earlier treatments that were used to break bud dormancy was presented in (Table 3). The data suggested that total carotenes in the fruit was also influenced by early treatments last January in two seasons. Again, the greatest magnitude of the increase was achieved with the combination of mineral oil plus ammonium nitrate related to the control. And many other individual treatments in both seasons. Such treatment was followed by mineral oil plus potassium nitrate that was similar to mineral oil plus urea at low pyrite. Moreover, all used treatments individually did not result in greater carotene content except with potassium nitrate or ammonium nitrate as compared with mineral oil or low pyrite urea individually and separately in both seasons.

Discussion

It was logical to find a greater bud break in the southern half of the tree as compared with the northern half. The exposure of buds to the sun is for a longer duration and the exposure to a higher temperature which means the tissue temperature which means a rising bud temperature. Such an increase is synergistic with the rise of respiration gained with the application of summer oil at 2%. The combination of the summer oil plus potassium nitrate and tween 80 means more penetration of the sprayed formulation across the buds cuticle since the summer mineral oil has a hydrophobic (lipophilic) nature. No wonder to find that the highest effectiveness was found with the combinations of either summer mineral oil plus either potassium nitrate or ammonium nitrate followed by the combination of the summer oil plus low pyrite urea at 2% (w/v) (Erez, 2020; Singh, 2020; Çalışkan & Kılıç, 2022; Luna et al., 1993; Uber et al., 2020).

Conclusion

The results provided evidence about the possibility of controlling bud break of apricot trees especially some formulations such as summer mineral oil at (2%) plus either potassium nitrate at (2%) or ammonium nitrate at (1.5%) in the presence of the surfactant tween 80.

With regard to bud break, the results indicated the highest bud break with the application of mineral oil at 2% (v/v) plus potassium nitrate at 2% (w/v). The greatest advancement in the rate of fruit growth was achieved with

the application of mineral oil plus potassium nitrate as revealed by the TSS percentage, the TSS to acidity ratio, the sugar or even the carotenoid content.

The use of the formulation containing mineral oil plus potassium nitrate plus the surfactant tween 80 appeared as a suitable alternative to Dormex whether economically, environmentally or with any health concerns.

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Mineral Composition of Feed and Mineral Soil Utilization in Haramaya District, East Hararghe Zone, Ethiopia

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ARTICLE INFO

ABSTRACT

Research Article

Received : 12.07.2024

Accepted : 25.08.2024

Keywords:

Mineral soil
Mineral concentration
Natural pasture
Ruminant
Seasonal variation

This study was carried out to assess feed resources mineral composition and utilization of mineral soil in the Haramaya district of the East Hararghe Zone, Ethiopia. The survey data was collected from four kebeles using a semi-structured questionnaire from 80 respondents (20 from each kebele) selected randomly. Two natural pasture samples across the wet and dry seasons were collected using a quadrant from each season. Two maize and sorghum stover samples were collected for analysis at the end of harvesting seasons. Two mineral soil samples were collected from Lake Adelle and Small Abaya. Table salt (50%), mineral water (30%), and mineral soil (20%) were used as mineral supplements for livestock. Farmers obtain mineral soil from the licking area of the mineral soil and feed animals by hiking them in the licking area and taking the mineral soil into the home. Maize stover was deficient in Ca, P, Na, Zn, and Cu, while sorghum stover was deficient in P, Na, Mg, and Cu. During the wet season, natural pasture consists of higher ($p < 0.05$) P, K, Cu, Zn, and Mn while Ca and Fe were higher ($p < 0.05$) in the dry season. During wet and dry seasons, the Ca, P, Na, Mg, and K content were 14.53 vs 20.47, 1.51 vs 0.82, 0.38 vs 0.45, 4.01 vs 3.84, and 16.50 vs 6.40 g/kg, respectively. The Mn, Fe, Cu, and Zn contents were 365.14 vs 415.22, 477.78 vs 336.11, 8.48 vs 7.38 and 42.74 vs 16.94 mg/kg in wet and dry seasons, respectively. The mineral soil of both lakes was deficient in P and K, but high in Na and Fe. Supplementation of animals with deficient minerals in their feed is necessary in the study area and the mineral soil can be used as a mineral supplement for ruminants. However, an extension service is essential to improve mineral soil utilization in the study area.

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Introduction

Ethiopia is home to a diverse range of Indigenous ruminant populations, contributing to food security and poverty alleviation. However, the livestock sector's potential remains underutilized, primarily due to inadequate forage supply in quality and quantity (Adujna & Aster, 2007), which is essential for providing necessary nutrients, including minerals. The mineral levels in the animals' bodies are influenced by the mineral levels in their feed (Tiwari et al., 2014). In tropical regions, low productivity and reproductive performance in grazing ruminants have been linked to mineral imbalances in soil and forage (McDowell, 1997).

In Ethiopia, many feed sources lack essential macro and micro minerals such as sodium (Na), phosphorus (P), zinc (Zn), and copper (Cu) (Fekede et al., 2013), with deficiencies often being location-specific (Tiwari et al., 2014). Consequently, the mineral content in the feed is

inadequate to support optimal ruminant productivity (Aschalew et al., 2006). Despite this, mineral nutrition has received limited attention, resulting in significant gaps in the nutritional profiles of local feed resources (Tikabo & Shumuye, 2021). The mineral composition of forages can vary based on factors such as plant age, soil type, fertilization practices, species, variety, seasonal changes, and grazing pressure (Aregheore, 2002). Minerals are supplied through feed, and their concentrations in body fluids are influenced by the mineral content of the feed, dietary intake levels, and mineral availability (Suttle, 2010). Therefore, conducting chemical analyses of forages is crucial to assess the adequacy of essential minerals in the major diets of grazing animals (Shakira et al., 2011). In the present study area, natural pastures, maize stover, and sorghum stover are the primary feed sources for grazing animals (Freweini et al., 2015). However, there is a lack of

information regarding the mineral composition of these feeds in the region. A comprehensive study of feed quality is necessary to document the nutritional values of available feed resources (Tikabo & Shumuye, 2021). Additionally, determining the mineral composition of these feeds is essential to address mineral deficiencies in animal diets.

Numerous studies have identified mineral soil as a significant mineral supplement for livestock (Muluken et al., 2015; Nderi et al., 2015; Wondewsen et al., 2019), particularly in developing countries. Mineral soil could serve as a viable intervention to mitigate mineral deficiencies in animal feed due to its availability and accessibility. In Ethiopia, similar to other regions, mineral soil is utilized for animal supplementation, especially in the study area. However, existing knowledge about mineral soil is largely based on indigenous practices. Therefore, it is essential to obtain comprehensive information about the mineral composition and uses of mineral soil to effectively integrate it into livestock diets for enhanced animal production. This study aims to assess the mineral soil utilization practices and to evaluate the mineral composition of the major feed resources and mineral soil in the study area.

Materials and Methods

Study Area

The survey was conducted in Haramaya districts in the East Hararghe zone of the National Regional State of Oromiya. The capital of the East Hararghe zone is Harar City, located 526 km east of the Ethiopian capital, Addis Ababa. The Haramaya district with a total area of 55,000 ha is located at a latitude of 9°9' to 9°32' N, at a longitude of 41°50' to 42°05' E, and at an altitude of 1600-2100 meters above sea level. Minimum and maximum temperatures are 10.8 and 24.62°C, respectively. The mean annual rainfall is 698 mm, ranging from 291 to 1104.2 mm. The area has a bimodal rainfall pattern, with small rains occurring from March to May and main rains from June to September (Shame, 2021).

Sample Size and Data Collection

Among districts of East Hararghe Zone, Haramaya district was purposively selected based on the availability of mineral soil. Accordingly, four kebeles (Adele, Iffaa Oromiya, Haroadii and Biftu Gedaa) were selected purposefully based on mineral soil availability and utilization practices. A total of 80 households from the four kebeles (20 households from each kebele) were selected randomly for survey data collection. A preliminary questionnaire was prepared, pretested, adjusted based on the feedback and used to collect data by interviewing individual farmers at their farm gates. The interview questions consist of demographic characteristics, types of mineral supplements used, methods and sources of mineral soil feeding, seasons of mineral supplement, reasons for mineral supplements, perceived mineral deficiency signs and use of mineral soil and other mineral sources as livestock feed. The data was also supplemented with information obtained from key informant interviews, focus group discussions and secondary data derived from the zonal and district offices of livestock production and health agencies.

Feed and Mineral Soil Samples Collection

Feed samples were collected from the selected kebeles of the study district. Based on their importance and contribution as reported by Freweini et al. (2015), three different feed samples (natural pasture, maize stover, and sorghum stover) were collected among the available feed resources. Natural pasture samples were collected during the dry (mid-February) and wet seasons (Mid-August). Natural pasture samples were collected from each selected representative site of the grazing lands by harvesting from 5 randomly positioned quadrants, spaced 20 meters apart, using stainless steel sickles. All harvested subsamples were thoroughly mixed to make a composite sample, from which a subsample of 1 kg was taken into labelled paper bags and transported to Haramaya University nutrition laboratory. On arrival, the samples were air-dried under shade to prevent spoilage of the samples before being placed into the oven. Maize and sorghum stover samples were collected at the end of harvesting seasons from each of the representative kebele, chopped, and thoroughly mixed to make a composite sample. For chemical analysis, three duplicate sub-composite samples were collected from the composite samples of each feed type. A mineral soil sample was collected from mineral soil licking areas of lakes Adele and Small Abaya located in Adele Kebele of Haramaya district and Gebibere Kebele of the Silte Zone, respectively. The samples were taken from three different points within the two lakes and each was placed into a polyethylene bag and transported to Haramaya University, stored in the open air on a bench at the nutrition laboratory until used for chemical analysis.

Chemical Analysis

The samples were ground in a Willey mill to pass through a 1mm sieve after drying in an oven at 60°C for 48 hours. The Ca, Mg, Mn, Zn, Cu, and Fe contents of the mineral soil and feed samples were determined by using an atomic absorption spectrometer (AOAC, 2000). The concentrations of Na and K were determined using a flame photometer (Black, 1965) and P was determined following the standard Olsen extraction method (Baruah, 1997). All chemical analysis were carried out at Haramaya University laboratories.

Statistical Analysis

The data was analyzed using the statistical package for social sciences (SPSS version 20) for Windows (SPSS, 2007). The mean mineral concentration of maize and sorghum stover, natural pasture and mineral soil were statistically analyzed by using the SAS general linear model procedure (SAS, 2007). The mean values were compared by using a list significant difference test ($p < 0.05$).

Results and Discussion

Demographic Characteristics of Households

The households' hhs average family size and age were 7.5 persons/hh and 48 years, respectively. This family size is greater than the national average value of 5.2 persons/hh reported by CSA (2007). The family size in the present study area is consistent with the previous report of 7.65 persons/hh in the Sekota district of the Amhara Regional State of Ethiopia (Zinash, 2015) and 7.5 persons/hh in the

Jimma zone of the Oromiya Regional State, Ethiopia (Zewdie, 2010). This large family size could be due to the relative labor-intensive diversified farming activities and weak family planning services (Zewdie, 2010). Most of the interviewed hhs were illiterate, which could be a gap for increased use of improved feeding practices such as knowledge-based extensive use of soil mineral lick. The proportion of male-headed households was about 85.0%. Most hhs relied on mixed agricultural practices for income (livestock rearing, and crop production (sorghum, maize, and khat), while the rest depend on off-farm income sources like the sale of khat (*Catha edulis*), which is considered as the major cash crop in the study area.

Types of Mineral Supplement

According to the result of the present study, table salt, mineral soil, and mineral water were popularly used as a mineral supplement (Table 1). Nearly, 50%, 30%, and 20% of the respondents used table salt, mineral water, and mineral soil, respectively. Consistent with this finding, table salt supplementation is common in other parts of Ethiopia (Bosenu et al., 2019; Wondewesen et al., 2019). Furthermore, 69.3% of the pastoralists used mineral soil as a mineral supplement for camels in the Somali region of Ethiopia (Bosenu et al., 2019). Moreover, mineral soil (bole) supplementation to dairy animals is common in the East Shoa Zone of Ethiopia (Wondewesen et al., 2019). In the study area, around 80% of the hhs were not providing mineral soils as supplements to their animals, which could be due to a lack of awareness of the importance of mineral soil.

Sources and Feeding Methods of Mineral Soils

Most hhs obtain mineral soil from the licking areas near Adele Lake (Table 2). Most (87.5%) farmers supplemented their animals by hiking them to the licking area, while the rest (12.5%) brought mineral soil in to the home. Similar to this result, pastoralists fetch mineral water and soil to the nearby fields when the licking and watering areas are

inaccessible to the camels (Temesgen, 2012; Bosenu et al., 2019). Studies have shown that salt licks are a natural gathering place for grazing animals to ingest as part of their feed and consume directly (Lameed & Adetola, 2012).

Seasons of Mineral Supplementation

The study showed that most of the respondents (87.5%) supplement mineral soil during the dry season, while the rest provide it during the wet season (Table 3). Although the intensity varies between seasons, the results show that livestock owners almost supplement table salt and mineral water throughout the year. Presence of seasonal variation in the frequency of mineral soil use was also indicated in a previous study (Muluken et al., 2016). Higher intensity of mineral supplementation during the dry season could be related to the better mineral content of forages and other feed resources available during the wet season and as a result of the lack of pronounced mineral deficiency in animals (Lameed and Adetola, 2012; Lengarite et al., 2013; Endale et al., 2015). Moreover, there is a greater loss of Na in hot weather due to respiration and perspiration (Shamat et al., 2009), hence animals show signs of need for salt to which owners respond by providing salt supplements frequently in dry than wet season. Mineral soil could supply adequate minerals for animals, especially when the mineral content of the forage is inadequate (Muluken et al., 2016), as tropical forages showed lower mineral content during the dry season (McDowell, 1997). The soil lick could provide the sodium, calcium, iron, phosphorus, and zinc necessary for the growth of animals (Lameed and Adetola, 2012). Therefore, mineral licks supplement minerals that are deficient in animal diets and maintain mineral equilibrium in the body of animals (Eksteen & Bornmann, 1990). About 60% of farmers supplement mineral soil in direct soil form and the rest (40%) mix with feed in the morning. Mixing mineral soil with feed increases fibrous feed intake and feeding mineral soil before grazing increases appetite and improves feed intake during grazing (Muluken et al., 2016).

Table 1. Mineral supplements used in the study area

Mineral Supplements	Frequency	Percentage
Table salt	40	50
Mineral water	24	30
Mineral soil	16	20
Total	80	100

Table 2. Sources and methods of feeding mineral soil in the study area

Mineral soil	Frequency	Percentage
Sources		
Soil lick area	16	100
Methods of feeding		
Trekking animals	14	87.5
Taking soil to home	2	12.5
Overall	16	100

Table 3. Season of mineral supplementation in the study area

Mineral type	Season	Frequency	Percentage
Mineral soil	Dry	14	87.5
	Wet	2	12.5
Table salt	All year round	80	100
Mineral water	Wet	14	58.3
	All year round	10	41.7

Table 4. Reasons of mineral supplementation in the study area

Variable	Frequency	Percentage
Increase intake	29	36.3
Increase weight gain	11	13.8
Clear and shiny hair coat	11	13.8
Increase milk yield	11	13.8
Increase disease resistance	10	12.5
Improve body condition	6	7.5
Increase conception rate	2	2.5
Overall	80	100

Table 5. Perceived mineral deficiency signs in the study area

Variable	Frequency	Percentage
Decrease intake	27	33.8
Abnormal skin color	15	18.8
Decrease weight gain	13	16.3
Decrease milk yield	13	16.3
Low disease resistance	7	8.8
Delayed estrus	3	3.8
Delayed maturity	2	2.5
Overall	80	100

Importance of Mineral Soil Supplementation

Almost all respondents believed that mineral soil supplementation provides better appetite, greater weight gain, good body condition score, greater resistance to disease, clear and shiny hair coat, higher conception rate, and higher milk production (Table 4). Similarly, farmers across different regions of Ethiopia use mineral soil to improve feed intake, induce heat in cows, promote weight gain and a shiny coat, and enhance milk production (Minyahel & Alemayehu, 2019). Additionally, pastoralists believe that supplementing with mineral soil leads to rapid weight gain in livestock, increases milk yield, encourages reproductive activity in cows, and possesses medicinal properties (Teshome, 2016). The consumption of mineral-rich soils is viewed as a method to address mineral deficiencies or imbalances in animal diets (Montenegro, 2004). The minerals found in lick soil can meet the seasonal nutritional needs associated with lactation, calving, and the growth of bones, horns, or tusks (Tracy & McNaughton, 1995), while also helping to mitigate gastrointestinal diseases in animals (Minyahel & Alemayehu, 2019).

Signs of Mineral Deficiency

Farmers observed that animals lacking essential minerals exhibited various symptoms, including reduced feed intake, unusual skin coloration, slower weight gain, decreased milk production, lower disease resistance, and abnormal behaviors like soil licking (Table 5). Supporting this observation, other studies revealed that farmers recognized mineral deficiency diseases and their associated symptoms, such as licking human urine, soil licking, delayed puberty, and extended calving intervals (Wondewsen et al., 2019). Research has demonstrated that mineral deficiencies can result in lower milk output, decreased water consumption, increased foraging for salty plants, bone chewing, and soil ingestion (Shah & Hussain, 2014). Sodium deficiency, in particular, can lead animals to develop unusual appetites, prompting them to consume soil, trees, stones, and even feces and urine (Robbins, 1993). To address this sodium deficiency, animals

often seek out areas rich in sodium or salt licks (Staal and White, 2001).

Mineral Composition of the Feeds

In the present study, the maize stover had sufficient Mg, K, Mn, and Fe content to meet the mineral requirements of ruminants (NRC, 1996; McDowell, 1997) (Table 6). The Ca content in the maize stover (MS) was lower than the 3.3 g/kg value while the P content was comparable with a report from the highlands of Ethiopia (Kabaija & Little, 1988). The mean Na content of maize stover was lower than the previous reports which were 2.2 g/kg (Garg et al., 2009) and 0.5 g/kg (Kabaija and Little, 1988). The Mg content in maize stover was lower than 2.5 g/kg (Kabaija & Little, 1988). The mean K content in the present study was lower than the values of 17.8 g/kg (Kabaija & Little, 1988) and 22.7 g/kg (Garg et al., 2009). Differences in Na, Mg, and K content among studies could be due to differences in cultivars, stage of harvest, soil, and climatic conditions in which plants are grown (Abarghani et al., 2013).

In the present study, the Ca, K, Mn, Fe, and Zn content of sorghum stover (SS) was sufficient to meet the mineral requirements of the ruminants (NRC, 1996; McDowell, 1997). The Ca content of the sorghum stover (SS) was comparable to the reports of Mirzaei (2012) which was 4.60 g/kg. The sorghum stover content of P and Cu were similar to the values of 0.9 g/kg P and 6.41 mg/kg Cu (Garg et al., 2009). The Na content of SS was similar to the value of 0.21 g/kg (Karbo et al., 2008). The K content in this study is lower than the values of 21.3 g/kg (Karbo et al., 2008) and 21.5g/kg (Garg et al., 2009). The contents of Mg and Fe were also lower than the reported values of 3.3g/kg and 434.7g/kg, respectively (Garg et al., 2009). The manganese content of SS was higher than the value of 31.12 mg/kg (Ramana et al., 2011). The differences in the mineral content of sorghum stover in the current study when compared with others could be due to variety, soil type and climate condition of the environment during plant growth (Abarghani et al., 2013).

Table 6. Mineral compositions of maize and sorghum stovers

Variable	Feed Type		SEM	Ruminant requirements †
	Maize Stover	Sorghum Stover		
Macro-minerals (g/kg)				
Ca	1.77 ^b	4.06 ^a	0.22	1.8 - 8.2
P	1.58 ^a	0.93 ^b	0.02	2.5 - 4.8
Na	0.22 ^a	0.16 ^b	4.94	0.6-1.8
Mg	1.56 ^a	0.60 ^b	0.04	1-2.5
K	10.23	10.37	0.32	5-10
Micro-minerals (mg/kg)				
Mn	63.5 ^b	70.20 ^a	0.62	40
Fe	225 ^a	177.5 ^b	9.96	30-50
Cu	5.41 ^b	6.21 ^a	0.04	8-14
Zn	16.67 ^b	41.13 ^a	2.64	20-40

^{ab} Means within a row with different superscripts differ significantly ($P<0.05$); Ca= calcium; P= phosphorous; Na= sodium, Mg= magnesium; K= potassium; Mn= manganese; Fe= iron, Cu = copper, Zn = zinc; mg= milligram; kg= kilogram; g=gram; SEM=standard error of mean; † Recommended mineral requirement for all classes of ruminants suggested by the NRC (1996) and summarized by McDowell (1997).

In the present study, maize stover exhibited significantly higher levels ($p<0.05$) of phosphorus (P), sodium (Na), iron (Fe), and magnesium (Mg) compared to sorghum stover. Conversely, sorghum stover had greater ($p<0.05$) concentrations of calcium (Ca), manganese (Mn), copper (Cu), and zinc than maize stover. In general, maize stover was deficient in Ca, P, Na, Zn, and Cu, while sorghum stover was deficient in P, Na, Mg, and Cu as compared to the level recommended for ruminants (NRC, 1996; McDowell, 1997). Similar to this finding, Cu, P, and Na deficiency in feed was reported in many parts of Ethiopia (Dermauw et al., 2014; Endale et al., 2015; Martne Sáez, 2015). Minerals are lost with seed shedding and the remaining stem or straw is low in most minerals (Suttle, 2010), which could be the reason for the low mineral content observed in this study. Furthermore, the deficiency of feed minerals in the present study area could be related to the mineral content of the soil, since the concentration of minerals in plants increases with the mineral content in the soil (Blake et al., 2000; Silvanus et al., 2014). Therefore, the main reason for mineral deficiencies in grazing animals is that soils are inherently low in plant-available minerals (Suttle, 2010).

Macro-Mineral Composition of Natural Pasture

In the current study, natural pastures in wet and dry seasons satisfy the minimum requirement of ruminants for macro-minerals such as Ca, Mg, and K (NRC, 1996; McDowell, 1997) (Table 7). The content of most minerals in natural pastures showed variation with season, with natural pastures having a higher content of minerals in the wet season (Lemma et al., 2002). The calcium (Ca) content of the natural pasture was higher ($p<0.05$) during the dry season than the wet season. The increased Ca content of natural pasture in the dry season is attributed to the lack of mobility of Ca, which tends to accumulate in old organs and stems as plants mature (Abarghani et al., 2013). Consistent with this finding, a higher Ca was reported in the dry-season natural pasture (Endale et al., 2015). The Ca content of the natural pasture in this study was higher than that obtained in other reports (Lengarite et al., 2013; Martnez Sáez et al., 2015). This difference could be due to higher levels of Ca content in the soil that increase the Ca content of the forage (Desjardins et al., 2018). Factors, such as soil acidity and season affect plant mineral uptake

(Soetan et al., 2010). The Ca absorption is impaired with increasing acidity in soils (Soder & Stout, 2003) due to the increases in concentration of hydrogen ions, which interferes with the uptake of Ca by the plant roots (Lawrence et al., 2021) and the availability of Ca in soil and plants were high with alkaline pH (Shisia et al., 2013) since the solubility or availability of Ca is high in neutral or slightly alkaline soils.

The wet season natural pasture had a higher content ($p<0.05$) of phosphorus (P). Consistent with this study, a higher P content in natural pastures during the wet season was reported in a previous study (Lengarite et al., 2013; Mokolopi, 2019). The P content of the natural pasture was similar to the value of 0.33-1.97 g/kg reported from Asela, Ethiopia (Martne Sáez et al., 2018), and the value of 1.7 g/kg reported from southern Ethiopia (Bezabih et al., 2016). The P content of plants is influenced by the availability of P in soil, soil pH, climate, and stage of plant maturity, and as grasses mature, P is transferred to the grain (Soetan et al., 2010), thus the content of P decreases as the plant increases in size and progresses to maturity (Rahim et al., 2008). With increasing soil pH, the content of P in the soil and the availability of P for forage increases (Soder & Stout, 2003).

The sodium content (Na) of natural pasture was not influenced ($p>0.05$) by the season. The Na content of the natural pasture was similar to the value of 0.4 g/kg (Márquez-Madrid et al., 2017). Similarly, 0.23-1.1 g/kg Na was reported from Asela, Ethiopia (Martnez Sáez et al., 2018). The Na content of the natural pasture was deficient in both seasons, which could be due to the physiochemical properties of the soil and the climate conditions (Khan et al., 2013). Sodium is generally poor in forage due to soil, plant, and husbandry factors and it is easily leached from soils with low cation exchange capacity (Suttle, 2010). Consistent with this study, all types of feed were deficient in Na in the central and western parts of Ethiopia (Aschalew et al., 2006). Generally, plant species in tropical areas accumulate less Na; therefore, animals grazing on pastures are more prone to sodium deficiencies (Khan et al., 2007) making its supplementation to animals a necessity.

Magnesium (Mg) content was not influenced ($p>0.05$) by season, which is consistent with previous reports (Lengarite et al., 2013). However, another report indicated that Mg levels in forages showed variation due to month, pasture, and

interaction between them (Zafar et al., 2007), which could be due to variations in forage species, climatic factors, and soil types (Mokolopi, 2019). The Mg content found in the current study agrees with the Mg content of 4.22 g/kg (Bimrew et al., 2018). Deficiencies in animals grazing tropical pastures are rare as forage contains sufficient amounts of Mg in these areas (Minson and Norton, 1984).

The potassium (K) content in the natural pasture was high ($p<0.05$) during the wet season, which could be related to the availability of water since the absorption of K is affected by the moisture content of the soil (McDowell, 2003). The decrease in K with increasing forage maturity was also reported in a previous study (Zafar et al., 2007). The loss of K as the plant matured was attributed to the translocation of K into the root system and then to the soil (Blue & Tergas, 1969). Similar to this result, a higher content of K in the wet season pasture was reported in Kenya (Lengarite et al., 2013). The K content was comparable to the value of 14.32-19.44 g/kg, as reported from Asela, Ethiopia (Martinez Sáez et al., 2018).

Micro-Mineral Composition of Natural Pasture

In the present study, the wet-season natural pasture had sufficient Mn, Fe, Cu, and Zn (Table 7), while the dry-season pasture had adequate Mn and Fe to meet the requirements of ruminant animals (NRC, 1996; McDowell, 1997). In the current study, the dry season natural pasture had higher manganese (Mn) ($p<0.05$) than the wet season, which could be due to plant maturity (Rahim et al., 2008). In agreement with the present study, Khan et al. (2009) showed influence of season on the content of Mn. The Mn content in this study was comparable to 369.77-417.72 mg/kg value (Endale et al., 2015). In contrast to this result, a higher Mn (753 mg/kg) was reported in natural pastures (Bezabih et al., 2016), which could be attributed to the mineral content of the soil.

The iron (Fe) content of the pasture was higher ($p<0.05$) during the dry season, which could be due to contamination of the forage with soil (Lemma et al., 2002), which is most likely to occur in soils prone to waterlogging (Suttle, 2010). Similar to the present finding, the Fe content of the natural pasture during the dry season was higher than in the wet season (Shamat et al., 2009). The Fe content of the natural pasture was comparable to the values of 225-812 mg/kg (Khan et al., 2009) reported from Pakistan. The

higher Fe content in the pasture in this study could be due to the higher Fe content of the soil in the area (Khan et al., 2006) as the Fe content in the soil influences the Fe content in the forage.

The wet-season natural pasture had a higher copper (Cu) content ($p<0.05$) than the dry-season pasture. The Cu content of the forage decreases with the maturity of the forage and is higher in the leaf than in the stem (McDowell, 1996). Cu is associated with a plant's new tissues and is translocated to the root system as plants mature (Sousa, 1978). Consistent with the present finding, forages in the highlands of Ethiopia were adequate in Cu during the wet season and deficient during the dry season (Lemma et al., 2004). The Cu content of the natural pasture from Southern Ethiopia consisted of 8.3 mg/kg (Bezabih et al., 2016) and it is similar to the amount reported in the present study.

The wet-season natural pasture had a higher ($p<0.05$) Zn content than the dry-season forage. This difference could be due to the maturity of the forage. Consistent with this result, a higher Zn content from the wet season forage was reported in a previous study (Shamat et al., 2009). The natural pasture reported from Pakistan contain Zn in the range of 25.88-42.24 mg/kg (Khan et al., 2009). The soil pH, soil texture, soil mineral content, and botanical composition are factors that mainly influence the content of Zn in herbage (Espen et al., 2005).

Mineral Composition of Mineral Soil

In the current study, the content of magnesium (Mg), sodium (Na), iron (Fe), and zinc (Zn) of the Lake Small Abaya mineral soil (bole) was higher ($p<0.05$) than that of the Lake Adele mineral soil (Table 8). However, the mineral soil of Lake Adele has higher ($p<0.05$) manganese (Mn) and copper (Cu) ($p<0.05$) than that of Lake Small Abaya mineral soil. The composition of the macro minerals such as Na, P, and K of Lake Small Abaya mineral soil was similar to other previous reports (Sisay et al., 2007). It was noted that mineral soil has a high content of Na (Holdo et al., 2002) and other elements, including Ca, Mg, P, and K (Lameed and Adetola, 2012). The presence of these essential minerals is the reason for livestock seeking natural licks for mineral supplementation (Nderi et al., 2015). Phosphorus and sodium are believed to be the main trace elements that cause animals to use salt licking (Cowan et al., 1949).

Table 7. Mean macro and micro mineral compositions of natural pasture in the study area

Variable	Season		SEM	Ruminant requirements †
	Wet	Dry		
Macro-minerals (g/kg)				
Ca	14.53 ^b	20.47 ^a	1.39	1.8 - 8.2
P	1.51 ^a	0.82 ^b	0.02	2.5 - 4.8
Na	0.39	0.45	0.50	0.6-1.8
Mg	4.01	3.84	0.39	1-2.5
K	16.50 ^a	6.40 ^b	0.17	5-10
Micro-minerals (mg/kg)				
Mn	365.15 ^b	415.22 ^a	1.35	40
Fe	336.11 ^b	477.78 ^a	8.79	30-50
Cu	8.34 ^a	7.38 ^b	0.05	8-14
Zn	42.74 ^a	16.94 ^b	2.08	20-40

^{ab} Means within a row with different superscripts differ significantly ($P<0.05$); Ca = calcium; P= phosphorous; Na= sodium, Mg= magnesium; K= potassium; Mn= manganese; Fe= iron, Cu = copper, Zn = zinc; mg= milligram; kg= kilogram; g=gram; SEM=standard error of mean; † Recommended mineral requirement for all classes of ruminants suggested by the NRC (1996) and summarized by McDowell (1997).

Table 8. Mean mineral concentrations in the mineral soil

Variable	Mineral soil type		SEM	Ruminant requirements †
	Mineral soil (Adele Lake)	Mineral soil (Lake Small Abaya)		
Macro-minerals (g/kg)				
Na	33.53 ^b	41.87 ^a	0.64	0.6 -1.8
P	0.21	0.25	8.61	2.5 - 4.8
Ca	5.57	5.84	0.17	1.8 - 8.2
Mg	1.51 ^b	2.89 ^a	0.07	1-2.5
K	3.13	3.23	0.07	5-10
Micro-minerals (mg/kg)				
Cu	12.53 ^a	10.35 ^b	0.04	8-14
Zn	23.23 ^b	52.68 ^a	0.55	20-40
Fe	647.22 ^b	819.4 ^a	79.9	30-50
Mn	461.77 ^a	450.29 ^b	0.76	40

^{ab} Means within a row with different superscripts differ significantly ($P < 0.05$); Ca= calcium; P= phosphorous; Na= sodium, Mg= magnesium; K= potassium; Mn= manganese; Fe= iron, Cu = copper, Zn = zinc; mg= milligram; kg= kilogram; g=gram; SEM=standard error of mean; † Recommended mineral requirement for all classes of ruminants suggested by the NRC (1996) and summarized by McDowell (1997).

This study showed that Na and Fe were the main minerals. Similar to the present finding, a higher Na in mineral soils was reported from the Somali region (Sisay et al., 2007). Mineral soils with a high Fe content could have an antagonistic effect on P and Cu (Kabaija, 1989), which could be the reason for the low P content observed in the mineral soil of the present study. In the present study, the mineral soils of both areas could not satisfy the phosphorus and potassium requirements for ruminants (NRC, 1996; McDowell, 1997), as they contain a lower amount of P and K. This agrees with another report (Sisay et al., 2007), in which mineral soils were low in phosphorus. The P deficiency in natural lick was also observed in Kenya (Nderi et al., 2015), and suggests that animals require phosphorus sources when fed on low-quality feed supplemented with mineral soil.

Conclusions

In the present study area, table salt, mineral soil, and mineral water were mineral supplements utilized for different ruminant animals. The soil mineral areas were one of the main sources of mineral soil for animals and farmers fed animals by hiking their animals to the licking area and taking the mineral soil in to the home. The current study revealed that the major feed's chemical composition of minerals was deficient and could not meet animal requirements. Maize stover was deficient in Ca, P, Na, Zn, and Cu, while sorghum stover was deficient in P, Na, Mg, and Cu. The content of minerals in the natural pasture varied with seasons. The wet season pasture was deficient in P and Na, while the dry season pasture was deficient in P, Na, Zn, and Cu. It is, therefore, concluded that animals in this area require the supplementation of deficient minerals in their diet, and mineral soil can mitigate this problem, as it contains adequate amounts of some essential minerals, except P and K.

Declarations

Acknowledgements

I would like to express my gratitude to the School of Animal and Range Sciences of Haramaya University for providing laboratory facilities, the Haramaya District Livestock and Fishery office for providing information

during data collection, and Wachemo University for the financial support.

Ethical Approval Certificate

This study was approved by the Haramaya University School of Animal and Range Science Research and Ethics Committee with decision number SARS/SGC180520185 and date of May 18, 2018

Conflict of Interest

The author(s) declare no conflict of interest concerning this article's research, authorship, and publication.

Fund Statement

Any profitable or commercial company did not fund this work.

Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

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The Effects of Starvation Duration on the Growth Performance, Feed Cost, and Water Quality in Common Carp (*Cyprinus carpio*)

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ARTICLE INFO

ABSTRACT

Research Article

Received : 25.07.2024

Accepted : 25.11.2024

Keywords:

Common Carp

Starvation

Feed Consumption

Feed Cost

Water Quality

The present study investigated the effect of starvation periods on growth performance, feed cost, and water quality in common carp (*Cyprinus carpio*). Two different starvation methods were implemented, with an average weight of 120.69±3.47g over a 45-day trial. Group D1 was subjected to a 1-day fasting/2-day feeding regimen, whereas Group D2 adopted a 2-day fasting/1-day feeding regimen. The control group (C) was fed twice daily to satiation. At the end of the trial, the average weight of the fish was 200.88±14.62g in the control group, 189.11±21.05g in Group D1, and 130.04±10.49g in Group D2. The specific growth rates were 1.13±0.08% (C), 1.00±0.05% (D1), and 0.17±0.06% (D2), respectively. Feed conversion ratios were 1.81±0.01 (C), 1.32±0.02 (D1), and 4.43±0.05 (D2), respectively. There were significant differences between the control group and Groups D1 and D2 in terms of dissolved oxygen (mg/L) and pH values of the water. Group D2 yielded lower feed costs due to reduced feed usage. The average weight gain analysis showed that the unit feed cost of Group D2 was 3.4-fold higher than that of Group D1 and 2.5-fold higher than that of the control group. The application of starvation periods in feeding common carp had significant effects on the growth, feed utilization, water quality, and feed cost.

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Introduction

Nutrition is a crucial activity that influences all vital functions of living organisms and significantly impacts growth and associated costs. Consequently, feeding practices are vital for sustainable aquaculture systems. Fish feeding aims to achieve the desired yield weight within an optimal timeframe and minimize feed and other costs while establishing economically viable and environmentally sustainable feeding protocols (Baki et al., 2020). Accordingly, research has been conducted on the most effective feeding models that affect feed conversion and fish growth (Føre et al., 2016; Foss et al., 2009).

In nature, fish can experience periods of starvation for various reasons at certain times of the year. In aquaculture settings, they may also face short or long-term feed deprivation due to adverse environmental conditions and production-related issues. Numerous fish species have evolved remarkable resilience to endure these starvation periods (Navarro & Gutierrez, 1995). Recent studies have examined the effects of starvation periods and restricted feeding regimes on growth performance (Baki et al., 2020; Chatakondi & Yant, 2001; Chen et al., 2022; Eroldoğan et al., 2008; Fang et al., 2017; Foss & Imsland, 2002; Heide et al., 2006; Reyes & Baker, 2017; Taşbozan et al., 2014;

Urbanati et al., 2014; Qian et al., 2000; Yengkokpam et al., 2013; Yılmaz et al., 2018; Zhu et al., 2001).

The present study aimed to assess the effect of starvation periods on the growth performance, feed cost, and water quality in common carp (*Cyprinus carpio*).

Material and Methods

Materials

In the study, 450 carp with an initial average weight of 120.69±3.47g and an average length of 19.04±0.16 cm were stocked into nine tanks (300 L each) with three replicates ($p>0.05$).

Methods

Two different fasting regimes were adopted for 45 days. The control group (C) was fed continuously, Group D1 was subjected to a 1-day fast followed by a 2-day feeding regimen, and Group D2 was subjected to a 2-day fasting followed by a 1-day feeding regime. All the groups were fed twice daily to satiation using a commercial carp grower feed containing 38% protein and 12% fat.

At the onset and the end of the experiment, weight and length measurements were taken for each group to determine the growth parameters of the fish (Antonelli et al., 2023; Cui et al., 2006; Skalli & Robin, 2004).

$$\text{SGR (\%, day)} = 100 \times (\ln W_f \times \ln W_i) / t$$

$$\text{Daily Growth Coefficient} = (W_f - W_i) / t$$

$$\text{Growth Ratio on Feeding Day} = (W_f - W_i) / t_f$$

$$\text{FCR} = \text{Feed Intake (g)} / \text{Weight gain (g)}$$

$$\text{SFR (\%, day)} = (\text{Food ingested (g)} / \text{day/fish weight}) \times 100$$

$$\text{FCR} = (\text{Daily Feed Consumption} / W, \text{ g}) \times 100$$

$$\text{PER} = [(W_f - W_i) / \text{Protein intake}] \times 100$$

$$\text{Condition Factor} = W/L^3 \times 100$$

$$\text{Feed cost (USD)} = \text{Cost diet} \times F_i$$

$$\text{FCG} = \text{Total feed cost} / \text{Total weight gain (kg)}$$

W_f = Final Weight (g),

W_i = Initial Weight (g),

FCR = Feed Consumption Ratio

PER = Protein Efficiency Ratio

FCG = Feed cost/kg gain (USD)

W = Fish weight (g),

L = Fish length (cm),

t = days,

t_f = The number of feeding days,

Cost diet = the cost of one kg of each diet

F_i = total feed intake (kg) during the experimental period (days).

Statistical Analysis

The data obtained from the research were analyzed using one-way ANOVA with SPSS 21 statistical software. Differences between the values were compared using Tukey's multiple comparison tests at a significance level of p<0.05.

Results and Discussion

Growth parameters determined in the study are given in Table 1.

At the end of the study, the average weights of the fish were found to be 200.88±14.62g (C), 189.11±21.05g (D1), and 130.04±10.49g (D2), with specific growth ratio (%) of 1.13±0.08 (C), 1.00±0.05 (D1), and 0.17±0.06 (D2) (p<0.05). Group D1 exhibited the highest weight gain and growth values following the control group. Also, according to the growth calculations based on the number of feeding days, Group D1 had the best growth ratio value (2.28±0.25) (p>0.05).

The groups subjected to starvation had lower final weight, specific growth ratio, and daily growth coefficient values compared to the control group, indicating that the duration of starvation significantly affected growth. Previous studies have reported that starvation has a significant effect on growth values (Abdel-Tawwab et al., 2006; Akpınar & Metin, 1999; Baki et al., 2013; Einen et al., 1998; Nikki et al., 2004; Tian & Qin, 2004), with significant reductions in specific growth values observed under starvation conditions (Kocabaş et al., 2013; Sevgili, 2007).

In terms of the feed conversion ratio (FCR), which expresses the efficiency of converting feed into biomass, the best value was in Group D1 (1.32±0.02) (p>0.05). In contrast, the lowest value was in Group D2 (4.44±0.05) (p<0.05). Regarding the specific feeding ratio (SFR), no significant differences were found between the control group (20.82±0.81) and Group D1 (20.75±0.78) (p>0.05), whereas the difference between these groups and Group D2 (25.75±1.53) was significant (p<0.05).

The study revealed that the average feed consumption during feeding days varied depending on the number of starvation days, with the best value in Group D1, and the difference between this group and the control group was not significant (p>0.05). In contrast, the difference with the D2 group was significant (p<0.05). Other studies have reported that fish experiencing starvation consume more feed compared to continuously fed fish (Bull & Metcalfe, 1997; Eroldoğan et al., 2006a, 2006b; Miglavš & Jobling, 1989; Nikki et al., 2004; Sevgili, 2007).

Table 1. Growth parameters obtained from the study

Group	Control	D1	D2
Final Weight (g)	200.88±14.62	189.11±21.05	130.04±10.49
Biomass	3013.22±158.26	2836.58±110.10	1950.62±127.30
SBO (% , day)	1.13±0.08	1.00±0.05	0.17±0.06
Daily Growth Coefficient	1.78±0.13	1.52±0.17	0.21±0.02
Growth Ratio on Feeding Day	1.78±0.13	2.28±0.25	0.62±0.05
FCR	1.81±0.01	1.32±0.02	4.43±0.05
SFR (% , day)	20.82±0.81	20.75±0.78	25.75±1.53
Feed cost (USD)	3.17±0.22	1.98±0.21	0.91±0.08
Feed cost/kg gain (USD)	0.04±0.01	0.03±0.01	0.10±0.01
Feed Consumption Ratio	1.60±0.13	1.59±0.17	2.13±0.18
Protein Efficiency Ratio	211.03±15.36	180.05±20.04	24.61±1.99
CF (%)	1.94±0.14	1.85±0.21	1.68±0.14

The each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other (p<0.05); a, b, c: The differences between the means with different letters on the same line within the group are statistically significant (p<0.05).

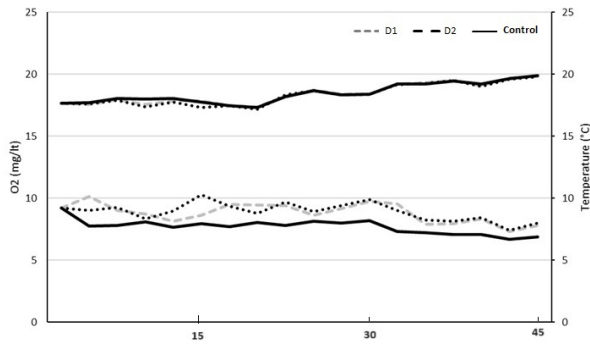


Figure 1. Dissolved Oxygen and Temperature Values

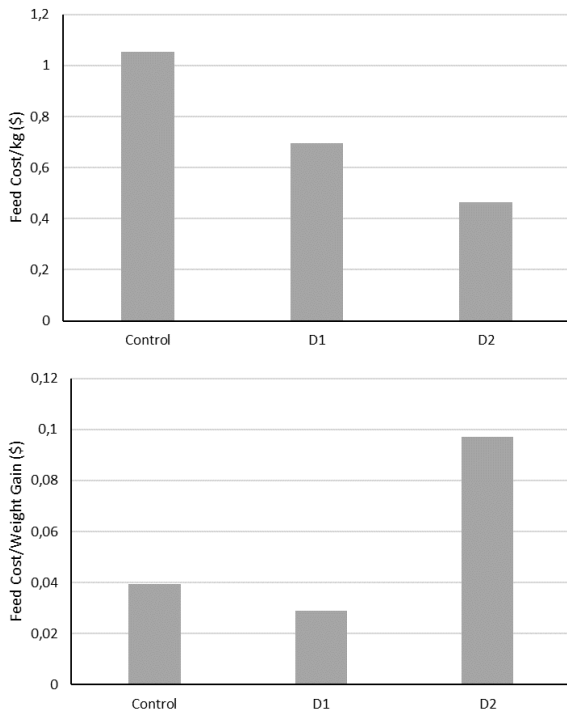


Figure 2. Feed Cost and Feed Cost/Weight Gain Values.

Regarding the protein efficiency ratio, the control group had the best performance, with significant differences from the other groups ($p < 0.05$). Other studies have reported no significant changes in the protein efficiency ratio between starvation and feeding groups (Sevgili, 2007). Heide et al. (2006) reported that the protein efficiency ratio in the control group was higher than in the starvation group.

In terms of the condition factor, the difference between the control group and Group D1 was not significant ($p > 0.05$), whereas the difference between the control group and Group D2 was significant ($p < 0.05$).

The water temperature values throughout the research ranged from 17.19 to 19.88°C, with an average of 18.45±0.19°C in the control group, 18.42±0.19°C in Group D1, and 18.35±0.20°C in Group D2 Group ($p > 0.05$). The dissolved oxygen values ranged from 8.70 to 12.27 mg/L, with an average of 7.70±0.14 mg/L in the control group, 8.81±0.18 mg/L in Group D1, and 8.79±0.19 mg/L in Group D2. The difference in dissolved oxygen values between Group D1 and Group D2 was not significant ($p > 0.05$), whereas the differences between the control group and the other groups were significant ($p < 0.05$) (Figure 1).

Dissolved oxygen is crucial for all aquatic organisms and is of great importance for aquaculture. Its value is inversely proportional to temperature values. The dissolved oxygen values in the groups in the present study did not fall below the critical level. However, the continuously fed control group had lower dissolved oxygen values than the starvation group.

The pH values in the study ranged from 7.12 to 7.63, with an average of 7.33±0.02 in the control group, 7.44±0.03 in Group D1 group, and 7.43±0.03 in Group D2. The difference in pH values between Groups D1 and D2 was not significant ($p > 0.05$), whereas the differences between the control group and both other groups were significant ($p < 0.05$). The oxidation-reduction potential (ORP) values during the study ranged from 204.9 to 270.4, with an average of 231.28±4.13 in the control group, 234.44±4.18 in Group D1, and 231.88±4.62 in the D2 ($p > 0.05$).

Feed costs constitute a significant portion of the total costs in fish production (Baki & Yucel, 2017; Das et al., 2018; Uddin et al., 2022). Therefore, in aquaculture, it is essential to evaluate the growth characteristics of the feed and the amount of feed given and to calculate the feed costs in production. In the present study, the total feed cost over 45 days was \$3.17 for the control group, \$1.98 for Group D1, and \$0.91 for Group D2. Examining the feed cost per unit fish weight (kg), the value for the control group was \$1, \$0.70 for Group D1, and \$0.47 for Group D2 (Figure 2).

In Group D2, less feed was used due to the hunger application, resulting in lower feed costs. However, when evaluated in conjunction with average weight gain, the unit feed cost was 3.4 times higher compared to Group D1 and 2.5 times higher compared to the control group.

Conclusion

Developing feeding strategies that can reduce production costs without compromising the growth performance of fish is crucial for the aquaculture industry. Also, ensuring the sustainability of these activities is equally important. The present study examined the effects of different fasting periods on the growth performance, feed cost, and water quality of common carp (*Cyprinus carpio*). Accordingly, temperature, dissolved oxygen, saturation percentage, and pH values were examined. Among these parameters, temperature is particularly vital as it can affect vital functions in aquatic environments and influence parameters like dissolved oxygen. Therefore, it requires regular monitoring. No significant changes were observed in temperature, dissolved oxygen, saturation percentage, and pH values throughout the study.

Examining the effect of fasting periods on growth parameters, the groups subjected to fasting had lower final weight, specific growth ratio, and daily growth coefficient values compared to the control group. Fasting periods have an effect on growth.

Starvation periods have been known to affect the growth performance of fish negatively. Also, a low feed conversion ratio (FCR) is targeted. In the present study, it was observed that Group D1 had the best FCR value, whereas longer periods of fasting, as indicated by D2, would have a negative effect on feed utilization.

Intermittent fasting and refeeding can be implemented in aquaculture practices, reducing labor and feed costs while minimizing feed waste. However, it is worth noting that improved protein retention rather than improved protein digestibility was attributed to compensatory growth in gibel carp (*Carassius auratus*), as Qian et al. (2000) reported.

Yengkokpam et al. (2013) stated that a 2-3 day fasting period per week with *Labeo rohita* fingerlings could induce some level of stress. Reduced feeding can lead to the depletion of organ antioxidant stores and increased oxygen free radicals in organs.

In conclusion, the practice of feed restriction in fish feeding activities does not necessarily compensate for the effects of prolonged fasting periods during subsequent feeding activities. This leads to a prolonged period of time for fish to reach market weight and affects the feed conversion ratio and feed costs.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of Sinop University, 30.03.2023 (Approval date and number: 2023/03).

Author Contribution Statement

Dr. Birol Baki: Data collection, investigation, formal analysis, and writing the original draft

Dr. Oylum Gökkurt Baki: Data collection, investigation, methodology, review and editing

Dr. Gülşen Uzun Gören: Data collection and investigation

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

In this section, you can acknowledge any administrative and technical support, or donations in kind (e.g., materials used for experiments).

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The Effects of Different Doses of Zeatin, Kinetin and Gibberellic Acid Biostimulants Applied during the Seedling Development Period of Peppermint (*Mentha Piperita* L.) on Growth and Biochemical Parameters

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ARTICLE INFO

Research Article

Received : 06.08.2024

Accepted : 09.11.2024

Keywords:

Antioxidant

Biostimulant

Seedling Development

Peppermint

Total Phenolic Content

ABSTRACT

This study aimed to evaluate the impact of varying doses of Zeatin, Kinetin, and Gibberellic Acid biostimulants on the growth and biochemical parameters of *Mentha piperita* L. Conducted in a greenhouse with three replications using a "Completely Randomized Experimental Design" design, the experiment assessed seedling and root lengths, fresh and dry weights of seedlings and roots, total phenolic content, and antioxidant activity (CUPRAC and FRAP). The results revealed that biostimulant applications significantly increased all growth and biochemical parameters compared to the control. Gibberellic acid at 200 mg/l produced the longest seedlings, while Kinetin at 50 mg/l resulted in the longest roots. The highest antioxidant activity (FRAP) and total phenolic content were observed with the 40 mg/l dose of Zeatin.

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Introduction

Commonly known as medical mint or peppermint (*Mentha × piperita* L.), with 28 synonyms, is a perennial aromatic herbaceous plant species that is 30-90 cm tall, has erect and quadrangular stems, generally in shades of purple or purplish tones. Its leaves are light green, oval-shaped, oppositely arranged, short-stalked, and serrated edges, 4 to 5 cm in length, and its flowers are purple or reddish in color, usually surrounded by inconspicuous bracts in false racemes, the fruit of the plant consists of four ellipsoidal seeds (Singh et al., 2015). The species peppermint (*M. × piperita*) is native to Europe but is widely found in Eastern and Northern Europe, the United States, and Africa. However, it is cultivated worldwide (Singh et al., 2015).

M. × piperita is a species belonging to the Lamiaceae family, known as the mint family, and is a natural hybrid of *M. aquatica* and *M. spicata*. The Lamiaceae family comprises about 7,200 species and around 260 genera of trees and shrubs. The genus *Mentha* contains about 61 species and 13 natural hybrids (Benabdallah et al., 2018; Kumar, et al., 2011).

Mentha piperita encompasses a wide array of secondary metabolites across numerous groups. Among

these, the principal ones include flavonoids (53%), phenolic acids (42%), different sub-groups such as lignans and stilbenes (2.5%), and terpenoids from monoterpenes (52%) and sesquiterpenoids (9%). Moreover, it is known that peppermint contains aldehydes (9%), aromatic hydrocarbons (9%), lactones (7%), and alcohol (6%) with menthone being the main component found in essential oils at a rate of (35–60%) (Mahendran and Rahman 2020). *Mentha piperita* is a widely used medicinal plant species proven by studies to exhibit antioxidant, antimicrobial, antiviral, anti-inflammatory, biopesticide, larvicidal, anticancer, radiation protective, genotoxicity, and anti-diabetic activities. It is used in folk medicine for fever, colds, mouth and throat inflammations, digestive system, anti-viral, anti-fungal treatment (Mahendran and Rahman 2020). The tea and essential oil derived from the leaves and flowers of medicinal mint are utilized in various fields.

Plant biostimulants are generally any substance (synthetic or natural) or microorganisms applied to plants in various forms and timings to increase nutrient content, improve abiotic stress tolerance, and/or enhance crop quality characteristics (Patrick 2015).

Zeatin belongs to a family of plant biostimulants called cytokinins and is known to play significant roles in plant growth and development. It aids in breaking apical dominance, supports the growth of leaves, contributes to the formation of chloroplasts, slows down the aging process, promotes seed germination, and helps regulate the cell cycle (Havlicek et al., 1997; Mok and Mok, 2001).

Kinetin, a member of the cytokinin biostimulant group, demonstrates effects such as delaying the aging process, promoting cell division, and thereby aiding in the plant's growth and development, regulating ethylene synthesis to slow down aging processes, and increasing chlorophyll synthesis (Toprak, 2019). Gibberellic acid (GA) is a phytobiostimulant produced by plants that plays crucial roles in various processes such as germination, water uptake, triggering of flowering, fruit development, shoot elongation, and metabolic functions. GA works in concert with other phytobiostimulants to regulate the development and growth of the plant (Zhu et al., 2019; Khan et al., 2020).

This study has been conducted to determine the effects of foliar applications of zeatin, kinetin, and gibberellic acid biostimulants at different doses on the growth and biochemical parameters of the medicinal mint plant during the seedling development phase.

Materials and Methods

Material

The study was conducted in the greenhouse within the Agricultural Sciences and Technologies Education Application and Research Center of Sakarya University of Applied Sciences, Faculty of Agriculture. The peppermint seedlings used in the research were procured from a commercial company.

Method

The trial was conducted with 3 replications according to the "Completely Randomized Experimental Design". In the study, peppermint seedlings were treated with zeatin (20, 40 mg/l), kinetin (50, 100 mg/l), and gibberellic acid (100, 200 mg/l) biostimulants, which are known to have effects on plant growth and development. A total of 21 pots with a capacity of 2 liters were used in the study. A homogeneous mixture prepared from finely sifted garden soil (3/4) and Klassman TS1 brand peat (1/4) was added to these pots. The ground where the pots were to be placed in the greenhouse for the study was leveled with a rake and then rolled, after which the pots were placed at a distance of 20 cm on top and 30 cm between rows.

After placing the pots in the greenhouse, five randomly selected pots were each watered with 500 ml of water. Plates were then placed under the bottoms of the pots to collect the water that drained through. After the drainage process was complete, an average of 215 ml of water accumulated in the plates from each pot. The water retention capacity of the pots was calculated by subtracting the drained water from the 500 ml of water added to each pot. Their water-holding capacities were measured to be 285 ml. Subsequently, the seedlings were planted in the pots at an approximate depth of 3 cm on November 1, 2023. During planting, the lower two leaves of the plants were removed by hand if they touched the soil to prevent fungal disease. Throughout the experiment, each pot was watered with approximately 100 ml of water once a week.

Kinetin and gibberellic acid hormones were dissolved in 96% ethanol, while zeatin hormone was dissolved in NaOH and completed to 1 liter with distilled water. The prepared biostimulant solutions were filled into 1-liter spray bottles, wrapped in aluminum foil to protect them from light, and stored in the refrigerator. The first foliar applications of the biostimulants were made on 05.01.2024 (approximately 2 months after planting). The study being conducted during the winter months, growth and development were slower compared to the summer months, hence the delay in biostimulant application. Biostimulant applications were made by spraying approximately 10 ml onto the leaves of the seedlings in each pot. The biostimulant applications were carried out three times, at 4-day intervals. The trial was concluded on 19.01.2024, after measurements of plant heights were taken. The trial lasted for an average of 2.5 months. During the period from the setup to the conclusion of the experiment, it was determined that the average daytime temperature was 15°C, while the nighttime temperature was 4°C (Anonymous 2024). The roots of the plants were softened and separated with water from the soil. Then, the root lengths were measured and recorded with the help of a ruler. The fresh weights of the seedlings and roots were measured on a precision scale. The aerial parts of the seedlings and the roots were placed in drying paper and put in an oven at 35 °C for 108 hours to dry. Afterwards, the dry weights of the seedlings and roots were measured.

Total Phenolic Content Analysis

The total phenolic content was assessed using the Folin-Ciocalteu method according to Waterhouse (2002). Initially, 250 µL of Folin-Ciocalteu reagent and 50 µL of the extract solution were added to a tube, with the total volume adjusted to 3 mL using distilled water. Following a 5-minute incubation, 750 µL of 20% (w/v) Na₂CO₃ solution was added and mixed. The mixture was then left in the dark at room temperature for 90 minutes before measuring the absorbance at 765 nm with a UV-Vis spectrophotometer (Agilent Cary-60, Santa Clara, CA, USA). A gallic acid standard curve was generated by repeating the procedure with concentrations of 50, 100, 150, 200, and 300 µg/mL. The total phenolic content was expressed as gallic acid equivalents using the standard curve (mg GAE/100 g of dry weight thyme).

Determination of FRAP Reducing Capacity

Initially, 0.3 M sodium acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution, 20 mM FeCl₃, and 2 mM FeSO₄ solutions were prepared. The working solution was obtained by mixing the buffer solution, TPTZ, and FeCl₃ solutions in a 10:1:1 ratio. Absorbance measurements were taken at 593 nm using a 2 mM FeSO₄ solution to create the standard curve, followed by measuring the samples at a minimum of three different concentrations. The results were reported as mg extract/µmol Fe²⁺ equivalents (Sachett et al. 2021).

Determination of CUPRAC Reducing Capacity

This method was based on a partially modified version of a previously reported procedure. Plant extracts were taken in different concentrations (10, 20, 40 µg) into tubes. Then, 0.25 mL of CuCl₂ solution (0.01 M), 0.25 mL of

ethanolic neocuproine solution, and 0.25 mL of CH₃COONH₄ buffer solution (1 M) were added. After incubating the mixtures in the dark for 30 minutes, absorbance values were measured at 450 nm against a blank (Ak and Gülçin 2008). The measurement results were evaluated by comparing them to trolox equivalents.

Statistical Analysis

Data analysis was conducted using COSTAT (version 6.03), and multiple comparisons were made using the Least Significant Difference (LSD) test at a 0.05 significance level.

Results and Discussion

Table 1 shows that the biostimulants applied during the seedling development stage of the peppermint plant have a statistically significant effect at the 0.05% level on all growth parameters. When examining Table 1, it can be seen that there are differences in growth parameters among the biostimulant applications. The highest seedling length was achieved with 17.70 cm from the gibberellic acid200 mg/l applications, the highest seedling fresh weight and root dry weight were achieved with 5.26 g and 1.34 g, respectively, from the zeatin40 applications, the highest root length was achieved with 33.25 cm from the kinetin50 applications, and the highest root fresh weight and seedling dry weight were achieved with 5.85 g and 0.94 g, respectively, from the kinetin100 applications, as shown in Table 1.

In bread wheat, foliar application of zeatin has been observed to shorten the maturation period, accelerate heading time, and increase main stem thickness. Additionally, it has been reported to increase spike length, spikelet number, grain weight per spike, and biomass yield, thereby enhancing agricultural productivity (Öztürk, 2023). It has been reported that the addition of zeatin to the medium in olive micropropagation increases callus formation compared to the control (Çiftçi, 2023). While zeatin40 application gave the best results in root dry weight compared to control and other applications, it gave higher results in seedling fresh weight compared to control and other applications. The increase in seedling fresh and root dry weights due to zeatin application from the leaf can be attributed to its promotion of cell division and growth, positive effects on the photosynthesis mechanism, and support for increased seedling and root development.

Studies conducted with different plants have reported that foliar applications of kinetin increase growth parameters (Tounekti et al., 2011; Tandel et al., 2018; Ghazy et al., 2023). Foliar applications of kinetin to *Ervatamia coronaria* plants have been reported to increase seedling fresh and dry weights, root fresh and dry weights, and root length compared to the control (Ashour et al., 2023). Kinetin biostimulant promotes the production of photosynthetic proteins by increasing chlorophyll content in plants, accelerates cell division, breaks apical dominance in plants, thereby increasing lateral branch and root formation, and consequently leads to an increase in both above-ground and below-ground biomass (Lazar et al., 2003; Bielach et al., 2017).

Foliar applications of GA3 have been reported to increase the height and quality of *Araucaria heterophylla* plants (Gul et al., 2006), increase seedling length in *Hibiscus sabdariffa* L. and wheat plants (Alharby et al. 2021), and enhance plant growth parameters when sprayed with 100 or 200 ppm GA3 in *Dahlia pinnata* plants (Yousef and Gomma, 2008), similar studies have been conducted by Santos et al. (1998) and Srivastava and Srivastava (2007). Application of gibberellic acid in thyme increases growth parameters, chlorophyll pigments, and volatile oil content (Dadkhah et al., 2016), and gibberellic acid applications have been reported to have positive physiological, morphological, and biochemical effects on plants (Taiz and Zeiger, 2010). The increase in growth parameters due to gibberellic acid applications in plants is reported to be associated with increased activity of enzymes such as carbonic anhydrase, nitrate reductase, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Yuan and Xu, 2001; Afroz et al., 2005; Aftab et al., 2010). Additionally, GA3 is reported to stimulate cell growth and division, thereby supporting growth and development (Taiz and Zeiger, 2010). Previous study findings support our results.

It is seen in Table 2 that the applied biostimulants have a statistically significant effect on CUPRAC, FRAP and total phenolics at the 0.05% level. Table 2. The highest values for FRAP antioxidant activity and total phenolic compound parameters were obtained from Zeatin40 applications, with values of 1.72 mM/g AAE and 0.50 mg/g GAE, respectively (Table 2). The CUPRAC antioxidant activity value of 6.98 mM/g TE obtained from Kinetin50 applications stands out compared to other applications

Table 1. Effects of Some Synthetic Biostimulants on Growth Parameters of *Mentha piperita* Plants

Biostimulants	Seedling length (cm)	Seedling fresh weight (g)	Root length (cm)	Root fresh weight (g)	Seedling dry weight (g)	Root dry weight (g)
Control	15.73 ab	4.82 a	25.60 b	5.46 a	0.89 a	1.29 ab
Zeatin20	14.23 b	4.43 ab	30.50 ab	3.99 bc	0.67 bc	1.09 abc
Zeatin40	15.50 ab	5.23 a	31.50 ab	5.08 ab	0.81 ab	1.34 a
Kinetin50	14.17 b	4.55 ab	33.25 a	4.09 bc	0.65 bc	1.17 abc
Kinetin100	15.80 ab	3.46 b	29.00 ab	5.85 a	0.94 a	0.97 bc
Gibberellic acid100	14.30 b	4.65 a	30.25 ab	3.75 c	0.55 c	1.13 abc
Gibberellic acid200	17.70 a	3.94 ab	30.75 ab	5.16 ab	0.67 bc	0.93 c
LSD (0.05)	3.29	1.17	6.2	1.21	0.19	0.34
CV (%)	12.26	14.96	11.76	14.52	14.88	17.58

Table 2. Effects of Some Synthetic Biostimulants on Biochemical Parameters of *Mentha piperita*

Biostimulants	CUPRAC (mM/g TE)	FRAP (mM/g AAE)	Total Phenolics (mg/g GAE)
Control	5,10 bc	1,24 c	0,37 bc
Zeatin20	4,94 bc	1,32 bc	0,37 bc
Zeatin40	6,97 a	1,72 a	0,50 a
Kinetin50	6,98 a	1,63 a	0,48 ab
Kinetin100	6,36 ab	1,58 ab	0,46 ab
Gibberellic acid100	6,44 ab	1,55 ab	0,38 abc
Gibberellic acid200	3,66 c	1,20 c	0,28 c
LSD (0.05)	1,68	0,29	0,12
CV (%)	16,61	11,62	17,25

Table 3: Correlation Table of Some Synthetic Biostimulants on Investigated Parameters of *Mentha piperita*

	1	2	3	4	5	6	7	8	9
1	1								
2	-0.382	1							
3	-0.265	0.063	1						
4	0.718	-0.360	-0.533	1					
5	0.349	-0.197	-0.583	0.895**	1				
6	-0.391	0.920**	-0.125	-0.096	0.153	1			
7	-0.631	0.334	0.352	-0.186	0.067	0.488	1		
8	-0.472	0.241	0.526	-0.140	0.027	0.344	0.950**	1	
9	-0.492	0.271	0.330	0.047	0.325	0.492	0.923**	0.903**	1

* Correlation is significant at the 0.05% level; ** Correlation is significant at the 0.01% level; 1: Seedling Length, 2: Seedling Fresh Weight, 3: Root Length, 4: Root Fresh Weight, 5: Root Dry Weight, 6: Seedling Dry Weight, 7: CUPRAC, 8: FRAP, 9: Total Phenolic Content

Santos-Gomes et al. (2003) reported that doses of zeatin and kinetin hormones applied to medicinal sage increased both antioxidant activity and total phenolics compared to the control. Similarly, Ravanfar et al. (2020) reported that zeatin hormone applied to purple cabbage increased total phenolics and antioxidant activity compared to the control, while Yousaf et al. (2024) reported that zeatin hormone applied to corn plants increased antioxidant activity compared to the control. Günaydin et al. (2017) reported that kinetin hormone application in thyme plants increased total phenolic, flavonoid, and antioxidant parameters compared to the control, while Acidri et al. (2020) reported that kinetin hormone in coffee plants was effective in scavenging free radicals and increased both total phenolics and flavonoids as well as phenolic components compared to the control. Brenner and Schmulling (2012), Bhargava et al. (2013), Kocsy et al. (2013), and Reguera et al. (2013) stated that the effects of cytokinins, including zeatin and kinetin hormones, on plant development, especially on phenolic compounds and associated antioxidant defense systems, are dependent on the regulation of gene expression related to secondary metabolism, including flavonoid and phenylpropanoid biosynthesis, glutaredoxin, peroxidase, glutathione transferase, and antioxidant enzyme genes, under normal conditions or stress.

Table 3 shows a strong positive correlation at the 0.01% level between seedling fresh and dry weights, as well as between root fresh and dry weights. Additionally, root dry weight is positively correlated with CUPRAC and FRAP antioxidant activities and total phenolic content, and there is a positive correlation between FRAP activity and total phenolic content.

Conclusions

It has been observed that synthetic biostimulants applied foliarly have significant effects on plant growth, and that there are differences in hormones in terms of growth parameters. While gibberellic acid200 mg/l application stands out as the best application for seedling length, Zeatin40 application is prominent in increasing seedling fresh weight and root dry weight compared to the control. It has been observed that foliarly applied synthetic biostimulants have a significant positive effect on antioxidant activities, total phenolic content, and total carotenoid content. Particularly, while kinetin50 applications show high CUPRAC antioxidant activity, zeatin40 applications have been the most effective biostimulant in terms of FRAP antioxidant activity and total phenolic content. These findings indicate that hormone applications may contribute to increasing plant health and antioxidant capacity, enhance growth efficiency through synthetic biostimulant use, and be beneficial in optimizing growth parameters. However, in order to make sustainable recommendations for the hormones applied, it is recommended to perform residue analyses, different doses and field trials.

Declarations

We would like to thank the employees of TABTEM Institution affiliated with SUBU for providing the environment for the conduct of the study.

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The Use of The Ancient Amaranth (*Amaranthus*) Grain in Traditional Turkish Cuisine

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ARTICLE INFO

Research Article

Received : 10.08.2024

Accepted : 22.09.2024

Keywords:

Amaranth
Pseudocereal
Ancient Grain
Gluten-Free
Local Food

ABSTRACT

With its culinary use dating back more than six thousand years, amaranth is known as the ancient grain and the food of the future. Recently recommended for consumption by FAO/WHO, the amaranth plant is a prominent, “forgotten,” functional food that can be used in human nutrition because of its drought-resistant cultivation, gluten-free, and protein and fiber-enriched content. This study evaluates amaranth's botanical character, functional properties, impacts on health, preparation-cooking methods, and use in local and traditional Turkish cuisines. Numerous studies have indicated the association between amaranth's chemical composition and its anti-oxidative, anti-tumor, gluten-intolerance, and cholesterol-lowering properties, and its assistance with intestinal flora and protein digestibility. Manifold dishes can be made using amaranth seeds and flour in traditional cuisines. Raw and cooked amaranth grains are used in rice, soup, and breakfast cereal. In the food industry, it is a crucial grain alternative to various bakery products, such as bread, pasta, cookies, manti, noodles, biscuits, and crackers, made from amaranth flour. There is a need for alternative recipes to increase the use of amaranth in the kitchen. In this context, it is thought that awareness should be increased by applying it to recipes that can replace semolina and bulgur in traditional cuisines. For this purpose, this study aims to increase the consumption of the ancient grain amaranth by including it in recipes in traditional Turkish cuisine.

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Introduction

Growing population, drought concerns, and increasingly tricky climatic conditions worldwide have led producers to search for new crops. In line with all these concerns, amaranth has recently been considered a crucial food in the pseudocereal group by producers because of its drought resistance and easy cultivation (Alvarez-Jubete, Arendt & Gallagher, 2009). Dicotyledonous plants such as amaranth (*Amaranthus sp.*), quinoa (*Chenopodium quinoa willd.*), and buckwheat (*Fagopyrum sp.*) are known in botany as “pseudocereals or grain-like” unlike most monocotyledonous grains such as wheat, rice, and barley. Since the functions and composition of these plants are similar to true cereals, they are categorized as cereals but are called pseudocereals (Köten, Karahan & Satouf, 2002). Carbohydrates are stored in the endosperm of these plants, and grains and pseudocereal plants are energy sources in the human diet (Pickersgill, 2007). The high nutritional value of amaranth, one of the most popular and edible

natural grains such as quinoa and buckwheat, has led to it being called the “new millennium food” (Rastogi & Shukla, 2013).

With consumers' and producers' growing interest in protecting health, nutritional trends have changed, and consumers' interest in functional foods has increased. Recent publications have focused on buckwheat, amaranth, quinoa, mung bean, and soybean as nutritional alternative products. In this context, amaranth is a “new food” that can be used as a functional food in human nutrition (Alandia, et al., 2021; Kumari, et al., 2023; Paško, et al., 2009). Studies are, therefore, needed to increase its use in the kitchen. In this regard, raising awareness of ancient grains by supporting them with traditional recipes is essential (Onur & Ceylan, 2023). To this end, this study aimed to increase the consumption of the ancient grain amaranth by including it in traditional recipes.

Method

Research on pseudocereal amaranth use in gastronomy is limited. In this sense, the study aims to explain the history, rituals, use, recipe examples, and health effects of amaranth, an ancient grain, in culinary culture. For this purpose, the study utilized document analysis for data collection. Documents were analyzed using databases such as Google Scholar, Scopus, Elsevier, Directory of Open Access Journals (DOAJ), BioMed Central, Ulakbim, and Dergipark. At the same time, the authors prepared sample recipes by adapting them to traditional Turkish dishes to promote their use in the national cuisine.

Conceptual Framework

Amaranth's General Properties

Amaranth derives from the Greek ἀμάραντος (amárantos), "unfading, endless, eternal," and originated from the Greek word ἄνθος (ánthos), meaning flower (Lucian, et al., 2018). Amaranth is a plant belonging to the *Amaranthus* genus, with more than 70 species (Wyatt, 2002). Some of the species produce edible seeds and leaves. The seeds of some species, such as *A. caudatus*, *A. cruentus*, *A. hypochondriacus*, and *A. mantegazzianus*, are rich in starch, protein, and other nutrients (Zhu, 2023).

Evidence shows that the cultivation of American-originated amaranth dates back to 6700 BC. Amaranth, one of the world's oldest food crops, was consumed by hunter-gatherers in North and South America. These civilizations developed the first cultivation techniques of amaranth (Sauer, 1950; Sauer, 1967). Amaranth is widely cultivated in countries such as China and India since it is a drought-tolerant plant (Schmidt, 2023).

Amaranth is a crucial product with high nutritional value for celiac patients and consumers due to its gluten-free, rich protein and fiber content (Arendt, et al., 2009; Martinez, et al., 2014). It also has properties that can be an alternative to vegan, vegetarian, and ketogenic diets (Özçelik & Yılmaz, 2022). With more than six thousand years of culinary use, amaranth is also called "ancient grain" (Kahlon & Chiu, 2015). Despite its significant place in the African diet, Amaranth's use in global and traditional cuisines has remained limited (Olufolaji & Tayo, 1980). However, some countries, such as Peru, have recently launched projects encouraging amaranth's reuse (Early, 1990). In addition, amaranth is among the foods recommended for consumption by FAO/WHO (Maurya & Arya, 2018).

Amaranth's circular seed structure differs from other cereal varieties (Taylor & Parker, 2002). Approximately 70 species and 400 varieties of amaranth worldwide, and only a few have been domesticated and used in different countries. The most common domesticated species are *Amaranthus cruentus*, *Amaranthus caudatus*, and *Amaranthus hypochondriacus* (Aderibigbe, et al., 2022). Flower, leaf, and stem colors vary in species grown for their seeds, but they are chestnut or dark red. Amaranth, generally a tall plant with broad leaves, resembles the red-rooted cockscomb (*Celosia*) plant in shape. The plant bears large, colorful spikes and has a robust, hairy stem like a sunflower. The species grown for their seeds are 1.5-2.1 m long and have a hairy hard body. Their leaves vary in size and are purple, green, or red, and the seeds of species

grown for cereal are generally white-grey. Figure 1 presents Amaranth plant and seed.

Amaranthus tricolor, *Amaranthus husblitum*, *Amaranthus dubius*, *Amaranthus spinosus*, and *Amaranthus viridis* are the most common species grown mainly as leafy vegetables. Among the various amaranth species domesticated for use in different parts of the world, *Amaranthus cruentus*, *A. hybridus*, and *A. dubius* are commonly grown in West Africa, while *A. tricolor* is mainly grown in East Africa, China, and India (Aderibigbe, et al., 2022). It is not grown for agricultural purposes in Türkiye but grows spontaneously in the fields as a weed (Ergun, et al., 2014). Amaranth seeds grown as vegetables are tiny and bright black (Kahlon & Chiu, 2015).

With its ability to tolerate biotic and abiotic stress conditions, amaranth has the potential to be an alternative crop in drought- and salt-affected areas. Many consider amaranth the future crop due to its C4 photosynthesis system and its productive structure proliferating in dry regions or poor soils with high soil salinity (Barba De La Rosa, et al., 2009; Hilou, et al., 2016). It is possible to procure a grain yield of 450-700 kg/ha in arid regions or 900-2000 kg/ha in areas with high rainfall (Williams & Brenner, 1995). It is an essential plant, especially for countries with dense populations, as it requires little water to cultivate. For this reason, amaranth cultivation has become widespread, especially in China, Russia, South America, and India, as a precaution against the threat of global famine and hunger (Aderibigbe, et al., 2022; Ruth, et al., 2021).

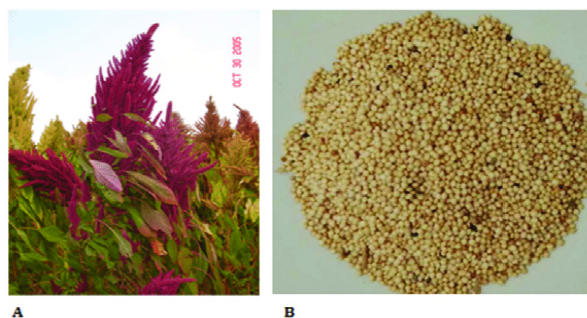


Figure 1. Amaranth plant (A) and seed (B) (Soriano-García, et al., 2018).

Amaranth's Nutritional Value and Functional Properties

Amaranth is utilized in food production, the pharmaceutical industry, and various industrial fields due to its functional properties (Rastogi & Shukla, 2013). Amaranth grains are gluten-free and contain many nutrients such as starch, protein, fat, dietary fibers, unsaturated fatty acids (linoleic acid), squalene, phenolic compounds, betalains, minerals, phytosterols and vitamins. Due to these properties, it is a promising product compared to other herbal products (Baraniak & Kania-Dobrowolska, 2022; Hilou, et al., 2016; USDA, 2023). Table 1 presents a comparative summary of amaranth with pseudocereals concerning some nutrients (USDA, 2023).

Amaranth also contains many essential micronutrient components such as calcium, magnesium, iron, vitamin C, β -carotene, and folic acid (Priya, et al., 2007). It is also the only grain that contains vitamin C, with its young leaves consumed (Tang, et al., 2016).

Table 1. A comparison of nutritional values of pseudocereals and amaranth (per 100 g)

Energy and Nutrients	Buckwheat	Quinoa	Amaranth
Energy (kcal)	343	368	371
Protein (g)	13.25	14.12	13.56
Carbohydrate (g)	71.50	64.16	65.25
Fat (g)	3.40	6.07	7.02
Saturated fat (g)	0.741	0.706	1.459
Single SFA (g)	1.040	1.613	1.685
Multiple SFA (g)	1.039	3.292	2.778
Pulp (g)	10.0	7.0	6.7
Sodium (mg)	1	5	4
Potassium (mg)	460	563	508
Calcium (mg)	18	47	159
Iron (mg)	2.20	4.57	7.61
Zinc (mg)	2.40	3.10	2.87
Magnesium (mg)	231	197	248
Folate (μ g)	30	184	82
Thiamine (mg)	0.101	0.360	0.116
Niacin (mg)	7.020	1.520	0.923
Riboflavin (mg)	0.425	0.318	0.200
Vitamin A (IU)	0	14	2
Vitamin E (mg)	-	2.44	1.19
Lysine (mg)	672	766	747

Source: (USDA, 2023)

Amaranth contains high amounts of protein (average 13-14%). The energy and nutrients of amaranth and other products are higher in nutritional quality, protein content, and balanced essential amino acid content of amaranth seed (Table 1). It is also rich in lysine, missing in amaranth cereal grains. The mineral content is generally higher than cereal grains, especially calcium and magnesium. Additionally, it has higher dietary fiber and lipid content than most grains. Furthermore, regarding the mineral content of cereal-like products, amaranth has a higher content of manganese, iron, zinc, magnesium, calcium, and phosphorus than quinoa and buckwheat (Sanz-Penella, et al., 2013).

Amaranth's small polygonal starch structure gives it properties different from other starch sources, such as corn, rice, and wheat, contributing significantly to the properties and functionality of starch. The core and shell parts of amaranth grains contain vitamin E and polyphenols with high antioxidant capacity (Barba De La Rosa, et al., 2009). Protein digestibility is 0.40 in wheat flour, 0.57 in oat flour, and 0.64 in amaranth flour. Therefore, amaranth is an essential source of protein digestibility (Bejosano & Corke, 1998).

Amaranth, quinoa, and buckwheat are alternatives to grain varieties because they do not contain gluten protein. These grains are preferred as they produce gluten-free products and improve their nutritional value (Yaver & Bilgiçli, 2020). Amaranth contains albumin and globulin proteins. It does not contain prolamin proteins, which have a toxic effect on celiac patients with gluten allergy. Therefore, it is an alternative product to help celiac patients eliminate malnutrition problems (Alvarez-Jubete, et al., 2010).

The fat in amaranth's composition contains high levels of natural organic compounds tocotrienol and squalene, which are involved in cholesterol metabolism and significantly lower LDL-cholesterol in the blood. In addition, phytosterol in amaranth seed is a beneficial fatty

acid that reduces cholesterol absorption and effectively lowers LDL cholesterol. In addition to being a source of energy, the fat in amaranth's composition plays a crucial role in fat-soluble vitamins (Sanz-Penella, et al., 2013). Clinical studies have determined that various enzymes, unsaturated fatty acids, and amino acids in amaranth seeds have a cholesterol-lowering effect (Ajayi, et al., 2021; Hosseintabar-Ghasemabad, et al., 2022).

The glycosamine streptomycin, quercetin glycoside, and rutin flavonoids in amaranth cause it to show antibiotic properties (Filipčev, et al., 2011; Iqbal, 2012). Also, the increased antioxidant activity of amaranth after digestion may help tumor treatment (Barrio & Añón, 2010; Silva-Sánchez, et al., 2008). Amaranth seeds and leaves have anti-cancer properties. The seeds are rich in amino acids such as lunasin, peptides, and lysine that help the body fight cancer (Orona-Tamayo & Paredes-Lopez, 2024). Amaranth is also a suitable plant for cancer patients as it stops the irregular growth of cancer cells in the breast, colon, and liver (Hongyan, et al., 2015).

Lunasin is a unique substance found extensively in amaranth seeds and consists of 43 amino acid elements. Amaranth, rich in beneficial fatty acids and amino acids, can reduce the risk of serious diseases such as cancer, diabetes, cholesterol, and heart attack (Hernández-Ledesmaa, et al., 2013).

Amaranth seed is an oily seed that does not contain gluten and has a high protein value (Skwarylo-Bednarz, et al., 2020). For this reason, dishes made with amaranth seeds are essential for people who want to lose weight and diabetic patients. Rich in vitamin K, this oilseed is especially recommended for the nutrition of people with weak bones (Ruth, et al., 2021).

Amaranth in rituals

Amaranth was first utilized as a grain in Central America over 6,000 years ago (Kahlon & Chiu, 2015). Civilizations such as the Aztecs, Mayas (Central America),

and Incas (South America) used this product, frequently consumed in their daily diets, to cook various dishes in their kitchens. The Aztecs not only cultivated the plant for food but also used it in religious rites and rituals. Although it spread from colonial times to tropical and subtropical regions of the Old World, the dietary consumption of amaranth declined significantly after the collapse of Central American culture (Chemutai, et al., 2019; Kahlon & Chiu, 2015).

Amaranth was also considered an important ritual and cash crop in Mesoamerica (Sauer, 1950; Sauer, 1967). Another ritual in which amaranth was used was religious rituals. Amaranth flour "tamales" and a mixture of "popped amaranth" and sweet maguey syrup called "tzoali" were offered to specific deities (Wyatt, 2002). The amaranth seed was considered sacred by ancient civilizations because of its unique properties. For the Aztecs, amaranth was as holy as cacao beans and honey. They combined honey and amaranth to form the figure of a god in their ceremonies (Dietz, 2022).

Amaranthus caudatus and *Amaranthus hypochondriacus* were used for decorative purposes. Especially in Victorian times, *A. caudatus* conveyed the meaning of despair or hopeless love (Jerome, 2001). Amaranth has a special significance in Mexico, where the bright red seeds, similar in color to blood, were considered the holiest of human substances (Wyatt, 2002).

Use of Amaranth in Gastronomy and Food Industry

Use in breadmaking: Bread is a traditional and religiously valued food commonly consumed in the culinary cultures of many countries. It is a staple food, although daily bread consumption varies according to individuals' characteristics, eating habits, living, working, and dietary patterns (Ardıç Yetiş, 2020). The nutrients found in wheat pass into the bread since bread's raw material is generally wheat flour. However, the flour's nutritional content decreases during the milling stage, depending on the purification state, since the vitamins and minerals in wheat are mainly found in the core (embryo) and outer shell (bran) of grain. Therefore, a product that can enrich bread's nutritional content is crucial in increasing the public's nutrient intake (Ayo, 2001). Recent studies have also focused on using pseudo-cereal grains such as buckwheat and quinoa to enrich bread's nutritional content. In this context, amaranth flour is richer in micro and macro elements such as energy, protein, fat, calcium, iron, magnesium, and lysine than other pseudocereal grains. In addition, among the quality characteristics of flour used in bread making, the water retention capacity of the flour is expected to be high (Ozan & Karababa, 1997). The starch structure of amaranth flour has small granules (average diameter 1 mm) and high water absorption capacity (Singhal & Kulkari, 1988).

In a study, amaranth-wheat blended breads were developed using different mixture formulations. Amaranth flour was added to wheat flour at different rates, such as 5, 10, 15, 20, and 30%, and the prepared mixtures were baked at 220°C for 18 minutes. It was found that bread's nutritional value increased as the amount of amaranth increased, and adding up to 10% of amaranth flour improved bread's sensory and quality properties (Emire & Arega, 2012). Sanz Penella et al. (2013) found that adding

amaranth flour to wheat flour in bread making significantly increased the bread's protein, lipid, ash, dietary fiber, and mineral contents (Sanz-Penella, et al., 2013). Since the phytic acid naturally present in amaranth's composition must be reduced or removed by various technological processes, it was determined that lactic acid fermentation might be successfully applied to eliminate the negativities due to the natural composition of pseudocereals containing phytic acid, such as amaranth (Özçelik & Yılmaz, 2022).

Machado Alencar et al. (2015) reported that the volume, texture, and water activity values of bread made with amaranth and quinoa flour showed similar results with standard bread (Machado Alencar, et al., 2015). Ayo (2001) found that adding 15% amaranth flour had no adverse effect on wheat bread's physical and sensory properties and recommended the consumption of amaranth bread for infants, children, and pregnant and lactating women. Another study found that the protein content of bread with 25% amaranth flour added increased, and the staling process slowed down (Miranda-Ramos, et al., 2019). It was found that breads made from amaranth flour were more nutritious and contained higher levels of fiber and minerals than breads made with wheat. It was also reported that they had less volume increase and darker color (Özçelik & Yılmaz, 2022).

Use in biscuit making: Biscuits are bakery products frequently consumed in almost every society. They can be eaten on the go and are considered healthier for consumers than chocolate. With an essential place in non-meal nutrition, biscuits are increasingly consumed daily for their long storage time without staling (Demir, 2015). Flours used in biscuit production are generally of *Tr. aestivum* species, which is too weak to be used in the bread industry. The protein content and quality of the grain are low. The particle fineness of biscuit flour is a significant factor in biscuit quality. A crisp and good quality biscuit is made from fine particle flour. Since excessive swelling and volume are not desired in biscuits, wheat with a soft grain structure, high starch content, and low gluten content are the properties of wheat accepted for the biscuit industry. The flour used for biscuits must also have a controlled and average spread (Bilgiçli & Soylu, 2016). Biscuits and cookies are dough products prepared by mincing. The starch structure forming the bulk of amaranth flour provides the desired flour quality properties for the biscuit industry because of its granular structure (average diameter 1 mm) (Singhal & Kulkari, 1988) gluten-free nature, and high spreading rate. Therefore, as consumers become healthier and more conscious, research is conducted on alternative products to enrich the nutritional content of biscuit production. While they focus on using pseudocereal grains such as corn, buckwheat, and quinoa as gluten-free products, research seems to focus on whole-grain wheat flour regarding its rich nutritional content. However, although amaranth flour meets the desired flour criteria in biscuit production and is rich in nutrients, the literature contains only limited studies.

A study stated that amaranth flour can be used in products with high viscosity and dry matter, especially in gluten-free breakfast products prepared for celiac patients, such as biscuits and crackers, since it can absorb more water during kneading than wheat flour (Boz, 2013). In this context, Sindhuja, Sudha, and Rahim (2005) replaced

wheat flour with up to 35% amaranth flour and found that the product's color, taste, flavor, and appearance improved positively. In another study, cookies were made with a 25% amaranth flour mixture, and it was determined that the product with amaranth flour substitute was the best in terms of organoleptic properties and color (Sindhuja, et al., 2005). The products were also reported to be sensory acceptable when 100% was added (Renu & Anirban, 2015). Schoenlechner, Siebenhandl, and Berghofer (2008) used amaranth, buckwheat, and quinoa to produce gluten-free biscuits and found that amaranth is suitable for replacing wheat flour (Schoenlechner, et al., 2008). Chauhan et al. (2016) produced gluten-free biscuits with raw and sprouted amaranth flour and found that adding up to 60% was acceptable (Chauhan, et al., 2016).

Use in pasta, noodle and manti: The flour used in the dough for making traditional foods such as pasta, noodles, manti, pastry, and baklava should be high-yield, first-quality durum wheat flour and semolina. These flours yield 70-80% and are full white. The starch content of wheat used in pasta production is also an essential criterion in determining pasta quality. In particular, since damaged starch constitutes a substrate for amylases during pasta making and increases the amount of solids that break down and pass into the cooking water, the flour used should be low in damaged starch to avoid disintegration after cooking (Bilgiçli & Soylu, 2016). The flour criteria required for preparing pasta, noodles, and manti, especially water-boiled, are explained above. Durum wheat's protein content is another desired factor. In pasta production, wheat with a protein content above 13% is preferred because durum wheat's protein content and quality significantly determine the cooking quality of pasta produced from this wheat. However, various studies have recently been conducted in the literature to increase the nutritional value of the foods consumed. They have mainly focused on dishes at the forefront of world cuisine, such as pasta, noodles, and manti. Manifold studies have been conducted in the literature on making pasta, noodles, and manti using amaranth grain.

Cárdenas Hernandez et al. (2016) cooked pasta with dried amaranth leaves and amaranth seed flour. They found that adding amaranth to pasta samples could increase the product's functional properties compared to the control but also reduce the cooking time, increase cooking loss, and decrease the brightness value (Cárdenas-Hernández, et al., 2016). Another study reported that amaranth-based pasta samples had higher cooking loss and lower stickiness than durum wheat pasta, while no difference was observed in taste perception (Chillo, et al., 2008). While protein, fat, ash, phytic acid, phenolic matter, and mineral matter content increased, the brightness value of noodle samples produced with pseudocereal (e.g., amaranth, quinoa,

buckwheat) flours added to wheat flour in different combinations at a rate of 30% decreased (Öncel & Demir, 2019). As the amount of amaranth increased, the baked dough became softer, and the disintegration properties increased. Cárdenas -Hernandez reported the same findings as a result of sensory analysis.

Additionally, researchers investigated the color and sensory analysis characteristics of pasta samples containing 30% amaranth and revealed a passing grade (Fiorda, et al., 2013). Martinez et al. (2014) examined the technological and sensory quality of pasta made from bread wheat flour substituted with *Amaranthus mantegazzianus* at 15, 30, 40 and 50%. Findings indicated the nutritional and functional properties of the pasta with 30% amaranth flour were acceptable when compared with the pasta made from bread wheat flour in the other control groups (Martinez, et al., 2014). In a study by Aygün (2021), nine different manti doughs were obtained using 0, 5, 10, 15, and 20% quinoa and amaranth flours. They determined that manti containing 20% amaranth and quinoa flour increased the amount of fat, protein, phenolic substances, and cooking time and were perceived positively. An examination of the weight increase in amaranth and quinoa manti samples indicated that those containing amaranth flour had a higher weight rise after cooking (Aygün, 2021).

Research has commonly shown that pasta, noodles, and manti produced by adding 20-30% amaranth flour increase nutritional benefits and have acceptable sensory and taste quality. Table 2 summarizes the disadvantages and solutions for using amaranth in making bread, biscuits, pasta, noodles, and manti.

Amaranth's Traditional Culinary Uses

More than 70 species of amaranth are used as food in both tropical and temperate regions of the world (Olufolaji & Tayo, 1980). In different cultures, amaranth is also called purple amaranth, red amaranth, red stem, bush greens, African spinach, and Indian spinach (Chemutai, et al., 2019). It has been named differently in different countries: bayam (Indonesia, Malaysia), kalunay (Philippines), chaulai (Uttar Pradesh, Bihar-India), chua (Uttarakhand), harive (Karnataka-India), cheer (Kerala), mulaikkira (Tamil; Nadu), shravani math (Maharashtra), khada saga (Orissa), rau dền (Vietnam) (Jerome, 2001).

Amaranth is a leafy vegetable in the Nigerian diet, and the soft parts of the stems and leaves of wild amaranth are used as ingredients in green salads and stews in African cuisine (Olufolaji & Tayo, 1980). In Mexico, amaranth grains are mixed with honey to make "alegría" candy. In Peru, "turron," made by mixing amaranth seeds with molasses, is consumed as a snack. Obtained by grinding amaranth seeds on stone, amaranth flour (pinol in Mexico; mash'ka in Peru) is widely used.

Table 2. Challenges in amaranth use in bakery products and proposed solutions

Challenges	Proposed Solutions
Reduction in bread volume	Usage up to 25%
Disintegration of pasta and noodles when cooked in water	Preference in breads that do not require volume, such as lavash and phyllo
Disintegration of unleavened dough when cooked in water	Mixing with wheat flour at a maximum of 30% in pasta and noodle dough
	Preferring different baking methods (oven) for unleavened doughs

Source: Author's work

Table 3. Uses of amaranth species in traditional cuisine

Amaranth Species	As a vegetable	As a grain	Uses in the Kitchen	Countries of Cultivation	Reference
<i>Amaranthus cruentus</i>	X	X	As an ingredient in food, snacks, soups, vegetable dishes or sauces	West Africa	Grubben & Denton, 2004
<i>Amaranthus caudatus</i>		X	Crackers, gluten-free brown bread, biscuits, cookies	South America, Argentina, Peru, Bolivia	Mekonnen et al., 2018
<i>Amaranthus hypochondriacus</i>		X	Marinating bread and fish	South America, Asia	Mlakar et al., 2010 He et al., 2002
<i>Amaranthus tricoloris</i>	X		Dishes and salad	East Africa, China and India	Herbst, 2001
<i>Amaranthus dubius</i>	X	X	Potherbs sauce	West Africa Caribbean and China	Herbst, 2001; Aderibigbe et al., 2022
<i>Amaranthus hybridus</i>	X		Dishes and salad	West Africa, Indonesia, Malaysia, Mexico, Thailand, Philippines, Nepal	Herbst, 2001

Source: Author's work

Table 4. Cooking methods using amaranth used as a vegetable

Cooking Method	Procedure
Boiling	Wash the amaranth, place the banana leaves in the pot to prevent burning, sprinkle salt between the unchopped amaranth, and boil without turning (about 1 hour) until the water dries up to prevent steam from escaping.
Sauteing	Fry the onion and tomatoes in oil. Add the chopped amaranth and cook for about 10 minutes while stirring.
Frying	Wash the amaranth, dry it in the sun to drain the water, then fry it with oil, onions, and tomatoes.
Steaming	Wash the vegetables and place in sufuria. Add chopped onions, tomatoes, salt, and steam for 10-15 minutes.
Boiling and fermenting	Boil amaranth for 20 minutes, add salt to taste, and heat once a day while adding milk each time for 2 to 3 days, (mostly done in combination with other vegetables).

Source: (Nyonje, et al., 2022).

In Peru, the seeds are used as a fermenting agent in beer making, while the red flowers of amaranth are added to color corn and quinoa in local cuisines (Early, 1990). In Northern India, amaranth (*rājgeerā*) is mixed with palm sugar (*jaggery*) and used to make “laddoos,” a sweet (Kahlon & Chiu, 2015). Amaranth flour is used to make flatbread in Latin America and the Himalayas, to prepare different types of curries (*Yulee*, *palya*, *majjigay-hulee*) in India, and as an ingredient in soups in China. In Greece, green amaranth (*A. viridis*) is used to prepare *βλήτα*, *vlita*, or *vleeta* (a type of salad served with fish) (Lucian, et al., 2018). Amaranth is essential in people's nutrition in the Ouagadougou Region of Africa (Hilou, et al., 2016). Called “huautli” in Nahuatl, amaranth is ground into flour, similar to corn, and used to make tamales and amaranth tortillas, specially prepared as a gourmet meal for nobles (Wyatt, 2002). The Indians, on the other hand, used amaranth seeds whole in drinks, sauces, and porridges and powder form in different formulations in bread, cereal products, and medicinal areas (Mlakar, et al., 2010). The literature review reveals this plant's use mostly in various dishes in African cuisines.

Specific beliefs and negative attitudes have led to the neglect of the amaranth plant over the years. Such attitudes include considering the plant a weed and a poor person's food. In particular, a study in Africa found that rural communities

in parts of Kenya and Tanzania consumed leafy amaranth at higher rates than urban dwellers. Factors such as amaranth's unfamiliar taste, small leaf size, short shelf life (leaf parts), and seasonality were found to reduce consumption levels (Nyonje, et al., 2022). *A. caudatus* is consumed as a grain crop in South America, with *A. dubius* as a vegetable in the Caribbean and China. *A. hybridus* is also used as a vegetable in countries such as Indonesia, Malaysia, Mexico, Thailand, the Philippines, and Nepal in the southwestern United States (Table 3), (Aderibigbe, et al., 2022).

Amaranth's Preparation and Cooking Techniques

In traditional cuisine, amaranth's leaves and stems are used as vegetables and seeds as grains. Amaranth is added to various salads and casseroles as a vegetable. The type of amaranth used as a vegetable does not contain poisonous species. However, since its leaves are known to contain oxalic acid and nitrate, it is generally recommended to be used after soaking in boiling water. Table 4 presents cooking methods using amaranth used as a vegetable.

As a grain, amaranth seeds are used in raw and cooked forms to prepare various dishes. Raw amaranth seeds are often ground and utilized to make gluten-free bread and pasta and traditionally to produce a sugar-type product from the grains (Martinez, et al., 2014).

Table 5. Forms of raw and cooked amaranth seed use in foods

Product	Amaranth's Usage Forms
Sweets	Popped grain
Bread	Ground form (from raw amaranth seeds)
Soup	Grain form (from cooked amaranth seeds)
Rice	Grain form (from cooked amaranth seeds)
Couscous	Grain form (from cooked amaranth seeds)
Baby foods	Grain and ground forms (from raw or cooked amaranth seeds)
Breakfast cereals	Grain form (from raw or cooked amaranth seeds)
Ready sauces	Ground form (from cooked amaranth seeds)
Pasta, manti	Ground form (from cooked amaranth seeds)
Cookie/cracker/biscuit	Ground form (from raw amaranth seeds)
Cake/pancake/crepe/muffin	Ground form (from raw amaranth seeds)
Beverages	Grain and ground forms (from raw or cooked amaranth seeds)

Source: (Arendt & Zannini, 2013).

Table 6. Nutrient content of wheat and amaranth flour

Compounds	Amount ^a	Wheat Flour	Amaranth Flour
Ash	g/100 g	0.53 ± 0.01	2.44 ± 0.08
Microelements			
Cu	ug/g	1.83 ± 0.03	6.94 ± 0.01
Mn	ug/g	5.82 ± 0.01	36.55 ± 0.12
Zn	ug/g	7.35 ± 0.10	42.08 ± 0.32
Fe	ug/g	12.66 ± 0.04	82.13 ± 0.17
Macroelements			
Ca	mg/g	0.22 ± 0.01	2.04 ± 0.01
Mg	mg/g	0.25 ± 0.01	2.69 ± 0.01
P	mg/g	1.11 ± 0.22	5.30 ± 0.02
Na	mg/g	112.4 ± 1.4	8.21 ± 0.27
K	mg/g	1.56 ± 0.01	4.70 ± 0.03

a: Units expressed in terms of dry matter Source: (Yaver & Bilgiçli, 2020)

Cooked amaranth seeds are added to soups, curries, sauces, and stews to enhance the dish's sensory and nutritional profile and are also used as an alternative thickener to replace cream, eggs, cornstarch, and wheat flour. Cooked amaranth seeds can be used instead of couscous, rice, or pasta in porridges, pastries, and stews. The weight of raw and cooked amaranth seeds in grams is 200 g for 1 cup of raw amaranth seeds and 245 g for cooked amaranth seeds. Table 5 presents culinary usage forms for amaranth seeds.

As with any food, the nutritional benefit of amaranth depends on many factors, including the species, production method, and cooking techniques (Nyonje, et al., 2022). In particular, amaranth grains must be cooked for nutritional benefit and flavor. They must be soaked, rinsed, and boiled to obtain cooked amaranth grains.

Amaranth does not contain gluten and increases the nutritional content of the foods, making it a preferred alternative product in bakery products (Kılınççeker & Büyük, 2019). It has been described as a “super grain” with great prospects and high commercial potential for product development in the food industry, especially in the bakery sector. *A. spinosus*, *A. hypochondriacus*, and *A. cruentus* species are generally preferred in baking (Ramos, et al., 2019). Amaranth grain is used alone or as mixed flour in making bread, biscuits, pasta, crackers, pancakes, noodles, cakes, pancakes, pancakes, crackers, muffins, and breakfast cereals (Aderibigbe, et al., 2022; Alvarez-Jubete,

et al., 2010). Unlike wheat, amaranth is a potential substitute raw material in gluten-free products. The small structure of starch granules contributes to amaranth use in film coating and emulsified formations (Lindeboom, et al., 2004) and its use in various products such as salad dressings and milk alternative beverages (Özçelik & Yılmaz, 2022). Oil is also obtained from amaranth seeds (He, et al., 2002). Amaranth flour is richer in macro and microelements than wheat flour in terms of dry matter (Table 6).

Amaranth is a plant frequently consumed, especially in its homeland, South American countries. It is possible to see this grain popped and sold like corn on the streets. It is also used to prepare breakfast porridge in regions such as Nepal, India, Mexico, and Peru (Aderibigbe, et al., 2022) “Dulce de alegria,” a popular Mexican food, is made by mixing popped amaranth with honey or sugar. Amaranth grain can also be used in popular foods such as bread, crackers, muffins, and pancakes (Dietz, 2022; Wyatt, 2002).

Amaranth seed dishes are varied. Amaranth is a plant that can be consumed in salads or mixed with rice. The seeds of amaranth, of which soup is also made, can be cooked with legumes, and dishes with olive oil can be made from fresh leaves (Arendt & Zannini, 2013). Amaranth malt has been used to make gluten-free beer. In recent years, the demand for high-quality gluten-free food, including gluten-free beer, has increased rapidly, and

gluten-free, healthy, and tasty beer has significantly improved people's well-being and perception of ordinary social life. Therefore, it can also produce gluten-free alcoholic beverages (Dabija, et al., 2022; Manassero, et al., 2020). It is also possible to prepare amaranth tea. That tea is known as cockscomb and is straightforward to make. It is obtained by finely pounding fresh ginger, masala, and amaranth seeds and drying them under the sun for two days (Tibagonzeka, et al., 2014).

Amaranth in Traditional Turkish cuisine

Turkish cuisine harbors more than 2500 types of food (Ceyhun-Sezgin & Sanlier, 2019), Turkish culinary culture includes various food types, such as soups, pastries, desserts, and meat dishes (Güler, 2010). The Mesopotamian grains, the Mediterranean vegetable and fruit culture, and the South Asian spices have influenced Turkish culinary culture (Öncel & Demir, 2019). Grains are particularly an essential part of Turkish culinary culture. Throughout history, agriculture and animal husbandry have been crucial sources of income in Turkish culinary culture.

For this reason, many types of dishes are made from grains and grain products in Turkish culinary culture (Karaman & Ceyhun-Sezgin, 2021). In Turkish culinary culture, it is consumed in every type of food, such as soup, meatballs, rice, stuffed vegetables, desserts, salads, and breakfast. Grain consumption is, therefore, very high in Turkish culinary culture. Wheat and bulgur are Turkish cuisine's most consumed grain products (Kasar, 2021). Amaranth can be used as an alternative to bulgur, wheat, semolina, and rice in Turkish culinary culture. In Turkish cuisine, amaranth seeds can be boiled and mixed into salads and dishes, and the seeds ground into flour can be used in all pastries, including bread.

Carrot Soup

Soup is "a hot and juicy beverage made with meat, vegetables and grain products" (Turkish Language Association, 2023). It is also considered a main dish in Türkiye. Simple porridges made with flour and wheat in Central Asia are the first representatives of today's soups (Yerasimos, 2019). Soup is a type of meal that has always been on the table as the essential product from past to present and is consumed at breakfast, lunch, and dinner (Çolakoğlu & Sarıışık, 2023). Although approximately 300 soup recipes exist in Turkish cuisine, there are over 1000 soup types with various ingredients and cooking techniques varying from region to region (Özbey & Köşker, 2021). According to the Turkish Patent and Trademark Office (2022), dishes and soups rank second with a rate of 20.7% among foods divided into 13 registered subgroups (Turk Patent, 2022). In Turkish cuisine, most soups contain grains, grain products, and legumes as the main ingredients (Arlı & Gümüş, 2007).

Wheat was one of the traditional ingredients of Central Asian and Seljuk Turks, and soups with wheat were consumed frequently. However, the demand for wheat soups in Ottoman cuisine decreased over time. Nowadays, wheat is commonly used in local soup varieties. Many soup types are included in the documents related to the seasonal food list given to palace employees, estimated to date back to the 16th century (Topkapı Palace Archive No. D.9599). Carrot soup is one of the soups found in the relevant documents (Yaman, 2022). In Turkish cuisine, carrot soup is among the vegetable soup groups. Since soups are consumed as main meals in Turkish cuisine, they are considered not only as a beverage but also as a food group. Therefore, wheat flour, bulgur, barley, and rice can thicken vegetable soups. The recipe for carrot soup, given below, was prepared using amaranth instead of wheat.

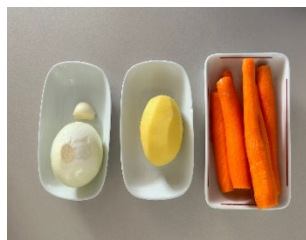
Carrot Soup



Procedures



Add 1 cup of boiling water to 1 cup of amaranth seeds and cover it for 30 minutes.



Prepare the carrots, potatoes, onions and garlic. Dice them later.



Fry the vegetables and bay leaf in olive oil.

Ingredients

- 5 medium-sized carrots (200 g)
- 1 medium-sized potato (170 g)
- 1 large onion (90 g)
- 1 clove of garlic
- 1 cup of amaranth seeds (170 g)
- 2 bay leaves
- 5 cups of vegetable fondue (1 lt/1000 ml)
- 2 teaspoons of salt (12 g)
- 4 tablespoons of olive oil (40 g)



Add the amaranth seeds previously soaked with hot water and mix. Boil for 10 more minutes and then remove from the stove. It is prepared to serve.



Crush the cooked vegetables using a blender.



Add 1 liter of previously prepared vegetable fondue and let it boil over medium heat for 10 minutes covered.

Source: Author's work

Halva

Dessert is an essential part of Turkish cuisine. Halva is one of the crucial desserts of today's Turkish cuisine. Halva is a dessert made with oil, honey/sugar, semolina/flour/starch, and water/milk (Turkish Language Association, 2023; Yerasimos, 2019). Halva is consumed and distributed on special occasions (important days, feasts, holidays, deaths, weddings) and dates back to ancient times. Cooking halva is one of the most well-

known traditions of Turkish cuisine, and it has been practiced as a ritual since Central Asian times. Since Göktürks believed in the afterlife and that souls would exist forever, they would make halvah and distribute it after the dead people (Bozagicı, 2023). As of February 2023, there are 32 different types of halva with geographical indications belonging to Turkish cuisine (Turk Patent, 2023). In the following halva recipe, some semolina is removed and replaced with amaranth.

Halva



Procedures



Prepare the ingredients.



Melt two tablespoons of butter in a pan. Add one cup of amaranth seeds and half a cup of semolina to the butter and roast them.



Add a glass of milk to the roasted amaranth seeds and semolina.

- Ingredients*
- 1 cup of amaranth seeds (170 g)
 - Half a glass of semolina (90 g)
 - 1 cup sugar (170 g)
 - 2 tbs of butter (15 g)
 - 1 glass of milk (200 ml)



Place the prepared amaranth halva into molds and serve.



Cover the lid and wait for the milk to absorb over low heat.



Add sugar to the mixture of milk, semolina, and amaranth seeds.

Source: Author's work

Salad



Procedures



Prepare and chop the ingredients.



Add one cup of hot water.

- Ingredients*
- 6 cherry tomatoes
 - 4 leaves of lettuce
 - 1 small onion (45 g)
 - ½ lemon
 - ½ pomegranate
 - Olive oil (5 g)
 - ½ teaspoon salt (2 g)
 - 1 cup of amaranth seeds (170 g)
 - 1 glass of water (200 ml)



Serve the salad with boiled amaranth seeds.



Boil for 30 minutes.

Source: Author's work

Hasıda

Hasıda is a type of dessert known as hesida, hasude and hasita in the Eastern Anatolia Region of Türkiye. Hasıda is one of the traditional halvas of Erzurum's local cuisine. Its main ingredients are wheat starch, white sugar, butter, and

water. Erzurum Hasıta / Erzurum Hasuta is a dessert registered by the Turkish Patent and Trademark Office and received a geographical indication (Turk Patent, 2022). Amaranth seeds are used instead of wheat starch in the recipe for hasıda dessert.

Hasıda



Procedures



Place 1 teacup of amaranth seeds into the saucepan and add hot water. Simmer for 20 minutes over low heat until the water is absorbed.

Add butter to the cooked amaranth and melt it.

Ingredients

- 1 teacup of amaranth seeds (90 g)
- 1 teacup of sugar (100 g)
- 1.5 tbs of butter (70 g)
- 3.5 teacups of hot water (250 ml)
- Walnut



After it turns into mush, add water and mix for 3 minutes. Serve with walnuts.



Add sugar and stir.

Source: Author's work

Conclusion

Having been used as a livelihood and commercial crop in the past, amaranth is a crucial ancestral but forgotten food of the future with good potential for third world agriculture. Amaranth can grow in arid and barren lands with minimal water requirement, does not contain gluten, and is richer in nutritional value than other grain products, increasing its use in the food industry and traditional cuisines. It is, therefore, widely used in content enrichment of food formulations not only for celiac patients but also for consumers. This study sought to show that amaranth might be an alternative source to other grains, especially in traditional recipes. The study also provided information on cooking methods to increase the applicability of amaranth in various culinary cultures.

One disadvantage of products made with amaranth is disintegration during cooking in local products with amaranth flour added, such as pasta, noodles, and manti. Prospective researchers might use different cooking methods (first in dry heat, then boiling in water or steaming) for products made with unleavened dough, such as pasta, baklava, and pastry. In addition, since breads to which more than 25-30% amaranth is added lose volume and have a hard structure, new research is needed to increase the use of amaranth in formulations, especially for

traditional bread types (lavash, phyllo, cornbread, flatbread, basmati, fetal, güdül, kartalaş, kömeç, tortilla bread) where rising is not desired.

Future studies revealing the culinary practices of amaranth and their recipes might be crucial to increasing its awareness, recognition, and consumption. In addition, qualitative studies should be conducted to increase the number of written sources in the literature, revealing the recipes used in the past and ensuring that amaranth is transferred to future generations as cultural heritage. Amaranth can be used as a substitute for cooked grain-like foods, and its awareness can be heightened by ensuring that flour and raw grain forms are included in the food industry and market shelves. Additionally, increasing the number of dishes containing amaranth as an ingredient in the local culinary culture can grow its use at home. Projects for functional food studies should be conducted, emphasizing that amaranth is gluten-free.

Implications for Gastronomy

Amaranth is an ancient and forgotten grain. Today, however, it is considered the food of the future. Adapted to arid soils and requiring a small amount of water for

cultivation, it is an essential product that can be evaluated as an alternative to cereals commonly grown in regions where climate crisis is predicted in the future. Therefore, this study aims to raise awareness of amaranth, which can be adapted to today's culinary culture, by creating various recipes by adapting it to modern recipes. On the other hand, it is thought that awareness will be raised to increase the use of amaranth, which is unknown in the culinary cultures of different countries, as a grain alternative in recipes. This study includes examples of healthy and alternative recipes using amaranth as an ingredient for consumers on a gluten-free diet.

Highlights

- Amaranth can be grown anywhere because it can adapt to ecologically difficult conditions.
- Amaranth is a plant beneficial for health, thanks to the components it contains.
- Gluten-free grains have an important place in the treatment of celiac disease.
- Flour and grain forms of buckwheat have been consumed with increasing interest in traditional foods in different countries for a long time.
- It is a good alternative for enriching menus in commercial kitchens.

Declarations

Ethics approval and consent to participate not applicable. Consent for publication All authors agree for this publication. Competing interests All authors declare there are no competing interests regarding this publication.

Acknowledgements

Not applicable.

Author Contributions

First and second author contributed for the writing main draft, concept, structure, resources, reviewing and editing, reviewing.

Funding

There is no funding resource that could be reported for this publication.

Availability of Data and Materials

Not applicable.

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The Comparison of Weight and Shape-related Traits in Eggs from Different Chicken Genotypes

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ARTICLE INFO

Research Article

Received : 05.11.2024
Accepted : 27.11.2024

Keywords:

Egg weight
Discriminant analysis
Egg shape
Genotype
Yield-type

ABSTRACT

Although there are intensively selected lines and non-selected standard breeds in terms of production characteristics in the chickens, there is limited information on the comparing their egg shape-related traits. This study aimed to compare using the parameters of weight, width, length, shape index-L/W (Length/Width), and shape index-W/L of egg in some meat-type (Anadolu-T, Ross 308, Dam Line and Sire Line), egg-type (Atak-S, Lohmann Brown and Lohmann Selected Leghorn), and standard breeds (Sultan and Ameraucana). The data from 2476 eggs from 9 genotypes obtained from 50-55 weeks hens and classified under 3 main types were analyzed with univariate and multivariate methods. The mean egg weights of Sire Line, Ross 308, Anadolu-T, Lohmann Brown, Atak-S, Dam Line, Lohmann Selected Leghorn, Sultan and Ameraucana genotypes were 69.89^f, 69.10^f, 62.84^e, 59.59^d, 59.58^d, 59.51^d, 56.81^c, 45.87^b and 43.03^a g, respectively (P<0.05). In the same order, the mean egg width was determined as 44.72^f, 45.61^g, 43.41^{de}, 43.32^{de}, 43.62^e, 43.17^d, 42.46^c, 39.90^b and 39.17^a mm (P<0.05). The mean egg length was found to be 61.97^g, 58.80^e, 59.72^f, 56.12^{bc}, 57.58^d, 56.59^c, 56.00^b, 51.30^a and 51.47^a mm (P<0.05). The egg shape index-W/L and egg shape index-L/W was calculated to be 74.78^a, 76.50^b, 77.55^c % and 138.67^c, 128.99^a, 137.59^c, 129.45^a, 132.16^b, 131.12^b, 131.93^b, 128.68^a, 131.47^b %, respectively (P<0.05). The egg weights of meat-type, egg-type, and standard breeds were 64.61^c, 58.36^b, and 45.42^a g, respectively (P<0.01). The egg width was found to be 44.06^c, 42.97^b and 39.78^a mm (P<0.05). The egg length was 59.05^c, 56.20^b, and 51.35^a mm (P<0.05). We also detected significant positive correlations (P<0.01) between the egg weight and the egg width (r=0.88), and the egg length (r=0.83). In the discriminant analyses, the success of assigning eggs to their groups was relatively low (52.4%) in terms of genotypes, but high (78.1%) in the type groups. The significant changes in the egg weight and shape-related traits were determined according to chicken genotypes and types. It was observed that intensive selection in chickens, especially in egg-type genotypes, had a strong effect on egg weight and shape-related traits.

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Introduction

In chicken breeding, commercial stocks consist of lines improved from certain breeds according to their egg and/or meat characteristics. However, there are also standard chicken breeds that are partially studied. Each breed or line has unique characteristics regarding egg quality traits (Zita et al. 2009; Hejdysz et al. 2024). The external quality traits of eggs are an important characteristic that is affected by the biological processes of egg production. The main external egg quality traits are egg weight, eggshell thickness and, egg shape index (Leeson and Summers 2010; Batanov et al. 2024). External quality traits such as egg weight and shape index can be affected by the genotypes and yield types of the hens (Hrncar et al. 2016; González et al. 2022; Assefa et al. 2023). The heritability

of these traits is relatively high compared to other egg-quality traits. The heritability of egg weight is in the range of 0.32-0.70 (Gervais et al., 2016a; Wan et al., 2019) and the heritability of shape index is in the range of 0.35-0.47 (Zhang et al., 2005; Blanco et al., 2014; Narinç et al., 2015).

External egg quality characteristics, especially egg weight and egg shape index, are important in both table and hatching production (Ayeni et al. 2020; Nowaczewski et al. 2022; Uçar et al. 2022). When compared to standard or local chicken breeds, it is seen that commercial genotypes have heavier eggs generally (Sokołowicz et al. 2019; Hejdysz et al. 2024). Although the improved materials have the same yield direction, egg weights vary

considerably according to genotypes (Hristakieva et al. 2014; Kraus et al. 2020). While there is generally a high and positive correlation between egg weight and egg width and length, it is generally reported that there is a low and negative correlation between egg weight and shape index. Although it varies according to genotypes, average correlations are between egg weight and egg width in the range of 0.400-0.839, between egg weight and egg length in the range of 0.510-0.820, and between egg weight and shape index in the range of -0.440-0.310 (Abanikannda et al. 2007; Shaker et al. 2020; Imouokhome and Omastuli, 2022; Tyasi et al. 2022). In a study conducted on a meat line belonging to the White Plymouth Rock breed, it was emphasized that there was a significant positive correlation of 0.734 between egg width and 28th-day broiler live weight, and therefore egg width could be a selection criterion in terms of live weight (Dymkov et al. 2020).

Different chicken breeds naturally produce eggs of varying shapes and sizes. For example, some breeds, like the Leghorn, are known for producing elongated eggs, while others might lay more rounded eggs. It is known that some genotypes have more oval eggs, while others have more spherical eggs (Parkhurst and Mountney, 2012; Gervais et al., 2016b; Rizzi, 2020). In a study conducted on 14 different genotypes, the average shape index was reported to be 75%, although it varied according to genotypes (Hejdysz et al. 2024). Although the shape index of eggs is desired to be between 72-76%, higher rates of shape index can be seen in meat-type chickens (Altuntaş and Şekeroğlu, 2008; Elibol, 2018). Younger hens tend to lay eggs that are more irregular in shape as their reproductive systems are still maturing. As hens age, their egg shape typically becomes more consistent and closer to the ideal oval shape (Nikolova and Kocevski, 2006; Crosara et al. 2019). It is observed that eggs obtained from hens with the same genotype in different weeks of the egg production period are more homogeneous in terms of shape (Shaker et al. 2017). The characteristics of egg shape data are an important criterion for the prediction of eggshell. Chicken eggs have an optimum egg shape without any defects, which is indispensable for the effective hatching of chicks, good packaging of eggs, and safe operation to market (Nikolova and Kocevski, 2006; Gervais et al., 2016b; Shaker et al. 2017).

Certain external quality traits, such as the egg shape index, can be easily measured without having to break the egg shell, providing us with a large amount of information that allows us to correctly classify eggs of different genotypes (González et al. 2022). The egg shape traits play a crucial role in incubation (Onasanya and Ikeobi, 2013; Jabbar et al. 2018). The egg shape index is important to achieve high hatchability rates in incubation. Because deviations from the standard egg shape index can cause a decrease in hatchability rates as a result of embryos taking a bad position, especially an increase in the number of embryos that stick to the shell and die. An ideal chicken egg is typically elliptical, with a slightly rounded end and a smoother, tapered opposite end. Variations in egg shape can occur, but significant deviations from the standard oval shape may lead to higher hatchability (Jabbar et al. 2018). In conclusion, egg shape quality is a vital aspect of poultry production that can significantly impact the economic success of production or incubation.

This study aimed to compare using the parameters of egg weight, egg width, egg length, and egg shape index in some meat-type (Anadolu-T, Ross 308, Dam Line and Sire Line), egg-type (Atak-S, Lohmann Brown and Lohmann Selected Leghorn), and standard breeds (Sultan and Ameraucana).

Materials and Methods

Materials

It used 9 genotypes Anadolu-T, Ross 308, Dam Line, Sire Line (Meat-type), Atak-S, Lohmann Brown, Lohmann Selected Leghorn (Egg-type), Sultan and Ameraucana (Standard breeds). It was collected 2476 eggs from 9 genotypes at 50-55 weeks old. All the hens were kept under similar standard environmental conditions and fed with the same ration. While meat and egg-type breeds had similar management practices under commercial conditions, similar practices had been applied to standard breeds in controlled poultry houses. The content of laying feed given to all breeds was 16-17% crude protein, 3.2-3.5% calcium, and 2750-2850 kcal/kg metabolic energy. Although there are some differences in meat and egg types, all these genotypes are raised in environmentally controlled poultry houses. Although environmental conditions are effective in the characteristics related to egg weight and shape, it is known that genotype is the main factor (Goto & Tsudzuki, 2017).

Methods

Each egg was coded from the small end and its measurements were made separately. Egg weight was measured with a precision scale to the nearest 0.01 g. Egg width and length were measured with a precision digital caliper to the nearest 0.01 mm. The Egg Shape Index was calculated from both the Egg width / Egg length \times 100 (W/L) and Egg length / Egg width \times 100 (L/W) formulas (Sarica et al., 2012; Uçar et al. 2022).

Statistical Analysis

Before the statistical analyses, the 9 genotypes of classified as meat-type, egg-type, and standard breeds. The Anadolu-T, Ross 308, Dam Line, and Sire Line genotypes were classified as meat-type, Atak-S, Lohmann Brown and Lohmann Selected Leghorn as egg-type, and Sultan and Ameraucana genotypes as standard breeds groups.

ANOVA was used to compare some external quality traits according to 9 genotypes and 3 types, and Duncan's multiple range test was used to determine which groups have significant differences in multiple comparison tests. The Discriminant Analysis (DA) was used in multivariate analysis. SPSS 20 statistical package program was used in all statistical analyses.

Results

Significant differences ($P < 0.01$) were found in all external egg traits of nine different chicken genotypes. The differences in the egg weight, egg width, egg length, egg shape index-W/L, and egg shape index-L/W were statistically significant ($P > 0.05$) between some genotypes (Table 1). The egg weight ranged from 36.00 to 96.74 g (Table 1 and Table 2).

Table 1. The external egg traits (n, standard deviation, minimum and maximum) of different chicken genotypes*

Traits	Genotypes	N	Mean	Std. Dev.	Min	Max.
Egg Weight (g)	Anadolu-T	408	62.84 ^e	4.545	43.00	77.00
	Dam Line	595	59.51 ^d	3.952	47.00	73.11
	Sire Line	437	69.89 ^f	5.639	50.52	96.74
	Ross 308	327	69.10 ^f	4.443	58.00	82.00
	Ameraucana	30	43.03 ^a	3.518	36.00	51.00
	Sultan	149	45.87 ^b	2.659	40.00	53.00
	Atak-S	53	59.58 ^d	4.012	53.00	68.00
	Lohmann Brown	238	59.59 ^d	3.893	51.00	70.00
	Lohmann Selected Leghorn	239	56.81 ^c	3.312	48.00	67.00
	Total	2476	61.88	7.829	36.00	96.74
Egg Width (mm)	Anadolu-T	408	43.41 ^{de}	1.267	39.00	47.00
	Dam Line	595	43.17 ^d	1.138	40.00	47.00
	Sire Line	437	44.72 ^f	1.308	40.67	49.28
	Ross 308	327	45.61 ^g	1.354	41.00	49.00
	Ameraucana	30	39.17 ^a	0.986	37.00	41.00
	Sultan	149	39.90 ^b	0.985	37.00	42.00
	Atak-S	53	43.62 ^e	1.924	41.00	49.00
	Lohmann Brown	238	43.32 ^{de}	1.095	40.00	47.00
	Lohmann Selected Leghorn	239	42.46 ^c	0.994	40.00	46.00
	Total	2476	43.52	1.862	37.00	49.28
Egg Length (mm)	Anadolu-T	408	59.72 ^f	2.343	52.00	67.00
	Dam Line	595	56.59 ^c	2.014	51.68	63.00
	Sire Line	436	61.97 ^g	2.725	50.48	71.52
	Ross 308	327	58.80 ^e	2.156	49.00	66.00
	Ameraucana	30	51.47 ^a	1.525	48.00	54.00
	Sultan	149	51.30 ^a	1.807	47.00	57.00
	Atak-S	53	57.58 ^d	2.274	54.00	65.00
	Lohmann Brown	238	56.12 ^{bc}	1.822	52.00	61.00
	Lohmann Selected Leghorn	239	56.00 ^b	1.522	51.00	61.00
	Total	2475	57.88	3.526	47.00	71.52
Egg Shape Index (% Width/Length)	Anadolu-T	408	72.82 ^a	3.195	63.27	81.16
	Dam Line	595	76.38 ^b	2.896	67.49	85.79
	Sire Line	436	72.27 ^a	3.437	62.66	92.04
	Ross 308	327	77.68 ^c	3.482	70.11	93.81
	Ameraucana	30	76.11 ^b	1.936	70.92	79.30
	Sultan	149	77.84 ^c	3.123	67.10	86.46
	Atak-S	53	75.75 ^b	2.599	69.27	84.97
	Lohmann Brown	238	77.32 ^c	2.351	68.78	83.00
	Lohmann Selected Leghorn	239	75.85 ^b	2.062	69.51	83.55
	Total	2475	75.35 ^b	3.681	62.66	93.81
Egg Shape Index (% Length/Width)	Anadolu-T	408	137.59 ^c	6.091	123.21	158.06
	Dam Line	595	131.12 ^b	4.993	116.57	148.17
	Sire Line	436	138.67 ^c	6.523	108.65	159.60
	Ross 308	327	128.99 ^a	5.635	106.60	142.63
	Ameraucana	30	131.47 ^b	3.395	126.10	141.01
	Sultan	149	128.68 ^a	5.277	115.66	149.04
	Atak-S	53	132.16 ^b	4.474	117.69	144.37
	Lohmann Brown	238	129.45 ^a	3.984	120.48	145.39
	Lohmann Selected Leghorn	239	131.93 ^b	3.565	119.69	143.86
	Total	2475	133.03	6.583	106.60	159.60

*Different small letters denote significant differences (P<0.05) between the means for each trait

Table 2. The External egg traits of different chicken types*

Traits	Types	N	Mean	Std. Deviation	Min.	Max.
Egg Weight	Meat-type	1767	64.62 ^c	6.452	43.00	96.74
	Egg-type	530	58.34 ^b	3.902	48.00	70.00
	Standard	179	45.40 ^a	3.005	36.00	53.00
	Total	2476	61.88	7.829	36.00	96.74
Egg Width	Meat-type	1767	44.06 ^c	1.574	39.00	49.28
	Egg-type	530	42.96 ^b	1.252	40.00	49.00
	Standard	179	39.78 ^a	1.020	37.00	42.00
	Total	2476	43.52	1.862	37.00	49.28
Egg Length	Meat-type	1766	59.05 ^c	3.095	49.00	71.52
	Egg-type	530	56.21 ^b	1.803	51.00	65.00
	Standard	179	51.33 ^a	1.760	47.00	57.00
	Total	2475	57.88	3.526	47.00	71.52
Egg Shape Index (Width/Length)	Meat-type	1766	74.78 ^a	3.896	62.66	93.81
	Egg-type	530	76.50 ^b	2.367	68.78	84.97
	Standard	179	77.55 ^c	3.023	67.10	86.46
	Total	2475	75.35	3.681	62.66	93.81
Egg Shape Index (Length/Width)	Meat-type	1766	134.08 ^c	7.008	106.60	159.60
	Egg-type	530	130.84 ^b	4.048	117.69	145.39
	Standard	179	129.15 ^a	5.112	115.66	149.04
	Total	2475	133.03	6.583	106.60	159.60

*Different small letters denote significant differences (P<0.05) between the means for each trait.

The mean egg weights of Sire Line, Ross 308, Anadolu-T, Lohmann Brown, Atak-S, Dam Line, Lohmann Selected Leghorn, Sultan and Ameraucana were 69.89, 69.10, 62.84, 59.59, 59.58, 59.51, 56.81, 45.87, and 43.03 g, respectively. The lowest mean in the egg weight was observed in the Ameraucana genotype (43.03 g), and the highest mean was observed in the Sire Line (69.89 g) and Ross 308 (69.10 g) genotypes (P<0.05). The egg width that ranged from 37.00 to 49.28 was determined to have a mean of 44.72, 45.61, 43.41, 43.32, 43.62, 43.17, 42.46, 39.90, and 39.17 mm, in the same genotype order. The widest mean in eggs was measured from Ross 308 (45.61 mm), and the narrowest mean was from the Ameraucana (39.17 mm) genotype (P<0.05). The minimum and maximum values of the egg length were determined to be 47.00 and 71.52 mm. The mean egg length was 61.97, 58.80, 59.72, 56.12, 57.58, 56.59, 56.00, 51.30, and 51.47 mm. The lowest means of egg length were determined in the Sultan (51.30 mm) and Ameraucana (51.47 mm) genotypes and the longest mean was in the Sire Line (61.97mm, P<0.05). The egg shape index-W/L and egg shape index-L/W were determined to be 72.27, 77.68, 72.82, 77.32, 75.75, 76.38, 75.85, 77.84, 76.11 %, and 138.67, 128.99, 137.59, 129.45, 132.16, 131.12, 131.93, 128.68, 131.47 %, respectively (P<0.05).

The differences between meat-type (Anadolu-T, Ross 308, Dam Line, and Sire Line), egg-type (Atak-S, Lohmann Brown, and Lohmann Selected Leghorn), and the standard breeds (Sultan and Ameraucana) in the egg

weight, egg width, egg length, egg shape index-W/L, and egg shape index-L/W were statistically significant (P<0.05, Table 2). The eggs from meat-type were heavier, and bigger than egg-type and standard breeds. The mean egg weights from meat-type hens (64.61 g) were heavier 10.70 and 42.25 % than egg-type (58.36 g), and standard breeds (45.42 g), respectively (P<0.01). The differences in mean egg widths (44.06, 42.97, and 39.78 mm) and lengths (59.05, 56.20, and 51.35 mm) were also statistically significant (P<0.05) between the meat-type, egg-type, and standard breeds. The differences in the egg shape index-W/L (74.78, 76.50, and 77.55 %) and egg shape index-L/W (134.08, 130.84, 129.15 %) were found significant between type groups (P<0.01).

Significant positive and negative correlations (P<0.01) were found between the all external egg traits (Table 3). The egg weight was significantly correlated with the egg width (0.878), the egg length (0.825) the egg shape index-W/L (-0.254) and the egg shape index-L/W (0.264). It was also observed significant correlations between the egg width and the egg length, and the egg shape index-W/L and the egg length diameter, 0.578 and 0.719, respectively.

The discriminant analysis successfully reallocated 52.4% (Figure 1, Table 4) and 78.1% (Figure 2, Table 5) of the eggs to their pre-assigned genotype and type groups, respectively. Interestingly, the majority of eggs (77%) obtained from the egg-type hen genotypes were classified as the eggs from the meat-type genotypes.

Table 3. The correlation coefficient (r) of external egg traits

Traits	Egg Weight	Egg Width	Egg Length	Egg Shape Index (Width/Length)	Egg Shape Index (Length/Width)
Egg Weight	1				
Egg Width	0.878**	1			
Egg Length	0.825**	0.578**	1		
Egg Shape Index (Width/Length)	-0.254**	0.137**	-0.719**	1	
Egg Shape Index (Length/Width)	0.264**	-0.128**	0.727**	-0.997**	1

** The Correlation is significant at the 0.01 level (2-tailed).

Table 4. Classification success of external egg traits to genotypes*.

Ratio	Genotypes	Estimated Group Distribution									
		An T	Da L	Si L	Ross	Amer	Sult	Atak	L Br	LS L	Total
Number	An_T	190	122	74	17	0	2	0	0	3	408
	Da_L	62	462	11	38	0	3	2	1	16	595
	Si_L	63	41	273	57	0	0	2	0	0	436
	Ross	16	51	48	211	0	0	1	0	0	327
	Amer	0	0	0	0	3	26	0	0	1	30
	Sult	0	3	0	0	1	138	0	0	7	149
	Atak	7	34	2	3	0	0	7	0	0	53
	L_Br	10	197	5	18	0	3	1	0	4	238
	LS_L	8	209	1	1	0	5	1	0	14	239
%	An_T	46.6	29.9	18.1	4.2	0	0.5	0	0	0.7	100
	Da_L	10.4	77.6	1.8	6.4	0	0.5	0.3	0.2	2.7	100
	Si_L	14.4	9.4	62.6	13.1	0	0	0.5	0	0	100
	Ross	4.9	15.6	14.7	64.5	0	0	0.3	0	0	100
	Amer	0	0	0	0	10	86.7	0	0	3.3	100
	Sult	0	2	0	0	0.7	92.6	0	0	4.7	100
	Atak	13.2	64.2	3.8	5.7	0	0	13.2	0	0	100
	L_Br	4.2	82.8	2.1	7.6	0	1.3	0.4	0	1.7	100
	LS_L	3.3	87.4	0.4	0.4	0	2.1	0.4	0	5.9	100

* An_T: Anadolu-T, Da_L: Dam Line, Si_L: Sire Line, Ross: Ross 308, Amer: Ameraucana, Sult: Sultan, Atak: Atak-S, L_Br: Lohmann Brown, LS_L: Lohmann Selected Leghorn

Table 5. Classification success of external egg traits to types.

Ratio	Types	Estimated Group Distribution			Total
		Meat type	Egg type	Standart breeds	
Number	Meat-type	1654	106	6	1766
	Egg-type	408	118	4	530
	Standart breeds	0	18	161	179
%	Meat-type	93.7	6.0	0.3	100.0
	Egg-type	77.0	22.3	0.8	100.0
	Standart breeds	0.0	10.1	89.9	100.0

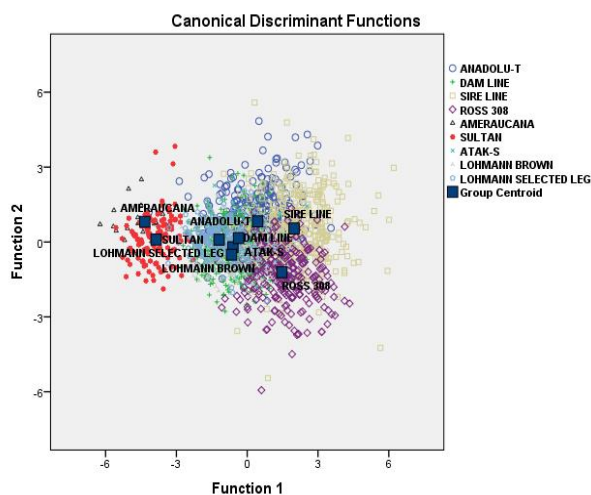


Figure 1. Scatter plot of the external egg traits to genotypes

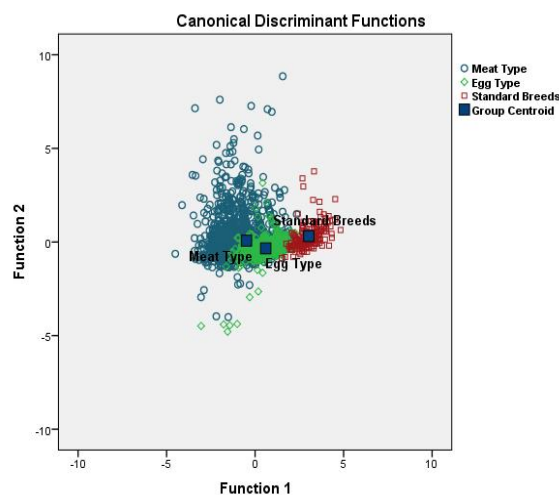


Figure 2. Scatter plot of the external egg traits to types

Discussion

Successful results have been obtained from studies on increasing egg weight and decreasing live weight in commercial white and brown layers. Thus, a certain average egg weight has been provided in line with consumer demands (Sarica et al., 2024). Similar to results of this study, it is known that commercial layer hens that lay white-shelled (such as Lohmann Selected Leghorn) eggs are approximately 2-3 g lower on average than those that lay brown-shelled (such as Lohmann Brown and Atak-S) eggs (Sarica & Uçar, 2024). It is also seen that the dam line within the meat-type has similar egg weights to brown layers. It has been determined that the egg weights of unimproved standard genotypes are quite low compared to the other genotypes and the egg weights of the Sire line, Anadolu-T and Ross 308 are high compared to the others. In our study, Ross 308 had eggs approximately 6 g heavier than Anadolu-T, and in another study, it was determined that Cobb 500 eggs were 2 g heavier than Ross 308 eggs (Hristakieva et al., 2014). Differences in hatching egg weight create variation in daily chick weight. As the egg weight changes, the weight of the chicks obtained from these eggs also changes and as a result, broiler performance is affected. Moreover, it can be said that the egg weight increases in meat type hens with high live weight and decreases somewhat compared to other meat breeds in the dam line where egg yield is taken into account (Ulmer-Franco et al., 2010; Duman and Şekeroğlu, 2017; Iqbal et al., 2017; Sarica et al., 2024).

Many studies have reported differences between genotypes in terms of egg weight, similar to our study results (Isidahomen et al. 2013; Hrnčar et al., 2016; Rehman et al., 2017; Oleforuh-Okoleh et al., 2018; Kraus

et al., 2020; Özentürk & Yıldız 2020; Nwoga et al. 2021; Tadele et al. 2023). However some studies, unlike our study, it was reported that there was no difference between genotypes (Olawumi and Ogunlade, 2009; Hrnčar et al. 2015). In a study conducted in accordance with our study results, it was observed that brown commercial layers had higher egg weights than white layers (Özentürk & Yıldız 2020). In one study, it was determined that Sasso, although a slow-growing meat-type, had heavier eggs than Ethiopian local chicken breeds (Assefa et al., 2023). Similarly, in our study, it was observed that meat-types had higher egg weights than both egg-types and standard breeds including our local breed (Sultan). It should be considered that in this study standard breeds are classified as light standard breeds according to body weight.

But unlike our study, Shaker et al. (2020) conducted on Black (Domestic), Black Brown neck (Domestic), Isa Brown (Layer) and Ross (Brewer) genotypes, the lowest average egg weight was determined in the meat-type. Again similar to our study, a study conducted with local, Sasso, Bovans Brown and Koekoek genotypes, it was determined that the local genotype had the lowest average egg weight (Assefa et al. 2019). In a study conducted with Atabey, Supernick, Atak, Brownnick and Atak-S genotypes, the highest egg weight (67.60 g) was measured in the Brownnick genotype, the lowest (61.14 g) was in Atabey (Sarica et al. 2012). In this study, it was observed that commercial genotypes widely used in the world have higher egg weights compared to hybrids developed in Türkiye. In our study, it was observed that our local hybrid Atak-S (59.58 g) had the same egg weight as Lohmann Brown (59.59 g) and higher egg weight than Lohmann

Selected Leghorn (56.81 g) genotype according to egg-types. Again similar to our study, Hejdysz et al. (2024) determined that the highest average egg weight was in Hy-Line Brown eggs and the lowest in Barbud'Anvers eggs among eggs obtained from hens of the genotypes Araucana, Ayam Cemani, Barbud'Anvers, Cochin Miniature, Faverolles, Green-legged Partridge, Hy-Line Brown, Italian Chickens' Gold, Italian Chickens' Silver, Leghorn, Marans, Rhode Island Red, Sultan and Sussex. However unlike our study, Hammershøj et al. (2021) used eggs produced from four different genotypes, including two dual-purpose genotypes, a local breed and a commercial layer genotype, and the highest egg weight was obtained in local breed and the lowest in commercial layer genotype.

Egg width and length and, depending on these, shape index may vary depending on hen genotypes and yield types (Parkhurst and Mountney, 2012; Gervais et al., 2016b; Rizzi, 2020). In a study (Shaker et al. 2017), it was reported that the shape index was more homogeneous within the lines at different ages (week) and that the shape index was different between the lines, which could facilitate the estimation of eggshell quality depending on the shape index. It is stated that there are many external egg traits that allow eggs to be distinguished according to their weight and shape in two Italian local breeds. It has been determined that the eggs of the Ermellinata di Rovigo breed are more oval, while the eggs of the Pepoi breed are more spherical (Rizzi, 2020). Hejdysz et al. (2024) reported that the egg shape index in eggs obtained from hens of Araucana, Ayam Cemani, Barbud'Anvers, Cochin Miniature, Faverolles, Green-legged Partridge, Hy-Line Brown, Italian Chickens' Gold, Italian Chickens' Silver, Leghorn, Marans, Rhode Island Red, Sultan and Sussex genotypes varied among groups, but the average was 75%. Hy-Line Brown had the highest egg shape index (78.9%), while both Marans (72.1%) and Leghorn (72.2%) had the lowest index. Similar to the findings of most studies, in our study, differences were detected in terms of egg width, length, and shape index traits according to both genotypes and types (Khan et al. 2004; Rehman et al. 2017; Hussien et al. 2019; Assefa et al. 2019; Özentürk & Yıldız 2020; Rakonjac et al. 2021; Assefa et al., 2023). According to our data, the average shape index W/L and L/W were determined as 75.35% and 133.03%, respectively. In a study (Olawumi and Ogunlade, 2009) conducted with two different layer genotypes (Isa Brown and Bovan nera), no difference was found in the egg width, while it was determined that the Isa Brown genotype had a higher average in egg length and shape index. In a study conducted with normal feathered and naked-necked local breeds of Nigeria (Oleforuh-Okoleh et al. 2018), it was reported that the normal feathered genotypes had higher average in egg width and egg length than the naked-necked genotypes, but there was no difference between them in terms of shape index.

In a study (Rahn et al., 1975) conducted on over 800 bird species, there is a relationship between female body weight and the egg weight, although it varies by species. The eggs from meat-type hens were heavier and bigger (wider and longer) than the egg-type and standard breeds. Moreover, it was determined that the genotypes had differences in terms of egg weight and size. Although not in the same scope as our study, some of the studies

conducted support these results (Hammershøj et al., 2021; Assefa et al., 2023; Hejdysz et al., 2024). Although egg-type hens to meat-types are smaller in body size, because heavy eggs are preferred for table use, selection has made eggs from egg-type hens heavier over time, approaching the egg sizes of larger meat-types (Thiruvankadan et al., 2010). The observed reclassification success of eggs obtained from meat-type and standard breeds in their groups was higher (93.7% and 89.9%, respectively) than the success of egg-type in its group (22.3%). The fact that the majority (77.0%) of the eggs obtained from egg-type hens were classified in the meat-type group supports the idea mentioned above regarding the selection process of the egg-type genotypes.

Conclusion

Our results reveal that the genotypic trend of selection programs conducted on egg quality traits in chickens caused significant positive changes in egg weight and shape. The results of this study show that discriminant analysis is a good tool to reflect or reveal shape-related egg traits of different genotypes and types. The results of this study are important for understanding the effect of selection or selective breeding on egg traits. We showed that egg weight and shape-related traits can be changed by genotype and type in this study.

Declarations

Ethical Approval Certificate

Since the research is conducted only on eggs and not on live animals, no ethics committee approval is required.

Author Contribution Statement

Ahmet Uçar: Data collection, investigation, formal analysis, and writing the original draft

Yasin Kahya: Supervision, conceptualization, statistical analysis, review and editing

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The Effect of Irrigation on Crop Yield Change in Some Cereals in Drought Conditions Determined Using SPI and PNI: Ankara Province Example

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ARTICLE INFO

ABSTRACT

Research Article

Received : 31.10.2024

Accepted : 27.11.2024

Keywords:

Irrigation

Drought

SPI

PNI

Grain

In this study, Standard Precipitation Index (SPI) and Percent of Normal Index (PNI) values were found in order to determine the drought conditions between 2012-2020 in Ankara province. Drought severity interpretations were made according to the index values obtained and these values were evaluated together with some grain yields grown in the region. Separate regression analysis was performed between the different drought index values obtained within the scope of the study and the irrigated and dry agricultural yield values of wheat, barley and triticale. According to the index values calculated between 2012 and 2020 according to the SPI method, drought conditions are generally close to normal in the region. According to the PNI values, it was determined that 2013 had mildly dry conditions and other years had near-normal drought conditions. According to both indices, a drought close to normal was observed during the research period. In the regression analysis made according to wheat, barley and triticale yields, the highest linearity was obtained in barley, and values close to barley were found in triticale. Wheat is the cereal with the lowest linearity. The regression coefficients obtained as 0.4294 for barley, 0.3331 for triticale, and 0.0502 for wheat were found to be 0.0584 for SPI and 0.0013 for PNI. According to the results obtained, it can be said that statistically linearity is in barley and triticale. In average yields, an increase of 47% in wheat, 55% in barley and 34% in triticale was observed with irrigation. In order to ensure sustainable grain cultivation in drought conditions, it is recommended to expand modern irrigation practices in coordination with drought analysis studies and to increase scientific studies on this subject.

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Introduction

Drought occurs as a result of less precipitation than normal values or expected in a region. This condition develops over time and does not occur suddenly. However, its calculation and forecasting processes are complex events. The occurrence of drought conditions reveals the absence of sufficient moisture in the root zone, which is necessary for the growth and development of plants. As a result of this situation, product yields decrease significantly. In addition, drying winds and high temperature values cause increased drought effects (Wilhite, 2000).

There are many methods for the determination of drought, SPI and PNI methods are the leading among these methods. The Standard Precipitation Index (SPI) is generally used to reveal and follow the current situation of drought in regions where the climate varies according to precipitation data by McKee et al. (1993). Another common method, PNI, is known as the percent-of-normal index and provides the determination of drought for periods of 12

months or less in time series. In this method, a percentage value is obtained by dividing the total precipitation in a certain period by the average precipitation. (Werick et al., 1994).

The effect of drought on crop yields affects the economic development of countries as well as meeting the need for healthy nutrition. For this reason, studies for the accurate determination of drought in today's conditions have gained momentum. One of the comprehensive studies on this subject in Türkiye was conducted by Şimşek and Çakmak (2010). The drought experienced in 2007-2008 Agricultural Year of Turkey was analyzed and some recommendations for future production activities. The drought was evaluated by using Standardized Precipitation Index (SPI), Percent of Normal Index (PNI) and the analyses of precipitation and temperature analysis. Among these studies, drought analysis of Kırıkkale province was made between 1950 and 2007, and analysis was made with SPI method by using monthly precipitation data. (Oğuztürk

and Yıldız, 2014), In another study, Hezarani determined the drought conditions in the Yeşilirmak Basin with SPI and PNI methods. (Hezarani et al., 2021)

The process of giving the plant the amount of water required for the development of the plant, which cannot be met naturally in the required time and amount by precipitation, is called irrigation. In agricultural terms, irrigation is an important factor in terms of productivity increase, economic development and sustainable development. The inability of existing water resources to meet the required amount of water has brought water management to a very important point. Population growth will be the most important factor affecting the use of water resources in the coming years. In this respect, the effective management of water resources will directly affect the water resource. (Aydoğdu et al., 2015).

Obtaining the highest yield and quality product from the unit area is the main purpose of agricultural production. Today, due to the rapid depletion of natural resources and environmental concerns, maximum product per unit area can no longer be targeted; rather, optimum yield should be targeted. In this respect, irrigation activities gain importance with the amount of precipitation. As a result of the researches carried out in different ecosystems, it has been observed that high rates of yield and quality increases occur in cereals under sufficient precipitation conditions or when the required water needs are met by irrigation (Spehar and De Barros Santos, 2005; Razzaghi et al., 2012), in which the amount of change in triticale yield with irrigation was investigated. In one study, while 187 kg grain yield was obtained per decare with irrigation, 169 kg grain yield was obtained under dry farming conditions. In a different study conducted in Bolivia, where the yield differences between irrigated agriculture and dry agriculture were investigated, it was stated that while 204 kg of seed yield was obtained per decare by irrigation, it decreased to 168 kg/da in dry farming conditions (Geerts et al., 2008).

Grains, which are of great importance in terms of healthy nutrition, are one of the main nutrients alternative to animal proteins. (Ertas, 2016). Some of the most cultivated cereals can be listed as wheat, barley, oat, rye, triticale, corn, paddy. Wheat, which is one of the most important cereals for our country, is a cereal that has great importance in our country and all over the world in terms of nutrition, field agriculture, economy and culture. When the nutritional conditions in Türkiye are examined, 36% of the total calories consumed are met from wheat and its products. At the same time, most of the producers who make a living from agricultural production are engaged in wheat cultivation (Anonymous, 2016). In terms of healthy nutrition, it is a source of protein, carbohydrates and fat, and it is one of the main nutritional sources due to the dietary fibers, vitamins and phytochemicals it contains. These substances in wheat provide protection against coronary heart diseases, cardiovascular diseases and cancer diseases (Shewry and Hey, 2015).

Barley is one of the first cereals cultivated. It has the most cultivation area worldwide after wheat, corn and paddy. Although it is mainly used as animal feed, it is also used in beer and flour production and takes place in human nutrition (Kün, 1996; Sezer, 2007). In Türkiye, barley is cultivated on approximately 3.2 million hectares of land

and 8.5 million tons are harvested annually. (TÜİK, 2023-a). Compared to wheat, barley harvested earlier is also more resistant to salinity and drought. It is also more suitable for crop rotation than wheat (İlker, 2006).

The first studies for triticale, which was produced with a wheat/rye hybrid, started in Scotland and entered the literature in Germany in 1935 (Stallknecht et al., 1996). Currently, triticale is grown mainly in Germany, Poland, Canada, China, Australia, France and Mexico (Ammar et al., 2004; McGoverin et al., 2011). These studies continued with summer varieties made by İbrahim Demir in the 1970s (Demir et al., 1986). Tatlıcak 97 is the first triticale variety registered in Türkiye (Kınacı and Kınacı, 2000).

When evaluated in terms of water and soil resources, Ankara, which has resources below the average of Türkiye, is located in the center of the Sakarya Basin. It is also located within the borders of Kızılırmak and Konya Closed Basin, in small proportions. These basins, within the borders of Ankara, constitute approximately 11% of Turkey's water potential (Köle, 2012).

The total agricultural area of Ankara is 11 594.222 decare, and its agricultural production potential is mainly based on grain farming. Approximately 73% of the agricultural products produced are field crops, with grains having the highest share in this ratio. Vegetable farming is approximately 3.5%, fruit and spice crops are grown at 2%. (TÜİK, 2023-b).

The aim of this study is to make a comparative statistical analysis of irrigated agriculture and dry agriculture yield values for wheat, barley and triticale in the drought conditions determined by SPI and PNI methods in Ankara, where grain production is intense in Turkish conditions, and to evaluate the obtained data in a comprehensive manner. For this purpose, drought index values calculated with the help of SPI and PNI methods using precipitation data (MGM, 2021) for Ankara province between 2012 and 2020, and irrigated agriculture and dry agriculture yield values (TÜİK, 2021) for wheat, barley and triticale were analyzed with regression analysis. were compared and correlation coefficients were obtained. According to the correlation coefficients obtained, the effect of drought conditions on yield and the effects of irrigation were revealed. Within the scope of the research, action plans for future periods according to drought conditions and results and suggestions have been created for the development of irrigation opportunities.

Materials and Methods

Research Area

Ankara, the second most populous city and the capital of Turkey, is adjacent to Çankırı in the northeast, Kırıkkale in the east, Bolu in the northwest, Eskişehir in the west, Konya in the south, Kırşehir and Aksaray in the southeast. The geographical location of Ankara in Türkiye is given in Figure 1. and its total surface area is 25.632 km². According to the 2023 census, 5 803.482 people live. (TÜİK, 2024). The total surface area of Ankara province is 25 632 decare, and the total agricultural area is 11 594.222 decare. In addition, the total area of pastures is 461.927 hectares, fallow land is 2.583 hectares and forest area is 28.846 hectares. (TÜİK, 2023-b)

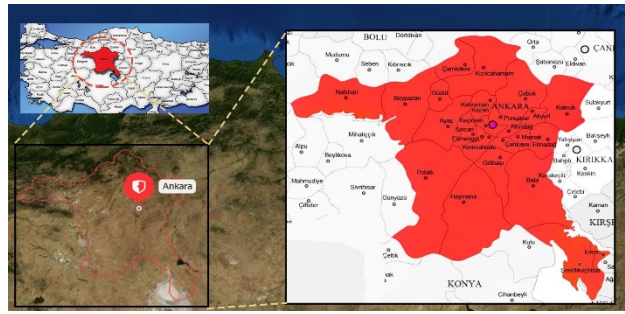


Figure 1. Location of the research area

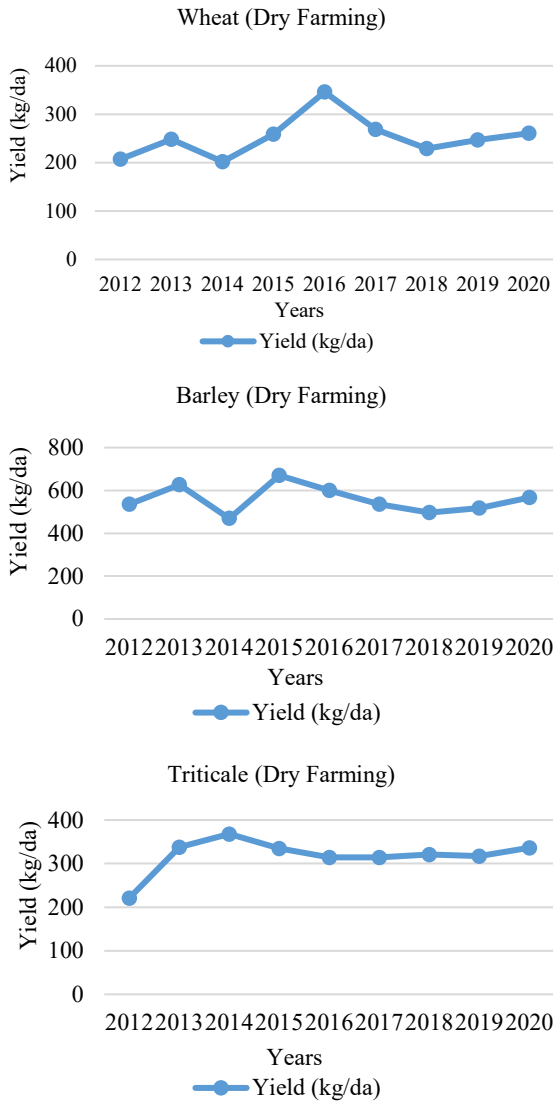


Figure 2. Yield of some cereals grown under dry farming conditions in Ankara between 2012 and 2020

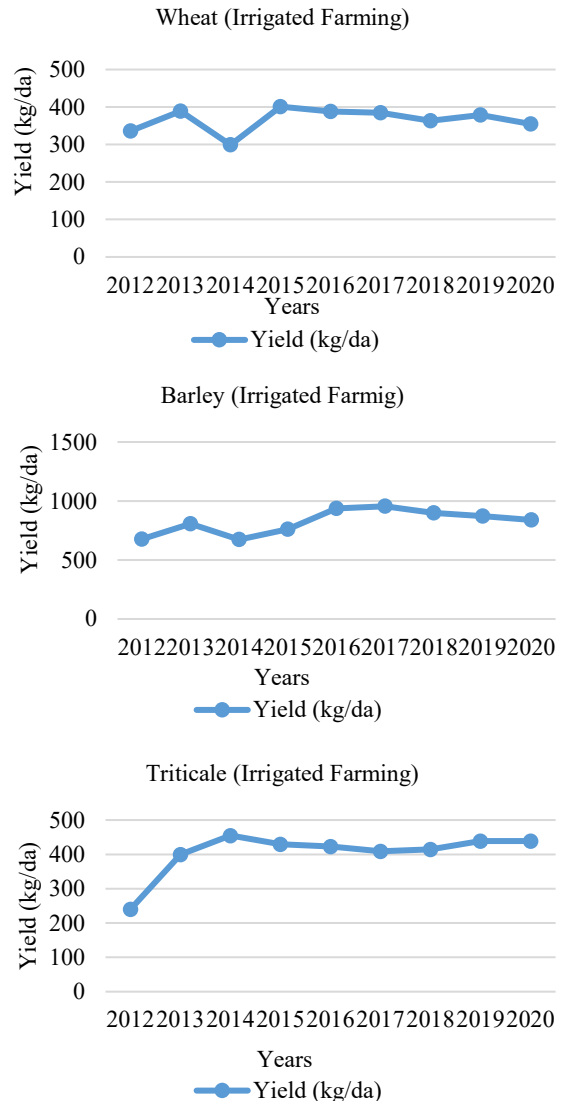


Figure 3. Yield of some cereals grown under irrigated farming conditions in Ankara between 2012 and 2020

Agriculture has gained importance in Ankara in terms of livelihoods and meeting the country's nutritional needs. The most important field products are wheat and barley. 5.77% of Turkey's total wheat production and 8.79% of barley production are produced in Ankara province. (TÜİK, 2023-b). For example, Polatlı district has one of the busiest grain exchanges in our country, as it is the second largest "granary" of Türkiye. The yield to be obtained in cereals varies with irrigation opportunities. In Ankara province, irrigation is carried out on a total area of 17.898 hectares. (DSİ, 2021) Since the total irrigated area occupies

very little space in the irrigable land, it adversely affects the grain cultivation, which is at the forefront in the region.

The material of the research is wheat, barley, triticale, which are the most cultivated cereals in Ankara, the yield values formed as a result of dry (Figure 2.) and irrigated (Figure 3.) agricultural activities between 2012-2020 and the SPI values of these yield values. The precipitation amounts between 2012-2020 were obtained to determine the change due to drought calculated with the SPI and PNI methods. Irrigated and dry farming yield values of wheat, barley and triticale analyzed in the study were obtained from the Central Distribution System (TÜİK, 2021).

Table 1. Spatial information of Ankara Regional Station (MGM,2021)

Specifications	Value
Latitude	39° 58' 21.7"
Longitude	32° 51' 49.3"
Altitude	950
Data period	2012-2020

Table 2. Annual average climate values of Ankara (1991-2020) (MGM, 2022)

Temperature (°C)	Max. Temperature (°C)	Lowest Temperature (°C)	Sunbathing Time (hour)	Number of Rainy Days	Average Total Rainfall Amount (mm)
12.6	18.4	7.3	6.3	103.7	413.6

Table 3. SPI values and drought classifications (Mckee et al., 1993)

SPI	Drought Class
> 2	Extremely wet
1,50 ~ 1,99	Very wet
1,00 ~ 1,49	Moderately wet
0,99 ~ -0,99	Near-normal drought
-1,00 ~ -1,49	Moderately drought
-1,50 ~ -1,99	Very drought
< - 2	Extremely drought

Table 4. PNI values and drought classes

Period	Normal and Above Drought	Mild Drought	Moderately Drought	Severely Drought
1	>75	65-75	55-65	<55
3	>75	65-75	55-65	<55
6	>80	70-80	60-70	<60
9	>83,5	73,5-83,5	63,5-73,5	<63,5
12	>85	75-85	65-75	<65

Table of precipitation and temperature data. It was obtained from the Ankara Regional Unit with the location and general characteristics of the station numbered 17 130 in 1. (MGM, 2021). In addition, the long-term climatic averages of the province of Ankara Table. It is given in 2. (MGM, 2022)

Standard Precipitation Index (SPI) Calculations

SPI (Mckee et al., 1993) calculations were made in order to determine the drought severity of Ankara province between the years 2012-2020. The SPI calculations were calculated separately for each year. The Standard Precipitation Index (SPI) is a drought index calculated with precipitation data in order to determine drought conditions and monitor future periods, especially in regions with climatic changes. The calculation is carried out by dividing the value obtained by subtracting the average precipitation from the total precipitation in a certain region by the standard deviation of the precipitation data (Mckee et al., 1993), (Yacoub and Tayfur, 2017).

$$SPI = \frac{x_i - x_i^{ort}}{\sigma} \tag{1}$$

Xi given in the equation; current precipitation amount and xi avg ; mean precipitation amount and σ; The drought classes obtained according to the SPI value calculated according to the standard deviation data are given in Table 3. The SPI values determined for Ankara province were interpreted according to the relevant drought classes.

Percent of Normal Index (PNI) Calculations

In the drought calculations made with the PNI method, the result is obtained by dividing the total precipitation amount at a certain time by the average precipitation and multiplying the value by 100. The obtained value is taken into account as a percentage, and the calculations made for 12 months and shorter time periods give accurate results.

$$PNI = \frac{x_i}{x_i^{ort}} * 100 \tag{2}$$

Xi given in the equation for the province of Ankara; actual precipitation amount and xi avg ; PNI values were calculated by using the average precipitation data. The obtained values were interpreted according to the drought classifications given in Table 4 (Werick et al., 1994).

Regression Analysis

In the research, first of all, the yield values obtained without irrigation were analyzed according to temperature and precipitation changes. Yield values were compared for each grain separately according to the years examined within the scope of the research, as a percentage according to the effect of irrigation. Regression and correlation analysis were carried out in order to reveal the bilateral relations between irrigated agriculture and dry agriculture. Regression and correlation analyzes are performed in order to determine the effect of a change in one of the variables on the other variable, the change at the same rate and indirectly the predicted situations that will occur in the future.

Table 5. Correlation coefficient and correlation strength

Correlation Coefficient (r)	Correlation Strength
<0.2	Very low correlation
0.2-0.4	Low correlation
0.4-0.6	Moderately correlation
0.6-0.8	High correlation
0.8>	Extremely correlation

Table 6. SPI values and drought classes of Ankara province between 2012-2020

Year	SPI Value	Drought Class
2012	- 0.9219	Near-normal drought
2013	-0.9276	Near-normal drought
2014	-0.5006	Near-normal drought
2015	-0.4984	Near-normal drought
2016	-0.5106	Near-normal drought
2017	-0.6451	Near-normal drought
2018	-0.8012	Near-normal drought
2019	-0.7129	Near-normal drought
2020	-0.6714	Near-normal drought

Correlation analysis is performed to determine the direction (positive or negative) and intensity (full relationship or incomplete relationship) of the relationship between variables, and Regression analysis is performed to determine the cause-effect relationship (formula of the relationship) between variables. The correlation coefficient is a value used to show the relationship between these variables (Orhunbilge, 2002). The equation used for the correlation coefficient calculation is given below.

$$r = \frac{\sum(xy) - (\sum x)(\sum y)/n}{\sqrt{(\sum x^2 - (\sum x)^2/n)} \sqrt{(\sum y^2 - (\sum y)^2/n)}} \quad (3)$$

The values obtained as a result of the correlation coefficient calculations Table. It was interpreted according to the intervals given in 5. (Yıldız et al., 1999)

The formula used for the linearity test between two variables, with y as the dependent variable and x as the independent variable, in the equations to be created for the regression analysis is given below. α and β show the parameters of the model, and ε the error value.

$$Yx = \alpha + \beta x + \varepsilon \quad (4)$$

While creating the regression equation, calculations were made using the least squares method so that the sum of the squares of the errors, which are the differences of the x and y values, is at a minimum value. The equation (3) necessary for the margin of error to be minimum is given below. As a result of the calculations, the regression equations (5;6), in which a and b parameters are obtained by using the least squares method, are given below.

$$\sum ei = \sum yi - yi = yi - a - bxi \quad (5)$$

$$y = ax + b \quad (6)$$

Within the scope of the research, the regression curve, equation and correlation coefficients were calculated for wheat (durum), barley, triticale grown in the drought conditions determined by SPI and PNI methods in Ankara,

by using the above-mentioned regression and correlation formulas. analysis was made.

Results and Discussion

Within the scope of the study, the drought conditions obtained according to the SPI and PNI calculations determined according to the climate data of Ankara province between the years 2012-2020, together with the analysis of the yield in dry agriculture and the yield as a result of irrigation, were presented separately for each product. Then, binary statistical analyzes, regression graph and coefficients between dry farming and irrigated farming yield values according to SPI and PNI values are explained separately for each product.

Standard Precipitation Index (SPI) Results

Within the scope of the research, the 12-month SPI values of Ankara province between the years 2012-2020 were determined between -0.4984 and -0.9276. These obtained values show that the drought conditions in the research period constitute a drought conditions close to normal. According to the results given in Table 6, it is seen that humid conditions were not observed during the research period and a climatic situation close to drought occurred with the presence of normal precipitation conditions.

In a similar study, it was conducted by Mehr et al. to reveal the drought forecast of Ankara province, and the study was conducted using SPI and SPEI. Within the scope of the study, it was concluded that relatively close to normal drought conditions could occur between 2016-2040. (Danandeh Mehr et al., 2019). In another study, duration, intensity and trend studies were conducted in order to determine the drought characteristics in Ankara province. As a result of trend analyses within the scope of the study, it was determined that the drought characteristics of Ankara province increased. (Danandeh Mehr and Vaheddosti, 2020) In another study, drought conditions for Ankara province were similarly conducted using SPI. It was concluded that extremely dry conditions would decrease relatively in the coming years. (Afshar et al., 2020)

Percent of Normal (PNI) Results

According to the results of PNI, another drought analysis of Ankara province, the 12-month values obtained between 2012 and 2020 ranged from 76.12% to 135.79%. Drought conditions, which were in the mild arid class in 2013, took normal values in other years. This showed that results close to drought conditions obtained according to SPI values were obtained. Obtained PNI values and drought classes are given in Table 7.

A study conducted using PNI together with SPI to analyze agricultural drought across Turkey concluded that drought will increase in the coming periods and that this situation will create a negative situation for many field crops, including wheat (Şimşek and Çakmak, 2010) In a similar study, PNI was used and drought analysis was conducted for Samsun province. As a result of the study, it was determined that drought gave a value close to normal with the trend analysis conducted. (Beden et al., 2020)

Yield Changes Between Irrigated and Dry Farming According to Drought Conditions and Comparative Analysis According to SPI and PNI

Wheat

According to the yield values of wheat in dry farming conditions given in Figure 2, the highest yield was obtained in 2016. In 2016, when the highest yield determined in dry farming conditions was achieved, an increase of approximately 12% was observed with irrigation (Figure

4). This rate rises to 62% in 2012. The lowest yield in dry farming was realized in 2012 and 2013. Especially, 2013 showed a difference compared to other years according to PNI values and it was determined that drought conditions increased. In 2020, there was a 36% increase in yield with irrigation.

When the change in wheat yield with irrigation between 2012-2020 in Ankara province conditions is examined, an average yield increase of 47.2% is observed. According to the SPI and PNI values, this yield increase was generally obtained in near-normal drought conditions. It can be mentioned that the increase in yield can be more with irrigation for the periods when the severity of drought will increase.

Equations, regression trend lines and correlation coefficients created to statistically analyze the bilateral relations of wheat with varying irrigation yield under dry farming conditions Figure 5 and Figure 6. According to the results of the analysis, correlation coefficients of 0.0502, 0.0891, 0.0584 and 0.0013 were found according to irrigated agriculture-dry farming yields, SPI and PNI drought conditions. This situation shows that there is no linear relationship between dry farming and irrigated farming in wheat yield under current drought conditions for Ankara province. There is a very weak relationship between dry farming and irrigated farming conditions at a level that cannot be correlated.

Table 7. PNI values and drought classes of Ankara province between 2012-2020

Year	PNI Value	Drought Class
2012	110.48	Normal and Above Drought
2013	76.12	Mild Drought
2014	135.79	Normal and Above Drought
2015	132.49	Normal and Above Drought
2016	122.06	Normal and Above Drought
2017	97.39	Normal and Above Drought
2018	126.77	Normal and Above Drought
2019	105.98	Normal and Above Drought
2020	97.49	Normal and Above Drought

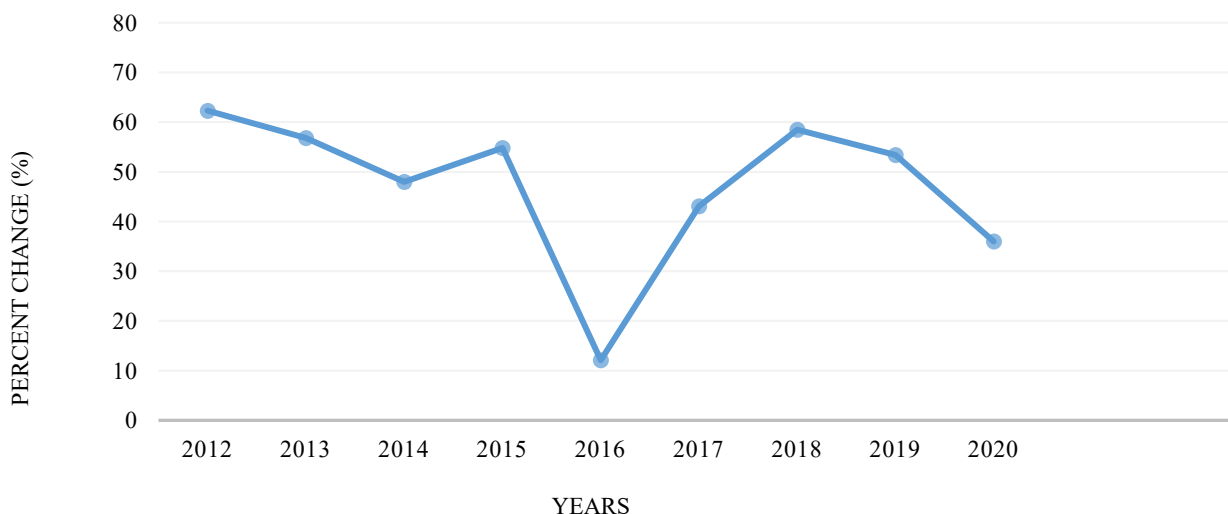


Figure 4. Yield change percentages of wheat in irrigated agriculture compared to dry agriculture

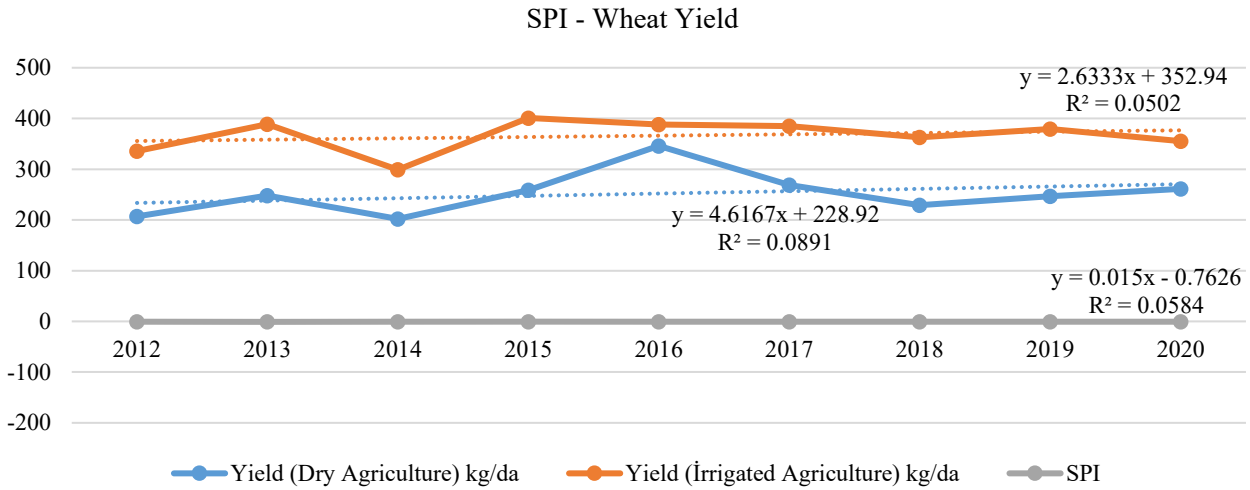


Figure 5. Regression Analysis of Wheat Yield and SPI Drought Conditions

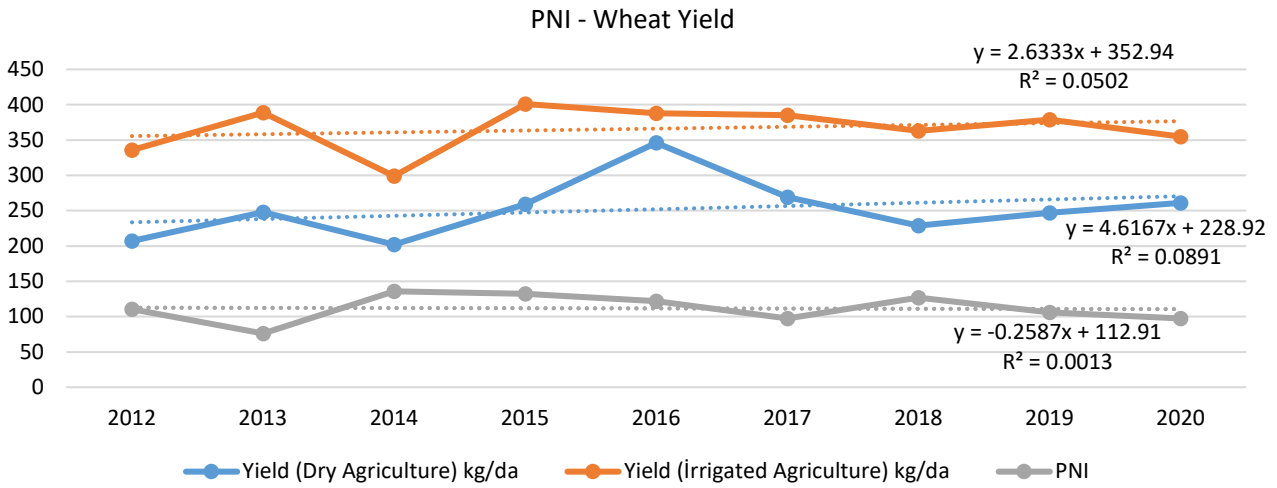


Figure 6. Regression Analysis of Wheat Yield and PNI Drought Conditions

A study conducted in the Adana region examined the effect of irrigation on yield changes in wheat. As a result of the study, it was concluded that 326.66 kg/da more wheat yield was obtained in the flat planting method, 267.46 kg/da more in the ridge planting method and 140.91 kg/da more in wide ridge planting. (Aykanat and Barut, 2018) In a study comparing the wheat yields of Hatay and Şanlıurfa provinces, it was determined that there were significant increases in wheat yields with irrigation, especially in Hatay province. (Tiryakioğlu et al., 2017)

Barley

According to the yield values given in Figure 2 and Figure 3 for barley, the lowest yield was experienced in dry farming in 2014, while the yield obtained with irrigation in the same year was 43.5% higher. This year was determined as the year in which the index value of -0.5006 was obtained according to SPI calculations. Between 2012 and 2020, which was examined within the scope of the research, an average of 46% yield increase was experienced with irrigation in barley cultivation in Ankara

province conditions (Figure 7). This situation is similar to wheat. According to SPI and PNI calculations, 55% increase in yield in years with near-normal drought indicates that irrigation will be more important in case of a change in drought severity.

Equations, regression trend lines and correlation coefficients in which barley yield was established according to irrigated farming - dry farming variables in SPI and PNI drought conditions Figure. It is given in Figure 8 and Figure 9. The correlation coefficients found according to the equations created are 0.4294, 0.0402, 0.0584 and 0.0013. Although a linear relationship is not provided, it has been observed that there is a statistically weak relationship. This shows that there is a relatively better statistical relationship in barley compared to wheat. Although the yield obtained with irrigation is much higher in percentage, it is seen that the yield difference will increase in the following planting periods in terms of statistics.

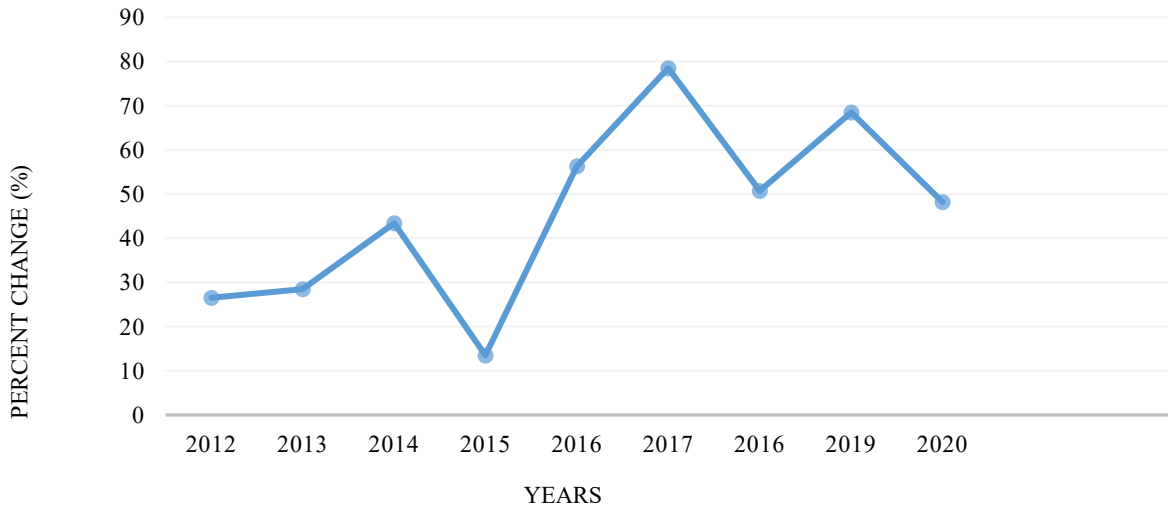


Figure 7. Yield change percentages of barley in irrigated agriculture compared to dry agriculture

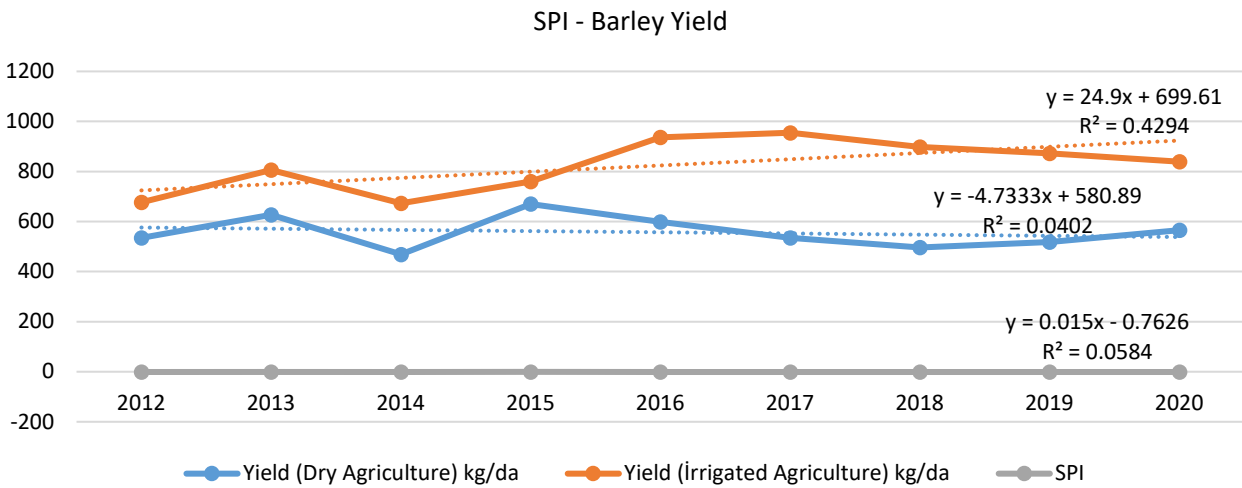


Figure 8. Regression Analysis of Barley Yield and SPI Drought Conditions

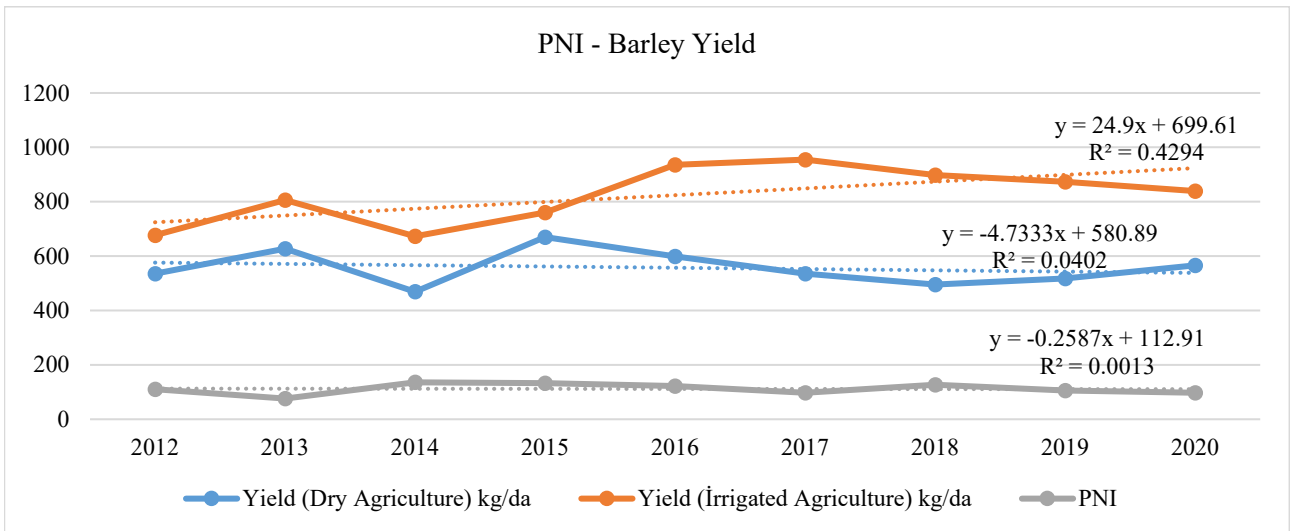


Figure 9. Regression Analysis of Barley Yield and PNI Drought Conditions

In a study examining the effect of irrigation and timing on yield in barley, it was seen that irrigation provided positive effects on quality characteristics as well as yield increase. (Gültekin and Tokgöz, 2008) A similar study investigated the relationship between irrigation and barley

yield in Harran Plain conditions. As a result of the study, positive results were obtained in yield and quality with irrigation applied in all growing periods of barley. (Yıldız and Tari, 2018)

Triticale

According to the graph given in the Figure 10 of triticale, in which the yield and percentage changes are examined, the lowest yield was obtained in dry agriculture in 2012, and 26,9% more efficiency was achieved with irrigation in this year. The yield of Triticale, a hybrid of wheat and rye, increased by 34% on average with irrigation facilities during the period of near-normal drought conditions, according to SPI and PNI values, between 2012 and 2020. This situation is at a lower rate than wheat and barley, and it can be mentioned that the increase in yield with irrigation may also change with the change of drought conditions. The best correlation coefficient after barley among the grains within the scope of the study was

calculated in triticale. Shape. The regression analysis given in Figure 11 and Figure 12. According to the equations created, correlation coefficients of 0.3331, 0.1022, 0.0013 and 0.0585 were obtained. The results show that there is a better statistical relationship in triticale than in wheat.

In a study investigating the effect of different nitrogen doses on yield and quality of triticale with irrigation, it was determined that irrigation opportunities had positive effects on yield and quality characteristics. (Takıl and Olgun, 2020) A similar study conducted in the Konya region on triticale showed that there was an increase in yield and quality characteristics during irrigated growing periods (Özer et al., 2010).

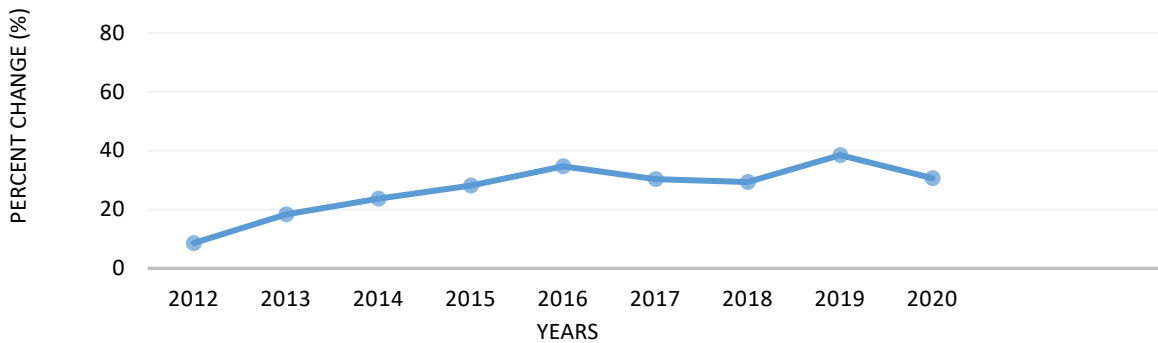


Figure 10. Yield change percentages of barley in irrigated agriculture compared to dry agriculture

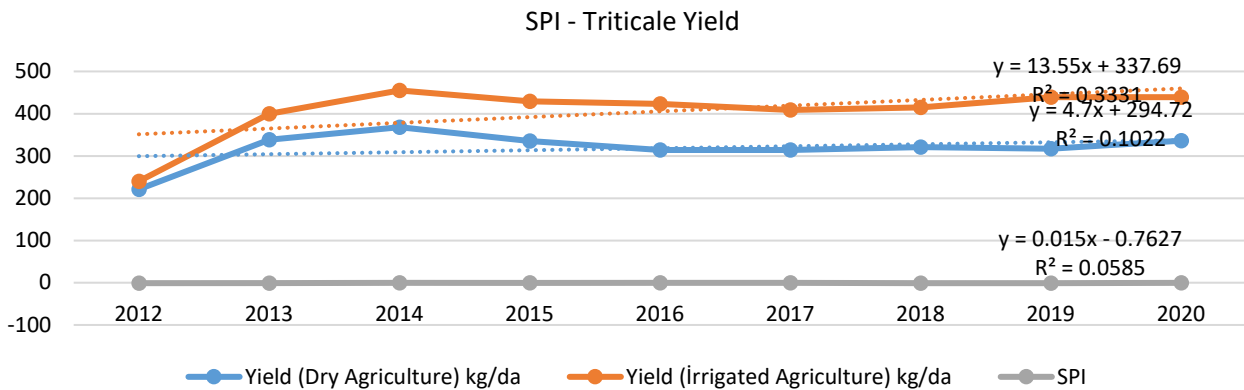


Figure 11. Regression Analysis by Triticale Yield and SPI Drought Conditions

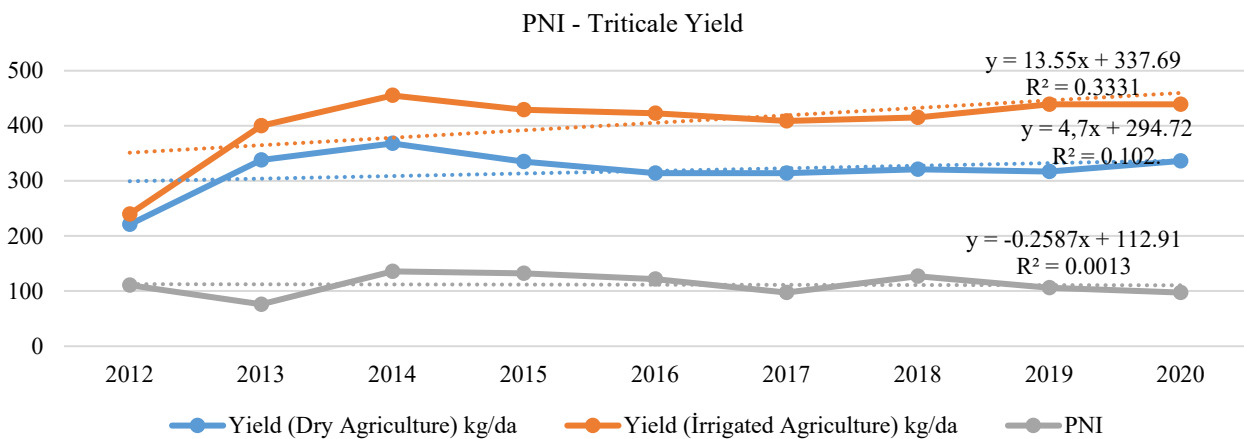


Figure 12. Regression Analysis by Triticale Yield and PNI Drought Conditions

Conclusion

In this study, in which the yield change of wheat, barley, triticale, determined by SPI and PNI methods between 2012 and 2020, was analyzed in the conditions of Ankara, which is one of the most important provinces where grain cultivation is carried out in our country, due to irrigation, it was observed that the harvest amount of all products increased with irrigation. According to the analysis, when the years within the scope of the research are examined, the highest yield increase with irrigation was experienced in barley with 81%, triticale with 75% and wheat with 62%. According to the correlation coefficients as a result of the regression analysis, barley and triticale are the products with the highest linearity between irrigated agriculture and dry agriculture with coefficients of 0.4294 and 0.3331. Correlation coefficients obtained for SPI and PNI were 0.0584 and 0.0013. When evaluated in terms of wheat, their coefficients were calculated as less than 0.1. It is seen that statistically higher values will be obtained with irrigation in the following planting periods of the cereals examined within the scope of the research compared to dry farming yields.

According to the SPI and PNI results obtained, it was determined that Ankara had a mild drought in general between 2012-2020 and a near-normal drought conditions. According to the research, it is seen that there is a decrease in yield when the grains are not irrigated, even during periods of climatic conditions with values close to normal. In this respect, urgent priority should be given to the development of irrigation facilities in regions where grain cultivation is carried out in the form of dry farming. Better results can be obtained by carrying out this situation together with the studies of determining the drought with different methods. In this context, the process and risk status should be determined and combated in drought control studies. In this respect, drought determination studies and trend analysis studies of drought severity in the region are of particular importance. Then, according to the results to be determined, risk assessment studies should be emphasized. In case of high drought severity in some regions in the province, crisis assessment and management studies should be considered in detail. It is extremely important to prepare action plans for the upcoming periods, especially according to the trend analysis of drought conditions in Ankara province. In this context, it is recommended to increase the potential water holding capacity, increase the number and capacity of water storage structures, recycle wastewater and ensure water harvesting practices as much as possible. In addition, R&D studies in combating drought, training and publication services to be provided to farmers in combating drought and afforestation studies should be taken into consideration.

In order to improve irrigation opportunities, instead of surface irrigation systems such as spine and furrow irrigation, irrigation systems such as pressurized sprinkler and drip irrigation should be used in regions where irrigated agriculture is practiced. Farmers should be informed about the use of pressurized irrigation systems and the increase in their yield and income. Providing public incentives as much as possible for the widespread use of pressurized irrigation systems will accelerate the transition to modern irrigation systems. Along with the transition to

modern irrigation, studies should be conducted on the use of wastewater in irrigation. In irrigation systems to be created, water saving and especially applications such as solar panels that can reduce energy costs and increase efficiency should be preferred, and farmers' expenses should be reduced.

Declarations

Ethical Approval Certificate

Not applicable

Author Contribution Statement

Murat Ozocak: Data collection, investigation, statistical analysis, and writing the original draft, administration, supervision, conceptualization, methodology, review and editing analyzed the data, and wrote the manuscript

Fund Statement

There is no financial support.

Conflict of Interest

The author declare no conflict of interest.

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Seasonal Distribution of Births in Anatolian Buffaloes and Effects of the Season on Some Milk and Reproductive Traits of Cows and Growth Traits of Calves

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ARTICLE INFO

Research Article

Received : 23.09.2024

Accepted : 18.10.2024

Keywords:

Anatolian buffalo

Calving season

Growth

Milk yield

Reproductive traits

ABSTRACT

The aims of this study were i) to evaluate the seasonal changes in births of Anatolian buffaloes, ii) to examine the changes of some milk [lactation milk yield (LMY) and lactation length (LL)] and fertility [first calving age (FCA) and calving interval (CI)] according to seasons and iii) to investigate the effects of seasons on the growth traits of calves [birth weight (BW₀), live weight at 6 mo (LW₆) and live weight at 12 mo (LW₁₂)]. A total of 8614 to 15605 yield records were evaluated including milk yield and fertility traits of buffalo cows and growth traits of calves between 2012 and 2023. The mean temperature, relative humidity, the duration of sunshine, and temperature humidity index (THI) values were calculated for these years. While the highest birth rate was observed in the summer season (34.27%), the lowest birth rate was determined in the winter season (11.99%). The BW₀, LW₆ and LW₁₂, LMY, LL, FCA, and CI values of the calves were determined as 30.8±0.04 kg, 107.6±0.23 kg, 172.6±0.27 kg, 994.7±2.05 kg, 262.6±0.22 d 1175.5±3.46 d and 561.7±1.84 d, respectively. Except for CI, all characteristics were significantly affected by seasonal changes (P<0.001). The growth traits of the calves born in autumn were higher than the other seasons. The LMY values in spring and winter were higher than those determined in the different seasons. Also, the highest FCA was obtained in heifers born in the winter. It was concluded that the season could affect both some fertility and milk yield characteristics of buffalo cows and the growth performance of calves.

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Introduction

Buffalo breeding is a livestock activity with very old history and stands out with its traditional features. Today, buffalo has mostly been raised in the Asian continent and has also been raised on a significant scale in some European, African, and South American countries (Minervino et al., 2020). Buffaloes, which are spread over a wide geography in the world, consist of different breeds that are distinguished from each other by different adaptation and productivity aspects according to the countries in which they are located. It has long been known that these breeds are significantly affected by the climate and geography of the countries or continents where they are located.

The climate characteristics of the regions have different effects on the ecosystem and vegetation, and depending on this situation, different feeding conditions and productivity levels can be observed (Marai and Haebe, 2010a). Although there are a total of 123 buffalo breeds in the world today, three breeds (Murrah, Nili-Ravi, and

Mediterranean Buffaloes) are widely raised due to their high milk yields (Petrocchi Jasinski et al., 2023).

The Anatolian buffalo is a milk and meat-producing breed that is raised in Türkiye and is included in the Mediterranean buffalo group. Anatolian buffalo raised in different regions of Türkiye are mostly raised in the provinces of Samsun, Diyarbakır, and Istanbul. Seasonal differences and vegetative changes in pasture resources observed in the regions where this breed has been raised may cause the differences in production. This may cause births as well as reproductive traits in buffaloes to change according to seasonal conditions (Şahin et al., 2013; Ermetin, 2017).

It has long been known that climatic factors have an important influence on the regulation of reproductive traits in farm animals. Environmental stress factors such as changes in day length or increased temperature cause reproductive organ growth or functional decline in many animal species (Vale, 2007). While Vale, (2007)

emphasizes that buffaloes are a non-seasonal polyestrous species, many authors report that buffaloes have a seasonal breeding characteristic (Singh et al., 2000; Hassan et al., 2007; Presicce, 2007; Mondal et al., 2008; Vijayakumar et al., 2011; Hozyen et al., 2016; Phogat et al., 2016; Petrocchi Jasinski et al., 2023). It has been pointed out that this feature is closely associated with ambient temperature, photoperiod, and feeding conditions (Phogat et al., 2016; Petrocchi Jasinski et al., 2023), and hormonal control of estrus is suggested by Singh et al. (2000) to eliminate the adverse cases. Photoperiod effects (duration and intensity of light) impacts pineal gland activity (Das and Khan, 2010; Kushwaha et al., 2011) and melatonin secretion (Chandra Prasad et al., 2014). This effect can alter the estrous cycle and estrous intensity in buffaloes in many countries (Chandra Prasad et al., 2014; Verma et al., 2021), except in regions close to the equator where photoperiod remains almost similar throughout the year (Vale, 2007; Verma et al., 2021). Petrocchi Jasinski et al. (2023) reported that temperature increases by regional conditions also causes the decline in milk yield, composition, and reproductive functions in buffaloes. Several authors reported that the thermo-neutral zone of buffaloes is 13.0 – 18.0 °C, with relative humidity between 55% and 60% (Marai and Haegeb, 2010b; Napolitano et al., 2023a). When the body temperature of buffaloes exceeds the normal values of 37.5 °C to 39 °C, they show a number of physiological and behavioral reactions to return these values to normal and maintain their health and productivity (Napolitano et al., 2023b).

There are some studies on the effects of seasonal changes on the reproductive traits (Tekerli et al., 2001; Koçak et al., 2019; Akyol, 2023) and milk yield traits of buffaloes (Akyol, 2023; Kul et al., 2016; Soysal et al., 2018), as well as the growth traits of calves (Alkoyak and Öz, 2022). In a study on Purnathadi buffaloes, Thokal et al. (2004) reported that calving during the rainy season (June to September) was higher than calving during winter (December to January) and summer (February to May). While the effect of seasonal changes on calving interval (CI) was found to be insignificant in a study conducted on buffaloes (Soysal et al., 2018), some studies also reported that this reproductive trait was affected by seasonal changes (Tekerli et al., 2001; Koçak et al., 2019; Alkoyak and Öz, 2020; Akyol, 2023; Alkoyak et al., 2023). Several authors reported that first calving age (FCA) in buffaloes varies due to seasonal effects (Akyol, 2023; Penchev et al., 2014). Similarly, a large number of authors emphasized that the milk yield traits [lactation milk yield (LMY) and lactation length (LL)] of Anatolian buffaloes change due to the temperature stress created by seasonal differences in the breeding regions and the changes in feeding adequacy, especially in pasture conditions (Kul et al., 2016; Koçak et al., 2016; Soysal et al., 2018; Alkoyak and Öz, 2020; Akyol, 2023). In a study conducted on Anatolian buffaloes (Alkoyak and Öz, 2022), it was determined that the growth traits [birth weight (BW₀), live weight at 6 months (LW₆), and live weight at 12 months (LW₁₂)] of buffalo calves were affected by the calving season.

To our knowledge, there are several studies on the effects of calving season on milk yield and fertility traits of buffalo cows as well as growth traits of calves. However, studies those evaluating climatic changes according to

seasons are limited. Therefore, the results obtained from this study will offer significant contributions to filling the gap in this field. Our hypothesis was that calving season affects i) CI and FCA values of buffaloes cows, ii) LMY and LL values of buffalo cows, and iii) BW₀, LW₆, and LW₁₂ values of calves. The aims of this study were i) to evaluate the seasonal changes in births of Anatolian buffaloes, ii) to examine the changes in some milk (LMY and LL) and fertility traits (FCA and CI) according to seasons, and iii) to investigate the effects of seasons on the growth traits (BW₀, LW₆, and LW₁₂) of calves.

Material and Method

This study was conducted in Samsun province, located in the Black Sea region of Türkiye. The Samsun province is located at 40° 50'-41° 51' N, 37° 08'-34° 25' E, and generally has a mild climate. However, the climate in the coastal and inland areas shows different characteristics. On the coast (Central district, Terme, Çarşamba, Bafra, Alaçam, 19 Mayıs, Tekkeköy, and Yakakent), the effects of the Black Sea climate are observed. Summers are hot and humid, and winters are warm and rainy on the coastline. Mean values of some climate indicators between the years 2012-2023, when the data were collected, are presented in Tables 1 and 2. The climatic indicators in the study were calculated from the data of the General Directorate of Meteorology.

The Community Based Animal Breeding Project that started in Samsun in 2011, is still proceeding. Within the scope of the relevant project, records are kept by breeders and the technical staff of the Buffalo Breeders Association in the province. There are a total of 93 farms and 3300 buffaloes in the project (in 5 districts; Bafra, 19 Mayıs, Çarşamba, Vezirköprü, and Ladik), and the calves born from these animals are used in herd renewal. Although pasture resources are mainly used in feeding of the animals, additional feeding, including silage, straw, and concentrate is offered due to the winter season and insufficient pasture.

All data on buffalo cows and their calves are recorded in the database called "Manda Yıldızı". Buffalo cows are milked once a day in the morning. Milking process is maintained with portable machines on most farms, but in small-scale farms, this procedure is performed manually. A total of 8614 to 15605 yield records obtained between 2012 and 2023 were evaluated.

The BW₀, LW₆, and LW₁₂ values of the calves and the lactation and birth information of the buffalo cows were recorded in the program called "Manda Yıldızı". The data were evaluated in the Excel program with permission from the General Directorate of Agricultural Research and Policies of the Turkish Ministry of Agriculture and Forestry.

Groups were formed by determining the year and season (Winter: December, January, February; Spring: March, April, May; Summer: June, July, August; Autumn: September, October, and November) according to their calving information. Seasonal calving rates (%) were determined by dividing the calving number in each season by the total calving number for that year. The total calving number for the years 2012-2023 was also divided into seasons, and proportional distributions were determined.

Table 1. Temperature and relative humidity values in the study region according to years and seasons*

Years	Temperature means (°C)					Relative humidity means (%)				
	Winter	Spring	Summer	Autumn	Means	Winter	Spring	Summer	Autumn	Means
2012	7.1	12.2	23.7	18.5	15.4	65.6	74.4	68.4	70.3	69.7
2013	8.7	13.8	23.3	16.3	15.5	61.3	71.3	66.6	64.7	66.0
2014	10.1	13.1	23.9	17.0	16.0	66.0	74.1	66.4	68.8	68.8
2015	8.3	11.8	23.5	18.4	15.5	62.1	72.5	66.9	66.4	67.0
2016	8.2	13.6	24.2	16.6	15.7	61.5	70.4	67.1	63.2	65.6
2017	8.6	11.6	23.5	17.3	15.3	57.8	73.5	67.1	62.2	65.2
2018	9.6	14.1	24.6	17.9	16.6	67.2	72.9	64.5	68.5	68.3
2019	9.6	12.5	23.9	18.3	16.1	64.2	73.2	73.6	73.0	71.0
2020	9.7	12.5	23.7	18.7	16.2	67.9	77.5	72.4	73.9	72.9
2021	10.4	11.9	23.7	16.6	15.6	61.7	78.4	76.3	72.2	72.2
2022	9.4	11.2	23.7	17.8	15.5	66.0	72.3	72.8	70.5	70.4
2023	10.3	12.4	23.7	18.8	16.3	63.8	81.1	72.7	69.3	71.8
Means	9.2	12.6	23.8	17.7	15.8	63.8	74.3	69.6	68.6	69.1

* Calculated from the data of the General Directorate of Meteorology, Türkiye

Table 2. THI and means of monthly sunshine duration in the study region according to years and seasons*

Years	THI					Monthly means of sunshine duration (h)				
	Winter	Spring	Summer	Autumn	Means	Winter	Spring	Summer	Autumn	Means
2012	47.3	54.5	71.7	64.1	59.4	86.4	160.8	268.2	161.0	169.1
2013	49.9	57.0	71.0	60.7	59.5	93.1	172.4	279.7	162.2	176.8
2014	51.6	55.9	71.8	61.8	60.3	94,6	173,8	274,2	158,5	174,9
2015	49.3	54.0	71.3	63.8	59.5	99.8	155.6	236.4	145.7	159.4
2016	49.1	56.7	72.3	61.1	59.8	73.9	174.0	257.1	155.1	165.0
2017	49.9	53.6	71.3	62.0	59.2	112.4	161.4	248.5	173.6	174.0
2018	50.9	57.5	72.7	63.1	61.2	61.8	187.4	304.9	137.7	173.0
2019	51.0	55.0	72.5	63.9	60.5	105.9	191.2	276.8	184.2	189.5
2020	51.0	54.9	72.1	64.5	60.7	92.2	199.8	303.0	174.3	192.3
2021	52.3	54.0	72.5	61.3	59.7	125.9	184.2	275.2	135.2	180.1
2022	50.6	53.0	72.1	63.0	59.6	94.2	183.0	262.1	145.0	171.1
2023	52.0	54.7	72.1	64.5	60.8	98.4	126.6	288.5	162.3	168.9
Means	50.4	55.1	72.0	62.8	60.0	94.9	172.4	272.8	157.8	174.5

* Calculated from the data of the General Directorate of Meteorology; Temperature Humidity Index (THI), Türkiye

Temperature Humidity Index (THI) is a unitless index that includes the effects of ambient temperature and relative humidity. This index is widely used to indicate heat stress in dairy cattle (Jeelani et al., 2019). Using temperature (°C) and humidity (%) values from meteorological data, THI was calculated separately for each year and for each season in the same year with the help of the following formula (Kalyan et al., 2022; Yadav et al., 2022; Aatralarasi et al., 2024).

$$THI=(0.8 \times Tdb)+[(RH/100) \times (Tdb-14.4)]+46.4 \quad (1)$$

Where;

THI is Temperature Humidity Index (THI)

Tdb is air temperature in a dry-bulb thermometer (°C)

RH is the air relative humidity (%)

The data consists of LMY and LL values of cows calving between 2012 and 2023, as well as BW₀, LW₆, and LW₁₂ values of calves. Moreover, the birth dates of cows calving between these years were also recorded. FCA and CI values were calculated from these data. The following formulas were used in these calculations:

$$FCA(\text{mo})=\text{Cow's first calving date}-\text{Cow's birth date} \quad (2)$$

$$CI(\text{d})=\text{Last calving date}-\text{Previous calving date} \quad (3)$$

Statistical Analysis

To investigate the effect of calving season on growth traits (BW₀, LW₆, and LW₁₂) of calves as well as lactation traits (LMY and LL) and reproductive traits (FCA and CI) of cows, variance analysis (General Linear Model) was performed. Statistically significant differences among the means were determined according to Duncan's multiple comparison test (P<0.05).

The effect of season was evaluated with the following linear model:

$$Y_{ij}=\mu + a_i + e_{ij}$$

Where Y_{ij} = an observed value of LMY, LL, FCA, and CI in cows, BW₀, LW₆, and LW₁₂ in calves; μ = the overall mean; a_i = the effect of calving season (j = Winter, Spring, Summer, Autumn); and e_{ij} = the random error. All statistical analyses were performed using the SPSS 21.0 (IBM Corp., Armonk, New York, USA) package program.

Results

The seasonal distribution of Anatolian buffaloes calving between 2012 and 2023 is presented in Figure 1. As seen, 34.27% of calving occurred in the summer season, and this rate is higher than the calving rates in other seasons. The calving rates in spring and autumn are very close to each other. The season with the least calving is winter, and 11.99% of the total calving occurred in this season.

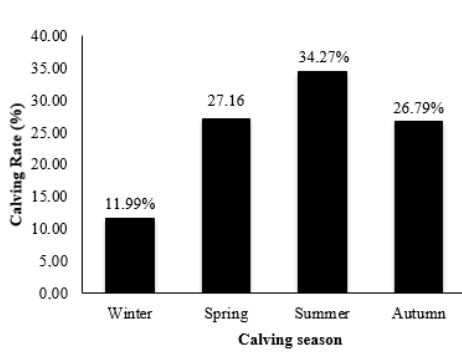


Figure 1. Seasonal distribution of calving between 2012 and 2023

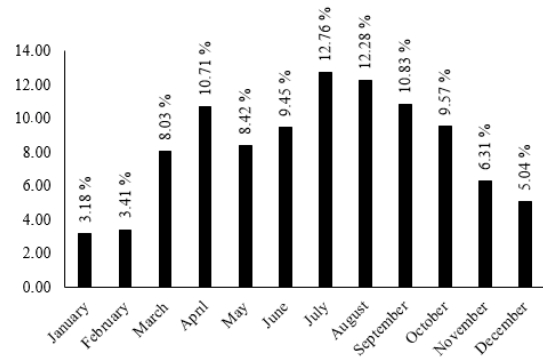


Figure 2. Calving rates by month between 2012-2023

Table 3. Distribution of seasonal calving rates by year (%)

Years	Calving Seasons				
	n	Winter	Spring	Summer	Autumn
2012	533	5.07	25.52	42.03	27.39
2013	583	10.12	32.42	38.25	19.21
2014	814	11.29	27.18	40.66	20.87
2015	1441	11.38	23.39	42.96	22.28
2016	1251	12.23	21.66	36.93	29.18
2017	1333	13.43	25.51	34.73	26.33
2018	1701	12.99	29.39	33.63	23.99
2019	1419	12.68	28.82	34.39	24.10
2020	1518	13.11	32.15	31.36	23.39
2021	1801	11.38	25.26	34.43	28.93
2022	1445	10.93	27.27	25.74	36.06
2023	1756	10.08	28.19	30.13	31.61

Table 4. Seasonal changes of live weight values of buffalo calves

Seasons	BW ₀ (kg)		LW ₆ (kg)		LW ₁₂ (kg)	
	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$	n	$\bar{X} \pm S\bar{x}$
	***		***		***	
Winter	1815	31.0±0.12 ^b	1440	108.4±0.69 ^b	1288	175.3±0.82 ^b
Spring	4238	30.1±0.07 ^a	3626	104.5±0.40 ^a	2945	170.8±0.55 ^a
Summer	5383	30.8±0.06 ^b	4844	106.6±0.38 ^{ab}	3854	171.5±0.45 ^a
Autumn	4169	31.6±0.07 ^c	3289	112.1±0.46 ^c	2908	174.7±0.49 ^b
Mean	15605	30.8±0.04	13199	107.6±0.23	10995	172.6±0.27

^{a-c}Mean values in the same column with different superscripts differ (P<0.001); ***P<0.001; BW₀: Birth weight; LW₆: 6th month live weight; LW₁₂: 12th month live weight

Similarly, the distribution of calving rates by month in each season is shown in Figure 2. The months with the highest calving between 2012 and 2023 are July and August (12.75% and 12.28%, respectively). It has been observed that these two months are quite close to each other, and the sum of the two months corresponds to approximately ¼ of the total calving. Likewise, it was determined that calving in April and September (10.71% and 10.83%, respectively) and in June and October (9.45% and 9.57%, respectively) were quite close to each other.

As shown in Figure 1, when the distributions are examined by month in the winter season when births are the least, January and February are quite close to each other (3.18% and 3.41%, respectively), but December is slightly higher than other winter months (5.04%).

The changes in the seasonal distribution of calving from year to year are presented in Table 3. In 2012, it was observed that the calving rate in winter was quite low (5.07%) compared to the general mean (11.99%). The rate

of calving in summer in 2012, 2014, and 2015 (42.03%, 40.66%, and 42.96% respectively) was found to be higher than other years and the general mean (34.27%). In 2022 and 2023, the calving rate in the autumn season (36.06% and 31.61%, respectively) was higher than in the other seasons. In 2020, the rate of calving in the spring season was found to be 32.15%, which was higher compared to the different seasons.

Seasonal means of BW₀, LW₆ and LW₁₂ values of the calves and differences between groups are presented in Table 4. In the conditions of Samsun province, BW₀, LW₆, and LW₁₂ means were determined as 30.8±0.04 kg, 107.6±0.23 kg and 172.6±0.27 kg, respectively. It was observed that there were seasonal differences between BW₀, LW₆ and LW₁₂, which are considered important indicators of the growth characteristics of the calf (P < 0.001). The highest BW₀ value was observed in those born in autumn (31.6±0.07 kg), while the lowest BW₀ value was determined in those born in spring (30.1±0.07 kg).

Table 5. Seasonal changes of milk and reproductive traits of buffalo cows

Seasons	LMY (kg)		LL (d)		FCA (d)		CI (d)	
	n	$\bar{X}\pm S_x$	n	$\bar{X}\pm S_x$	n	$\bar{X}\pm S_x$	n	$\bar{X}\pm S_x$
		***		***		***		NS
Winter	1563	1002.6±6.12 ^{bc}	1561	261.3±0.72 ^{ab}	290	1230.7±11.09 ^b	1036	569.4±5.42
Spring	3849	1022.1±3.69 ^c	3835	266.8±0.39 ^c	778	1173.5±6.55 ^a	2371	561.2±3.53
Summer	4464	967.5±3.40 ^a	4460	261.8±0.35 ^b	989	1166.8±5.96 ^a	3010	560.2±3.11
Autumn	3294	995.9±4.28 ^b	3293	259.3±0.48 ^a	929	1169.1±6.36 ^a	2197	560.5±3.56
Mean	13170	994.7±2.05	13149	262.6±0.22	2986	1175.5±3.46	8614	561.7±1.84

* $P<0.001$; NS: Not significant; ^{abc} Mean values in the same column with different superscripts differ ($P<0.001$); LMY: Lactation milk yield; LL: lactation length; FCA: first calving age; CI: calving interval

A similar distribution was observed for LW_6 and the highest value was in those born in the autumn season (112.1 ± 0.46 kg). The lowest LW_6 value was determined in those born in spring (104.5 ± 0.40 kg). Also, no statistical difference was observed between the LW_6 value of those born in spring and summer. While the highest LW_{12} mean value was observed in calves born in winter, the lowest LW_{12} value was determined in calves born in spring.

The LMY value examined was significantly affected by seasonal factors ($P<0.001$; Table 5). The mean LMY value was determined as 994.7 ± 2.05 kg, the highest LMY value was in those born in spring and winter (1022.1 ± 3.69 kg and 1002.6 ± 6.12 kg, respectively). Also, the mean LMY value was the lowest in those born in summer (967.5 ± 3.40 kg). The average value of the total 13149 calculated LL values was determined as 262.6 ± 0.22 d. The longest LL value was observed in cows calved in spring, while the shortest period was determined in cows calved in autumn ($P<0.001$).

While the FCA value of buffalo cows was affected by seasonal conditions ($P<0.001$), the effect of the season on CI was statistically insignificant (Table 5). The average FCA of the cows was determined as 1175.5 ± 3.46 d, and the average CI was determined as 561.7 ± 1.84 d. The FCA (1230.7 ± 11.09 d) value of buffalo cows born in winter was higher than those born in other seasons ($P<0.001$). It was observed that the FCA values of cows born in spring, summer and autumn were similar ($P<0.001$). Similarly, it was observed that the CI means obtained from the data of the all populations in four different seasons were quite close to each other and there was no statistical difference by birth season conditions.

Discussion

In water buffaloes, the reproductive cycle has seasonal traits, and reproductive traits associated with day length, defined as photoperiod, as well as temperature and feeding conditions (Vijayakumar et al., 2011; Hozyen et al., 2016) may show regional differences (Phogat et al., 2016; Petrocchi Jasinski et al., 2023). The results obtained in this study also support the above information. According to 12-year data analysis collected from Anatolian buffaloes, the highest birth rate was observed in the summer season (Figure 1). The increase in births in the summer season indicates that estrus also increases in the autumn season and the pregnancy rate increases in this season. Examining the results of many studies, Marai and Haebe (2010a) and Vale (2007) reported that buffaloes tend to reproduce less in hot summer months and there is an increase in estrus signs in cooler seasons. The results obtained from the data of these studies also support our facts. Apart from the

summer season, the birth rates in spring and autumn were close to each other, while the birth rate in winter was determined to be the lowest at 11.99%. These results also reveal that the reproductive characteristics of the Anatolian buffaloes show seasonal changes. Marai and Haebe (2010a) emphasized that there is a decrease in ovarian activity in the summer season, while this activity rate increases in cooler seasons. Another author has noted that seasonal changes in photoperiod have a decisive effect on reproductive activity (Vale, 2007). In addition, Ryan et al. (2007) underlined that the highest birth rates were in January, February, and March in their study on African buffalo. Barile (2005) reported that calving in Mediterranean buffalo cows occurs mostly in July and December, with a longer calving interval for births between February and June. The author related these findings with a decrease in conception rates in the spring-summer seasons. It was also noted that this result is related to climate, especially photoperiod (melatonin secretion). Similarly, in the study conducted by Hussain (2007) on Nili-Ravi buffaloes, seasonal fluctuation in births was observed; the highest birth rate was in August (34.46%) and the lowest in April (0.14%).

As observed in similar previous studies (the above mentioned studies), buffaloes generally appear to have a seasonal reproductive characteristic. Reproductive seasons may vary by region depending on a number of reasons, such as temperature, humidity, sunshine duration (or day length) and seasonal feeding conditions. When the climate data of the region where the current study was conducted were examined, as expected, the hottest season was summer; the average temperature was 23.8°C and the relative humidity was 69.6% (Table 1). The THI value calculated from these data was 72.0 and the monthly average sunshine duration was 272.8 days in summer (Table 2). The high birth rate in summer means that estrus mostly occurs in autumn, in which temperature, relative humidity THI and monthly average sunshine duration were determined as 17.7°C , 68.8%, 62.8 and 157.8 h, respectively. It is thought that the decrease in the duration of sunbathing in particular stimulates the onset of estrus in buffaloes. Indeed, it was stated above that this effect of the season was also emphasized in previous studies. Phogat et al. (2016) reported that in buffalo, estrus signs are shorter and less obvious in hot summer months, and the months when estrus signs are seen more (59%) are between September and February. These findings seem to be consistent with the result that estrus also occurs in autumn for the higher proportion of summer births determined in this study. It was reported that the THI value at which heat stress begins in buffaloes is 68-69, and that changes in

biochemical parameters in animals begin at these values (Umar et al., 2021). However, it was also stated that more significant changes in biochemical parameters are observed when the THI value is 73-76. In the distribution of births by month, it was observed that the highest birth rate was obtained in July and August (Figure 2), and in this case, it could be said that inseminations become more frequent in the autumn months. These results are partially similar to the findings of Barile (2005) and Hussain (2007), but differ from the results of Thokal et al. (2004), Ryan et al. (2007) and Kushwaha et al. (2011). It can be said that inseminations for the months of January and February, when the lowest birth rates are obtained, are in the spring season, and the estrus rates in this season are considerably reduced.

There are some changes in the seasonal distribution of birth rates for each year compared to the average of all years, and these data are given in Table 3. While the summer season had a higher rate in terms of birth rates in the years 2012-2019 and 2021, the birth rates in spring in 2021 and in autumn in 2022 and 2023 were slightly higher than other seasons. It is thought that these changes mainly depend significantly on the climatic changes seen from year to year as well as the care and feeding conditions during the pregnancy period for each birth. Although the changes in the last four years deviated from the averages of all years, it was observed that the change intervals were not too much in other years except for 2022. In 2022, autumn births were 36.06%, while the following season was spring with 27.27%. These changes are thought to be due to climatic changes and variations in animal feeding practices (or: conditions/regime). Such that, it was emphasized by Vijayakumar et al. (2011), Hozyen et al. (2016), Phogat et al. (2016) and Petrocchi Jasinski et al. (2023) that these changes are related to the feeding conditions as well as the ambient temperature and photoperiodic changes.

The growth status of calves in BW_0 , LW_6 and LW_{12} phases was also evaluated. As a result of weighing 15605, 13199 and 10995 calves in these periods, respectively, the average values were determined as 30.8 ± 0.04 kg for BW_0 , 107.6 ± 0.23 kg for LW_6 and 172.6 ± 0.27 kg for LW_{12} . The BW_0 value was higher than the values determined for the same breed by Alkoyak and Öz (2022), Uğurlu et al. (2016), Yilmaz et al. (2017), and Kul et al. (2018), but was consistent with the values reported by Akkulak and Kul (2023) and Kaplan and Tekerli (2024). On the other hand, Genç et al. (2019), who worked on Anatolian buffaloes, found the BW_0 (34.62 ± 0.11) value higher than the results of the current study and similar studies. Different results were obtained in similar studies for LW_6 and LW_{12} values. For example, Kaplan and Tekerli (2024), who studied the same breed, found the LW_6 value higher than the result of this study, while the LW_{12} value was lower than the result of this study. Yilmaz et al. (2017) found the LW value in both growth periods to be lower than the result of this study. In addition, the LW_6 value found by Alkoyak and Öz (2022) was higher than the current study result, but the LW_{12} values were similar. The variation among the results of studies conducted on the same breed indicates that the growth characteristics of calves can vary significantly depending on the rearing conditions.

The effect of calving season on three growth periods of calves was significant in this present study. The highest value for BW_0 was obtained in those born in autumn

(31.6 ± 0.07 kg), while the lowest value was determined in those born in spring (30.1 ± 0.07 kg). This case largely shows that the dam benefits better from pasture conditions during pregnancy. This difference in BW_0 value continued for the following periods, and the highest value for LW_6 (112.1 ± 0.46 kg) was obtained in those born in autumn. As for LW_{12} value, calves born in both autumn and winter had higher growth performance compared to those born in other seasons. In summary, the lowest values for all periods were determined in those born in spring. Our hypothesis was that calves were less able to benefit from pasture in spring and summer and that their nutrition was inadequate in barn conditions during the pasture period. In addition, we concluded that calves born in spring and summer may be adversely affected by hot and humid climate conditions. In a similar study, Alkoyak and Öz (2022) reached similar results for the effect of season on calf growth. Kaplan and Tekerli (2024) stated that there was no effect of season on BW_0 , but LW_6 and LW_{12} values were statistically different according to seasons; the highest value was in those born in winter in both periods. Contrary to the current study, Kul et al. (2018) reported that the lowest BW_0 was in the autumn season. This effect of the season also varies regionally, the climate characteristics of each growing region and the feeding conditions created by these characteristics significantly affect the growth characteristics of the buffaloes. The season affects the milk production and feed intake of pregnant buffaloes after birth, the buffaloes' ability to give enough milk, and the conditions for the buffaloes to benefit from the pasture.

We focused specifically on some productivity traits, such as milk and reproductive traits, that are most affected by seasonal changes. It is known that these traits are largely affected by environmental factors. As seen in Table 5, the average values for LMY and LL were determined as 994.7 ± 2.05 kg and 262.6 ± 0.22 d respectively. The LMY value determined by Soysal et al. (2018), who worked on Anatolian buffaloes in Istanbul province, is higher than the result of this study, but the LL value is lower than the study's mean. LMY and LL values were significantly affected by seasonal conditions. The highest milk yield was in cows calving in spring and winter. Although the LMY values of cows calving in spring appeared numerically higher than those calving in winter, there was no statistically significant difference between the two seasons. In a previous study on Anatolian buffaloes (Kaplan and Tekerli 2024), the highest LMY was found in cows calving in winter and autumn. Similarly, in our study, the longest LL was determined in cows calving in spring with 266.8 ± 0.39 d. These results could result from the positive or negative effects of seasonal differences on the nutrition of animals rather than the climatic effects of the season. By the reason of region where the study was conducted, buffaloes have generally been raised in extensive conditions and pastures. It is thought that LMY and LL were higher than other seasons due to the encouraging effect of pastures in spring and summer on milk yield of buffalo cows calving in spring and winter. It is also emphasized by many authors that milk yield changes positively due to the improvement of feeding conditions in pastures in spring and summer (Kul et al., 2016; Soysal et al., 2018; Koçak et al., 2019; Alkoyak and Öz, 2020; Akyol, 2023). The effects of seasonal differences on milk

yield of buffaloes have also been reported in previous studies. Similar to our findings, Uğurlu et al. (2016) reported that LMY and LL values of cows calving in spring-winter months were higher than those calving in summer-autumn months. While Koçak et al. (2019) reported that the longest LL value was in those calving in winter, Soysal et al. (2018) determined the highest LMY value in winter and autumn, and the longest LL value in autumn. In a previous study on Egyptian buffaloes (Basant et al., 2017), it was reported that cows calving in winter had higher 305-day milk yield, similar to the current study's findings.

When the FCA and CI values were examined in the study, the FCA (mean 1175.5 ± 3.46 d) value was affected by seasonal changes ($P < 0.001$), but the CI (mean 561.7 ± 1.84 d) value was not affected by this environmental factor. The FCA value of heifers born in spring was higher than the FCA value of those born in other seasons. The average FCA value for this season (winter) was determined as 1230.7 ± 11.09 days, i.e. approximately 41 mo. Regarding this issue, it was reported that when the age of sexual maturity coincides with the seasons when estrus is less in terms of reproduction, the first pregnancy may extend until the next season, which will increase the FCA value (Verma et al., 2021). The same authors emphasized that there may be large variations in FCA in buffaloes according to countries, population rearing conditions and breeds. The authors reported that in Indian conditions, 70-80% of buffaloes conceived between July and February and less insemination was needed during this period. Therefore, it was stated that FCA value for calves born in winter months was lower than those born in summer months. On the contrary, Penchev et al. (2014) emphasized that the FCA value of those born in winter is longer. It is thought that the FCA value determined in the current study can be reduced by improving the rearing and feeding conditions.

It was concluded that seasonal changes did not affect the CI value and the mean value was 561.7 ± 1.84 day. However, it might be expected that a buffalo cow's re-estrus and re-pregnancy after giving birth would vary due to seasonal climatic and nutritional differences. Such an effect has not been observed under current population and rearing conditions. In a previous study, the average CI value for Anatolian buffalo raised in Istanbul province conditions was determined as 417 ± 1.73 d, which is considerably lower than the average of this study (Soysal et al., 2018). The authors reported that the season did not affect the CI value, in line with the results of this study. In contrast to the current study, Kaplan and Tekerli (2024) reported that the CI value was affected by seasonal changes, with the highest CI values reported in buffaloes calving in summer and autumn. Similarly, El-Wakeel et al. (2013) noted that the season on CI, with the highest CI values determined in spring calving. In contrast to the current study, Kaplan and Tekerli (2024) reported that the highest CI value was in buffaloes calving in summer and autumn. In a previous study, El-Wakeel et al. (2013) noted that the highest CI value was determined in buffaloes calving in spring. Koçak et al. (2019) determined the highest CI value in buffaloes calving in winter. In the current study, the determined CI value is high, and reducing this value is important for profitable production.

Conclusion

As a result, the reproductive, milk and growth characteristics of Anatolian buffaloes are affected by seasonal conditions; therefore, in planning herd management and feeding requirements, the climatic characteristics of the region and the feeding opportunities provided by these climatic conditions should be carefully examined. As determined in many breeds and different regions, the reproductive characteristics of Anatolian buffaloes also show seasonal changes, and births increase in certain seasons. In addition, milk yield and growth characteristics in buffaloes are also affected by these climatic changes. Although this situation can be partially overcome in intensive farms, it does not seem possible to eliminate climatic factors such as seasonal temperature, humidity and sunshine duration in the near future. Therefore, a selection program that can increase tolerance to seasonal factors and herd management practices that will reduce the effect of the season are needed.

Declarations

Ethical Approval Certificate

In the current study, previously collected data and records from the Samsun Province Buffalo Breeding Project carried out by the Turkish Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies, and other project stakeholders were used. Therefore, Ethics committee approval was not required for this study.

Author Contribution Statement

İ.C.O., S.A. and H.E.: Data collection, investigation, formal analysis, and. S.A. and H.E.: Conceptualization, methodology, review, and editing S.A. and İ.C.O.: Data collection and investigation

Fund Statement

This work was not funded by an institution/organization.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank the Turkish Ministry of Agriculture and Forestry, the General Directorate of Agricultural Research and Policies and grateful to the technical staff of Samsun Buffalo Breeders' Association.

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Examining Heavy Metal Transfer from Soil to Bread

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ARTICLE INFO

Research Article

Received : 17.09.2024

Accepted : 26.10.2024

Keywords:

Bread

Soil

Flour

Heavy metals

Food Safety

ABSTRACT

For Turkish society, bread has been an indispensable part of the kitchen and daily life throughout history. Due to its high consumption in Turkish society, it plays an important role in terms of both health and nutritional habits. Contamination from the soil where wheat is planted to the bread making process is of great importance for health. In this study, the amounts of heavy metals such as aluminum, copper, nickel, chromium, manganese, iron, lead and cadmium in the soil of an agricultural land, in flour obtained from wheat grown there and in bread made from this flour were investigated using ICP-MS. The average levels of Al, Cr, Cu, Mn, Ni, Cd, Fe, Pb and As in soil samples were 120.46, 12.23, 44.9, 93.46, 10.83, 2.06, 196.87, 1.96 and 0.21 mg/kg, respectively. In flour samples, these levels were 17.20, 2.03, 28.93, 26.3, 3.37, 0.09, 30.93, 1.37 and 0.03 mg/kg, respectively. In bread samples, 11.27, 0.77, 8.27, 18.63, 0.4, 0.02, 12.76, 0.04 and 0.001 mg/kg, respectively. The results obtained show that high metal levels in the soil are also found in bread. This indicates that heavy metal levels in bread may pose health risks in long-term consumption. Especially levels of aluminum, nickel, chromium and cadmium metals can cause serious health problems. Therefore, it is important to reduce heavy metal contamination in agriculture and production processes and to conduct regular inspections. Compliance with maximum limits set by health authorities and regulatory agencies is also critical for public health.

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Introduction

Bread is a food item that has an important place in our daily diet and is consumed most frequently by consumers. It has an indispensable importance in terms of nutrition as it contains basic nutrients such as carbohydrates, proteins, vitamins and minerals. In Turkey, an average of 65% of an individual's daily energy intake is obtained from grains, and 55% of this is obtained from bread. Approximately 50% of the daily protein intake is obtained from bread consumption (Zioła- Lebbos et al. 2019; Frankowska et al. 2021; Yurt and Bayraklı 2022; Basaran 2022). This rate is 90% in poor countries and 40% in developed countries. The fact that people with limited income meet their nutritional needs from cheap grain group foods, especially bread, causes this rate to increase. Bread is not only a source of calories, but also provides important nutrients for human nutrition, such as essential amino acids, minerals, vitamins, beneficial phytochemicals and dietary fiber (NDND 2014; Shewry & Hey 2015; Onur & Ceylan, 2023). The bread industry is shaped by the contributions of various businesses consisting of local and national businesses of different sizes. In recent years, the variety of bread produced by the food industry has increased as a result of the development of consumer awareness and

increasing population rates (Sundkvist et al. 2001; Dewettinck et al. 2008). There is therefore currently more than one bread brand operating in the market (Li et al. 2019). The bread production process involves a series of complex operations, from the cultivation and storage of grains to their production and sales and marketing, which can lead to increased food-borne health risks (Hembrom et al. 2020).

One of these threat factors is heavy metals, which attract the attention of both consumers, manufacturers and scientists. Therefore, the processes from bread production to consumption need to be carefully managed and supervised to reduce potential health risks, especially heavy metals (Pirsaheb et al. 2021; Basaran 2022). Heavy metal pollution resulting from population growth, natural events and industrial activities mixes with air, water and soil in various ways and contaminates food (Belhaj et al. 2016; Chaza et al. 2018; Garg et al. 2019; Affourtit et al. 2020). Published tests on the toxicity of metals in soil to microorganisms, plants and animals date back approximately 30-40 years. Most older studies are of limited use in defining allowable metal concentrations in soil because they lack a wide enough range to define a

complete dose-response, examine only one or a few soils, do not use standard methods, or do not report important properties of the soils examined (Rooney et al. 2006; Criel et al. 2008; Li et al. 2019). Solid waste disposal, wastewater irrigation, sludge applications and industrial activities are the major sources of soil heavy metal pollution, and increased metal uptake of food products grown in such contaminated soils is quite high (Chaza et al. 2018; Affourtit et al. 2020). Environmental pollution caused by heavy metals causes great negativities on ecosystems. These negativities can be easily carried from one ecosystem to another, directly or indirectly. Heavy metals reaching the atmosphere, rain, snow and hail can pollute surface water resources such as lakes, streams and rivers, and also cause pollution of underground water resources by leaking from the soil. Heavy metals can be transported from the soil to the human body through food, to animals through plants that serve as feed sources, and to humans through the meat and milk of animals. When water with high amounts of heavy metals is used in agricultural areas, it causes many negative effects on plants, animals and humans (Yerli et al 2020). Heavy metal accumulation in the soil can be reduced thanks to the buffering capacity of the soil and the organic matter, clay, iron and aluminum oxides found in the soil. In addition, many factors such as soil texture, organic carbon, soil water content, soil temperature, phosphorus, clay type, carbonates and bicarbonates can affect the movement of heavy metals in the soil. The most abundant heavy metals in soil are arsenic, mercury, zinc, cadmium, chromium, lead and nickel (Wuana & Okieimen 2011). Heavy metals can easily pass from soil to seeds in agricultural lands, from seeds to bread or baked goods, and then to the human body. Excessive accumulation of heavy metals in agricultural soils not only causes soil contamination but can also lead to increased uptake of heavy metals by crops, threatening food quality and safety (Muchuweti et al. 2006). Between 2018 and 2019, samples were collected from Shenzhen city and region to investigate heavy metal pollution in rice, flour and products in Shenzhen. Flour and flour products have been tested for lead, cadmium, total mercury and total arsenic. The results show that the cadmium detection rate in flour and flour products is 98.13%, over 50% (Yang et al., 2020). In the study conducted by Mahdi and Omran (2021), the levels of toxic heavy metals such as Cd, Pb, Cr, Ni, Zn, Cu, Fe, Co, As were determined using X-Ray fluorescence in flour made from wheat samples collected randomly and seasonally from three silos in Baghdad and kept for a long time. The results obtained showed that the levels of Cd, Pb and Cr exceeded the permissible safe limits according to FAO/WHO. Although it is thought that storage conditions, as well as heavy metal pollution from the soil, affect this situation.

Effects of Heavy Metals on Human Health

Heavy metals that can be easily transported between ecosystems can cause serious damage to the environment and human health (Yerli, Çakmakçı, Sahin, & Tüfenkçi, 2020). Arsenic causes liver enlargement, skin staining and different skin diseases, respiratory cancer and bone disorders. High exposure to mercury metal causes kidney damage, damage to the nervous system and miscarriage

during pregnancy (Çağlarımak, & Hepçimen, 2010). While copper is one of the heavy metals necessary for the body, high amounts of copper intake cause brain damage and regression in body development (Özbolat, & Tuli, 2006). Boron causes bone damage. Boron poisoning causes nausea, headache, diarrhea, muscle spasms, skin lesions, irregularities in the digestive mechanism and glands (Velioglu & Şimşek, 2003; Turkez, Geyikoglu, Tatar, Keles, & Kaplan, 2012). Mercury poisoning causes psychological disorders (Bakar & Baba, 2009). Iron plays an important role in maintaining various metabolic processes in the body. In case of deficiency, symptoms such as shortness of breath, weakness, fatigue and anemia may occur. However, excessive iron accumulation can lead to health problems such as liver failure, dizziness, and stomach disorders (Finkelman, Skinner, Plumlee, & Bunnell, 2001; Atıçlı, Gültekin, Şen, & Elp, 2016). Zinc causes ulcers, pulmonary edema, irritation of mucous membranes and respiratory tract (Vural, 1993). Although lead is one of the first metals used since ancient times, it is known as an important heavy metal that causes serious toxic effects on human metabolism (Kara, Pırlak, & Özdilek, 2004; Asri & Sönmez, 2006).

In this study, the amounts of heavy metals in the soil of an agricultural land, in the flour obtained from wheat grown there, and in the bread made from that flour were examined. For the purpose of this study, the changes in Cr, Al, Cd, Mn, Pb, As, Fe, Cu and Ni grades in soil, flour, and bread were examined as shown schematically in Figure 1.

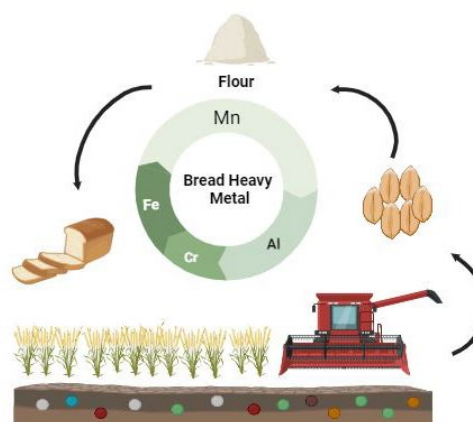


Figure 1. Schematic illustration of farm to table

Experimental

Material and Methods

All chemicals belonged to the category of analytical reagents. %65 HNO₃ and %48 HF, %30 H₂O₂ were purchased from Sigma-Aldrich and Merck, respectively. Multi-element standard solutions were obtained from Agilent (Santa Clara, USA) and were based on Merck standard mixture solutions. Ultrapure deionized water was used for the preparation of standard and sample solutions. Heavy metal analyses were performed using an ICP-MS 7900/ASX 500 instrument (Agilent Technologies, Japan). In addition, the Microwave Digestion System (CEM Mars5, USA), wheat grinder Perten Instrument (LM-3100, Sweden), an ultra-distilled water device (Millipore, USA) were used.

Three soil samples were taken from different parts of the cultivated field. It was taken from the flour coming out of the wheat mill and samples were taken from the bread whose making is given in detail below using this flour. The samples were first dried in an oven at 70 °C for 3 h. Later, a method that could address all sample types and ensure complete dissolution was created in the CEM Mars 5 Microwave Disintegration System. 0.5 g of each samples were weighed and 12 mL of HNO₃ and 3 mL H₂O₂ were added on it, and a closed system was created. The segments were pulled out at the end of the 30 min. method (Temperature: 240 °C, Gradient temperature-time: 30 min, Holding time: 15 min) in the device. An extra 3 mL of HF was added to soil samples only to dissolve silicon. After cooling to room temperature, the samples were passed through a filter with a pore size of 0.45 µm and then analyzed by ICP-MS. The analyses were performed in three replicates and three parallel runs.

Bread Making

The wheat obtained from the Bolu region was cleaned and ground in a wheat grinder to obtain flour. After the necessary resting process of the flour is done, it is ready for use. In the laboratory selected for bread making, 1 kg of flour obtained from ground wheat was kneaded with 700 ml of water in a spiral mixer with a capacity of 5 L for 2 min. at a slow speed and 3 min. at a fast speed. Afterwards, 25 g of salt and 40 g of yeast were added and the kneading process was continued for another 2 min.. In order for the yeast to become active in the dough, the internal temperature of the dough must be 21-27 °C. After the internal temperature of the dough reached +22°C, the dough was placed in a lightly oiled storage container and pre-fermented for 30 min.. At the end of the pre-fermentation, the dough was divided into two 500 gr pieces and shaped into bread. Then, for the final fermentation, the bread was placed in the baking container and kept in the fermentation cabinet at 30 °C and 80% humidity for 60 min.. At the end of the fermentation period, the breads were baked in the oven at 220 °C for 40 min.. At the end of the baking time, the breads were removed from the oven and

allowed to cool. Cooled breads were analysed without waiting.

Results and discussion

Within the scope of the study, soil samples taken from the field where wheat was planted, flour obtained from the wheat grown and harvested after planting, and finally samples from the bread made from this flour were taken to determine the levels of 9 different heavy metals. Findings regarding heavy metal levels are given in table 1. Additionally, heavy metal distribution in each of the soil, flour and bread samples is shown in Figures 3-5. The change in heavy metal content in the process from soil to bread making has not been examined in the literature. Each of the sample types mentioned in the literature was examined and evaluated separately. An attempt was made to compare and examine the distribution of heavy metals in bread, which is among the most basic food sources that pose a risk to health. When the studies in the literature are examined, it has been seen that the heavy metal content in the soil is in accordance with the standards and even insufficient in terms of metals that will be beneficial for the plant depending on the type of product to be grown. The average Al, Cr, Cu, Mn, Ni, Cd, Fe, Pb and As heavy metal levels obtained in the soil samples were found to be 120.46 ± 2.7 mg/kg, 12.23 ± 0.7 mg/kg, 44.9 ± 4.6 mg/kg, 93.46 ± 2.3 mg/kg, 10.83 ± 2.8 mg/kg, 2.06 ± 0.06 mg/kg, 196.87 ± 7.8 mg/kg, 1.96 ± 0.11 mg/kg and 0.21 ± 0.04 mg/kg, respectively.

Average Al levels in flour and bread samples were found to be 17.2 ± 1.6 and 11.7 ± 1.5 mg/kg, respectively. It is seen that the Al level is very high, especially in soil samples. This situation was also seen in the amount of Fe. Fe levels were found to be 196.8 ± 7.9, 30.9 ± 3.0 and 12.8 ± 1.5 mg/kg, respectively. It has been observed that these two elements pass into wheat from the soil at high levels. It has been shown that the amount of iron in food products is beneficial for health and is found at high levels, starting from the soil and ending with bread.



Figure 2. Demonstration of bread making.

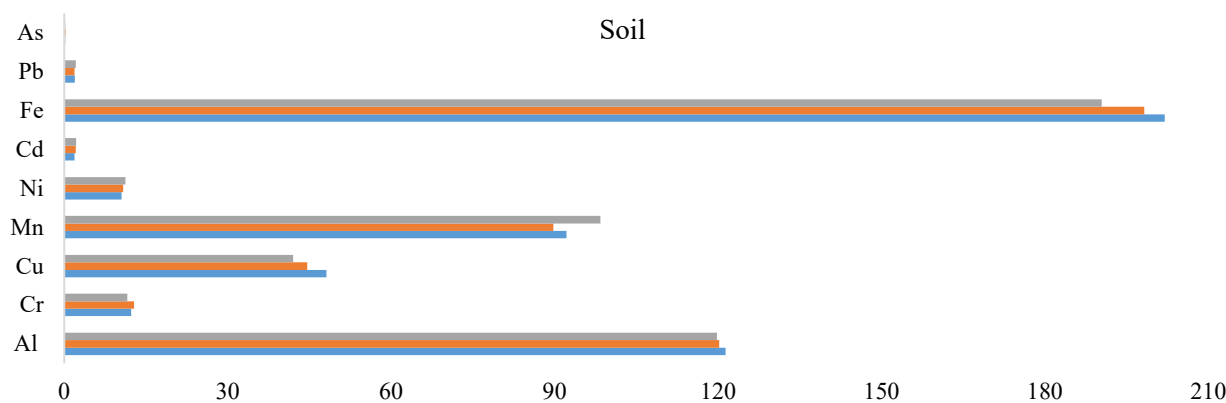


Figure 3. Illustration of heavy metal contents of soil samples.

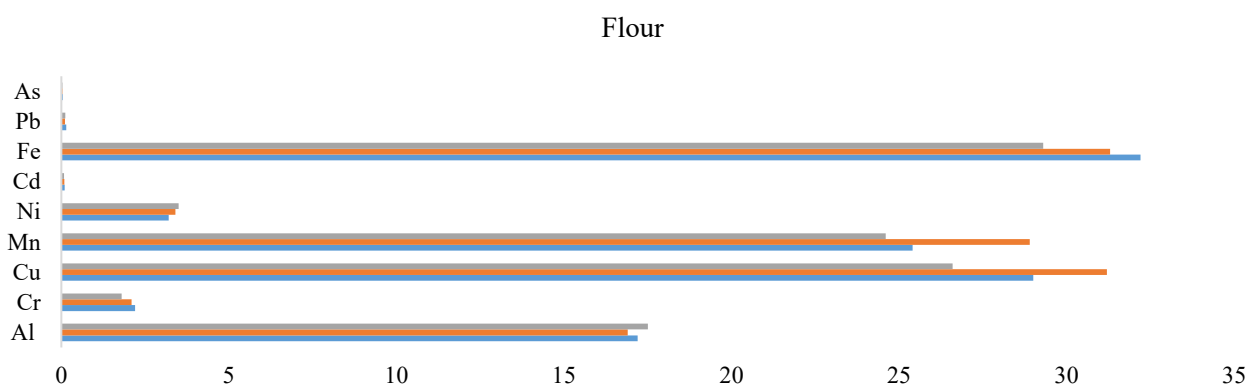


Figure 4. Illustration of heavy metal contents of flour samples.

Table 1. Mean heavy metal levels of samples(mg/kg)

Samples	n	Al	Cr	Cu	Mn	Ni	Cd	Fe	Pb	As
Soil 1	3	121.4±2.3	12.3±0.6	48.1±3.5	92.2±4.2	10.5±1.2	1.9±0.4	202±8.8	1.92±0.5	0.2±0.02
Soil 2	3	120.2 ±2.4	12.8±1.1	44.6±4.2	89.8±4.3	10.8±0.8	2.1±0.2	198.2±5.6	1.86±0.4	0.24±0.07
Soil 3	3	119.8±3.5	11.6±0.4	42±6.3	98.4±6.3	11.2±1.5	2.2±0.3	190.4±9.2	2.1±0.8	0.18±0.04
Flour 1	3	17.2±1.3	2.2±0.5	29±2.4	25.4±2.4	3.2±0.6	0.1±0.008	32.2±4.6	0.15±0.06	0.04±0.003
Flour 2	3	16.9±1.8	2.1±0.3	31.2±2.1	28.9±5.2	3.4±0.9	0.088±0.004	31.3±2.4	0.11±0.004	0.03±0.006
Flour 3	3	17.5±1.9	1.8±0.2	26.6±4.3	24.6±2.1	3.5±0.8	0.08±0.002	29.3±2.1	0.12±0.002	0.03±0.002
Bread 1	3	11.8±2.1	0.7±0.08	9.2±2.2	18.8±1.4	0.36±0.03	0.025±0.012	13.2±0.9	0.046±0.006	0.0012±0.00008
Bread 2	3	11.1±1.6	0.8±0.06	8.9±2.1	19.6±1.2	0.44±0.08	0.024±0.01	12.4±2.5	0.042±0.009	0.0011±0.0002
Bread 3	3	10.9±0.8	0.8±0.08	6.7±2.6	17.5±3.2	0.38±0.04	0.012±0.002	12.7±1.2	0.032±0.003	0.0009±0.0001

n: Number of sample replicates

Therefore, it is seen that bread has higher levels than other heavy metals. In addition, when the average Al level in the literature was examined to determine the heavy metal contents in different types of bread, Arnich et al. (2012), Wang et al. (2020) and Ziola-Frankowska et al. found it to be 2.6 mg/kg, 4.79 mg/kg, 3.62 mg/kg, respectively. It seems that the average Al value obtained in this study is higher. It is seen that the amounts of Cd, As and Pb in all soil, flour and bread samples are in trace amounts, as seen in Table 1. It is seen that the average values of Cr amounts in all flour and bread samples, are 2.0 ± 0.3 and 0.76 ± 0.07 mg/kg, respectively. It is seen that the average values of Ni amounts in all flour and bread samples, are 3.37 ± 0.37 and 0.39 ± 0.076 mg/kg, respectively (Table 1).

Average Cu levels in soil, flour and bread samples were found to be 44.9 ± 4.7 , 28.9 ± 2.9 and 4.7 ± 2.3 mg/kg, respectively. The fact that the amount of copper in wheat is high compared to the amount of copper in the soil suggests that wheat receives external copper supplements.

External copper supplementation is generally applied to wheat during its development period. In addition, the average Cu level in flour was found to be higher than the studies conducted in the literature and was found to be 28.93 ± 2.9 mg/kg. In the study conducted by Lei et al. (2015), it was observed that the amount of Cu in the soil of a roadside field was 28.1 mg/kg, while the amount of Cu in the flour obtained from there was 2.66 mg/kg. It is seen that the Cu level is very high, especially in flour samples. In copper deficiency, wheat ears do not form well, the stem bends and there is a tendency to lean sideways. Resistance to diseases decreases. In this study, the average Mn level in bread was found to be 18.63 mg/kg; however, it corresponds to a higher value than the literature in general. Shokunbi et al. (2019) stated that the average Mn levels in bread were 7.32 mg/kg (5.08–9.57 mg/kg); Ziola-Frankowska et al. (2021) stated that the average level in different types of bread is 7.28 mg/kg.

Bread

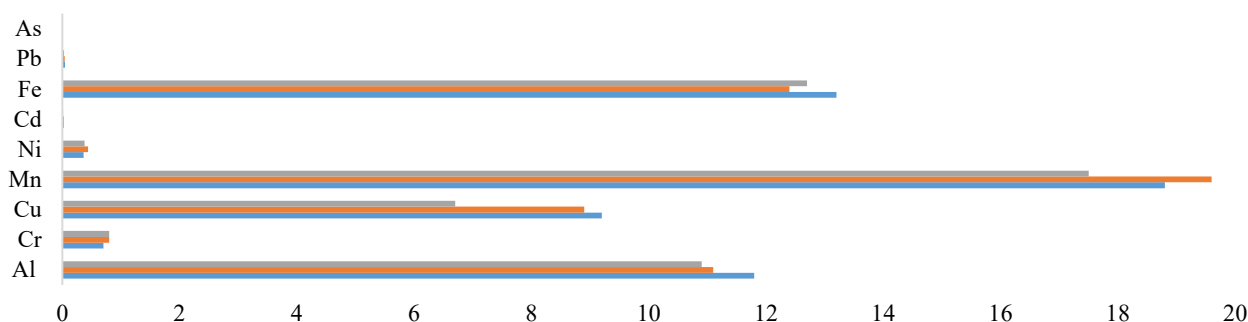


Figure 5. Illustration of heavy metal contents of bread samples.

When all the results were examined, it was seen that the amount of Al and Fe was at high levels in the samples collected during the process from soil to bread, and the levels of Mn and Cu were also at high levels. It has been observed that toxic metal levels other than this do not pose any threat to health. It is known that the reason why these values are at these levels is not only due to the metal content of the soil but also to the conscious use of pesticides and water use. Studies show that the accumulation of heavy metals in the soil will increase day by day due to factors such as unconscious tillage, incorrect irrigation, pesticide application and fertilization, and as a result, it will become increasingly difficult to obtain healthy products.

Aluminum (Al): High intake of aluminum is associated with neurotoxic effects and Alzheimer's disease (Odukoya, et al, 2022). The presence of 11.27 mg/kg Al level in bread poses health risks in the long term.

Bakır (Cu): Although copper is an essential trace element, excessive intake can be toxic and cause gastrointestinal disorders (Zhao, et al, 2020). The 0.77 mg/kg Cu level in bread appears to be within acceptable limits.

Nikel (Ni): Nickel can cause allergic reactions and chronic exposure can increase the risk of cancer. The presence of a Ni level of 7.39 mg/kg in bread may pose a potential health risk.

Demir (Fe): Iron is an essential mineral for the body, but excessive intake can be toxic and cause liver damage (Odukoya, et al, 2022). The 12.76 mg/kg Fe level in bread seems to be within acceptable limits.

Kurşun (Pb): Lead is toxic and can cause developmental problems in children and various health problems in adults (Román-Ochoa, et al, 2023). Although the level of 0.04 mg/kg Pb in bread is low, caution should be exercised as there is no safe level of lead exposure.

Kadmiyum (Cd): Cadmium can cause kidney damage, osteoporosis, and cancer (Özden, & Erkan, 2016). The presence of a Cd level of 0.02 mg/kg in bread is noteworthy and may pose health risks in long-term consumption.

Arsenik (As): Arsenic is toxic and classified as a carcinogen (Sarwar, et al, 2021). The level of 0.001 mg/kg As in bread is low, but it may cause health problems with long-term intake.

Conclusions

In this study, the stages of the production process of bread, one of the basic foods of our daily life; The changes in heavy metal amounts in the soil in the cultivated field, the flour obtained from the wheat grown there, and the bread made from this flour were examined. The results obtained show that the variable parameters in the production process of the raw materials used, rather than the bread consumed, change the levels of heavy metals and that they are found at a certain level in the final consumer product, bread. While it is necessary to constantly check whether these levels are harmful to human health, it is known that these controls are not carried out in the world. With this study, authorities need to develop a strategy by creating awareness about following this process. It is important to be compatible and follow not only with bread but also with all the basic food products in our daily lives. As a result of the data obtained, the presence of heavy metal levels in bread poses various health risks and some risks in long-term consumption. In particular, levels of metals such as aluminum, nickel, chromium and cadmium cause significant health problems with long-term consumption.

It has also been determined that exposure to high doses of Cu can cause irritation in the nose, mouth and eyes and cause headaches (Lei et al. 2015). Cd causes both acute and chronic toxicity in the human body and in some parts of the human body (such as the kidneys, lungs and bones) (Oteef et al., 2015). High concentrations of Ni are said to cause cancer, fatigue, headache and dizziness, skin rash, heart and respiratory diseases (Khan et al., 2013). One of the most important ways of exposure to environmental pollutants is through the consumption of contaminated foods. Especially in Turkey, wheat and its products are important nutrients because they constitute a significant part of the daily diet. Therefore, consumers have a high potential for exposure to environmental pollutants due to contaminated wheat products.

Therefore, it is important to use appropriate agricultural and production methods to minimize heavy metal contamination in the bread production process, as well as to carry out regular inspections. Compliance with the maximum limits set by health authorities and regulatory bodies is also critical for public health.

Organic Farming and Green Fertilization Methods: Organic farming protects soil health by replacing chemical fertilizers and synthetic pesticides with natural animal or plant fertilizers such as compost. These methods can reduce the accumulation of heavy metals in the soil.

Phosphorus and pH Management: Balancing phosphorus and pH levels can affect the mobility of heavy metals in soil. Phosphorus deficiency can lead to greater absorption of heavy metals by plants, so a balanced phosphorus management is important.

Phytoremediation: Plant species that absorb heavy metals from the soil can absorb and store heavy metals from the soil and reduce heavy metal pollution. However, the use of these methods requires careful selection of plants and careful determination of growing conditions.

Education: In addition to these practices, another important factor to reduce heavy metal pollution in soil is to ensure the development of conscious farmers. Conscious farmers contribute to healthy and safe food production.

Soil Analyses and Management Plans: Regular analysis of samples taken from fields is very important for the health of the practices. These analyses show which areas are at risk of heavy metal contamination and management plans can be created accordingly. At the same time, these analyses ensure the safety of products reaching human consumption along the food chain.

Declarations

Acknowledgements

I would like to thank Dr. İlker AKIN for his help in the analysis.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations/Conflict of Interest

I have no conflict of interest to declare.

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Impact of Boron Toxicity and Humic Substance Applications on Cotton Fiber Quality and Yield

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ARTICLE INFO

Research Article

Received : 28.08.2024

Accepted : 03.11.2024

Keywords:

Boron
Humic Substance
Cotton
Toxicity
Hyperaccumulator

ABSTRACT

This study investigated the effects of boron toxicity and humic substance applications on cotton fiber quality and yield over two consecutive years, targeting boron toxicity issues in soils affected by agricultural and geothermal activities. The experiment evaluated varying concentrations of boron (0.6–1.8–5.4–16.2 mg B l⁻¹) and humic substances (0–200–400 kg ha⁻¹), with a focus on their effects on seed cotton yield, fiber length, fineness, strength, and gin efficiency. In the first year, the highest seed cotton yield was recorded at 452.5 kg da⁻¹ with the B1 application, followed by 428.3 kg da⁻¹ with B2. In the second year, increased boron application led to a notable decrease in seed cotton yield, with the lowest yield at 99.3 kg da⁻¹ for the B4 application. The highest dose of boron also significantly reduced fiber strength, with the lowest recorded at 31.57 g/tex, and gin efficiency, which dropped to 37.98%. Humic substance applications showed limited influence on fiber quality parameters; however, the highest dose (H3) led to a significant increase in fiber strength to 33.47 g/tex in the second year. Cotton leaves accumulated substantial amounts of boron, reaching concentrations of 2048 mg B kg⁻¹ during the flowering period of the second year, suggesting that cotton could serve as a hyperaccumulator in phytoremediation efforts for boron-contaminated soils. The study further determined that cotton can tolerate boron concentrations in irrigation water ranging from 1.8 to 5.4 mg B l⁻¹, making it a viable crop in boron-affected regions. These findings provide critical insights into the potential of cotton as a resilient crop in environments with elevated boron levels, underscoring the need for further research to optimize cotton cultivation under such conditions.

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Introduction

Boron (B) is an essential micronutrient for plants, typically found in combination with oxygen and widely distributed in the Earth's crust, where it has an average concentration of 8 mg kg⁻¹ (Anonymous, 2010). For optimal plant growth, boron concentrations in soil generally range from 0.1 to 0.5 mg kg⁻¹, depending on the species (Butterwick et al., 1989). Boron plays a crucial role in regulating plant hormone levels, flower production and retention, pollen tube elongation and germination, as well as seed and fruit development. However, due to the narrow range between deficiency and toxicity, boron concentrations that fall outside of the optimal range can lead to significant yield losses in crops (Chapman et al., 1997). Boron is naturally released into the soil and water through various processes, including rainfall, the weathering of boron-containing minerals, desorption from clays, and the decomposition of organic matter. Anthropogenic activities, such as the application of boron-

containing fertilizers, the use of fly ash as a soil amendment, irrigation with wastewater, and the discharge of industrial and geothermal wastewater, further contribute to boron levels in the environment (Butterwick et al., 1989; Mumma et al., 1984; Koç, 2011). Geothermal water resources, which often contain high levels of boron along with other potentially harmful substances like heavy metals, pose a significant risk to agricultural production (Gemici & Tarcan, 2002). These geothermal waters emerge at varying temperatures (51-163°C), depending on the geological reserve and geographical conditions. The elevated temperatures of geothermal waters increase the solubility of boron, making it a potent pollutant. One of the primary sources of high boron concentrations in irrigation water is the contamination of these waters with boron-rich geothermal effluents. Soil type significantly influences the impact of boron on plant health. For example, sandy soils tend to exhibit faster and more severe damage from high

boron irrigation water compared to loamy or clayey soils, due to the differing boron binding characteristics of these soils (Keren & Bingham, 1985). Boron compounds in the soil are transformed into borates, which do not degrade further and thus accumulate over time, leading to boron toxicity (Bradford, 1966). This issue has become particularly pronounced in regions where geothermal water sources have been used for agricultural irrigation over the past 2-3 decades. In the Büyük Menderes River basin, for instance, the discharge of wastewater containing boron has led to significant pollution. Prior to these discharges, boron levels in irrigated soils were around 0.15 mg kg^{-1} , but have since increased to 13.90 mg kg^{-1} at a depth of 0–20 cm (Akar, 2007). Similarly, soil boron levels have been found to range from 0.43 to 2.34 mg kg^{-1} (Aydın et al., 2010). Cotton (*Gossypium hirsutum* L.), a major crop in the region, exhibits remarkable resistance to boron toxicity. For instance, the permissible boron concentration in irrigation water is 0.33 mg l^{-1} for sensitive plants and about 0.67 mg l^{-1} for semi-tolerant plants like cotton (US Salinity Laboratory, 1954). This resilience is largely due to the plant's anatomical adaptations, such as a well-developed root system that efficiently excludes and sequesters boron, preventing its excessive uptake and translocation to the aerial parts of the plant. Nevertheless, there is a threshold beyond which boron toxicity can impair cotton growth and yield. To mitigate this, farmers in the region have increasingly applied humic substances, which enhance nutrient availability in the soil and facilitate nutrient uptake and transport. Humic substances are characterized by their dark brown to black color, high molecular weight, large specific surface area, and stable molecular structure, which does not easily degrade in the soil (Stevenson, 1994). Boron is a crucial micronutrient for plants, yet its narrow effective concentration range in soil makes both deficiency and toxicity significant concerns for crop production. The issue of boron toxicity is particularly acute in regions where geothermal waters, rich in boron, contaminate agricultural lands. The Büyük Menderes River basin serves as a prime example, where the discharge of boron-laden geothermal wastewater has led to elevated boron levels in soils, threatening agricultural productivity. Cotton, a key crop in the region, has shown a notable resilience to boron toxicity due to its anatomical adaptations. However, there are limits to this resilience, and once surpassed, boron toxicity can lead to reduced growth and yield. Farmers in the region have increasingly turned to the application of humic substances to mitigate the adverse effects of boron

toxicity and enhance nutrient availability and uptake in soils. Given the growing importance of sustainable agricultural practices, this project aims to investigate the combined effects of boron and humic substances on cotton growth dynamics, yield, and fiber quality. By exploring the potential of humic substances to alleviate boron toxicity, this research seeks to contribute to the development of effective soil management strategies that can sustain cotton production in boron-affected areas. The findings will have implications not only for the Büyük Menderes River basin but also for other regions facing similar challenges, thereby supporting broader efforts to enhance agricultural sustainability in the face of environmental stressors.

Material and Methods

Experimental Site

The experiment was carried out at field conditions at University of Adnan Menderes, Aydın, Turkey (Figure 1). The site is located in the western regions of Turkey ($37^{\circ} 45' \text{ N}$, $27^{\circ} 45' \text{ E}$, 34 m).

The site receives a long-term seasonal rainfall 106.9 mm and an average temperature of 24.05°C . According to climatic data, the season of 2012 was drier and warmer than in 2011. Between May and October 2011, there were 210.2 mm and 109.6 mm rainfall in the same period of 2012 (Table 1).

The soil (*Typic Xerofluvent*) is sandy loam in texture that facilitates leaching and no drainage problem occurs even with heavy rains. In addition, there is no salinity problem observed in the soil. A bulk of silt loam was collected from Ap horizon. The soil was air-dried, crushed and sieved through 2 mm sieve. The soil analyses were carried out by the methods of Ryan et al. (2001). The soil was deep, well-drained, coarse silty, moderately calcareous, hyperthermic, Typic Haplocambids. The chemical analyses revealed that soil had: pH 8.31; organic matter content: 0.90%; Total N content 0.10%; NH_4Oac extractable-K, 173 mg kg^{-1} ; NaHCO_3 available-P, 26 mg kg^{-1} ; and extractable B, 0.92 mg kg^{-1} (Table 2).

Upon examining the properties of the irrigation water, it was found that the pH was slightly alkaline 7.75, EC was within usable limits 0.97 dS m^{-1} , SAR value was low 1.04, B was satisfactory 0.6 mg l^{-1} , Cl^{-1} and SO_4^{-2} were within acceptable levels 0.024 , 0.86 me l^{-1} , HCO_3^{-1} was concerning 4.69 me l^{-1} , and the irrigation water was classified as C_3S_1 (Table 3).

Table 1. Some seasonal climatic conditions on experimental site

Year	Parameters	May	June	July	August	September	October
2011	Average Temperature ($^{\circ}\text{C}$)	19.18	24.72	27.49	26.87	23.49	15.43
	Relative Humidity (%)	71.55	56.70	54.58	53.23	58.93	74.48
	Precipitation (mm)	49.00	50.00	0.40	0.00	38.40	72.40
2012	Average Temperature ($^{\circ}\text{C}$)	20.08	27.02	29.60	27.89	22.69	19.88
	Relative Humidity (%)	73.39	55.30	50.74	45.32	52.70	72.39
	Precipitation (mm)	43.60	2.40	3.20	0.00	0.00	60.40
Long-Term Average	Average Temperature ($^{\circ}\text{C}$)	20.90	25.90	28.40	27.40	23.30	18.40
	Relative Humidity (%)	54.00	46.50	43.40	46.00	51.70	62.50
	Precipitation (mm)	34.00	13.40	3.30	2.00	12.30	41.90

Table 2. Soil chemical properties sampled at 0-30 cm depth, during experiment

B rates mg l ⁻¹	Years	Period	pH	OM	N	B	P	K
				%			mg kg ⁻¹	
B1 0.6	2011	initial	8.31	0.90	0.10	0.92	26	173
	2012	final	7.99	1.68	0.09	1.84	18	127
B2 1.8	2011	initial	8.31	0.90	0.10	0.92	26	173
	2012	final	7.93	1.68	0.10	4.28	20	134
B3 5.4	2011	initial	8.31	0.90	0.10	0.92	26	173
	2012	final	7.90	1.64	0.10	8.49	19	139
B4 16.2	2011	initial	8.31	0.90	0.10	0.92	26	173
	2012	final	8.13	1.44	0.10	21.04	21	145

OM: % Organic matter; N: % Total Nitrogen; B: Available Boron; P: Available Phosphor; K: Extractable Potassium.

Table 3. Some chemical properties of the irrigation water

Class	pH	EC	TDS	B	TH	SAR	HCO ₃ ⁻¹	SO ₄ ⁻²	Cl ⁻¹
		dS m ⁻¹	mg l ⁻¹		°d			me l ⁻¹	
C ₃ S ₁	7.75	0.97	0.80	0.6	32.48	1.04	4.69	0.86	0.024

EC: Electrical Conductivity, TDS: Total dissolved solids, B: Boron, TH: Total Hardness, SAR: Sodium absorption ratio, HCO₃: Bicarbonate, SO₄: Sulphate, Cl: Chlorine.



Figure 1. A satellite image of the experimental site. It was taken from Google Earth software in 2013

The Experiment

Irrigation water containing four different B levels (0.6–1.8–5.4–16.2 mg B l⁻¹), three different humic substances doses applied to the soil before sowing (H1:0 – H2: 200 – H3: 400 kg HS ha⁻¹) and the commercially renowned Carmen cotton variety in the region (*Gossypium hirsutum* L. Carmen) were determined as the subject of the study. The experiment was set up in a split-plot design with 4 replications, using the same parcels pegged to the same coordinates. Boron treatments were designated as main plots, while humic substance treatments constituted the sub-plots. The material used as a source of humic substance was sprinkled on the soil surface by hand and then mixed with a rake and disc harrow on May 16, 2011 in the first year of the experiment and on May 05, 2012 in the second year of the experiment. Cotton plant was sowed on May 20

in 2011 and on May 23 in 2012, with a planting density of 70×3.5 cm. The distance between rows was adjusted to 70×20 cm and above to have a plant density of 70.000 plants/hectare by making rarefy and single treatment hoe. Irrigation was conducted using the drip irrigation method, with a schedule of every other day, while also considering the daily evaporation losses. This method ensured a more consistent and efficient application of water to meet the specific water requirements of the crops, thus optimizing irrigation practices and resource utilization. In the first year of the experiment, the amount of water applied to the soil through the drip irrigation system was 4874 tons ha⁻¹, while in the second year, it was 5525 tons ha⁻¹. The boron quantities applied along with the irrigation water are provided in Table 4.

Table 4. The amounts of boron applied to the soil with irrigation water throughout the experiment (kg B ha⁻¹)

B rates (mg l ⁻¹) in irrigation water	Amount of Applied Boron (kg B ha ⁻¹)		
	First year	Second year	Total
B1 0.6	3.0	3.3	6.3
B2 1.8	8.7	10	18.7
B3 5.4	26.3	29.8	56.1
B4 16.2	79.0	89.5	168.5

Table 5. Seed cotton yields according to different boron and humic substance applications (kg da⁻¹)

kg HS ha ⁻¹ mg B l ⁻¹	First year				Second year			
	H1	H2	H3	Average	H1	H2	H3	Average
B1	448.3	450.7	458.4	452.5 a	377.3	398.0	341.7	372.3 a
B2	430.9	416.3	437.7	428.3 ab	353.7	377.7	415.3	382.2 a
B3	428.9	400.2	399.8	409.6 bc	314.7	336.3	265.7	305.6 b
B4	385.5	408.5	376.8	390.3 c	94.7	112.0	91.3	99.3 c
Average	423.4	418.9	418.2	420.2	285.1	306.0	278.5	289.9
LSD B	34.51				54.93			
LSD HM	-				-			
LSD B × HM	-				-			

Sampling and Analysis

The samples were collected 3rd true leaf, squaring, blooming, and harvest period in accordance with the phenological stages of cotton. 10 plants were randomly selected for observations and analyses of plant samples in each parcel and in each period (Oosterhuis et al., 1983). These randomly selected plants were removed from the soil along with their roots. Plant samples taken from each parcel were brought to the laboratory straight away in perforated plastic bags and first carefully washed with tap water to remove surface contamination and then passed through pure water twice. Plant samples were dehydrated with drying paper and divided into components such as root, stem, leaf, petiole, square/boll and kept in the drying-oven at 70 °C for 48 hours. Boron concentrations of plant components determinations were made by dry ashing 0.5 g of dry tissue material, placing it in porcelain crucibles, and heating a muffle furnace at 500 °C for 6 h (Kacar & Inal, 2008). The ash was dissolved in 0.1 N H₂SO₄ and B was determined colorimetrically (430 nm) by the Azomethine-H method (Wolf, 1974). Fiber length (mm), fiber fineness (micronaire), fiber strength (g/tex) and gin efficiency (%) on fiber cotton obtained after the ginning of 20 boll samples that reached harvest maturity collected from each parcel have been examined using the High Volume Instrument (HVI) device.

Statistical Analysis

The analysis of variance was performed among different treatments. The significant differences between treatments were evaluated by LSD multiple range tests (P<0.05) using the SPSS statistical software (PASW Statistics 18).

Results and Discussion

Seed Cotton Yield (kg da⁻¹)

In the first year of the study, a significant reduction in seed cotton yield was observed with increasing boron levels. The highest yield was recorded in the B1 treatment (452.5 kg da⁻¹), followed by B2 (428.3 kg da⁻¹), while the

lowest yield was observed in B4 (390.3 kg da⁻¹), indicating a 5.34% decrease in B2, 9.47% in B3, and 13.75% in B4 compared to B1. Although humic substance application slightly improved yield, the differences were not statistically significant. No positive interaction between boron and humic substances on yield was detected in the first year. In the second year of the study, a significant decline in seed cotton yield was also noted with higher boron doses. The highest yield was in the B2 treatment (382.2 kg da⁻¹), followed by B1 (372.3 kg da⁻¹), with the lowest in B4 (99.3 kg da⁻¹), representing a decrease of -2.66% in B2, 17.93% in B3, and 73.32% in B4 relative to B1. Humic substance application outcomes differed from the previous year, with the highest yield was obtained from H2 (306.0 kg da⁻¹) and a statistically insignificant decrease in yield with higher doses of humic substances. No significant interaction between boron and humic substances was found in the second year (Table 5).

These results indicate that increasing boron application reduces unginning cotton yield, with particularly severe losses at doses above the irrigation water toxicity limit (Oertli & Roth, 1969; Ahmed et al., 2008). The residual boron in the soil from the first year contributed to more pronounced yield losses in the second year, leading to plant death in some cases. While cotton exhibited some tolerance at the B3 dose, it failed to tolerate the B4 dose. The highest yield in the first year was achieved with B1, while in the second year, it was with B2, likely due to cotton's higher boron demand and the B2 concentration being at the critical level for optimal yield (Nable et al., 1997; Reid, 2010; Kumar et al., 2018). Humic substance applications showed limited beneficial effects, with no statistically significant impact observed. In the first year, while yields increased in control plots, other plots showed fluctuations, suggesting humic substances may have been ineffective due to interactions with other soil minerals and incubation time (Evangelou et al., 2004). In the second year, H2 increased yield while H3 decreased it, possibly due to humic acid enhancing boron uptake to toxic levels (Ören & Başal, 2006; Karakaya & Paksoy, 2008). These findings align with previous research.

Plant Boron Content (mg kg^{-1})

In the first year of the study, boron content in plants varied according to sampling times and plant organs. Boron levels in all plant organs were similar before and during squaring, increased during flowering, and peaked at harvest. Post-harvest, boron content decreased due to the cessation of irrigation and boron application. The highest boron content was observed in the leaves (B4; 1020 mg B kg^{-1}), with other organs showing negligible differences (Figure 2). The ranking of boron content among organs was leaf > petiole > boll > root > stem. The increase in boron content compared to B1 was 1.45% in B2, 79.10% in B3, and 468.56% in B4. Regarding humic substance applications, significant increases in boron content were noted only in the leaves during the squaring period. The distribution of boron by sampling periods and organs was similar, and the impact of humic substances was statistically insignificant. Interactions between boron and humic substances in the first year did not show significant effects. In the second year, boron accumulation began during pre-squaring and continued similarly during

squaring, peaking during flowering and declining post-harvest. The highest boron content was recorded in the leaves (B4; 2048.4 mg B kg^{-1}), followed by the square/boll organs (659.1 mg B kg^{-1}). Boron content ranking was leaf > boll > petiole > stem > root (Figure 3). Increases compared to B1 were 79.46% in B2, 423.29% in B3, and 1152.08% in B4. Notably, square/boll boron content increased by 147.45% in B2, 403.00% in B3, and 1879.28% in B4 during flowering. Humic substance applications showed varying effects. During the pre-squaring period, significant increases in boron content were observed in the stem, while during squaring, significant increases were noted in leaves and stem. The highest boron content in leaves was from the B4H2 application (2090 mg B kg^{-1}), followed by B4H3 (2039 mg B kg^{-1}). Increased boron doses significantly elevated boron content in organs, but the effect of humic substances varied across organs and sampling periods. In the flowering period, humic substances increased boll boron content up to B3 but decreased it at B4.

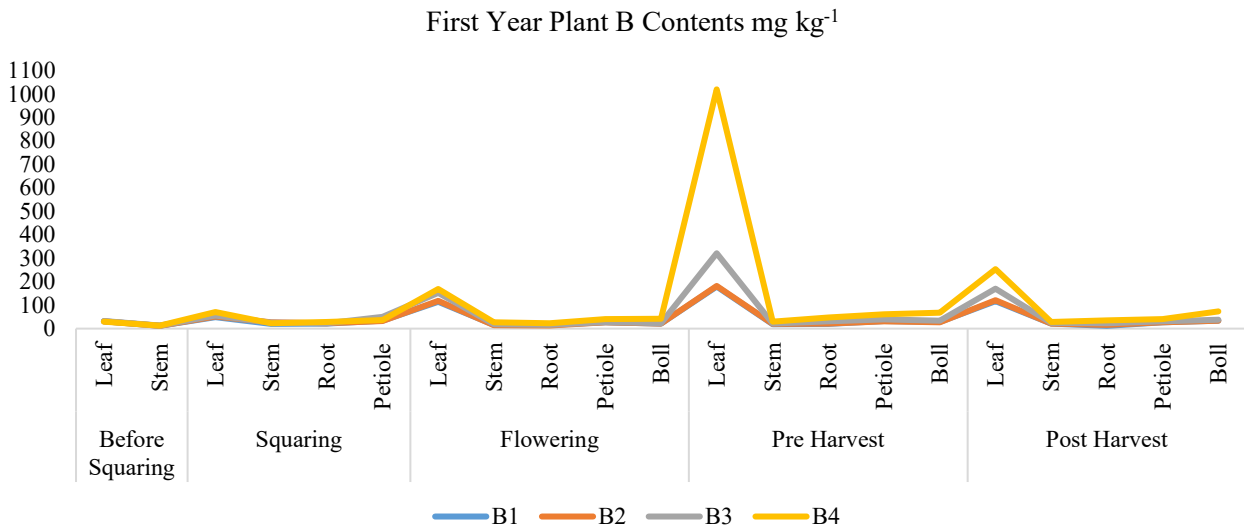


Figure 2. Distribution of plant boron content across phenological stages and plant components in the first year

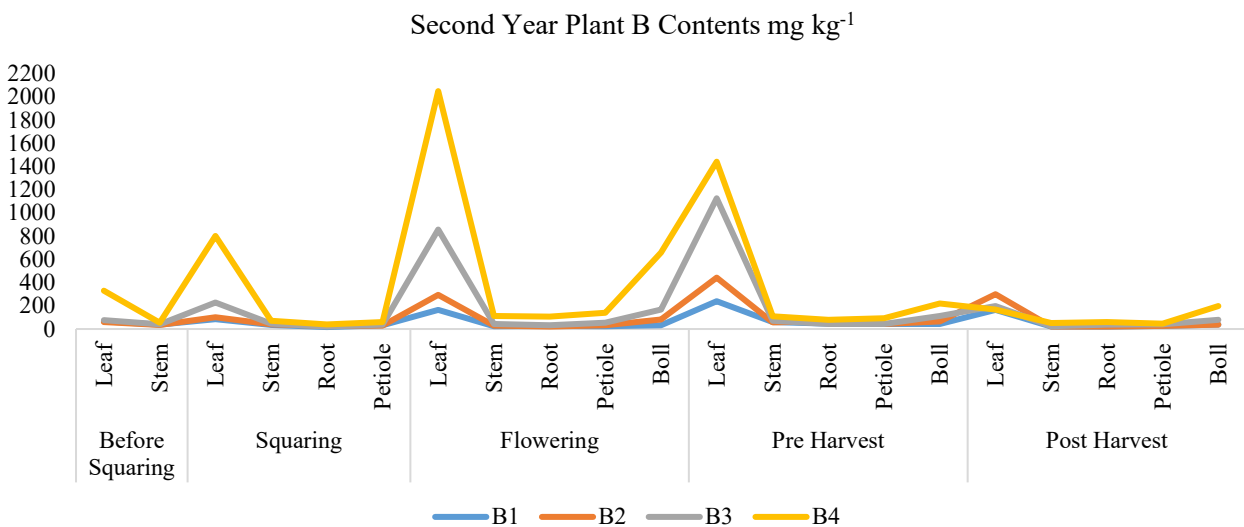


Figure 3. Distribution of plant boron content across phenological stages and plant components in the second year

Overall, increased boron applications were positively correlated with plant boron content, leading to boron toxicity symptoms such as chlorosis and necrosis, especially in older leaves and in severe cases, plant death. In the first year, toxicity was evident only at the B4 dose, while in the second year, both B3 and B4 showed symptoms. The highest accumulation of boron was in leaves, with minor accumulation in generative organs. These observations are consistent with previous studies (Bergmann, 1992; Ahmed et al., 2008; Chatzissavvidis et al., 2008; Chatzissavvidis & Therios, 2010; de Souza Júnior et al., 2022). Humic substance applications improved soil boron availability, likely due to their chelating properties and hormone-like effects on nutrient uptake. However, this positive effect was not clearly reflected in plant boron content, possibly due to interactions with biomass yield and boron content relationships. These findings align with research by Evangelou et al. (2004), Turan & Angin (2004), and Angin et al. (2008).

Fiber Length (mm)

In the first year, the boron treatment B4 achieved the highest fiber length at 28.82 mm, with B2 closely following at 28.64 mm. The lowest fiber length was recorded in the B3 treatment at 28.43 mm. While fiber length tended to increase with higher boron doses, these differences were not statistically significant. Among humic substance applications, the H3 treatment resulted in the longest fiber length at 28.79 mm, followed by H2 at 28.63 mm, though these increases were also statistically insignificant. Interactions between boron and humic substances revealed variations, but these differences did not reach statistical significance. In the second year, the B3 treatment yielded the highest fiber length at 27.86 mm, with B1 at 27.65 mm, and B4 producing the shortest fiber length at 27.38 mm. The impact of increasing boron doses on fiber length was

inconsistent (Table 6). For humic substance applications, the H3 treatment provided the greatest fiber length at 27.68 mm, followed with H1 at 27.67 mm. Fluctuations in fiber length due to varying humic substance doses were noted, but these differences were statistically insignificant. The interaction between boron and humic substances showed that the B3H3 treatment achieved the longest fiber length at 28.24 mm, followed by B4H1 at 28.13 mm. The lowest fiber length, 26.85 mm, was observed with the B4H2 treatment, and these results were statistically significant. In conclusion, Boron applications did not significantly affect fiber elongation or length. These observations are consistent with findings by Eleyan et al. (2014), Ahmed et al. (2010) and Rosolem & Bogiani (2011), which suggest that fiber quality is primarily influenced by genetic factors, with environmental and climatic conditions having a secondary effect. The role of boron toxicity in fiber development is likely modulated by genetic factors. Although humic substance applications showed a positive trend in fiber length, this effect was not statistically significant, in line with Ören (2007).

Fiber Fineness (micronaire, mic)

In the initial year of boron applications, the B1 and B3 treatments yielded the highest fiber fineness at 5.23 mic. The B4 treatment recorded the lowest fineness at 5.00 mic. Although an overall decrease in fiber fineness was observed with increasing boron levels, these variations were not statistically significant. Regarding humic substance applications, the H1 treatment produced the highest fiber fineness at 5.24 mic, followed by H2 at 5.18 mic. Despite a slight reduction in fineness with higher humic substance doses, these differences also lacked statistical significance. When evaluating the interaction between boron and humic substances, no significant statistical differences were detected, although some variability was present (Table 7)..

Table 6. Fiber length measurements according to different boron and humic substance applications (mm)

kg HS ha ⁻¹ mg B l ⁻¹	First year				Second year			
	H1	H2	H3	Average	H1	H2	H3	Average
B1	28.33	28.61	28.71	28.55	27.23	28.06	27.67	27.65
B2	28.40	28.58	28.95	28.64	27.76	27.21	27.65	27.54
B3	28.44	28.70	28.14	28.43	27.54	27.79	28.24	27.86
B4	28.47	28.64	29.36	28.82	28.13	26.85	27.17	27.38
Average	28.41	28.63	28.79	28.61	27.67	27.48	27.68	27.61
LSD B	-				-			
LSD HM	-				-			
LSD B × HM	-				0.99			

Table 7. Fiber fineness measurements according to different boron and humic substance applications (mic)

kg HS ha ⁻¹ mg B l ⁻¹	First year				Second year			
	H1	H2	H3	Average	H1	H2	H3	Average
B1	5.28	5.19	5.24	5.23	4.86	4.87	4.94	4.89a
B2	5.21	5.18	5.08	5.16	5.01	4.99	4.83	4.94a
B3	5.38	5.38	4.93	5.23	5.14	4.58	4.77	4.83a
B4	5.08	4.97	4.96	5.00	4.22	4.62	4.69	4.51b
Average	5.24	5.18	5.05	5.16	4.81	4.76	4.81	4.79
LSD B	-				0.24			
LSD HM	-				-			
LSD B × HM	-				0.52			

Table 8. Fiber strength measurements according to different boron and humic substance applications (g/tex)

kg HS ha ⁻¹ mg B l ⁻¹	First year				Second year			
	H1	H2	H3	Average	H1	H2	H3	Average
B1	33.00	33.45	33.08	33.18	33.43	33.73	33.77	33.64a
B2	33.33	33.10	32.13	32.85	34.47	32.17	33.70	33.44a
B3	33.05	32.83	33.25	33.04	32.70	32.23	34.28	33.07a
B4	33.58	32.28	33.50	33.12	32.05	30.53	32.12	31.57b
Average	33.24	32.91	32.99	33.05	33.16a	32.17b	33.47a	32.93
LSD B	-	-	-	-	1.12	-	-	-
LSD HM	-	-	-	-	0.94	-	-	-
LSD B × HM	-	-	-	-	-	-	-	-

Table 9. Gin efficiency measurements according to different boron and humic substance applications (%)

kg HS ha ⁻¹ mg B l ⁻¹	First year				Second year			
	H1	H2	H3	Average	H1	H2	H3	Average
B1	41.15	41.78	41.84	41.59	39.66	39.65	39.81	39.71ab
B2	41.84	41.67	41.54	41.68	40.26	40.62	39.35	40.08a
B3	42.06	41.17	41.04	41.42	39.88	38.59	38.59	39.02b
B4	41.15	40.90	40.75	40.93	37.11	38.18	38.65	37.98c
Average	41.55	41.38	41.29	41.41	39.23	39.26	39.10	39.20
LSD B	-	-	-	-	0.76	-	-	-
LSD HM	-	-	-	-	-	-	-	-
LSD B × HM	-	-	-	-	1.67	-	-	-

In the second year, the B2 application resulted in the highest fiber fineness at 4.94 mic, followed by B1 at 4.89 mic, with the B4 treatment showing the lowest value at 4.51 mic. This demonstrates a trend of decreasing fiber fineness with increasing boron doses. Among humic substance treatments, highest fineness value was achieved in H3 and H1 treatment at 4.81 mic. Despite observed fluctuations, the differences were again not statistically significant. Notably, in the interaction between boron and humic substances during this period, the B3H1 combination produced the highest fiber fineness at 5.14 mic, followed by B2H1 at 5.01 mic, while the B4H1 treatment resulted in the lowest fineness at 4.22 mic, with these findings were statistically significant. Humic substance applications revealed a decrease in fiber fineness in the first year and negligible impact in the second year. These outcomes contrast with Rosolem & Bogiani's (2011) findings, which suggest that fiber quality is predominantly genetically determined

Fiber Strength (g/tex)

In the first year, the B1 application resulted in the highest fiber strength at 33.18 g/tex, followed closely by the B4 application at 33.12 g/tex. The lowest fiber strength was observed in the B2 application, with a value of 32.85 g/tex. These results indicated that increasing boron doses led to fluctuations in fiber strength, making the effect of boron uncertain. Similarly, with humic substance applications, the H1 application yielded the highest fiber strength at 33.24 g/tex, followed by the H3 application at 32.99 g/tex. As with boron, increasing doses of humic substances caused variations in fiber strength, leading to inconclusive results. The interaction between boron and humic substances also did not produce statistically significant differences in fiber strength (Table 8). In the second year, the B1 application again produced the highest fiber strength at 33.64 g/tex, with the B2 application following at 33.44 g/tex. The lowest fiber strength was recorded in the B4 application at 31.57 g/tex. A decrease

in fiber strength was observed with increasing boron doses, and these differences were statistically significant. For humic substance applications, the H3 application resulted in the highest fiber strength at 33.47 g/tex, followed by the H1 application at 33.16 g/tex. While the H2 application showed a decrease in fiber strength compared to the control, the H3 application led to an increase. These differences were also statistically significant. However, the interaction between boron and humic substances in the second year did not yield statistically significant differences in fiber strength. The results indicate a negative relationship between boron application and fiber strength, with increasing boron doses leading to reduced fiber strength in the second year. Our findings do not align with the results of Eleyan et al. (2014), who reported that boron treatments increased fiber strength. The increase humic substance doses led to a decrease in fiber strength in the first year and had no significant effect in the second year. These findings contradict previous research by Grimes & El-Zik (1990) and Rosolem & Bogiani (2011), which suggested that fiber quality is predominantly influenced by genetic factors.

Gin Efficiency (%)

In the first year, the B2 application resulted in the highest gin efficiency at 41.68%, followed by the B1 application at 41.59%. The lowest value was observed in the B4 application at 40.93%. These findings suggest that increasing boron doses led to fluctuations in gin efficiency, making the impact of boron uncertain. Regarding humic substance applications, the highest gin efficiency was recorded in the H1 application at 41.55%, followed by the H2 application at 41.38%. Although there was a slight decrease in gin efficiency with higher humic substance doses, the differences were statistically insignificant. The interaction between boron and humic substances also did not yield statistically significant differences in gin efficiency (Table 9).

In the second year, the B2 application again produced the highest gin efficiency at 40.08%, with the B1 application following at 39.71%. The lowest efficiency was recorded in the B4 application at 37.98%. A decrease in gin efficiency was noted with increasing boron doses, and these differences were statistically significant. For humic substance applications, the H2 application had the highest gin efficiency at 39.26%, followed by the H1 application at 39.23%. As with boron, humic substance doses caused fluctuations in gin efficiency, leading to an uncertain effect. However, when examining the interaction between boron and humic substances in the second year, the B2H2 application yielded the highest gin efficiency at 40.62%, followed by the B2H1 application at 40.26%. The lowest efficiency was observed in the B4H1 application at 37.11%, and these results were statistically significant. The data indicate that boron toxicity tends to reduce gin efficiency, with the highest efficiency consistently associated with the B2 application in both years. Humic substance applications appeared to decrease efficiency in the first year and had no significant effect in the second year. Karademir and Karademir (2019) revealed that boron applications have non-significant effect on cotton ginning percentage. These findings align with the results of Ören (2007), although the specific impact of boron toxicity on ginning efficiency has not been widely studied.

Conclusion

In the first year of the experiment, boron toxicity effects were minimal, but they became more apparent in the second year due to reduced soil leaching and B accumulation. Boron toxicity most affected seed cotton yield, fiber fineness, followed by strength, gin efficiency, and length. Humic substances did not significantly affect fiber length, or gin efficiency in either year, although higher levels improved fiber strength and fineness in the second year. The interaction between boron and humic substances showed inconsistent results. Boron applications led to a significant increase in plant boron content, with the highest level observed in the leaves during the second year's flowering period (2048 mg B kg⁻¹). This high accumulation capacity in cotton leaves highlights their potential for use as hyperaccumulators in phytoremediation efforts targeting boron-contaminated soils. This experiment aimed to address boron toxicity issues in soils from agricultural and geothermal activities. The study further determined that cotton can tolerate boron concentrations in irrigation water ranging from 1.8 to 5.4 mg B l⁻¹, making it a viable crop in boron-affected regions. These findings provide critical insights into the potential of cotton as a resilient crop in environments with elevated boron levels, underscoring the need for further research to optimize cotton cultivation under such conditions.

Declarations

This manuscript was derived from PhD Thesis.

Ethical Approval Certificate

There is no need for ethical approval.

Author Contribution Statement

Mustafa Ali KAPTAN: Data collection, investigation, formal analysis, writing the original draft, and review and editing

Mehmet AYDIN: Project administration, supervision, conceptualization, methodology, review and editing

Fund Statement

This work was funded by Scientific Research Projects Coordination Unit of Aydın Adnan Menderes University as the project numbered ZRF-12012.

Conflict of Interest

The authors declare no conflict of interest.

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Effects of Different Zinc Concentrations on Culture Growth of *Spirulina platensis* and Its Production of Zinc Enriched as Superfood

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ARTICLE INFO

Research Article

Received : 27.08.2024

Accepted : 17.10.2024

Keywords:

Culture growth

Functional food

Phytochelatin

Supplement

Zinc deficiency

ABSTRACT

With its high protein, vitamin and mineral content, *Spirulina platensis* (SP) is the most widely used microalgae as a food supplement and the most cultivated microalgae for this purpose. Zinc is a regulatory microelement that is incorporated into the structure of many proteins in the cell and is particularly deficient in cereal-based societies. Due to the high adaptability of SP to environments with high metal concentrations and its high capacity to secrete substances called phytochelatin and metal-binding capacity, in this study zinc-enriched SP (ZnSP) was produced by binding metals to SP by organic means. For this purpose, modified media with 4 different Zn concentrations were prepared and SP was cultured in these media. Optical density, chlorophyll-a, phycobiliprotein and dry cell weight analyses were performed to monitor the culture. During the culture period, biomass and filtered culture medium were collected from logarithmic and stationary stages and Zn analyses were performed. The most suitable culture medium and growth conditions were determined to obtain Zn-enriched SP. 338.4 mg kg⁻¹ Zn was measured in SP biomass grown in Zn-3 medium containing 8 mg L⁻¹ Zn. It may be possible to obtain Zn-enriched SP in this medium and under the specified culture conditions, and even this ratio can be increased by adding Zn to the culture medium after the logarithmic stage.

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Introduction

Zinc is the second most abundant trace element after iron and is associated with many enzymes in the human body. It is involved in many metabolic processes such as DNA, RNA and protein synthesis, gene expression, synthesis and regulation of enzymes, release and storage of hormones, growth and development, healing of injuries, reception and transmission of impulses, regulation of central nervous system activities (Tapiero & Tew, 2003). It also regulates the functioning of the immune system and is important for the development and function of immune cells (Hojyo & Fukada, 2016). In addition, its antioxidant properties help to protect cells from oxidative damage caused by free radicals. Zinc deficiency has been shown to stimulate and accelerate apoptosis (Formigari et al., 2007).

Zinc is mainly found in red meat, chicken, fish, seafood, all cereals and dairy products. The bioavailability of zinc varies depending on the source and cooking technique (Lim et al., 2013). Zinc deficiency is common in our country, where we tend to eat a cereal-based diet. The phytate molecule in cereals binds zinc and reduces its

bioavailability (Gupta et al., 2013). Soya protein, which is often used for its high protein content, contains a significant amount of phytate, which has a negative effect on zinc absorption. Amino acids, especially cysteine, histidine and methionine, increase zinc absorption (Hall & King, 2023). The amount of protein in the diet is positively correlated with zinc absorption also.

The World Health Organization (WHO) reports that 800.000 people die each year from zinc deficiency. 450.000 of these are children under the age of five (Das & Green, 2013). Approximately 2 billion people worldwide are affected by zinc deficiency (Yokokawa et al., 2023). The United Nations (United Nations System Standing Committee on Nutrition, 2004) reported that more than half of the world's population is deficient in micronutrients and that the majority of this group are children and women. Zinc deficiency is observed in about 1/3 of the world's population (Walsh et al., 1994). The main reason for zinc deficiency is that the diet does not include foods of animal origin, which are rich in these minerals, and high amounts

of cereals, which are rich in phytate, which binds phosphate and other minerals. In our country, 37 per cent of the daily energy requirement is provided by cereals and cereal products, while only 6 per cent is provided by meat and fish (Güzelcan et al., 2011). The United Nations Food and Agriculture Organisation (FAO) has stated that cereal crops grown on more than 50% of the world's soils are deficient in zinc (FAO, 2023). In our country, about 50% of soils are deficient in zinc (Kınacı et al., 2010). Iron deficiency was found in 49.7%, zinc deficiency in 18.9% and subclinical vitamin A deficiency in 14.7% of 334 children included in the study (Ekemen et al., 2018). Zinc, which is very effective in the growth and development of children and in human health, should be supplied to the body through a balanced diet and its deficiency should be corrected.

Spirulina platensis (SP), a member of the Oscillatoriaceae family, is being touted as a functional food with a protein content of up to 50-70%, high levels of polysaccharides, essential fatty acids and amino acids, vitamins (vitamin B types, vitamin E, vitamin A precursors), minerals and antioxidant pigments (phycobiliproteins, carotenoids, chlorophyll-a). SP is the most cultivated microalgae in the world and is hailed as the superfood of the future. The Global Spirulina Market Size is estimated to be USD 480 Million in 2021 and is projected to reach a market size of USD 1.1 Billion by 2030, at a CAGR (compound annual growth rate) of 10.4% from 2022 to 2030 (Spirulina Market Size, 2022).

Microalgae have the potential to be used in the removal of heavy metals because of their high bioremediation capacity. The phytochelatins they produce and the exopolysaccharides they secrete bind the metal ions in the environment, thus removing these substances from the environment. The most used medium for the culture of SP is the Zarrouk culture medium. This medium contains 0.05 mg L⁻¹ Zn. According to FoodData central data 2 mg zinc 100 g⁻¹ *Spirulina*. On the other hand, it is known that SP can survive in high concentrations of zinc and to adsorb zinc (Zhou et al., 2018).

The high ratio of protein and essential amino acids (methionine (14 mg g⁻¹), cysteine (7 mg g⁻¹), histidine (10 mg g⁻¹)) and Vitamin B6 (8 µg g⁻¹) of *Spirulina* increases the bioavailability of zinc (Liestianty et al., 2019). In addition, there is no phytate molecule in SP, which is known to bind divalent cations and reduce their bioavailability (Ebid et al., 2022). These properties suggest the cultivation of SP in a high zinc medium and the production of zinc-enriched spirulina (ZnSP). People who consume such a product will benefit from the protein, vitamins and antioxidant pigments of SP and meet their zinc requirements.

Based on this information and considerations, the aim of this study was to optimize the culture of SP in Zarrouk media modified in terms of Zn concentration and to obtain ZnSP biomass. For this purpose, SP was cultured in media with four different Zn concentrations and culture growth parameters were monitored twice a week. Zn analyses of both culture medium and biomass were performed in logarithmic and stationary phases. The culture medium with the highest Zn concentration (Zn-3) was found to be the optimum growth medium for obtaining ZnSP.

Materials and Methods

Materials

For stock culture, SP was obtained from MAKUMACC with strain number MAKUMACC-093. SP was cultivated in the Zarrouk medium (Table 1) (Zarrouk, 1966). In 5 L flasks, 4 L medium was added and autoclaved at 121°C for 30 min. After cooling, SP was inoculated in the flasks which kept in an incubator at 30 °C, 12:12 photoperiod, for aeration gas flow rate was adjusted to 1Lmin⁻¹, pH value was 8.7-9 and a light intensity of 200 µmol photons m⁻²s⁻¹ of fluorescent lights. All process was carried out under sterile conditions.

Methods

Zinc Modifications and Culturing Methods

In this study, Zarrouk medium was determined as the control group, and also media with 3 different zinc concentrations were prepared. As totally 4 different culture media were created (Control, Zn-1, Zn-2, Zn-3) and SP was inoculated to each medium with the close initial OD values (Table 2). Cultures in all media were sustained for 37 days and culture growth parameters were detected every other day.

Detecting of Culture Growth Parameters

For the determination of the cell density, the optical density (OD) values were measured at 680 and 750 nm in the spectrophotometer (Shimadzu UV-1650). 1 mL of sample from each culture medium was taken homogenously and placed in the cuvette and measured in 3 replicates (Santos-Ballardo et al., 2015).

One of the parameters that gives information about the culture status in the growth process of the culture is the Dry Cell Weight (DCW, mg L⁻¹) in the culture. For this, on the same days when OD is determined, 40 mL of samples were taken from the cultures, filtered with a glass fiber filter (Whatman GF/C, 1.2 mm, UK), dried in an oven at 40°C and determined in the following equation (1) (Vonshak, 1986).

Table 1. Recipe of culture media (Zarrouk, 1966).

Zarrouk culture medium	
Nutrients	Amount (g L ⁻¹)
NaCl	1.0
CaCl ₂	0.04
NaNO ₃	2.5
EDTA (Na)	0.08
K ₂ SO ₄	1.0
MgSO ₄ .7H ₂ O	0.2
NaHCO ₃	16.8
K ₂ HPO ₄	0.5
FeSO ₄ .7H ₂ O	0.01 mL ^B
Trace metal mix ^C	1 ml L ⁻¹

B: 10 g of FeSO₄.7H₂O in 100 ml 1N HCl; C: ZnSO₄.7H₂O, 0.222 mg L⁻¹; CuSO₄.5H₂O, 0.079 mg L⁻¹; MoO₃, 0.015 mg L⁻¹; H₃BO₃, 2.88 mg L⁻¹; MnCl₂.4H₂O, 1.81 mg L⁻¹

Table 2. Zinc concentrations and initial optical density values of all culture media.

Media	Control	Zn-1	Zn-2	Zn-3
Zn Concentrations (mg L ⁻¹)	0.05	2	4	8
Initial OD ₆₈₀ values (abs)	0.391	0.405	0.377	0.356
Initial OD ₇₅₀ values (abs)	0.290	0.314	0.293	0.285

$$DCW = FFW - FIW \quad (1)$$

DCW: Dry cell weight (DCW)

FFW: Filter final weight

FIW: Filter initial weight (g L⁻¹)

Specific Growth Rate (SGR, μ), Doubling Time (Dt, day), and Biomass Productivity (BP, g L⁻¹day⁻¹) values were calculated separately for each type established. The calculations of these values were done as follows.

The SGR of SP was calculated using Equation (2) (Chia et al., 2013).

$$\mu = \frac{\ln\left(\frac{X_2}{X_1}\right)}{t_2 - t_1} \quad (2)$$

Where μ is specific growth rate (day⁻¹) and X_1 and X_2 are the OD₇₅₀ at time t_1 and t_2 (day), respectively.

Dt (Godoy-Hernández et al. 2006) and BP (Hempel et al. 2012) were calculated with the following equations.

$$Dt = 0.6931 / \mu \quad (3)$$

$$BP \text{ (g L}^{-1} \text{ day}^{-1}\text{)} = (X_2 - X_1) / (t_2 - t_1) \quad (4)$$

X_1 and X_2 are the dry weight of biomass at times t_1 (culture starting time) and t_2 (culture finishing time).

Chlorophyll a (chl-a) amount was detected according to (Lichtenthaler, 1987 and Wellburn, 1994) for observing culture growth. 1 mL culture sample was centrifuged, the supernatant was discarded. 2 mL methanol (99%) was added onto the pellet. It was incubated in a hot water bath at 60°C for 30 minutes. Then 10 min. incubated on ice and at 13000 rpm for 5 min. it was centrifuged. After centrifugation, the pellet should be completely white, if not, the extraction process was repeated. Methanol was used as blank, extraction was measured in the spectrophotometer at 665.2, 652.4 and 470 nm. Calculation was made using the formula below.

$$\text{Chlorophyll a (chl-a)} = 16.72(A_{665.2}) - 9.16(A_{652.4}) \quad (5)$$

To determine the amount of phycobiliprotein concentration, phycocyanin, phycoerythrin and allophycocyanin amounts were measured. For extraction 10 mM sodium phosphate buffer (pH 7.0) was used. All other processes were made according to Arashiro et al., (2020). The optical density of the extracts was measured in a spectrophotometer (Shimadzu UV-1280, Japan), 280, 562, 615 and 652 nm. The amounts of phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) were calculated according to formulas.

$$PC \text{ (mg / mL)} = [A_{615} - (0.474 \times A_{652})] / 5.34 \quad (6)$$

$$APC \text{ (mg / mL)} = [A_{652} - (0.208 \times A_{615})] / 5.09 \quad (7)$$

$$PE \text{ (mg/mL)} = [A_{562} - (2.41 \times PC) - (0.849 \times APC)] / 9.62 \quad (8)$$

Zn Measurement of Culture Media and Microalgae Biomass

To detect the Zn amount in biomass and in the culture media, biomass and also culture medium were harvested at two different times during the culture period. The first at the logarithmic stage of culture (16th day) and the second at the stationary stage of culture (37th day). Zn amounts of

microalgae biomass and filtered culture media were determined by Perkin Elmer ICPOES Optima 8000. Approximately, 0.5 g dried samples were dissolved in 9 mL nitric acid and 3 mL hydrochloric acid mixture (HNO₃: HCl=3:1,v/v). After preliminary digestion, the mixture was placed in a microwave digestion system (Milestone Stard-D) for further digestion. The oven temperature was 110 °C for 15 min. Then, the sample was cooled to room temperature and measured by ICPOES.

Statistical Analysis

The data consisted of the means and standard deviations of at least three replicates. The obtained data were statistically examined at 0.05 using the least significant difference (LSD).

Results and Discussion

Culture Growth Parameters

In microalgae culture studies, regular monitoring of culture growth parameters from the beginning to the end of the culture is of great importance in terms of determining the general condition of the culture and the harvest time. For this reason, optical density, dry cell weight, chl-a and total phycobiliprotein values were measured every other day during the culture period and culture growth curves were prepared with these data (Figure 3, 4, 5, 6 and 7).

Cultures in all media were started with equal initial OD values and optical density values of all culture media were measured every other day. It was observed that SP showed growth in all media but according to Figure 3 and 4, in general maximum OD values were recorded in Zn-3 medium. Maximum OD value is recorded as 1.38 (680 nm) and as 1.19 (750 nm) in Zn-3 medium on 30th day of culture. For the biomass that obtained from other media have lower OD values than the Zn-3 medium. This shows that increasing of Zn content in the culture medium causes a huge optical density. However, this result may be due to the intensive culture caused by long filaments, as shown in Figure 2. If the morphological characteristics are examined in detail, it can be seen that the longest filament is in SP in Zn-3 medium.

When examining the DCW results, it can be seen that the highest dry cell weight was measured in the Zn-1 medium (0.00126 g mL⁻¹) on day 27 (Figure 5). This was followed by Zn-3, Zn-2 and control groups on the same day. This shows that the Zn-3 medium with a high OD value does not have a high DCW value at the same time. In fact, the high OD value of Zn-3 medium is due to long filaments.

With using DCW values, BP and Dt values were estimated (Table 3). The lowest BP value was recorded in the Zn-3 medium. However, the highest OD values were also recorded in this medium. These results show that long filaments (Figure 2) in this medium caused high OD values, but due to high Zn concentrations there was a stress on the microalgae so that the same high biomass productivity values were not obtained in this medium. It is also known that filament elongation is related to abiotic stress in the culture medium (Faluweki & Lucas, 2022) and it was clear from these results that a high Zn concentration created a stressful situation for microalgae and there was no high biomass production

Table 3. The results of estimation of Specific Growth Rate (SGR), Doubling Time (DT) and Biomass Productivity (BP) values of all culture media.

Media	Control	Zn-1	Zn-2	Zn-3
SGR (day ⁻¹)	0.0458±0.001	0.0253±0.0003	0.0256±0.002	0.0385±0.01
DT (day)	15.1172±0.0001	27.3429±0.001	27.0310±0.01	17.9921±0.001
BP (gL ⁻¹ day ⁻¹)	0.0308±0.0021	0.0310±0.002	0.0490±0.001	0.0276±0.001

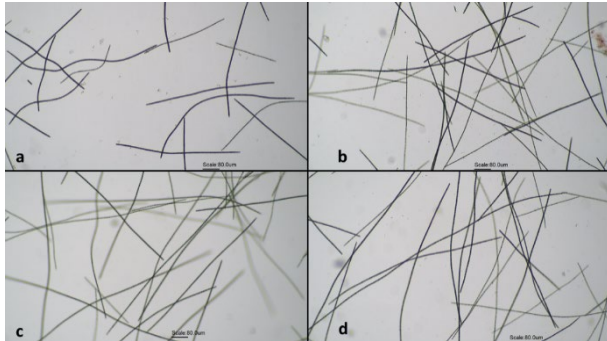


Figure 1. Light micrographs (10X) of *Spirulina platensis* in the four different culture media during the cultivation period (Scale bar: 80.0 µm). a: Zn Control, b: Zn-1; c: Zn-2; d: Zn-3.

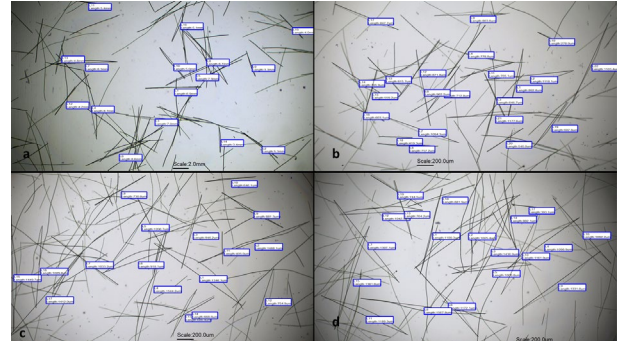


Figure 2. Filament measurements (4X) of *Spirulina platensis* in four different culture media during the cultivation period. a: Zn Control, b: Zn-1; c: Zn-2; d: Zn-3.

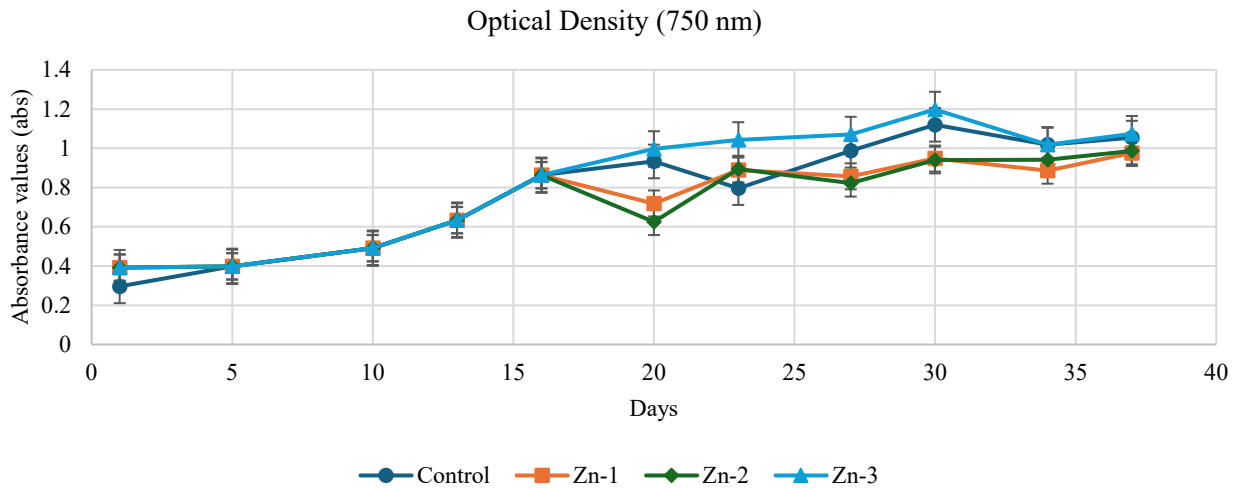


Figure 3. OD₇₅₀ of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05)

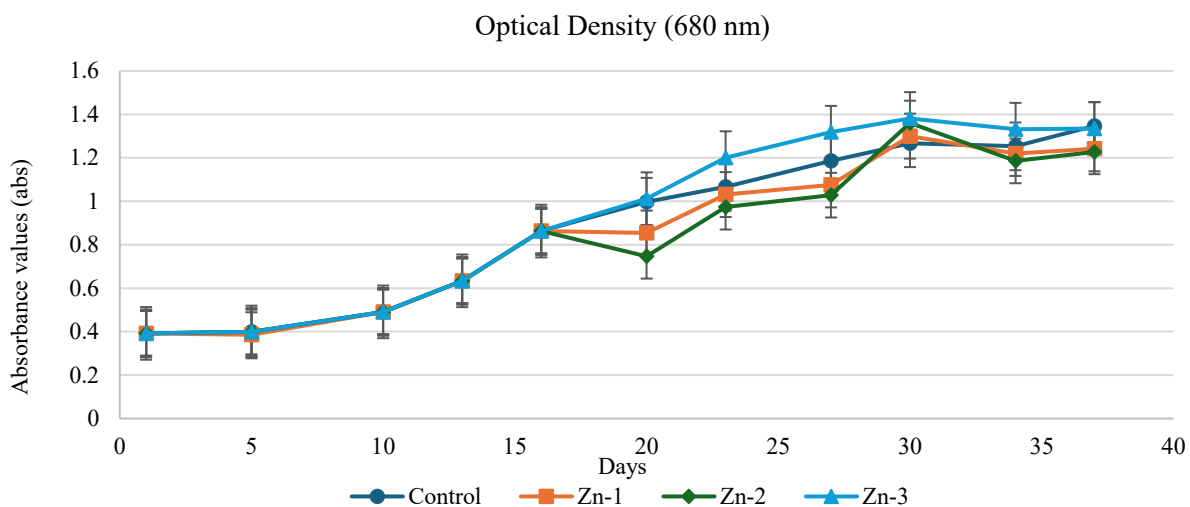


Figure 4. OD₆₈₀ of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05).

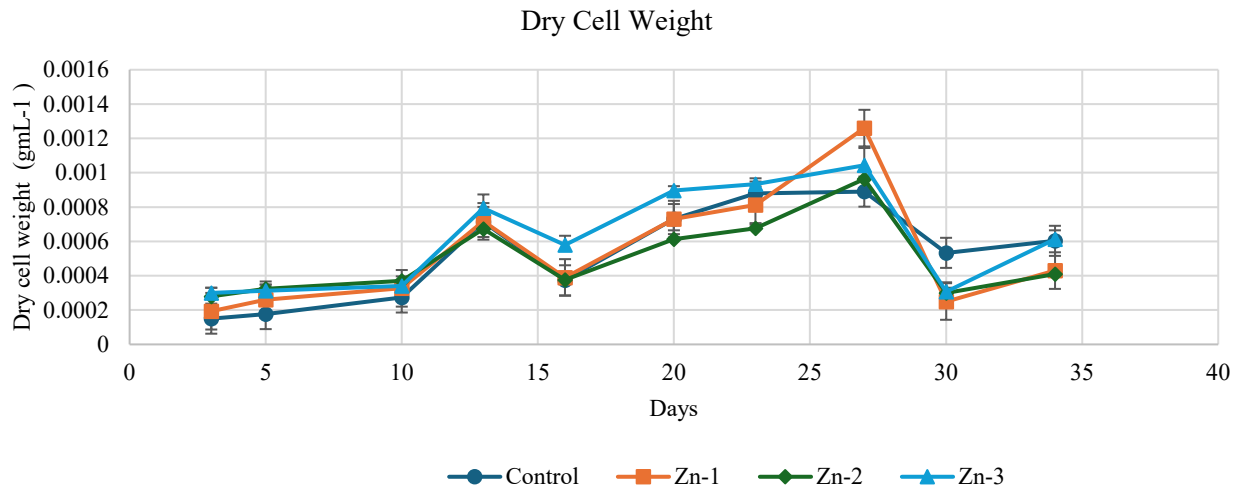


Figure 5. Dry cell weight (DCW) of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05).

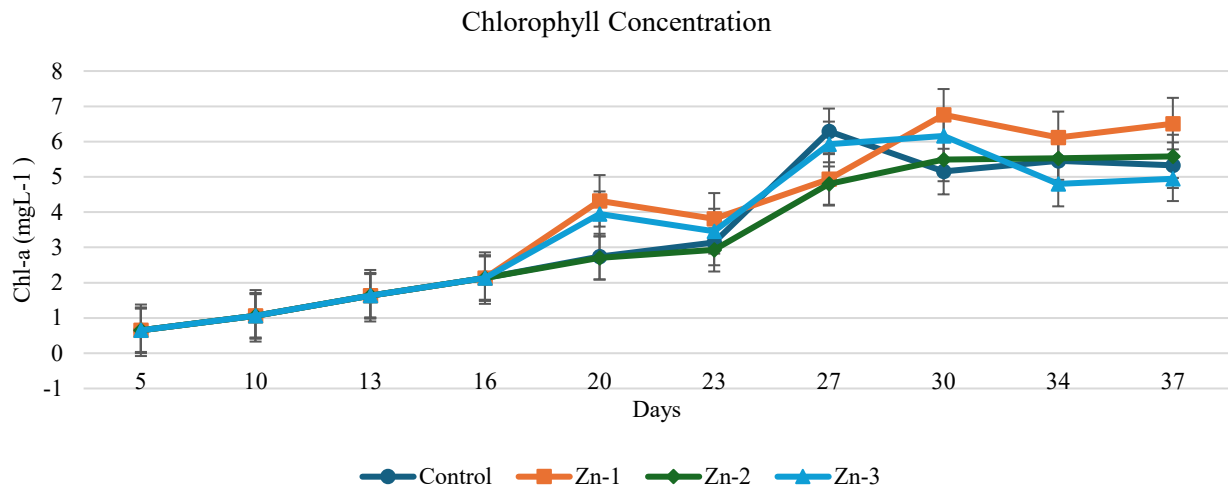


Figure 6. Chlorophyll-a (Chl-a) values of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05).

BP results show that for obtaining the high biomass production, the best medium is Zn-2 medium. Because the highest BP value ($0.049 \text{ g L}^{-1} \text{ day}^{-1}$) was recorded in this medium. On the other hand, SGRs were estimated with the OD values and showed in Table 3. The highest SGR value (0.0458 day^{-1}) and the shortest DT (15.117 day) were found in the control medium, which is the most commonly used medium in SP culture. This result is quite normal as this is an optimized medium. The aim of this study was not to monitor the survival of SP at optimum Zn concentrations, but to investigate its viability at increasing Zn concentration, to optimize this medium and culture conditions and to obtain ZnSP biomass. The second highest SGR (0.0385 day^{-1}) was observed in Zn-3. This result shows that SP can survive in culture medium with high Zn concentration, but the BP value of this medium does not increase in the same way. Considering the commercial production process of the ZnSP, high biomass productivity is required. Therefore, Zn-2 medium, which is the medium with both high BP and SGR, seems to be more suitable for commercial production. Or, with the different application

suggestions we have given below, this problem can be overcome and SP biomass containing more Zn can be obtained.

From the analysis of Figure 6 it can be seen that the highest chl-a value (6.12 mgL^{-1}) was in the Zn-1 medium on day 30. In fact, SP tolerated all Zn concentrations and adapted to all four media. However, the fact that the amount of chl-a was higher in the Zn-1 medium shows that SP lived in a healthier way in this medium. On the same day, the amount of phycobiliprotein was lowest (0.040 mgL^{-1}) in the Zn-1 medium and highest (0.065 mgL^{-1}) in the Zn-3 medium. On the 30th day it was observed that the phycobiliprotein value of Zn-3 medium was higher than the other media. This indicates that SP was stressed in the Zn-3 medium but adapted to this medium with high phycobiliproteins. It is known that the amount of phycobiliprotein in cyanobacteria increases under stress conditions (Carnicas et al., 1999). As can be seen from the above results, the reason for the higher OD value of the Zn-3 medium may be the high phycobiliprotein value as well as its long filaments.

Zn content of Microalgae Biomass and Filtrated Culture Media

As the Zn concentration in the culture medium increases, the amount of Zn in the SP biomass also increases (Figure 8). It can be seen that SP can easily survive in the medium containing higher concentrations of Zn compared to the control medium and can absorb Zn into the biomass. The logarithmic stage and the stationary stage were sampled during the culture period and it was observed that the amount of Zn in the biomass taken from the stationary stage was high in 4 different media. The highest Zn concentration (338.44 mgkg⁻¹) was reached in the biomass taken from the stationary stage in Zn-3 medium, and a 33.8-fold increase was observed compared to the biomass taken from the control group. Similarly, Zhou et al., (2018) reported a 27.2-fold increase in the total zinc content of the medium containing 4 mgL⁻¹ Zn compared to the control. In the same medium, the highest amount of Zn was observed in the biomass (211.33 mgkg⁻¹) taken at the

logarithmic stage. Analyzing the graphs of the culture growth parameters, it can be seen that the Zn concentration in the Zn-3 medium is not high for SP and SP tolerates this high Zn concentration by showing healthy growth. These results show that our modified Zn-3 medium is a very suitable medium for obtaining ZnSP and that our physical conditions are designed in such a way as to have a positive effect on growth. On the other hand, some studies indicate that increasing the amount of Zn in the culture medium does not always increase the amount of Zn in the biomass, and sometimes even high Zn concentration inhibits Zn accumulation (Zdziebłowska et al., 2024). Ghafari et al., (2015) explained that much higher Zn concentrations increased cell proliferation and lipid degradation in their study with different microalgal species.

The Zn content in the SP biomass was analyzed as well as the Zn content in the filtered culture medium remaining after removal of the biomass, and the results are shown in Figure 9.

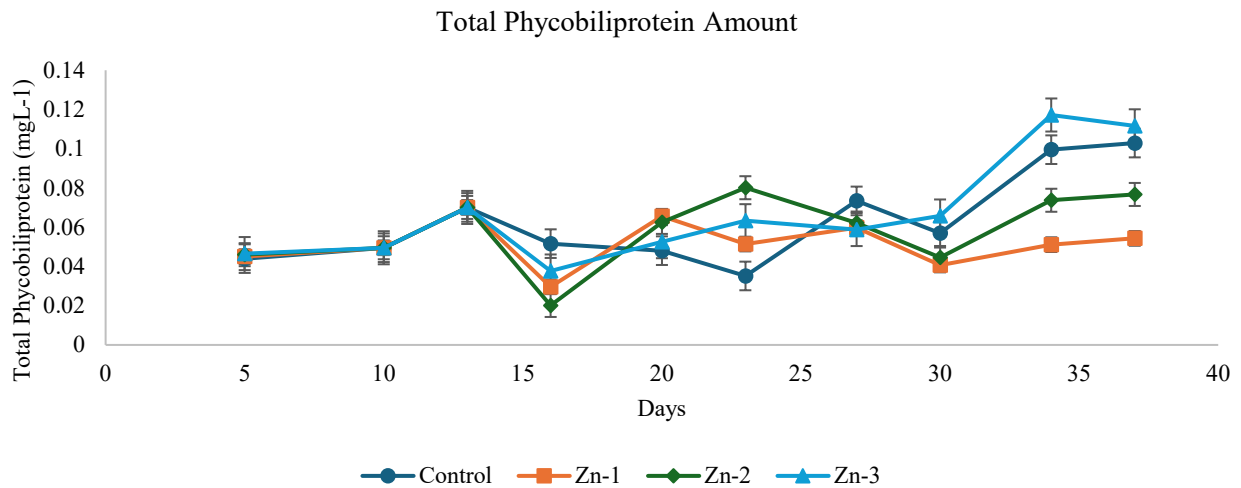


Figure 7. Total phycobiliprotein values of *Spirulina platensis* in four different culture media during the cultivation period. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05).

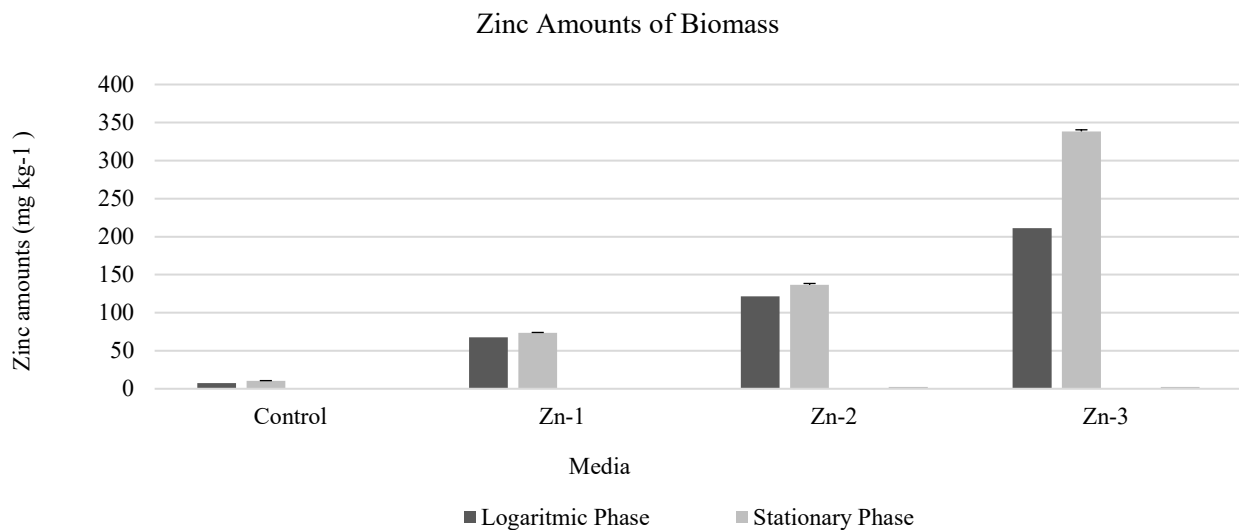


Figure 8. Zinc amounts of *Spirulina platensis* biomass were obtained from four different culture media and two different culture phases. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05)

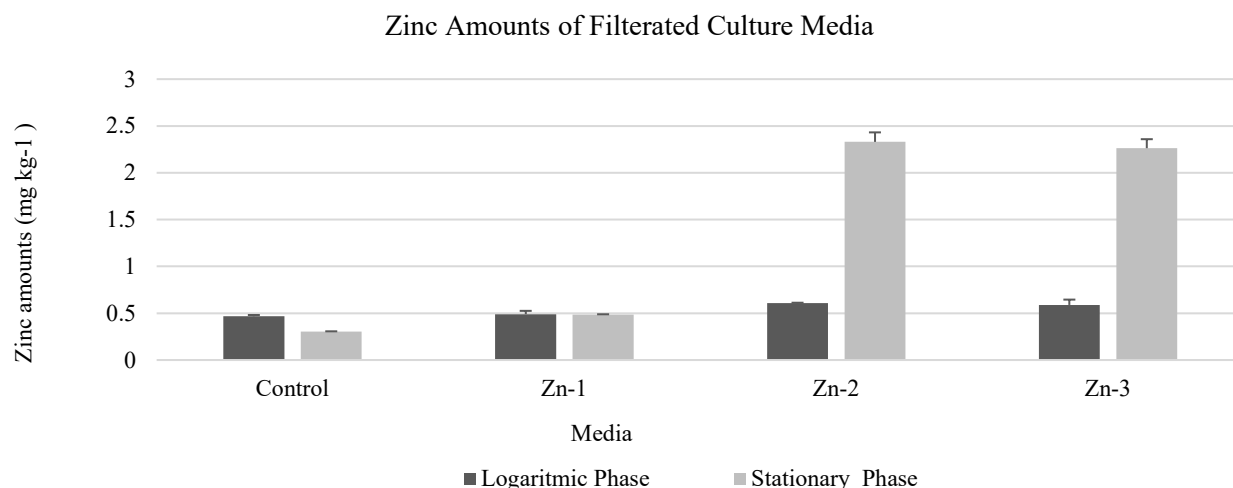


Figure 9. Zinc amounts of filtrated culture media were obtained from four different culture media and two different culture phases. Error bars represent the standard deviation among the three replicates of each culture condition (n=3, p<0.05).

In the control and Zn-1 media, since SP was able to completely consume the available Zn, there were no major differences in the amount of Zn between the logarithmic phase and the stationary phase. For example, the amount of Zn in the filtered culture medium obtained from the Zn-1 medium was 0.49 mgkg⁻¹ in the logarithmic phase and 0.48 mgkg⁻¹ in the stationary phase. However, since Zn concentrations were higher in Zn-2 and Zn-3 media, there was a difference between logarithmic and stationary phase in terms of Zn concentration. This situation shows that Zn in the culture medium can be taken up as much as it can be taken up by the SP, and increasing the amount of Zn further will not increase the amount of Zn in the SP and the cells will not be able to take it up. The results in Figure 9 also show that the Zn concentrations we modified in our study are very suitable for our purpose, and increasing the amount of Zn further may stress the cell. Furthermore, when analyzing the SGR and BP values (Table 3), it can be seen that the highest SGR value belongs to the control medium (0.045 day⁻¹), followed by the Zn-3 medium (0.038 day⁻¹). The fact that the Zn-3 medium with the highest Zn concentration has an SGR value close to that of the control medium indicates that this medium can be used to obtain ZnSP biomass.

On the other hand, Zn-3 medium had the lowest BP value (0.027 g L⁻¹ day⁻¹), suggesting that this medium cannot be used for this purpose. In Zn-3 medium, the cells were stressed, so they started to divide and elongate, and as a result the highest OD values were recorded in this medium due to elongated filaments (Figs 3 and 4). Again, the high levels of phycobiliprotein, which has a high antioxidant capacity, in the Zn-3 medium, especially in the stationary phase, indicate that the cells were stressed (Figure 7).

At this point it is necessary to consider how the metals can be used in the cell. Cyanobacteria take up metals either by binding them to functional groups in the cell membrane or by accumulating them in the interior of the cell (Pane et al., 2008.). High levels of metal uptake are associated with the production of substances called phytochelatin, which

are responsible for detoxifying heavy metals (Pawlik-Skowronska, 2001). These molecules are present in the cell as metal-binding proteins and provide resistance to high metal concentrations, especially in cyanobacteria (Harada et al., 2004). In fact, it is necessary to measure the phytochelatin concentration of the cell to make a more realistic inference as to which medium should be chosen.

When all these data are evaluated together, it can be seen that Zn-3 medium can be used for the production of ZnSP, but low biomass productivity will be encountered. To overcome this problem, starting the culture with a high OD value can shorten the culture period and more biomass can be obtained in a shorter time. Another method is to add Zn to the culture medium after the logarithmic phase. This practice also allows the cells to be exposed to a high concentration of Zn for a shorter period of time, thus preventing the cells from experiencing prolonged stress. Zinicovscaia et al., (2021) emphasised that the time of Zn addition to the culture is important in determining tolerance to metal ions. In such applications, the desired ZnSP production can be achieved with higher biomass efficiency. It is of great importance to carry out ZnSP, known as superfood, organically by biological processes and to determine the most suitable and efficient modified culture media and culture conditions.

In fact, there is not much data in the literature on the metal bioaccumulation capacity of microalgae and the factors that influence it. It is almost impossible to draw complete and accurate conclusions. This is because the rate of accumulation of a metal in a microalgae cell varies depending on the type of microalgae, the culture conditions, the concentration of the metal and other metals in the culture medium. For this reason, the determination of the bioaccumulation rates of microalgae in culture media containing the most deficient metal in society and at different concentrations of this metal is important both to fill the gap in the literature and to obtain a commercial product. Our study has been a study that serves this field and determines the most suitable Zn concentration and culture conditions for the production of ZnSP.

Conclusion

Determining which modified culture medium to use and under what conditions to produce ZnSP is a complex process with many parameters to monitor. The Zn binding capacity of SP varies depending on the concentration of Zn and other metals in the culture medium, the culture conditions and the time of addition of Zn to the culture medium. In this study, we added Zn at the beginning of the culture because we wanted Zn not only to bind to the cell membrane surface, but also to enter the cell and bind to proteins through organic processes. In our study designed for this purpose, we were able to achieve 338.44 mg kg⁻¹ Zn in Zn-3 medium, and most of this Zn is bound to metalloproteins in the cell due to prolonged treatment with the cell. In future studies, higher concentrations of Zn can be tested, and higher levels of Zn-bound SP production can be attempted. Considering that commercially produced ZnSP should provide 1/3 of the daily intake of 10 mg Zn with 3 tablets of 1 mg, the Zn-enriched SP to be produced should have a ratio of 1000 mg kg⁻¹.

Declarations

Author Contribution Statement

Fusun Akgül: Data collection, investigation, writing the original draft.

Rıza Akgül: Methodology, review and editing.

Fund Statement

This work was funded by KOSGEB Ar-Ge, Ür-Ge ve İnovasyon Destek Programı.

Conflict of Interest

The authors declare no conflict of interest.

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Combining Pasture- and Animal-Based Factors to Predict Herbage or Dry Matter Intake of Lambs Grazing on Cocksfoot, Meadow Fescue and Tall Fescue Pastures

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ARTICLE INFO

ABSTRACT

Research Article

Received : 27.08.2024

Accepted : 24.09.2024

Keywords:

Cocksfoot

Dry matter intake

Grazing

Meadow fescue

Tall fescue

In this study, it was aimed to establish the correlations between actual dry matter intake (DMI) and some animal (body weight, (BW)) and pasture (crude protein (CP); neutral detergent fiber (NDF); *in vitro* dry matter digestibility (IVDMD); dry matter yield (DMY); herbage allowance (HA); herbage mass (HM); metabolizable energy (ME); relative forage quality (RFQ); total digestible nutrients (TDN)) based factors to formulate precise regression equations for DMI prediction. For this purpose, data (n = 36, 2 years × 3 blocks × 6 data collection) were utilized for two grazing seasons (2020–2021) on cocksfoot (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) and tall fescue (*Festuca arundinacea*) mixed pastures with Karayaka male lambs at an average age of 2 months for 60 days in each season. Positive correlations were determined between DMI and BW (0.777), HA (0.814), DMY (0.844), and NDF (0.609), while DMI had negative correlations with IVDMD (-0.738), RFQ (-0.357), CP (-0.209), TDN (-0.177) and ME (-0.039). In addition, animal and pasture-based factors were evaluated by principal component analysis to determine the in-cooperating variables in variance. As a result, equations were developed by using parameters with high correlation coefficient and the best-fit 3 equations for predicting DMI of lambs grazing cocksfoot, meadow fescue and tall fescue pastures: (I) $-1224.09 + 39.90BW$ (kg) + 33.69HA (kg DM/ kg BW) + 8.22NDF (% of DM), $r^2=0.815$, II) $-701.47 + 18.96BW$ (kg) + 673.61DMY (kg/ per square meters) + 8.19NDF (% of DM), $r^2=0.807$, III) $-325.32 + 43.49HA$ (kg DM/kg BW) - 2.21IVDMD (%) + 8.57NDF (%), $r^2=0.786$.

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Introduction

Pastures and forage crops are still significant ingredients in the diets of domestic ruminants (Pollock et al., 2022). Moreover, grazing on natural grasslands and/or sown pastures is the most common practice in feeding small ruminants worldwide (Pulina et al., 2013). As such, the production performance of grazing small ruminants is highly dependent upon the intake of desirable forage crops on the pasture. Farmers and nutritionists should routinely observe the effect of forage quality on animal production. In pasture-based systems (PBS), voluntary herbage intake (HI) or dry matter intake (DMI) by domestic ruminants is controlled by a complex combination of external and internal physical and physiological factors that interact with various environmental, pastoral, and experiential influences on the animal (Decruyenaere et al., 2009; Roca

& Gonzalez, 2013; Galyean & Gunter, 2016; Pollock et al., 2022). Therefore, predicting HI or DMI intake on pastures (Akdağ & Ocak, 2019; Woli et al., 2023) is crucial in identifying if animals need supplementations to meet their nutrient requirements, tracking voluntary feed intake, achieving desired growth performance, and maintaining PBS. Because DMI is a function of both the intake potential of the pasture forage crops and the nutrient demand and/or requirement of animals, it can be affected by the dry matter (DM) content of the herbage on offer or the herbage mass (HM), which is the weight of the above-ground herbage DM (Tharmaraj et al., 2003). In the PBS, HI is, therefore, a function of animals' energy requirement or grazing behavior, or HM on pasture, which is unconnected to animal-related factors (Woli et al., 2023).

Contributions of pasture-based factors, such as nutritive value [crude protein (CP) and neutral detergent fiber (NDF) contents, *in vitro* dry matter digestibility (IVDMD), metabolizable energy (ME), total digestible nutrients (TDN), and relative forage quality (RFQ)] and quantity [dry matter yield (DMY) and herbage allowance (HA)] and animal-based factor [body weight (BW)] to variance in HI or DMI is not equal. However, the nature of grazing animals' responses to forage nutritive value (Sollenberger & Vanzant, 2011) or quantity regarding HM or HA has been well characterized (Sollenberger & Vanzant, 2011; Woli et al., 2023).

The widely utilized equations for approximating HI based only on pasture quantity and quality characteristics are $DMI (\% \text{ of body weight (BW)}) = 120/\% \text{ NDF}$ (Undersander, 2003), $DMI = -2.318 + 0.442 \times CP - 0.010 \times CP^2 - 0.0638 \times TDN + 0.000922 \times TDN^2 + 0.180 \times ADF - 0.00196 \times ADP^2 - 0.00529 \times CP \times ADF$ and $DMI = 120/\text{NDF} + (\text{NDFD} - 45) \times 0.374/1350 \times 100$ (Atalay & Kahriman, 2020; Aydin & Ocak, 2022). On the other hand, the herbage allowance (HA), forage DM (kg) per kg of animal, as defined by Sollenberger et al. (2005), enables the inclusion of indirect BW-related animal factors in predicting herbage or dry matter intake. Animal and pasture-based factors must be jointly considered in estimating HI and/or DMI of grazing ruminants, and precise equations should be formulated to this end.

In many studies conducted on dairy cattle, beef cattle, sheep, and lambs grazing on pastures, many methods have been compared for determining and/or estimating DMI (Piasentier et al., 1995; Malossini et al., 1996; Macoon et al., 2003; Smit et al., 2005; Undi et al., 2008; Decruyenaere et al., 2012). Among the methods used in these studies, some of them are prediction and some are determination; marker techniques, performance-based equations, nutrient requirements-based equations, and clipping method one of the most accurate methods (Decruyenaere et al., 2015) when used with short-term appropriate stocking rate. When scrutinizing the findings of these studies, it becomes apparent that discrepancies of up to 300% exist in the estimation and determination of DMI of grazing ruminants. It is believed that such discrepancies primarily stem from the utilization of techniques that are predominantly reliant on either animal or pasture-based factors, not integrated.

Integrating a continuously detected trait such as BW of grazing animals with the quality and quantity characteristics of the pasture and developing accurate equations will be important for meeting the nutrient requirements of animals, achieving the desired

performance, deciding the time and duration of grazing, and sustainable animal husbandry in pastures. Therefore, this study aimed (i) to determine the relationships between actual DMI of lambs grazing on mixed-grass pasture (MGP) (cocksfoot (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) and tall fescue (*Festuca arundinacea*)) and quality traits of these pastures, (ii) identify the most influential components of variation in a grazing-based lamb growth model in MGP using principal component analysis (PCA) and (iii) establish the correlations between dry matter intake (DMI) and pasture-based factors as well as components deemed influential in the lamb growth model, and subsequently, to formulate precise regression equations for DMI estimation.

Materials and Methods

The data used in this study were obtained from the project numbered TUBITAK 118O197, which took place in 2020 and 2021. The experimental protocol and implemented procedures were approved by the local Ethics Committee for Experimental Animals of Ondokuz Mayıs University and also, this committee ascertained that the experiment was not an unnecessary repetition of previous experiments (Protocol code 2016/44).

Materials

In this study, data ($n = 36$, 2 years \times 3 blocks \times 6 data collections) were utilized for two grazing seasons (2020–2021) on 33.3% cocksfoot, 33.3% meadow fescue and 33.3% tall fescue MGP with 18 Karayaka male lambs at an average age of 2 months for 60 days in each season. Analyzed or calculated nutrient compositions and some physical characteristics of the pastures and BW of lambs are presented in Table 1.

Methods

After dry samples were ground to pass through a 1 mm sieve, their nutrient contents (CP and NDF) were analyzed according to methods approved by AOAC International (AOAC, 2005). For this purpose, CP (method 954.01) analysis was performed. The NDF content was analyzed in accordance with the ANKOM A200/220 Fiber Analyzer filter bag technique (ANKOM Technology Corp., Fairport, NY, USA). *In vitro* dry matter digestibility (IVDMD) values of the cocksfoot, meadow fescue and tall fescue forages were determined by using the ANKOM Daisy Incubator Fermentation System (ANKOM Technology, Macedon, NY, USA) as described by Hervás et al. (2004).

Table 1. Some quality traits of the cocksfoot, meadow fescue and tall fescue pastures and body weights of lambs

Item	Mean	Minimum	Maximum
Body weight, kg/lamb	29.25	22.93	36.10
Crude protein, %	12.37	9.06	14.90
Neutral detergent fiber, %	60.88	49.24	73.76
In vitro dry matter digestibility, %	56.50	29.57	59.31
Dry matter yield, kg/m ²	0.788	0.514	1.065
Dry matter yield kg/da	788.3	514.5	1065.0
Herbage allowance, kg/lamb	16.39	10.97	21.22
Metabolizable energy, Mcal/kg	2.01	1.98	2.22
Total digestible nutrients, %	62.63	48.73	68.38
Relative forage quality	116.08	81.03	120.50

In addition, the actual DMI values used in the development of the equations were determined daily as defined by Burns et al. (1994).

The HM, which is the expression of total DM on the pasture, was calculated as;

$$HM = DMY \times 1000 \quad (1)$$

Where, HM is herbage mass, DMY is dry matter yield per square meter of pasture, and decare is 1000 square meters.

The HA differs from HM with the BW factor and has been calculated as;

$$HA = HM / (SR \times BW) \quad (2)$$

Where, HA is herbage allowance, SR is stocking rate and BW is the average body weight of the grazing animals.

The metabolizable energy represents the energy that can be used by an animal after accounting for energy lost in feces, urine and gases (such as methane in ruminants), was calculated as (Belyea et al., 1993);

$$ME \text{ (Mcal/kg)} = 0.17 \times (\text{DDM}\%) - 2.0 \quad (3)$$

Where, DDM is digestible dry matter.

Digestible dry matter was calculated as;

$$\text{DDM} (\%) = 88.9 - (0.779 \times \text{ADF}), \quad (4)$$

Where, ADF is acid detergent fiber.

Relative forage quality (RFQ) is a forage quality trait including potential dry matter intake and total digestible nutrients of forages, was calculated as;

$$\text{RFQ} = (\text{DMI}_c (\% \text{ of BW}) \times \text{TDN} (\% \text{ of DM})) / 1.23 \quad (5)$$

Where, DMI_c is the estimated dry matter intake potential of forages, TDN is total digestible nutrients.

The TDN, NDFn, and NFC values are calculated as stated below;

$$\text{TDN} = (\text{NFC} \times 0.98) + (\text{CP} \times 0.87) + \text{FA} \times 0.97 \times 2.25 + \text{NDFn} \times \text{NDFDp} / 100 - 10 \quad (6)$$

$$\text{NDFn} = \text{NDF} \times 0.93 \quad (7)$$

$$\text{NDFDp} = 22.7 + 0.664 \times \text{NDFD} \quad (8)$$

$$\text{FA} = \text{EE} - 1 \quad (9)$$

Where, NFC is non-fiber carbohydrates, FA is fatty acids, NDFn is nitrogen-free NDF and NDFD digestibility of NDF.

Both BW and pasture-based factors, presented in Table 1, were determined at 10-day intervals in two grazing seasons, 60 days for each season. Data from three MGPs that provided three observation opportunities (blocks) were used for the equations of herbage and DMI estimation. Initially, the relationship between DMI and animal and pasture-based factors in PBS lamb production was determined by Pearson's correlation (Figure 1). Principal

component analysis was then performed for both to strengthen the correlation coefficients and to identify variables that act together or in opposition to each other in generating variation in the existing system (Figure 2). After approximating the effects of variables using PCA and correlation analysis, equations were developed for estimating the DMI of lambs grazing on MGP through both single and multiple linear regression models (Table 2).

Statistical Analysis

To comprehend the impact of each factor within the lamb grazing model, considered simultaneously, principal component analysis (PCA) was applied. The data obtained from a lamb grazing study (n = 36, 1 treatment \times 2 years \times 3 blocks \times 6 data collection, BW of the lambs and pasture quantity and quality traits was determined 6 times with 10-day intervals) was used as the cases. The mean values of each replicate in each data collection time were used to perform PCA. Before performing PCA, the suitability of data for factor analysis was assumed using the Kaiser-Meyer-Olkin (KMO) test and Bartlett's test (KMO = 0.533; $\chi^2 = 197.8$, $p < 0.001$). Thus, a new set of 10 orthogonal variables was generated by PCA. Principal components (PCs) that had only eigenvalues of > 1.0 were accepted as significant to describe most of the total data variations. Pearson correlation test was used to determine the relationships between DMI and some animal and pasture-based factors. The DMI was estimated by linear multiple regression equation using BW, DMY, HA, NDF, ME, TDN, IVDMD, CP, and RFQ variables. Regression models of DMI were evaluated by the coefficients of determination (r^2) and standard error (S_y) of estimation. The regression model used was:

$$Y = a + b_1x_1 + b_2x_2 + \dots + b_nx_n \pm S_y$$

Where Y was the dependent variable (DMI) of grazing lambs; a was the regression constant; b_k was the regression coefficients of independent variables and x_n was the explanatory variables of independent variables. SPSS software was used in all analyses (Version 17.0).

Results

To determine the parameters to be used in the estimation of herbage and/or dry matter intake of grazing lambs, the relationships between the actual dry matter intake data and some animal and pasture-based factors were determined (Figure 1). Positive correlations were determined between DMI and BW (0.777), HA (0.814), DMY (0.844), and NDF (0.609), while DMI had negative correlations with IVDMD (-0.738), ME (-0.039), RFQ (-0.357), CP (-0.209), and TDN (-0.177).

Principal component analysis was also used to identify animal and pasture-based factors for estimating the DMI of lambs grazing on MGP. The PC1 and PC2 composed 67.36% of the variability in the data set from the grazing lamb model consisting of some animal and pasture-based factors (Figure 2). Factors in the model were distributed to all quadrants of the PCA. By considering this distribution, it may be possible to deduce which factors are more effective and important for developing estimation equations for DMI of lambs grazing on MGP.

Table 2. Regression equations developed to predict the DMI of lambs grazing on cocksfoot, meadow fescue and tall fescue pastures

Model	Regression equations	S _y	r ²
I	-1224.09+39.90BW+33.69HA+8.22NDF	83.504	0.815
II	-701.47+18.96BW+673.61DMY+8.19NDF	85.141	0.807
III	-325.32+43.49HA-2.21IVDMD+8.57NDF	89.784	0.786
IV	-674.96+44.91HA+9.75NDF+75.73ME	90.116	0.784
V	-4723.66+204.07BW+243.65HA-4305.22DMY	95.869	0.756
VI	-1485.51+64.98BW+35.37HA+1.04RFQ	101.400	0.746
VII	2471.13-41.71IVDMD-5.05NDF+4.94RFQ	125.848	0.609
VIII	516.55-19.17IVDMD+7.03NDF+12.36TDN	136.991	0.501
IX	739.00-9.54IVDMD+9.71NDF-6.03CP	145.694	0.436

Body weight (BW; kg); Herbage allowance (HA; kg/head); Dry matter yield (DMY; kg/da); Neutral detergent fiber (NDF; % of DM); *In vitro* dry matter digestibility (IVDMD; %); Metabolizable energy (ME; Mcal/kg); Relative forage quality (RFQ); Crude protein (CP; % of DM); Total digestible nutrient (TDN; %), S_y: Standard error of the estimation, r²: Regression coefficient.

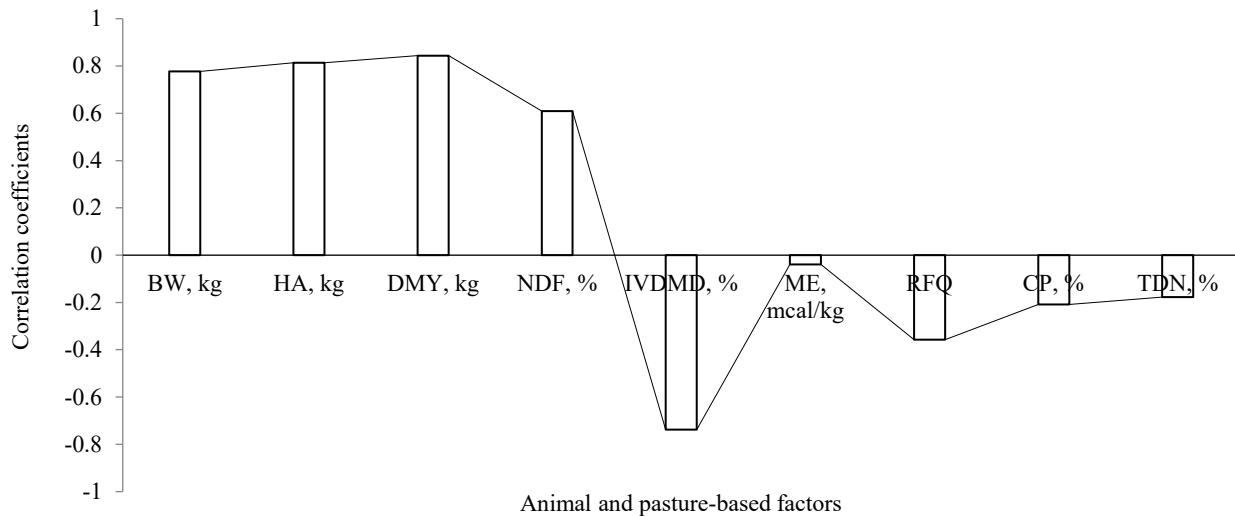


Figure 1. The relationships between the actual dry matter intake (DMI) data and some animal and pasture-based factors. Body weight (BW; kg); Herbage allowance (HA; kg/head); Dry matter yield (DMY; kg/da); Neutral detergent fiber (NDF; % of DM); *In vitro* dry matter digestibility (IVDMD; %); Metabolizable energy (ME; Mcal/kg); Relative forage quality (RFQ); Crude protein (CP; % of DM); Total digestible nutrient (TDN; %).

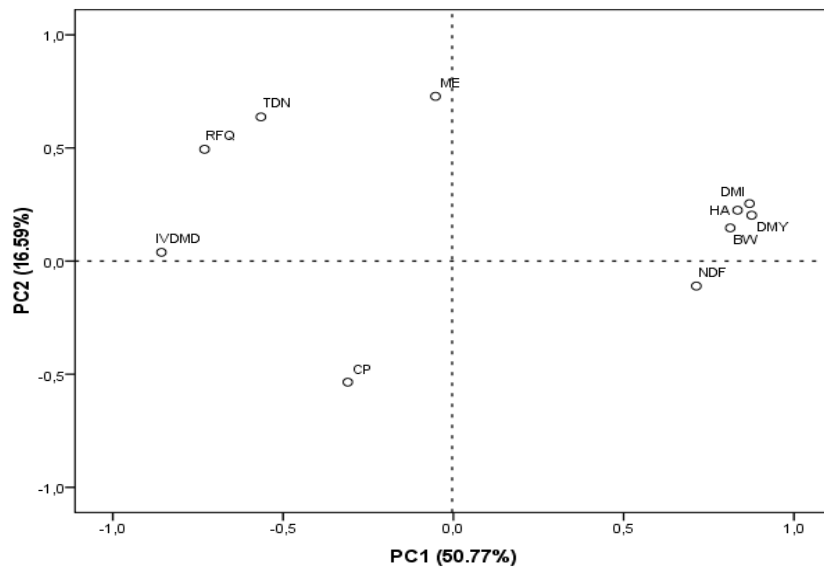


Figure 2. Loading plots of principal components (PC1 and PC2) for some animal and pasture-based factors to be used in the DMI prediction equations for lambs grazing on cocksfoot, meadow fescue and tall fescue.

Body weight (BW; kg); Herbage allowance (HA; kg/head); Dry matter yield (DMY; kg/da); Neutral detergent fiber (NDF; % of DM); *In vitro* dry matter digestibility (IVDMD; %); Metabolizable energy (ME; Mcal/kg); Relative forage quality (RFQ); Crude protein (CP; % of DM); Total digestible nutrient (TDN; %).

The correlation matrix loadings (or scores) from the PCs indicated that the main contributions derive from three groups. The first group was composed of factors with positive loadings for PC1 and PC2 (DMI [0.870 and 0.254], DMY [0.876 and 0.203], BW [0.813 and 0.147], and HA [0.835 and 0.225]). The second group contained NDF with positive loading for PC1 and negative loading for PC2 [0.714 and -0.110]. Group 3 was composed of factors with negative loadings for PC1 (CP [-0.310 and -0.535], IVDMD [-0.857 and 0.039], RFQ [-0.731 and 0.494], TDN [-0.565 and 0.637], and ME [-0.052 and 0.728]). Based on the correlation matrix loadings (≥ 0.70 and positive factor loadings) of the factors, it may be deduced that HA, DMI, DMY, BW, and NDF are cooperating in the herbage and/or dry matter intake estimation model and contributed most strongly to PC1.

Regression equations for DMI estimation were determined by using one animal-based (BW) and eight pasture-based factors (HA, NDF, DMY, IVDMD, ME, RFQ, TDN, and CP). The DMI was the dependent variable and three others (determined by using correlation coefficients and PCA's loading plots) were independent. The DMI of lambs grazing on MGP was estimated by model I ($-1224.09+39.90\text{BW}+33.69\text{HA}+8.22\text{NDF}$) with the highest r^2 value (0.815) and the lowest S_y (85.504). Since the HA value was indirectly calculated from DMY model II ($-701.47+18.96\text{BW}+673.61\text{DMY}+8.19\text{NDF}$) had similar r^2 (0.807) and S_y (85.141) to model I in the DMI estimation. This was followed by Model III, IV, V, and VI with the r^2 and S_y values (0.786–89.784), (0.784–90.116), (0.756–95.869), and (0.746–101.400), respectively. It has been observed that in the first six models where both animal and pasture-based factors were employed together, accurate predictions were achieved, whereas models (VII, VIII, and IX) relying solely on pasture-based factors were less accurate with 0.609–125.848, 0.501–136.991 and 0.436–145.694 $r^2 - S_y$ values, respectively.

Discussion

In this study, DMI prediction equations were developed by integrating animal and pasture-based factors using actual DMI of Karayaka male lambs grazing on MGP for 60 days each over two grazing seasons (2020 and 2021). Correlation analysis showed that there is a strong and positive relation between DMI and BW (0.777). This result is not surprising and is consistent with a significant body of research (Blümmel et al., 1997; Cannas et al., 2004; INRA, 2018; Oliveira et al., 2020). Increasing DMI with increasing BW is inevitable for healthy grazing animals to meet their requirements. Indeed, it should be noted that the relationships between animal and pasture-based factors of the lamb growth model in the present study are based on lambs growing due to grazing, i.e. gaining body weight, and pastures consisting of cocksfoot, meadow fescue, and tall fescue that mature and change in nutrient content during the grazing season. Also in all evaluations, DMI was used in grams, not as a percentage of BW.

It is generally accepted that there is a relationship between plant fiber structure and the amount of fibrous materials with the DMI of grazing animals. Dry matter intake is believed to be regulated by the NDF level and digestion rate due to the effects of NDF

digestion/degradation on rumen volume (Chen et al., 2023). In this context, numerous studies have demonstrated a negative correlation between NDF and DMI (Minson, 1990; Meyer et al., 2010; Chen et al., 2023). There are also variations between some results of different studies. Van Soest (1965) has reported that correlations between DMI and NDF can vary between 0.57 and -0.95 in a study with different forage crops. These variations here basically arise according to the animal-rearing model. This situation becomes complicated when we consider lambs grazing on MGP, increasing BW day by day, maturing, and changing in nutrient content of the pasture, which is the only source of nutrients. However, in our study, DMI is g, not a percentage of BW. Indeed, a correlation coefficient of 0.609, indicating that DMI in grams increased despite higher NDF content, is not abnormal. A study by Meyer et al. (2010) assuming that maximum HI occurs when herbage NDF content is 35%, showed how variation in NDF alters HI. According to the results of this study, even if NDF content is 60%, sheep can reach 85% of the potential HI. In addition, the average NDF content of the pasture in two grazing seasons during our study was 60.04%. In this scenario, employing NDF in the DMI prediction equation (Model I) in tandem with HA calculated from BW of grazing animals and pasture yield and BW is deemed acceptable.

A large number of regression models to predict DMI based on animal-based factors have been developed (Yungblut et al., 1981; Kertz et al., 1991; Roseler et al., 1997). On the contrary, many studies used only feed-related factors such as ADF, NDF, CP, and ME as the basis for predicting DMI (Yungblut et al., 1981; Harlan et al., 1991; Holter et al., 1996). In our study, defined as a lamb growth model on MGP, DMI was predicted by combining animal and pasture-based factors. After examining CP and ME, two factors related to pasture, they were deemed unworthy for use in developing equations to estimate DMI, based on correlation and PCA results. Although there is a low and negative relationship between DMI and ME, it was still possible to estimate DMI in a proper way ($r^2=0.784$) when ME was used with NDF and HA in the regression equation (Model IV). It should also be taken into consideration that, in our study, the ME value in MGP was in a very narrow range (1.98–2.22 Mcal/kg). Correlations between CP level of feed and DMI and ME and DMI have been highlighted in many studies (Yang et al., 2017; Xiao et al., 2020; Chen et al., 2023). It should be considered that, in our study, growing lambs graze on pastures that mature, fibrous structures increase in the pastures during the maturation process and CP and ME tend to decrease proportionally. Moreover, a high concentration of fibrous content (i.e., ADF and NDF) of forage reduces digestibility and affects grazing behavior, as reported by Xiao et al. (2020). Although there was a negative and strong correlation (-0.738) between IVDMD and DMI in this study, the NDF level was never high enough to reduce DMI dramatically. On the other hand, increasing BW prevented a decrease in DMI because of the increasing nutrient requirements. Furthermore, the slight negative correlations between DMI and TDN (-0.177) and RFQ (-0.357) back the hypothesis that a reduction in diet quality and composition in the animals' diet will lead to a rise in DMI (Meyer et al., 2010).

In PBS, it is believed that DMI and/or HI is regulated by energy requirements, grazing behavior, and HM which is a commonly used item because of the simplicity of obtaining data (Herrero et al., 1998; Woli et al., 2023). Herbage allowance (HA) refers to the amount of herbage, expressed in kg of DM per kg of body weight (kg DM/ kg BW) is directly related to DMY and BW of grazing animals. It is a significant factor in estimating herbage intake and animal performance on pasture. The HA can affect the potential DMI of grazing animals. Generally, it is accepted that higher HA results in higher intake levels (Sollenberger et al., 2005). It is important to consider the HA in grazing management as it impacts the nutritional intake and performance of grazing animals (Woli et al., 2023). As HA increases, animals have more opportunities to select and intake herbage, resulting in increased DMI. Besides, when HA decreases, animals have less access to herbage and face restricted nutrition with decreasing DMI and/or HI. In this study, results of Pearson correlation and PCA showed that HA and DMY can accurately be employed to estimate the DMI of lambs grazing on MGP. After all this inference and information, the correlation and PCA results for estimating DMI with HA and/or DMY seem to be quite accurate.

Conclusion

It is crucial to note that DMI is essential for meeting the nutritional requirements of animals and promoting their health and performance. Monitoring and considering DMI and factors such as grazing conditions and forage quality and integrating such factors with animal-based factors can help to ensure proper nutrition for grazing animals. This study found positive and negative correlations between DMI and animal and pasture-based factors as; positive correlations were determined between DMI and BW (0.777), HA (0.814), DMY (0.844), and NDF (0.609) while DMI had negative correlations with IVDMD (-0.738), RFQ (-0.357), CP (-0.209), TDN (-0.177), and ME (-0.039). By using correlation coefficients and PCA results, parameters to be used for estimation equations were selected. Prediction equations were developed and the best-fit 3 equations for predicting DMI of lambs grazing MGP were: I) $-1224.09 + 39.90BW \text{ (kg)} + 33.69HA \text{ (kg DM/ kg BW)} + 8.22NDF \text{ (% of DM)}$, $r^2=0.815$, II) $-701.47 + 18.96BW \text{ (kg)} + 673.61DMY \text{ (kg/ per square meters)} + 8.19NDF \text{ (% of DM)}$, $r^2=0.807$, III) $-325.32 + 43.49HA \text{ (kg DM/kg BW)} - 2.21IVDMD \text{ (%)} + 8.57NDF \text{ (%)}$, $r^2=0.786$.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee for Experimental Animals of Ondokuz Mayıs University (29.08.2016 and number: 2016/44).

Author Contribution Statement

Ahmet Akdağ: Data collection, investigation, formal analysis, methodology and writing the original draft

Nuh Ocak: Project administration, supervision, conceptualization, methodology, review and editing

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Fund Statement

The research was funded through the Scientific and Technological Research Council of Türkiye (TUBITAK) (Grant/Award Number: 118 O 197).

Conflict of Interest

There is no conflict of interest in this study.

Acknowledgments

The authors thank the Scientific and Technological Research Council of Türkiye, TÜBİTAK for financial support. We are grateful to MSc. Berat Bilik for his valuable contribution to laboratory work.

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Sustainable Agriculture and It's Practices: A Review

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ARTICLE INFO

Review Article

Received : 22.12.2023

Accepted : 20.11.2024

Keywords:

Organic farming
Food security
Environmental health
Precision farming
Eco-friendly agriculture

ABSTRACT

Sustainable agriculture, a holistic approach to farming, offers a promising solution to the global challenge of balancing food production with environmental preservation. Sustainability is based on the idea that we should fulfill current needs without jeopardizing the ability of future generations to fulfill their requirements. It involves the farming practices that maintain the health of our land, water, and air while producing sufficient food necessary for the growing population. This comprehensive review explores diverse sustainable agricultural practices essential for balancing productivity, economic viability, and social equity. Key principles of sustainable agriculture, emphasizing environmental health, financial feasibility, and social justice, underpin a multifaceted approach. Permaculture, emphasizing biodiversity and ecosystem regeneration, aligns with nature's principles. Crop rotation and diversification mitigate pests and diseases, and enhance soil health. Water management through techniques like drip irrigation and rainwater harvesting optimizes water usage. Innovative practices including aquaponics, hydroponics, vertical farming, and agroforestry ensure year-round, efficient food production. Climate-smart agriculture adapts to climate change, while precision agriculture enhances resource efficiency. Organic farming, relying on natural processes, offers a sustainable alternative to conventional methods. Challenges like excessive chemical usage, climate-related disruptions, and knowledge gaps persist despite promising outcomes. Overcoming these hurdles requires collaborative efforts, policy support, and education initiatives. Sustainable agriculture represents the path toward a resilient and food-secure future for our growing global population.

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Introduction

Food, an essential requirement for all living organisms, is intrinsically linked to agriculture which plays a central role in providing a wide range of foods we consume daily, including cereals, legumes, grains, fruits, tubers, and vegetables (Saikia & Laisharam, 2023). In many poor nations, the majority of their citizens reside in rural regions and derive their main source of income primarily from agricultural activities (Gollin, 2010). Agriculture stands as the primary source of livelihood and a cornerstone of economic stability, underscoring the critical importance of sustainability to fulfill its multifaceted role in meeting diverse human needs (Spiertz, 2009). As the global population is estimated to reach nine billion by the year 2050 (FAO, 2017), there is a growing need for agriculture to sustain food. This raises a critical question: Can traditional farming meet the increasing global food demand? The answer lies in adopting sustainable farming practices (Kumar, 2013; Calicioglu et al., 2019). Conventional agriculture diminishes saprophytes, humus

return, and nitrogen-fixing organisms, making the system reliant on more external inputs. Moreover, reduced diversification can result in higher instances of plant and animal diseases, as well as increased pest and weed issues (Worthington, 1980). To sustain agricultural growth, it is vital to encourage the preservation and responsible use of finite natural resources. Sustainable agricultural practices are essential to address global food security, without depleting food resources or causing environmental harm.

While sustainability definitions encompass various environmental, economic, and social aspects, many tend to emphasize specific areas such as environmental preservation, resource conservation, productivity, and the profitability of farms and businesses. Charles Francis, for instance, characterizes sustainable agriculture as a "management strategy" with the primary objective of lowering input expenses, mitigating environmental harm, and ensuring long-term production and profitability (Francis et al., 1998). It aims to produce abundant food

without harming the environment, following nature's principles for self-sustaining farming while fostering vibrant rural communities and providing wholesome food. However, in the 21st century, it remains in its early stages as commonly accepted practices and a model farm economy are still emerging (Earles & Williams, 2005). Another definition of sustainable agriculture is an integrated approach to farming that recognizes the interdependence of all components within a farming system, including the farmer and their family. It prioritizes the preservation of various biological balances within the system and underscores the significance of adopting environmentally responsible production practices to minimize harm while enhancing productivity (Allen et al., 1991). It seeks to fulfill the food and fiber needs of society in an environmentally responsible manner that preserves natural ecosystems and processes (Sow & Debnath, 2023). A universally accepted brief definition of sustainable agriculture has yet to emerge, primarily because it's often seen as a management philosophy rather than a fixed operational method. Acceptance or rejection of any definition is closely tied to one's values. However, regardless of the precise definition, most agriculturalists concur that sustainable agriculture is crucial for the long-term sustainability of our planet and its growing human population (Abubakar & Attanda, 2013). Global challenges like starvation in poor countries, obesity in wealthy nations, rising food prices, climate change, increasing fuel and transport costs, global market failures, pesticide contamination, disease resistance, soil fertility depletion, soil degradation, biodiversity loss, and desertification are the major issues in today's world. Despite scientific advancements, these issues reveal that conventional agriculture cannot adequately feed the population or preserve ecosystems (Lalrintluangi et al., 2021). As a result, sustainable agriculture will be of prime importance in the future. To ensure the long-term adoption of sustainable practices, it is essential that the approaches not only be suitable and appealing but also deliver tangible short-term benefits and market opportunities while considering local traditional knowledge and agroecological specifics (Silici et al., 2015). Sustainable agriculture improves soil health, conserves water, and boosts long-term productivity and climate resilience. It lowers production costs, offers market opportunities for organic products, enhances food security, and promotes community health by reducing chemical use. Additionally, it supports biodiversity and environmental conservation, ensuring ecosystem stability.

Principle of Sustainable Agriculture

A set of core principles guides sustainable agriculture, emphasizing environmental sustainability, economic viability, and social equity (Brodt et al., 2011). Achieving sustainable and resilient agriculture necessitates careful equilibrium among three critical pillars: environmental health, economic viability, and social equity. These facets collectively constitute the bedrock of sustainable agriculture, providing a robust framework that can withstand the challenges of an ever-changing world (Sow & Debnath, 2023). A fundamental concept of agricultural sustainability is that we should fulfill the requirements of

today while safeguarding the potential of future generations to fulfill their own necessities, emphasizing the importance of both short-term gains and long-term resource management. It holds the transformative potential not only to nourish millions but also to elevate human well-being (Saikia & Laisharam, 2023). Environmental sustainability in agriculture involves practices like maintaining healthy soil, efficient water management, reducing food waste, minimizing pollution, promoting biodiversity, limiting tillage, and integrating livestock and crops, all while improving the quality of life for farming communities (Karaka & Ince, 2023). Similarly, economic viability in sustainable agriculture focuses on practices and strategies that ensure farmers can make a reasonable income from their operations. Balancing economic viability with environmental and social concerns is a fundamental principle of sustainable agriculture. These principles form the foundation for implementing practices that ensure long-term food security without compromising the well-being of future generations.

Sustainable agriculture practices

Sustainable agriculture encompasses a diverse range of practices aimed at producing food, fiber, and other agricultural products while minimizing harm to the environment and promoting economic viability. These practices are rooted in principles that prioritize the long-term well-being of both people and the planet. Some of the practices are described below.

Permaculture

Permaculture, a term combining "permanent agriculture" and "permanent culture," represents an eco-friendly farming method deeply rooted in long-lasting sustainability (Vargas-Hernandez, 2021). It focuses on using management strategies and design-driven approaches to attain specific goals, like improving habitats or promoting sustainable living and food production (Alexandra, 2022). Inspired by nature's intricate designs, permaculture aims to create farming systems that sustain and regenerate the environment (Hathway, 2015). A key principle involves fostering biodiversity by cultivating diverse crops and nurturing symbiotic relationships between species. This diversity enhances the system's resilience, making it less susceptible to pests and diseases while promoting soil health and nutrient cycling (Alexandra, 2022). Permaculture design principles complement the practical experience and technical knowledge, guiding the creation of site-specific solutions that aim to unite culture and nature beyond the limitations of sustainable development (Holmgren, 2020). In permaculture, careful planning of zones and sectors optimizes energy and resource use while adhering to ethical principles that ensure sustainable resource utilization without harming the environment (Baradia, 2021). Farmers actively harvest rainwater, utilize composting to enrich the soil, and repurpose waste materials, reducing reliance on external resources and promoting sustainability. Permaculture offers a promising approach to soil protection and enrichment, emphasizing the importance of healthy soil for overall ecological well-being. Its sustainable practices, including increased

organic matter, enhanced soil structure, and efficient moisture retention, contribute to soil health and resilience, mitigating issues like erosion and nutrient loss (Korez, 2018). In Sotang Organic Farm switching from agrochemicals led to high yields, healthier crops, and a balanced ecosystem, producing 100% certified organic products and diversifying into various farming practices, thus contributing significantly to agricultural sustainability (Yadev et al., 2023). Moreover, permaculture extends its regenerative approach beyond the farm, employing reforestation and soil restoration techniques to revitalize degraded landscapes and ecosystems. Beyond its ecological focus, permaculture fosters community engagement, knowledge sharing, and equitable distribution of produce (Maye, 2018). For instance, the Gandaki Trout Farm in Kaski, designed for high trout fish production, also supports agritourism and agriproducts (Yadev et al., 2023). Whether applied in rural or urban settings, permaculture embodies sustainable agriculture's essence; an intricate interplay between humans, the environment, and nature's systems that ensures the well-being of present and future generations.

Crop Rotation and Diversification

Crop rotation and diversification represent fundamental components of sustainable agriculture, contributing significantly to both pest and disease management and soil health enhancement (Li et al., 2019; Shah et al., 2021). Crop rotation, a time-honored practice, involves the systematic alternation of crops in a specific sequence over a defined period. This strategy disrupts the life cycles of pests and diseases, reducing their buildup in the soil and minimizing the need for chemical interventions (Sharma, 2023; Ganesh et al., 2021). For instance, rotating nitrogen-fixing legumes like beans with nitrogen-demanding crops like corn can improve soil fertility while naturally managing pests. Crop rotation breaks the disease cycle in the soil, preventing pathogen buildup in monoculture farming, and ultimately reducing the severity of plant disease outbreaks. Rotations involving different plant families disrupt the pathogen cycle, leading to a rapid decline in the pathogen population in the soil (Shah et al., 2021). Alternating between brassica crops like broccoli and cauliflower with allium crops such as onions and garlic can help control soil-borne diseases like clubroot. The rotation strategy reduces the buildup of pathogens specific to each plant family, leading to healthier crops over time. This approach not only safeguards crop productivity but also aligns with sustainable agriculture by reducing the reliance on synthetic pesticides and fungicides and promoting long-term soil health and resilience. In Western Canada, rotating spring wheat with dry pea or lentil crops in a canola-spring wheat-spring wheat-spring wheat rotation significantly reduced environmental impacts (17-25% reduction in Global Warming and Resource Use, 1-24% in Ecosystem Quality, and 3-28% in Human Health). It also improved farm-level returns over variable costs, from \$20.43/ha to \$110.45/ha for dry pea and \$138.78/ha for lentil rotations (MacWilliam et al., 2014). Furthermore, diversifying crop species within a farming system has profound benefits for soil health and resilience. By cultivating a variety of crops with different growth patterns, root structures, and nutrient needs, farmers

enhance the overall vitality of their soil (Watson et al., 2006). For instance, integrating cover crops like clover or rye into a crop rotation plan not only prevents soil erosion but also adds organic matter, improving soil structure and moisture retention. Crop diversification also promotes biodiversity above and below ground, creating a more resilient and adaptable agricultural ecosystem (Tamburini et al., 2020). A research conducted by Bowles et al. (2020) states that crop diversification has enhanced sustainability by increasing maize yields by an average of 28.1%, improving resilience during drought years with yield loss reductions of 14.0%–89.9%, and serving as a key strategy in environmental risk reduction.

Water Management

Water is a critical resource in agriculture, and its responsible management is essential for the sustainability of farming systems. Sustainable agriculture employs a range of strategies to address water-related challenges and promote efficient water use. *Drip Irrigation* is a hallmark of sustainable water management. This technique delivers water directly to the root zone of plants, minimizing losses from evaporation and runoff (Nair, 2019). By providing precise control over water application, drip irrigation not only conserves water but also enhances crop productivity. A study by Narayanamoorthy et al. (2018) demonstrated that drip irrigation significantly outperforms flood method irrigation in brinjal cultivation, resulting in 1.5 times higher yields. This is due to reduced moisture stress, effective weed control, nutrient retention, and extended fruit harvesting, while also enhancing water and energy productivity. *Rainwater Harvesting* is another sustainable practice that has gained prominence. This method involves capturing and storing rainwater for agricultural use. Rainwater harvesting systems collect runoff from rooftops or designated catchment areas, storing it in tanks or reservoirs. This stored rainwater serves as a valuable resource during dry spells, reducing the reliance on freshwater sources and alleviating pressure on local water supplies (Lo, 2003). In-situ and ex-situ rainwater harvesting methods have demonstrated notable benefits, including enhanced soil moisture, reduced runoff, increased groundwater replenishment, and higher agricultural yields. These outcomes mitigate risks and have positive ripple effects on various ecosystems (Yosef & Asmamaw, 2015). Additionally, *Irrigation scheduling* is a crucial way to optimize agricultural production, conserve water, and ensure irrigation system sustainability. It relies on understanding crop water needs, soil characteristics, and efficient irrigation methods to prevent issues like water-logging and excessive water use (Chartzoulakis & Bertaki, 2015). Incorporating these water management and conservation practices into agriculture is fundamental to sustainable farming. By optimizing water use, and reducing wastage, sustainable agriculture ensures the longevity of farming systems while contributing to broader water resource conservation efforts.

Aquaponics and Hydroponics

Both aquaponics and hydroponics represent sustainable agricultural practices that offer significant advantages. *Aquaponics* is a modern food production technique that combines the cultivation of fish and aquatic animals with

the growth of plants, primarily vegetables and herbs, in a symbiotic system (Fruscella et al., 2021). In integrated aquaponic systems, fish waste is naturally converted by waterborne bacteria into plant nutrients; fish waste nourishes the plants, and plants naturally filter the water for the fish. This makes aquaponics a comprehensive and sustainable food production technology, addressing environmental, economic, and social aspects (König et al., 2016). Through this integrated approach there will be minimal water consumption, avoidance of synthetic fertilizers and pesticides, and efficient waste recycling. These aspects collectively address environmental concerns, particularly in mitigating eutrophication issues in aquatic ecosystems, thereby promoting sustainability (Kledal et al., 2019). *Hydroponics*, on the other hand, involves growing plants in a nutrient-rich water solution without soil (Naik et al., 2015). While it may seem like a departure from traditional farming, hydroponics offers its own sustainability advantages like efficient water utilization, reduced pesticide usage, and increased crop yields (Sridhar et al., 2023). It achieves these benefits by cultivating plants in a controlled environment with carefully managed nutrient-rich water solutions instead of traditional soil-based methods. This innovative approach is particularly valuable for urban agriculture, where available arable land is limited (Khan et al., 2020). Furthermore, the controlled environment in hydroponics systems means fewer pests and diseases, resulting in reduced reliance on chemical pesticides and herbicides. This not only prevents soil and water contamination but also fosters healthier ecosystems. Hydroponics can be implemented year-round and can adapt to various climatic conditions. By reducing the reliance on seasonality, it helps meet the demand for fresh, locally-grown produce throughout the year (Sheikh, 2006), reducing the environmental costs of long-distance transportation and cold storage. The results from the study by Sayara et al. (2016) indicate that the hydroponic system stands out as the most efficient, enabling rapid production while conserving minimal resources.

Vertical Farming

Vertical farming represents a unique and innovative approach to sustainable agriculture. It involves cultivating crops in stacked layers or inclined surfaces, often within controlled indoor environments (Jurkenbeck et al., 2019), which optimizes space utilization and resource efficiency. It maximizes land usage, making them particularly suitable for densely populated urban areas where arable land is scarce. Besides this, vertical farming seeks to enhance productivity while minimizing the unpredictability of outdoor weather conditions, and reliance on soil and sunlight, offering advantages like clean, local food production, biosecurity, freedom from soil-borne pests, and reduced transportation and fossil fuel use (Benke & Tomkins, 2017). Additionally, this method significantly reduces the need for chemical pesticides, as the controlled environment minimizes exposure to pests and diseases (Jukenbeck et al., 2019), aligning with the principles of sustainable agriculture that prioritize ecological balance. Furthermore, vertical farming has year-round production capability, which ensures a continuous supply of fresh produce. Introducing vertical farming has proven to be a powerful tool in combating food insecurity and promoting

environmental sustainability as well. For example, study by Zhang et al. 2018 show that vertical farms can become economically viable within 10-20 years, with a specific analysis indicating a breakeven point at around 11.5 years. After this period, vertical farms can generate annual profits of up to \$92,000, significantly reducing environmental stress and supporting sustainable agricultural practices (Zhang et al., 2018).

Agroforestry

Agroforestry has gained global recognition as a comprehensive strategy for sustainable land management due to its capacity to deliver both productive and ecological advantages (Nair et al., 2009). It is a farming system in which the soil is managed collaboratively and harmoniously by different participants, such as farmers, livestock, and plants. This approach provides a wide range of advantages, such as preserving biodiversity, controlling pests and diseases, enhancing soil, air, and water quality, optimizing nutrient cycling, and bolstering resilience in the face of climate change (Ramil Brick, 2022). It not only helps reduce poverty but also provides various ecosystem services and positive environmental outcomes (Jose, 2009). It holds the potential for significant transformation and presents a chance to enhance the sustainability of organic farming (Rosati et al., 2021). Agroforestry systems extend to arable lands, where trees and woody perennials are strategically planted on terrace risers, field edges, and as intercrops. This integration of trees in fields serves as a natural nutrient reservoir, enhances soil fertility with bio-fertilization, conserves moisture, and ultimately boosts overall system productivity. Furthermore, agroforestry diversifies production methods, including food crops, fruits, vegetables, legumes, and medicinal plants, helping rural communities meet urban market demands and ensure nutritional security. It also provides essential resources like timber, fiber, and medicine, promoting sustained productivity. (Ali et al., 2023). Research conducted by Triwanto et al. (2022) revealed that the integration of coffee agroforestry systems in Indonesia could yield a favorable benefit-to-cost (B/C) ratio of 2.98, indicating its positive impact on farmers. The adoption of coffee agroforestry substantially enhances farmers' overall income, accounting for 58.47% of their total earnings. The focus of coffee agroforestry development is on improving productivity and quality while preserving the well-being of shade plants, ensuring a sustainable approach.

Climate Smart Agriculture

Climate-Smart Agriculture (CSA) aims to address food security challenges and enhance rural livelihoods while mitigating adverse environmental effects (Azadi et al., 2021). Climate Smart Agriculture (CSA) encompasses a range of crucial dimensions, including practices that focus on efficient water management, adaptability to weather variations, responsible nutrient utilization, carbon footprint reduction, and energy-efficient strategies. These dimensions are indispensable for meeting the growing global demand for food in a manner that is both productive and profitable while also safeguarding environmental integrity (Chakraborty et al., 2023). Achieving a more productive and adaptable agriculture necessitates a significant shift in the management of land, water, soil

nutrients, and genetic resources through the application of climate-smart agricultural methods (Amin et al., 2015). CSA encourages collaboration among farmers, researchers, the private sector, civil society, and policymakers to pursue climate-resilient approaches in four key areas: (1) evidence building; (2) local institutional improvement; (3) alignment of climate and agricultural policies; and (4) integration of climate and agricultural financing (Lipper et al., 2014). The increasing water scarcity and mishandling of existing water resources pose significant challenges to sustainable development across various sectors, including domestic, industrial, and agricultural. With the help of CSA, we can enhance the efficiency of water utilization which results in fewer conflicts among different water usage sectors, improved local food security, increased availability of water for agricultural, household, and industrial purposes, and enhanced preservation of natural water sources (Hamdy et al., 2003). About 75% of the direct emissions from agricultural soil are attributed to the use of inorganic nitrogen (N) fertilizers (Zheng et al., 2004; Mohanty et al., 2017). In addition to their role in greenhouse gas emissions, nitrogen-based fertilizers also reduce soil microbial activity and diminish bacterial diversity (Ding et al., 2017). Conversely, employing organic compost represents a sustainable and climate-responsible method for improving soil fertility. The global interest in utilizing composted organic materials to boost soil productivity and fertility is on the rise (Goyal et al., 2005). It doesn't just eliminate waste; it also converts waste into a nutrient-rich organic product that can be utilized to improve soil fertility (Neher et al., 2013). In Oyo State, Nigeria, a study found that 99% of cassava farmers achieved higher agricultural output and significant benefits from using Climate Smart Agriculture Practices (CSAP). These benefits include increased income, food production, and enhanced pest management, along with improved farm planning and soil health. This demonstrates how CSAP contributes to sustainability by boosting productivity and environmental resilience (Victory et al., 2022).

Precision Agriculture

With a growing global population and limited resources, there's a pressing need for a fresh approach to farming that improves productivity, quality, and resource efficiency. Precision agriculture emerges as a contemporary management strategy capable of addressing these challenges. Precision Agriculture is a management strategy that gathers, processes, and analyzes temporal, spatial, and individual data and combines it with other information to support management decisions according to estimated variability for improved resource efficiency, productivity, quality, profitability, and sustainability of agricultural production (ISPA, 2021). Precision agriculture (PA) can assist farmers in making more effective decisions regarding resource allocation, which can lead to reduced production expenses or enhanced yields, and ultimately, the potential for increased profitability (Batte and Arnholt, 2003). The techniques for precision agriculture include Global positioning systems (GPS), Geographic information systems (GIS), Remote sensing, Variable rate technology (VRT), Site-specific Nutrient management (SPNM), Nutrient Expert systems, Real-time Nitrogen

management, and Laser-based land leveling (Biswas & Munnoli, 2023). Nonetheless, there is a lack of comprehensive understanding regarding how farmers employ precision agriculture technologies (PATs) to inform their managerial choices, and there is limited knowledge about the comparative scale of advantages and drawbacks associated with PATs at the level of individual farms (Batte and Arnholt, 2003). A study conducted by Rajiv (2022) in southern Alberta, the largest, most fertile, and most productive agricultural region in Canada, found that precision agricultural technologies tend to be spread across all the lands (both dry and irrigated) and crops (cereal crops, oilseeds, and specialty crops), and the benefits could be significant. Integrating technologies like variable rate seeding (VRS) using the soybean VRS simulator has shown considerable profit enhancements, ranging from \$5 to \$57 per hectare compared to conventional fixed seeding methods (Correndo et al., 2022). Furthermore, nanotechnology, which falls under Precision agriculture, reduces the environmental impact of traditional agriculture by minimizing the use of fertilizers, pesticides, and water. Nanopesticides target pests specifically, reducing the need for broad-spectrum chemicals and minimizing harm to non-target organisms. Additionally, nano remediation can mitigate pollution from agrochemical runoff in soil and water, promoting environmental sustainability (Thimmareddy et al., 2023). This involves the application of precise and well-regulated smart drip irrigation, the monitoring of soil moisture and crop conditions through robotic systems, and the utilization of remote sensing technology (Mulla, 2013).

Integrated Pest Management (IPM)

Integrated Pest Management (IPM) is characterized by its affordability, durability, minimal environmental impact, and potential for local enhancements, making it a sustainable component within a broader integrated crop protection framework. It is an environmentally friendly and cost-effective approach to crop protection. It emphasizes ecological considerations and consists of four key elements which are pest management options, the acquisition of knowledge and resources for crafting effective management strategies, information management and timely decision-making, the sharing and distribution of information to improve local practices (Niranjan et al., 2023). IPM operates on the principle that not all levels of pest populations pose a threat to crops. Its strategies for different field crops involve combining resistant plant varieties, cultural practices, mechanical and physical interventions, beneficial organisms, biopesticides, and pesticides in a balanced manner to control pest populations while safeguarding the environment (Chander, 2023). It helps in the control of weeds, pests, and diseases based on the introduction of natural enemies, pheromones, and the use of native species as a barrier to pests and diseases (Porcel et al., 2013; Rodr guez et al., 2014). It works on the principles of prevention and suppression, monitoring, and making decisions based on monitoring and thresholds, non-chemical methods, reduced pesticide use, and anti-resistant strategies (Bazok, 2022). It has seen significant advancements in recent years, leading to more effective pest control while reducing the reliance on chemical pesticides and promoting eco-friendly practices. These

developments encompass improved biological control methods that leverage natural predators, parasitoids, and diseases to manage insect populations (Patel et al., 2023). IPM provides better resilience to crop outbreaks, displacement of polluting and higher energy-consuming chemicals, and preservation of biodiversity (Shelef et al., 2018). Besides the ecological benefits, IPM also offers significant economic advantages. Implementing IPM practices for vegetables in Banke and Surkhet has shown potential economic benefits, estimated at \$1.06 to \$1.44 million across these districts (McGowan, 2023). This dual impact underscores the comprehensive value of IPM in agricultural sustainability.

Organic Farming

Organic farming is an agricultural practice that focuses on cultivating crops and raising livestock using methods and inputs that prioritize environmental sustainability and the well-being of the ecosystem. It adapts to local conditions and relies on agronomic, biological, and mechanical techniques instead of synthetic substances whenever possible to serve specific functions within the system (FAO, 1999). Organic farming aims to produce healthy, high-quality food while minimizing harm to the environment and reducing the use of synthetic chemicals in agriculture. It stands as a more sustainable option when compared to the prevailing agricultural practices. Giri and Pokhrel (2022) state that the adoption of organic farming systems holds promise as a potential approach to mitigate greenhouse gas emissions within the agricultural sector. Furthermore, organic farming serves not only as a blueprint for sustainable agriculture and food security but also offers opportunities for innovative combinations of organic and conventional farming techniques, which could contribute to boosting global agricultural productivity (Giri & Pokhrel, 2022). Techniques like crop rotation, cover crops, minimal tillage, and composting are used to boost soil fertility. Reduced tillage prevents soil exposure to the air, retaining more carbon and increasing soil organic carbon. This method also aids carbon sequestration, reduces greenhouse gas emissions, and mitigates climate change (Yadav et al., 2023). A study by Ramesh et al. (2010) found that Organic farming, despite lower crop yields in some cases, proved financially advantageous due to higher profits attributed to premium pricing and reduced cultivation costs and showed positive effects on soil quality and nutrient availability, promoting long-term sustainability in crop production.

Challenges

Sustainable agriculture holds the promise of addressing the world's food security needs while preserving the environment, yet it is not without challenges. The widespread adoption of sustainable agriculture is hindered by farmers' limited commitment to and focus on sustainable practices, along with institutional obstacles in providing customized support and expertise (Silici et al., 2015). Furthermore, adopting sustainable agricultural practices encounter significant challenges. These include traditional land management methods, limited knowledge, excessive fertilizer and chemical input usage, repetitive crop rotations, insufficient extension services.

Additionally, obstacles arise from agricultural policies at global, national, regional, and local levels, necessitating institutional reforms to address these complexities (Rehman and Farooq, 2023). Implementing knowledge-intensive practices like biological pest control and labor-intensive methods such as composting, IPM, and minimum tillage requires farmers to acquire technical skills and managerial expertise. In particular, tasks like weeding and mulching, which are integral to these practices, often place a heavier burden on women than men (Silici et al., 2015). Climate change would increase crop susceptibility to severe weather events, shifts in rainy day frequency, changes in rainfall volume and intensity, more frequent floods and droughts, temperature abnormalities, and humidity level shifts. These factors ultimately threaten the sustainability of agriculture (Das et al., 2020). While climate-smart agriculture (CSA) holds promise for achieving sustainable farming, there are weak linkages among its components at the field level. Furthermore, the concept is often not well comprehended by different stakeholders (Sarker et al., 2019). The second would be the use of pesticides. Pesticides are a fundamental support system for the agri-food industry in its mission to ensure food production, but their use is often viewed as a barrier to achieving sustainability. The primary concerns revolve around the negative impacts they have on both human health and the environment (Lykogianni et al., 2021). Extensive research has explored site-specific crop management strategies through precision agriculture in various agricultural systems, with varying degrees of success, but it has fallen short in delivering consistent enhancements in crop yields, profitability, resource efficiency, and environmental concerns. More effective, dynamic, and integrated site-specific management approaches are emerging, necessitating improved methods for understanding crop growth determinants at relevant spatial and temporal scales (Dobermann et al., 2004). Other limitations in organic agriculture pertain to the utilization of mineral fertilizers, making it difficult to ensure an appropriate nitrogen source and increasing the requirement for land (Muller et al., 2017).

Future Directions

In the future, addressing the challenges of sustainable agriculture requires a multifaceted approach. Research and development efforts should focus on enhancing farmers' technical and managerial skills through comprehensive training programs. Collaborative efforts between researchers, farmers, policymakers, and agricultural extension services are essential to bridge the gap between scientific knowledge and on-field implementation (Gupta et al., 2022). To drive the adoption of new agricultural practices, future interventions should focus on enhancing market access, encouraging farmer experimentation, and promoting the adaptation of technologies, while also considering market, policy, and institutional factors that facilitate smallholder investments (Shiferaw et al., 2009). To pave the way for a brighter future, it is equally essential to integrate CSA approaches into academic curricula, seminars, symposiums, and research initiatives, ensuring broader dissemination at the field level (Sarker et al., 2019). Encouraging the adoption of climate-smart

agricultural practices and promoting agroforestry systems can significantly enhance the resilience of farming systems against climate change impacts (Awazi et al., 2022). Moreover, fostering community engagement and knowledge sharing can empower local communities to develop context-specific sustainable agriculture solutions, ensuring the long-term sustainability of agriculture while addressing global food security needs.

Conclusion

In conclusion, sustainable agriculture represents a multifaceted approach to address the global demand for food while minimizing environmental impact. Various strategies exist within sustainable agriculture to uphold environmental sustainability, economic viability, and social equity. Practices such as permaculture, crop rotation, integrated pest management, water conservation methods, aquaponics, hydroponics, climate-smart agriculture, precision farming, and vertical farming prioritize biodiversity, resource efficiency, and resilience. Despite these promising strategies, the adoption of sustainable agriculture faces challenges including excessive chemical use, climate-related disruptions, the need for institutional reforms, limited knowledge dissemination, and market dynamics. Despite challenges, sustainable agriculture offers a crucial pathway to a resilient, food-secure future.

Declarations

Acknowledgement

The authors express their gratitude to the academic community for their invaluable contributions through research papers, publications, and scholarly works. These collective efforts served as the cornerstone for the development and enhancement of this review article.

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Evolution of Parasitoidism in Hymenoptera

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ARTICLE INFO

Review Article

Received : 15.07.2024

Accepted : 01.09.2024

Keywords:

Hymenoptera

Parasitoid

Parasitoidism

Predator

Biological control

ABSTRACT

Insects, the most diverse group of animals, are known to benefit society for a sustainable future. By focusing on the use of natural enemies of pests, including parasitoids and predators, the need for biological pest control for the conservation of agricultural crops has been emphasized. Parasites are organisms that live in the body of another organism and feed on it without killing it, while predators hunt, kill and eat their prey. Parasitoids, on the other hand, live in or on another organism and feed on it, ultimately killing the host. Our study highlights the use of parasitoids to control pests in agriculture and describes the parasitoid lifestyle as an evolutionary transition between parasitism and predation. It also notes that parasitoid larvae typically require only one host to complete their development and can be used to control a wide range of pests. The aim of this study was to determine the origin of the parasitic life form of order Hymenoptera, the mechanisms revealed by the parasitic life form, the importance of life strategies, the types of parasitism and to evaluate the important insect groups belonging to the order Hymenoptera used in biological control.

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Introduction

Insects, the most diverse group of animals in the world, provide many benefits to our society as we work toward a more sustainable future. Although they are terrestrial creatures, they can be found in all types of ecological environments except deep sea bottoms. Many of them are harmful, but some groups are extremely beneficial. Pests are responsible for about one-third of the world's crop losses. Due to the destructive environmental effects of pesticides and development of insect resistance to these pesticides, alternative control strategies have become crucial in the fight to preserve agricultural plants. The most important of these strategies is biological pest control, which employs natural enemies of pests. Beneficial organisms consisting of predator and parasitoid insect groups are used in this struggle. Certain species can be very important in controlling pest populations because many parasitoid hosts are insects and other arthropods that are, or have the potential to be, significant pests in the real world. In most cases, control occurs naturally, and the host only becomes a pest when the natural control agent is absent, such as when a potential pest moves artificially or naturally into an area lacking its natural enemies. However, the deliberate introduction of parasitoids—known as classical biological control—into new regions can have major positive effects on the environment and the economy (Polaszek and Vilhemsens, 2023).

What is Parasite, Predator and Parasitoid?

Predators and/or parasitoids have been used in biological control programs around the world to control a wide range of pests and have become an important tool in conservation efforts. Today, the terms parasitoid and parasite are often used interchangeably. Parasites are a group of organisms that live and feed on the body of another organism (host) (Fernandes and Waquil, 2018). Host acceptance, host suitability etc.. are important for host selection. Parasitic organism can rarely kill their hosts, but rather affect the health of the host. Predators are organisms that feed on another organism by hunting, killing, and eating it (Abrams, 2000). Since predators use their prey for food, the prey eventually dies. Many organisms have a predatory lifestyle and play an important role in the ecosystem.

The concepts of parasites and parasitoids in insects are important for ecosystem dynamics and interactions between organisms. The differences between these two terms help to understand the transition between parasitism and predation. Here are the main differences between parasites and parasitoids:

- Parasitoids can often be larger than their hosts. For example, some parasitoid insects can reach sizes larger than the host insect, whereas parasitoids are usually similar or smaller in size to the host (Price, 1980).

- While parasites spend some or all of their life cycle inside the host, parasitoids usually develop inside the host and eventually complete their life cycle by killing the host (Begon, Townsend, & Harper, 2006).
- Parasites usually have a long-lasting effect on the host and can weaken the host's health. Parasitoids, on the other hand, have a shorter but more destructive effect by killing the host (Godfray, 1994).
- While parasites live by taking nutrients from the host, parasitoids usually consume tissues inside the host, leading to the death of the host in the process (Eberhard, 1990).
- Examples of parasites include intestinal worms and some plant parasites, while examples of parasitoids include insect parasitoids (e.g. some species of flies) (Thacker, 2006).
- Parasites usually lay large numbers of eggs within the host, while parasitoids usually develop on a single host, causing the death of the host in the process (Price, 1980).
- Parasites play an important role for ecosystem balance, while parasitoids can serve a critical function in terms of population control (Begon et al, 2006).
- While parasites generally have a wide range of hosts, parasitoids can be specific to particular hosts and therefore have a more specialised life cycle (Godfray, 1994).

This transition between parasitism and predation is an important factor shaping ecosystem dynamics.

A parasitoid is a group of organisms that live inside the body of another organism (the host) and feed on that host but differs from a parasite in that it kills the host (Godfray, 1994). The parasitoid lifestyle can be viewed as an evolutionary transition between parasitism and predation. The parasitoid life cycle can be defined by the feeding behavior of the parasitoid larva. The larva feeds entirely on or in another arthropod, resulting in the death of the host. The parasitoid larva usually requires only one host to complete its development. Parasitoids can be found in many different species and are used to control many pests. The terms parasitic insect, parasitic Hymenoptera, and parasitic bee are used synonymously with the term parasitoid, although there are significant differences between parasitism and parasitoid life forms (Polaszek and Vilhemsen, 2023).

Parasitoid Groups in Insects

Parasitoidism occurs in the following seven holometabolous insect orders: Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera, Strepsirtera and Trichoptera (Yu et al., 2016; Labandeira and Li, 2021; Bolu et al., 2022). However, among all insect orders, Hymenoptera have the greatest species diversity and numerical abundance (Heraty, 2017). Parasitoids are present in almost every ecosystem in terms of species and numbers of individuals. Parasitoid bees make up 10% to 20% of the order Hymenoptera (Godfray, 1994; Oucike, 1997; Whitfield, 1998; 2003). Approximately 50,000 parasitoid species of Hymenoptera and 16,000 parasitoid species of Diptera are known, with a total of approximately 3,000 species in all other parasitoid insect orders (Godfray, 1994; Feener and Brown, 1997). The number of identified insect species in the world is more than one million, of

which approximately 68,000 species are parasitoids (Godfray, 1994; Stork, 2018; Bánki et al., 2023), representing 6.8% of insects, and Hymenoptera account for 80% of these parasitoid insect species (Oucike, 1997).

Hymenoptera

The incredibly diverse insect order Hymenoptera, with its wide range of life-history traits such as phytophagy, parasitism, predation, pollination, and eusociality, provides an excellent model for studying the evolutionary origin and transition of several important traits. Hymenoptera serves as an excellent model for studying the evolutionary transition between different lifestyles (Branstetter et al., 2017; Peters et al., 2017). It consists of two suborders, Symphyta (the basal lineage of the order) and Apocrita as a derived suborder. Orussidae is the only family in Symphyta that has the parasitic lifestyle. The derived suborder Apocrita is divided into two groups: Parasitica and Aculeata. Almost all members of the Parasitica and some members of the Aculeata have a parasitoid lifestyle. The parasitoid species of Hymenoptera play an important role in the control of agricultural pests and are therefore often used in biological control.

Diptera

The order Diptera contains an estimated 16,000 species as parasitoids, or about 20% of the total number of species with this lifestyle (Feener and Brown, 1997). The order consists of two suborders: Nematocera and Brachycera. The Dipteran parasitoid families are Cecidomyiidae, Acroceridae, Bombyliidae, Nemestrinidae, Phoridae, Pipunculidae, Conopidae, Sarcophagidae, and Tachinidae (Godfray 1994).

Coleoptera and Other Orders

Only five families in the order Coleoptera are parasitic: Carabidae, Staphylinidae, Ripiphoridae, Melonidae, and Stylopidae (Godfray, 1994). The parasitic lifestyle is also observed in two families of Lepidoptera and one family of Neuroptera.

Parasitoid Hymenoptera

Hymenoptera is one of the major orders of insects, including honeybees, wasps, and ants. It is called membranous winged insects because they have two pairs of membranous wings. In Symphyta, the 8th and 9th abdominal segments are transformed into a saw-shaped ovipositor. The female's ovipositor is specialized into a saw-like shape for depositing eggs in plant tissue. The internal component facilitates the expulsion of the egg from the body. The plant is damaged when the hatching larva feeds on the nearest plant tissue. They are therefore considered economically as agricultural and forest pests. In particular, the larvae of *Nematus* sp. (Tenthredinidae) use the leaves of plants as a food source. Although some of them lay eggs on a single plant species, others can lay eggs on many species. *Cephus pygmeus* (Cephididae), which is a widespread species, lays its eggs in Poaceae stems. The hatching larvae hollow out the stem from the inside and prevent grain formation in the plant. Even if grains are formed, they remain hollow. The only parasitoid family in Symphyta is the Orussidae (Godfray, 1994).

The suborder Apocrita consists of two groups, Parasitica and Aculeata. Their larvae are legless, i.e. they have no legs on the thorax. In Apocrita, the ovipositor has evolved into a needle or piercing organ. The diet of Apocrita is very diverse, ranging from an exclusively animal diet to an exclusively plant diet. The larvae of *Apis mellifera* (honeybees) feed solely on pollen and nectar, while the larvae of *Vespa* sp. (wasps) feed on a mixed diet. Still others eat only animal foods. Feeding takes the form of parasitism in or on some other insects and spiders. However, unlike other parasites in the animal kingdom, these parasites eventually kill their hosts, becoming parasitoid rather than parasitic.

When the evolution and some characteristics of parasitoid Hymenoptera and other orders of parasitoid insects are compared, significant differences are observed (Eggleton and Belshaw 1992, 1993). In Hymenoptera, the parasitoid lifestyle probably evolved first in families such as Siricidae and Xiphydriidae, which are related to Orussidae (the only known parasitoid Symphyta family) and exhibit an endophytic-mycophagous diet. However, the parasitoid lifestyle is thought to have appeared independently at least 14 times in Coleoptera and 21 times in Diptera. The main route to parasitism in Diptera is through saprophagous feeding, but it has also been shown to evolve from predation (Quicke 1997).

Although some Diptera species are parasitic on Gastropoda, Hirudinea and Centipedes, no Hymenoptera select these groups as hosts. It is noteworthy that almost all hymenopteran hosts are evolutionarily newer insect groups (e.g., Coleoptera, Lepidoptera, and Diptera). Many parasitoid Diptera (23 families), Coleoptera (11 families) and some Lepidoptera and Neuroptera lay their eggs in the host environment. Thus, the encounter between the egg and the host depends either entirely on chance or on the ability of the first larval stage of the parasitoid to find the host. Parasitoid Hymenoptera, however, take no chances and lay their eggs on, in, or very close to the host. While a few families in the order Hymenoptera have been observed to lay their eggs in the host environment, these are exceptions and represent a secondary specialization rather than an ancestral behavior.

The Origin of The Parasitoid Lifestyle in Hymenoptera

Except for the parasitic sawfly family Orussidae, the ancestral Hymenoptera, members of the suborder Symphyta, are phytophagous, so it is of great interest to discuss how parasitic and predatory life forms have evolved. Handlirsch (1907) was one of the first to discuss the evolution of the parasitoid lifestyle, and his hypothesis is generally accepted. According to this hypothesis, a primitive saw bee (Siricoidea or similar), which was phytophagous and laid its eggs in wood tissue, began to lay eggs in woodworms (Coleoptera larvae) in the same microhabitat. However, it did not explain what possible steps it went through during this transition. Today, two alternative scenarios have been developed based on this idea. According to one scenario, the parasitoid ancestor had the advantage of choosing an oviposition site close to the eggs or larvae of another insect. This is because it provides a more nutritious resource for its offspring than plant material. Over time, with some behavioral and biological

specialization, a dependency will evolve that allows the parasitoid to thrive on an increasingly large and valuable host. An alternative scenario can be suggested by observing the biology of members of the Orussidae, the only living parasitoid Symphyta family today. *Guiglia schauinslandi* (Ashmead), a species of Orussidae found in New Zealand, is parasitic on another Symphyta, *Sirex*, a member of the Siricidae (Nuttall 1980). Siricidae and Xiphydriidae, whose larvae feed on dead wood tissue, have a symbiotic relationship with fungi. They cannot feed on wood tissue that is not contaminated with fungi. Therefore, during oviposition, they inject fungal spores, which they carry in special sacs in their abdomen, into the tree tissue. The wood tissue digested by the developing fungus can be used as food by the hatched larvae of these families. Some members of these families do not carry fungi. In order for their larvae to develop, they must lay their own eggs in the wood tissue where the fungus-bearing species lay their eggs. Based on these data, a credible hypothesis for the evolution of the parasitoid life history can be formulated as follows:

- Of the larvae of fungus-carrying and non-fungus-carrying members of the Siricidae and Xiphydriidae families encountered in the tree tissue where they lay eggs, the larvae of the non-fungus-carrying species should have evolved to kill larvae of the fungus-carrying species.
- The second step in the parasitoid life path is not only to kill these larvae, but to start using them as food, which is an advantage in the competition for food, so there must have been selection in this direction.
- In the next stage, the egg must have been laid on the host larva, a mechanism that leaves the hatching larva no chance of finding its host.

In addition to Hymenoptera, many parasitoid Coleoptera are known to have descended from a mycophagous ancestor that fed on dead wood (Godfray 1994). The most important environmental pressure forcing this transition is the increase in the population of species that colonize wood tissue. This is because spawning, egg deposition, and subsequent larval development in tree tissue provide complete protection from predators and ensure the persistence of the species. However, the occupation of this microhabitat by an insect community beyond its carrying capacity has led to competition for food between species. Here, selection pressure would favor species whose survival depends on their ability to exploit the richer animal food (Rasnitsyn 1980).

This idea of Handlirsch is not accepted by everyone. There are few hypotheses as Eggleton and Belshaw 1992, Malyshev 1968. According to Malyshev (1968), Apocrita and the parasitoid lifestyle originated from a Cephidae-like ancestor that formed gal. A secretion released by the female bee during oviposition caused isolated but nutrient-rich deformations in plant tissues. The availability of such a rich food source gradually led the larvae to settle there. This nutrient-rich tissue became attractive to other insects that laid their eggs in this environment. These larvae, initially feeding only on plant tissue, gradually acquired a carnivorous or parasitoid lifestyle. However, there is serious debate about the validity of this hypothesis. Phylogeny of Hymenoptera shows that the closest group to Apocrita is Orussidae and Siricidae + Xiphydriidae clade.

For Malyshev's hypothesis to be valid, the parasitoid lifestyle must have evolved several times independently in Hymenoptera.

Mechanisms Revealed by the Parasitoid Lifestyle

Concurrent with or immediately following the parasitoid lifestyle, several mechanisms have evolved to make this lifestyle competent. These can be grouped under the following headings.

- Host Selection
- Egg Laying Strategy
- Reproductive Strategy

Host Selection

The place where the insect can lay its egg is the place where the larva will feed. Almost all ancestral Hymenoptera have phytophagous larvae (Gauld and Botton, 1988), meaning that the egg is laid in useful plant tissue and this ancestral behavior is still maintained. The host is usually phytophagous but can also be a predator or a scavenging arthropod.

Parasitoid eggs are laid by different species on different hosts, or the host may be specialized for different stages of a host's life cycle. At the same time, each natural enemy species can attack a series of hosts ranked from high quality to low quality. Some parasitic bees have evolved from predators rather than parasitoids, which make greater use of a host to complete their development. Presumably parasitoid life has evolved either by a decrease in the size of a predator or an increase in the size of a prey (Godfray, 1994).

Egg Laying Mechanism

The ovipositor is formed by differentiation of the 8th and 9th abdominal segments. In parasitoids, the host is usually found by females that lay their eggs directly in or on the host. The eggs are placed in the host by the ovipositor. The ovipositor has two functions. These are oviposition and venom injection. Both functions are seen in all Apocrita except the Aculeate group, but in later groups the ovipositor is not used for oviposition but only for venom injection. Many hosts are found under the bark of trees or between the leaves, where the parasitoid cannot reach them directly. For this reason, there has been a great deal of differentiation in the ovipositor. For example, many parasitic bees have specialized ovipositors that can easily pierce the host's cuticle and deliver their eggs to the host located between tree trunks or leaves (Onstad and McManus 1996; Strant and Obrycki, 1996).

The ovipositor is usually strong enough to pierce wood tissue and long enough to reach deep places. Studies of the order Hymenoptera have revealed the presence of zinc or manganese in the ovipositors and mandibles of species that can only bore holes. The presence of these metals has been associated with reduced abrasion by causing cuticular hardening (Quicke et al., 1998; Morgan et al., 2003). There are also various specializations in identifying egg-laying sites on the host, for example, it has been shown that members of the Orussidae use a vibration sensing method to locate suitable oviposition sites (Vilhelmsen, 2001). During oviposition on the host, the vibrations generated by striking the wood with the antennae are converted into nerve impulses that are picked up by the forelimbs via the

basitarsal spurs and transmitted along the basitarsi to thin-walled areas on the tibia and through the hemolymph to the subgenital organs.

At the same time, most of the venom injected into the host through the venom injecting ovipositor paralyzes the host without killing it. It is important for the parasitoid that the host be immobile. A moving host can harm the parasitoid. The immobilized host is vulnerable to all kinds of predatory attacks. To prevent this, the parasitic bee lays its eggs in a protected or semi-protected host.

Reproductive Strategy

Darwin observed that in animals, females do not immediately mate with the first males they meet but find a way to select high quality males. The selection of high-quality males has adaptive value because it allows more offspring to be produced and the offspring produced are of higher quality. If females are selective in mating, then males are high quality mates. Natural selection has favored the evolution of behaviors that maximize the reproductive success of males and females (Bahceci, 2000; Freeman and Herron, 2009). The recognition and selection of a superior male increases the chances of growth and survival of an animal's offspring (Bahceci, 2000).

Classification of Parasitoids

Parasitoids are classified according to the host in which they live as parasitoids and in which their offspring develop. Some parasitic flies (Diptera) lay their eggs directly in or on the host, while others lay their eggs close to the host. If the eggs are laid near the host, the host may eat the eggs, or the mobile larvae that hatch from the eggs may enter and infect the host. Many parasitoids attack the host only at a particular stage.

Species that lay egg, larva, pupal and complete their development in the egg stage of the host are called egg parasitoids. Parasitoids that attack in other life stages are called larval, pupal and adult parasitoids. In other words, those that complete their development in the larval stage are called "larval parasitoids", those that complete their development in the pupal stage are called "pupal parasitoids", and those that complete their development in or on an adult insect are called "adult parasitoids".

There are also parasitoids that lay eggs in one stage of the host but complete their development in another stage. These are known as egg-larval, larval-pupal parasitoids. In egg-larval parasitoids, the parasitoid lays its egg in the egg of the host, but the host completes its development when the host enters the larval stage. A similar situation occurs in the larval-pupal parasitoid. The parasitoid lays its egg in or on the larva of the host, and the host completes its development when the host reaches the pupal stage.

Parasitoids are also classified according to where the offspring feed. Species that develop inside the host are called "endoparasitoids", while those that feed outside the host are called "ectoparasitoids". Also, there are two types of parasitism, these are superparasitism and multiparasitism. Superparasitism is a form of parasitism in which the host (typically an insect larva such as a caterpillar) is attacked more than once by a single species of parasitoid. Multiparasitism or coinfection, on the other hand, occurs when the host has been parasitized by more than one species.

If only one individual develops in a host, it is called a solitary parasitoid; if more than one individual develops in a single host, it is called a gregarious parasitoid.

A primary parasitoid is a single individual that develops in the host. Secondary or hyperparasitoids are species that develop within or above the primary parasitoid and infect it. There are heterenome parasitoids in the family Aphelinidae of Hymenoptera. Among them, female wasps develop as primary parasitoids on Homoptera, while males develop as hyperparasitoids on females of their own or other species (Onstad and McManus 1996; Strant and Obryeki, 1996).

Today, two new categories of parasitoids have been added to the literature. These are coidiobionts and idiobionts. In the case of the coidiobiont, the host can continue to develop after being infested, whereas in the case of the idiobiont, the host cannot develop after being infested, i.e. the host is permanently paralyzed. In fact, it is more accurate to consider choidiobionts, idiobionts, ectoparasitism and endoparasitism as life strategies and the others as types of parasitism. Also, a feeding tactic known as kleptoparasitism involves an animal purposefully stealing food from another. Theft from individuals of the same species might be intraspecific, or it can be interspecific, including members of different species (Broom et al., 1998; Furness, 1987).

Life Strategies

The transition between ectoparasitism and endoparasitism is a critical stage in the evolutionary adaptation of parasitoids. Over time, ectoparasites can evolve into an endoparasitic lifestyle by moving into the internal organs of their hosts (Quicke, 1997). This transition allows parasites to exert more control over their hosts and develop a more efficient feeding strategy. Similarly, transitions between idiobiont and koinobiont play an important role in the evolution of parasitoids. While idiobiont parasitoids become more resistant to the defences of their hosts, koinobiont parasitoids have the opportunity to feed for longer periods of time, affecting the life span of their hosts (Vinson, 1990). These transitions can affect the balance in ecosystems by increasing the diversity of parasitoids. The evolution of parasitoidism in insects has been shaped by transitions between ectoparasitism and endoparasitism and between idiobiont and koinobiont. These transitions play an important role in ecosystems by determining the effects of parasites on their hosts and their feeding strategies. Future research will contribute to a better understanding of these processes and elucidate the evolutionary dynamics of parasitoids.

Idiobiont Strategy

In Hymenoptera, the ancestors of parasitoid species probably had larvae that fed endophytically (feeding within plant tissue) and consumed other larvae they encountered (Gauld and Bolton, 1988). The next evolutionary step toward a parasitic lifestyle may have been for the adult female to deliberately seek out shoots or plant stems containing other immature insects that would serve as a food source for her larvae, and to lay the eggs near that food source (Gauld and Bolton, 1988). This strategy probably evolved into the widespread and relatively

unspecialized parasitoid behavior seen today. The hymenopteran egg consumes its host to develop, then pupates and emerges from the host as a larva. Such a parasitoid, i.e. a parasitoid that stops host development when it lays its eggs on the host, is called an “idiobiont”. An idiobiont immediately paralyzes/kills its host and is estimated to have lower fecundity (Pennacchio and Strand, 2006; Yadav and Borges, 2018). Ectoparasitoids, endoparasitic eggs, and pupal parasitoids that develop (outside/on) the host by permanently paralyzing it are usually idiobionts. The idiobionts that attack larval host stages are almost always ectoparasitoids (Yadav and Borges, 2018). Egg, pupal and adult parasitoid are usually idiobionts. Egg-larval and larval-pupal parasitoid are koinobionts. Ectoparasitoids typically immunosuppress their hosts (Pennacchio and Strand, 2006). Most hosts in the idiobiont strategy are either hidden or protected. Specialization of the parasitoid ovipositor has occurred in order to reach the host. Thus, most ectoparasitic idiobionts are highly specialized. Ectoparasitic idiobionts inject venom into the host during oviposition, causing death, paralysis, or developmental arrest. This prevents the developing parasitoid from falling off the host and the host from harming or killing the parasitoid.

Some idiobionts may lay eggs on exposed hosts. Since the ectoparasitic larvae would be very vulnerable to crushing and damage in this situation, these idiobionts have an “endoparasitic” lifestyle. Recent studies have shown that the ancestor of Ichneumonoidea was an idiobiont ectoparasitoid (Sharanowski et al., 2021), in which the parasitoid larva feeds inside the host and secures itself. On the other hand, this strategy exposes them to the host’s immune defense system. Endoparasitic idiobionts stop or reduce host responses to their larvae. They use toxins or physically paralyze the host by laying eggs in vital organs such as the brain. In the case of egg parasitism, the host’s eggs are lysed. Except for this natural parasitism, there is kleptoparasitism. Kleptoparasitism, “parasitism” by theft (not true parasitism). Kleptoparasites steal food (e.g., prey) from another animal as their main feeding strategy, but do not feed directly from a host’s body.

In the idiobiont strategy, the parasitoid is usually outside the host and can be thought of as an unprotected piece of flesh. Idiobiont ectoparasitoid larvae are vulnerable because they are immobilized after venom injection and open to attack by many predators. For this reason, idiobionts are often found in places that are not easily accessible, such as between the bark of trees.

The parasitoid spends a lot of time searching for, finding, and laying eggs on these hosts. Because of this, the female idiobiont tends to live a long time. During their lifetime, these species lay many eggs to maturity. This is called “synovigenic”. In the idiobiont strategy, the parasitoid must complete its development on the host without being noticed by other organisms. The production of such eggs is more difficult for the adult female parasitoid. This is because the parasitoid female must feed more in order to produce nutritious eggs. These species generally exhibit feeding behavior on the host to provide protein for egg production. The family Dryinidae (Chrysidoidea) has several interesting biological features (Olm, 1984; Guglielmino, 2002). These wasps are both parasitoids and predators of Auchenorrhyncha hosts

belonging to the order Hemiptera. Female wasps lay eggs on the hosts and their pupa develop outside the hosts (ectoparasitoid). Female wasps also capture and feed on hosts. Male wasps, however, do not hunt or feed on hosts. Parasitoid wasps with both predatory and parasitoid behavior are rare in Hymenoptera. In addition, no other wasps with predatory behavior are found in the Chrysidoidea (Melo et al., 2011). Therefore, the origin of predatory behavior in Dryinidae is likely to be an independent trait that has gained events in the evolution of Hymenoptera (Yang et al. 2021). Some idiobiont parasitoids protect the host by hiding it after paralyzing it. For example, some bethylids protect their paralyzed host by dragging it into their shelter. Others, such as pompilids and sphecids, overcome this problem by building a nest before finding the host (Gauld and Bolton, 1988).

Koidiobiont Strategy

If the host can continue to develop after the eggs are laid, such parasitoids are called “choidiobionts”. Choidiobionts are thought to have higher fecundity (Pennacchio and Strand, 2006). Egg-larval, larval pupal koinobionts is proovogenic. Almost all choidiobionts are endoparasitoids. Endoparasites complete their development inside the host. They are expected to encounter a strong host immune defense response, but they usually overcome the immune defenses (Schmidt et al., 2001). Unlike the idiobiont strategy, hosts in the choidiobiont are not hidden. The host is usually unprotected on plants. Since it is easier to reach the host, i.e. to lay eggs, there is not much specialization here. Since the endoparasitic choidiobionts lay their eggs inside the host, the parasitoid is prevented from falling off the host or the host from directly damaging the parasitoid, but the host is open to predation. For this reason, choidiobiont ectoparasitoids can carry many eggs at the same time and lay them when they find a suitable host (Gauld, 1988; Sharanowski, 2009).

Types of Parasitoidism

Primary Parasitoidism

Primary parasitoids complete their development by laying their eggs inside the host. The best studied species is the aphid bee *Aphidius smithi*, which has demonstrated control of the exotic pea aphid *Acyrosiphon pisum* in North America. The female bee lays eggs inside the aphid, and over a period of about 8 days, the parasitic larvae gradually eat the aphid from the inside, killing it. The fourth instar larva weaves a cocoon inside the dead aphid while the outer shell forms and changes from green to light brown (this is called the “mummy”). After about 4 days when the larva pupates (or about 12 days after the original oviposition), the new adult primary parasitoid cuts the circular exit hole on the dorsal side of the mummy and expels itself.

Secondary Parasitoidism

It occurs when the primary parasitoid is parasitized by another parasitoids. Aphid hyperparasitism has been studied extensively. They divided aphid hyperparasitoids into two categories based on adult oviposition and larval feeding behavior.

- The female bee of the endophagous species lays her egg inside the primary parasitic larva while it is still developing inside the living aphid before the aphid mummifies. The egg does not open until after the mummy has formed and then the hyperparasitic larva feeds internally in the primary larval host.
- The female bee of the ectophagous species lays her egg on the primary parasitoid larva after the aphid has been killed and mummified. The hyperparasitic larva feeds externally on the primary larval host while both are still inside the host. The 8 taxonomically listed genera that are basic (essential) in these behavioral criteria are arranged as shown below. Endophagus hyperparasitoid species in *Alloxysta*, *Phoenoglyphis*, *Lytoxysta*, *Tetrastichus*, ectophagus species in *Asophes*, *Dendrocerus*, *Pachynwon* and *Conna*.

Tertiary Parasitoidism

A secondary parasite occurs when it is parasitized by another parasite. For example, the larvae of *Apanteles* inhabit caterpillars (primary parasite), while certain species of Chalcidoidea (*Mesochorus* and *Tetrastidus*) infest the larvae of the primary parasite and lay eggs (secondary parasite). The larvae of this secondary parasite develop on or in the larvae of the primary parasite. These secondaries are beneficial insects to humans (if the primary parasitized insect is harmful). Pleurotropis of Chalcidoidea also lives on this secondary parasite (tertiary parasite) and is considered harmful to humans (Demirsoy, 1995).

Facultative Parasitoidism

Some parasitoids behave as both secondary and primary parasitoids. These are called facultative parasitoids. *Pachyneuron coccolar* can develop as tertiary parasitoids on members of its own species or on other Chalcidoidea that develop as secondary parasitoids on encyrtid hosts. Despite this, an interesting feature that has been demonstrated is that *P. concolor* is a true facultative hyperparasitoid but develops as a primary parasitoid on butterfly pupae. The choice of host is based on the fly pupae in the puparium or the one localized on the soft body inside the hard dry container, regardless of whether it is the primary parasitoid on the mummified host. Unfortunately, when *P. concolor* acts as a primary parasitoid, it attacks the beneficial aphidophagous butterflies. *P. concolor* is therefore detrimental to biological control programs (Sullivan, 1987).

Parasitoids as Biological Control Agents

The history of parasitoids has progressed in parallel with the evolution of agricultural practices. The first applications of biological control date back to ancient times. The Egyptians used natural enemies in the control of pests. However, modern biological control practices started towards the end of the 19th century. The first systematic use of parasitoids occurred during the red spider (*Tetranychus* sp.) infestation in Australia in the 1880s. During this period, parasitoids brought to Australia were effective on pests. In the early 1900s, important developments were experienced in the field of biological control. In particular, *Trichogramma* sp. parasitoids were effective in the control of many pests by targeting eggs.

This species was widely used in the USA in the early 1900s (Van Driesche & Bellows, 1996). *Trichogramma* sp. has been recognised as an effective tool to reduce pest populations in agricultural fields.

In the 1950s, other parasitoid species, such as *Cotesia glomerata*, were also introduced for biological control. This species was effective against important pests such as cabbage moth (Godfray, 1994). In the same period, *Encarsia formosa* was also used in the control of whiteflies. These species contributed to the widespread use of biological control methods in agriculture. Today, the parasitoid species used in biological control have become more diversified. Research is focussed on the discovery of new species and increasing the effectiveness of existing species. The use of parasitoids has become an important strategy for sustainability in agriculture.

The superfamilies and families containing parasitoid species of the order Hymenoptera can be listed in order of availability as follows (Godfray, 1994; Quicke, 1997).

- Superfamily Ichneumonoidea: Ichneumonidae: Braconidae
- Superfamily Chalcidoidea: Eulophidae : Chalcidoidea
- Superfamily Cynipoidea: Cynipidae
- Superfamily Platygastroidea: Platygastriidae
- Proctotrupoidea: Proctotrupidae

Parasitoids are frequently used as biological control agents against harmful insects. *Torymus sinensis* (Hymenoptera: Torymidae), a solitary ectophagous parasitoid species that has recently been used in biological control, can be given as a good example of this control method. This species is used against the chestnut gall wasp (*Dryocosmus kuriphilus*), which causes loss of productivity and quality in chestnut trees, which has important economic and ecological importance. *D. kuriphilus* is a Chinese gall wasp that was introduced to the United States in 1974 (Rieske, 2007). It was first reported in Europe in 2002 (Brussino et al., 2017) and in Tukiye in 2014 (Cetin et al., 2014) from chestnut trees in forested areas in Yalova. In the following years, it was observed that the population density increased rapidly in the Marmara, Aegean and Black Sea Regions. *D. kuriphilus* is considered to be one of the most harmful organisms for plants of the genus *Castanea*, by causing galls on buds and leaves, reduced branch growth and fruit formation (Lobo et al., 2024). The resulting galls reduce photosynthesis and therefore can cause plant death in heavy infestation (Payne et al., 1975; Anagnostakis and Payne, 1993).

To control *D. kuriphilus* are used various methods such as biological, chemical and mechanical/cultural. However, since it lays its eggs inside the fruit, the success rate of chemical control is very low. In a mechanical control method, shoots with gal formation are cut. However, the most effective method on the pest species is biological control. The *T. sinensis* parasitoid bee is used as a biological control agent. This species was identified for the first time in Turkey in the Marmara region (Cetin et al., 2014). In the following years, the population increased rapidly and was recorded from different regions (Yıldız et al., 2020; Akyuz et al., 2022; Kok et al., 2023). These natural enemies are ectophagous parasitoids of the pest *D. kuriphilus* and have a solitary life form (Quacchia et al., 2013). *T. sinensis* females lay eggs on *D. kuriphilus* gals

(the body of the host *D. kuriphilus* or the wall of the larval chamber) in early spring. Several eggs per larva have been observed in a single compartment under natural conditions. However, only one parasitoid larva completes its development among *T. sinensis* larvae due to cannibalism (Amorim et al., 2022). The parasitoid larva feeds ectophagously on the host larva. In late spring, the mature larvae stop feeding, but do not pupate until winter, and adults emerge the following spring. By eating the gall wasp larvae, they help control the pest population.

Moriya et al. 2024 conducted a study on the long-term effect of *T. sinensis* on *D. kuriphilus* in Japan between 1982 and 2023. This study showed that the density of the pest fell from 42.5% to 0.7% in the first 10 years, a reduction of about 61-fold, and remained at low levels for the next 30 years. However, it was observed that pest density occasionally exceeded the economic damage threshold. Over the same time period, the density of *T. sinensis* was also found to be synchronized with fluctuations in pest density. These results show that *T. sinensis* has played an important role in suppressing *D. kuriphilus* for 40 years and that biological control is an effective method. In this context, effective control of the pest *D. kuriphilus* allows chestnut trees to produce more fruit (Luo et al., 2014) and minimizes the negative impact on the environment by reducing the use of chemical pesticides (Gehring et al., 2018).

Conclusion

The order Hymenoptera exhibits interesting evolutionary patterns and adaptations related to parasitism. The parasitoid insects within this order are organisms that live on or inside other organisms, feeding on them and ultimately killing the host organism. Hymenopteran parasitoids have developed various adaptations for locating their prey, accessing them, and laying eggs. For example, Hymenopteran parasitoids possess keen olfactory abilities to locate host organisms. They also have specialized structures and behaviors for oviposition. Some species deposit their eggs inside the host organism, while others lay their eggs externally, employing different adaptation strategies. For instance, some species secrete chemical substances to alter the behaviors of host organisms or suppress their immune systems. These adaptations enable the successful habitation and reproduction of the parasitoids on their host organisms. Furthermore, these adaptations are crucial for understanding the evolutionary relationships of parasitoids with host organisms. In addition, there are many parasitoid species used as biological control agents. It is especially important for economically important plant species.

Declarations

Ethical Approval Certificate

Not applicable.

Author Contribution Statement

Sevda Hastaoğlu Örgen: Methodology, Validation, Writing - original draft, Investigation, Review and editing.

Mehmet Gülmez: Investigation, Conceptualization, Data curation, Review and editing.

Fund Statement

No financial support was received for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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St John's Wort (*Hypericum perforatum* L.) in Broiler Nutrition

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ARTICLE INFO

ABSTRACT

Review Article

Received : 22.07.2024

Accepted : 06.08.2024

Keywords:

St John's Wort

Hypericin

Animal health

Broiler

Antibiotics

Nowadays, the concept of sustainability is important in poultry meat production as in every field. The health status of the birds must be well-stated for sustainable broiler production. Considering that synthetics attract reactions and some of them are banned or limited, the use of natural feed additives for health protection has been the focus of research attention. St John's wort is a plant that can positively affect the health of animals with bioactive components such as the hypericin it contains. St John's wort or hypericum (*Hypericum perforatum* L.) has antiviral and antimicrobial effects in broilers and positively affects on blood biochemical parameters. This positive effect on health level also improves performance. The review aims to give information about St John's wort and to examine a limited number of studies on their use in the diet of broilers.

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Introduction

Broiler production aims to obtain high quality and amount of animal protein within the optimum feed conversion ratio. Therefore, quite different feed additives are used and some of those are antibiotics which started to be used in poultry nutrition in the 1940's. However, due to the development of antibiotic-resistant bacterias in the animal-food-human cycle and residues in meat, their use as growth promoter feed additive is prohibited in the European Region (Castanon, 2007). Nowadays, animal health protection is very important for sustainable production.

Accordingly, the focus has been on the possibilities of using natural source feed additives such as probiotics, prebiotics, organic acids and herbal stuffs in poultry nutrition. Herbal origin feed additives are added in their extract or directly to the feed and they have various bioactive components which affect broilers' health or performance parameters. These quite diverse effects; increasing feed consumption, flavoring, stimulating digestive enzyme secretion, antimicrobial, antioxidative

activity, coccidiostat activity, antiviral and immune supporting and anti-inflammatory effects (Kırkpınar et al., 2011).

Hypericum perforatum L. is a species 0.3-1.2 m high perennial plant with the names of St John's wort, hypericum, millenpertuis ("sarı kantaron" in Turkish), in the family *Hypericeae* (*Clusiaceae*) (Southwell et al., 2001). It is native to Europe, West Asia, North Africa, Maderia and the Azores and has weed status in North America and Australia; besides it spreads by oellet or seeds and can be found at pastures, disturbed sites or side of the highways (Saddiqe et al., 2010). This plant grows naturally in Türkiye between the altitudes 750-3200 m; has been used for a long time in folk medicine (Cakir et al., 1997). It is believed that the name "hypericum" derived from the Greek words "hyper" (over) and "eikon" (image), St John's wort may have arisen as the flowers bloom around St John's Day, 24th of June (Barnes et al., 2001). This review aims to that give information about hypericum and indicate its effects on broiler nutrition.

The Use of Hypericum in Broiler Nutrition

Hypericum has variable bioactive components such as flavonoids, hyperforin, pseudohypericin, hyperprofin and hypericin (Landy et al., 2012; Davoodi et al., 2014). Hypericin and pseudohypericin are major bioactive components of hypericum called photodynamic pigments that have many positive effects (Gadzovska et al., 2005). These components have many effects (antiviral, antibacterial and antidepressant) on human and animal health. The main active compositions of hypericum extract are hyperoside, quercitrin, quercetin, pseudohypericin, and hypericin, and a combination of these compounds could mediate the antiviral activities in high-performance liquid chromatography/electrospray ionization–mass spectroscopy (Chen et al. 2019). Hypericum is known for its use in the treatment of mild-to moderately severe degenerative disorders (Barnes et al., 2001). Nonetheless, hypericum has valuable lipid content. The fatty acid composition of hypericum is shown in Table 1 (Bakır, 2018).

Table 1. The fatty acid composition of Hypericum (Bakır, 2018).

Fatty Acids	g/100g
Myristic acid	0.07
Palmitic acid	8.86
Margaric acid	0.04
Stearic acid	4.76
Cyclopropanoic acid	0.04
Arachidic acid	0.47
Tricosanoic acid	0.24
<i>Total Saturated Fatty Acids</i>	14.48
Palmitoleic acid	0.1
Linolelaidoyl chloride	0.1
Oleic acid	-
Petroselinic acid methyl ester	34.23
Gadoleic acid	0.38
Erucic acid methyl ester	0.11
<i>Total Mono Unsaturated Fatty Acids</i>	34.92
Hexadecadienoic acid methyl ester	-
Linoleic acid	44.35
11,14-Eicosadienoic acid methyl ester	1.07
<i>Total Poly Unsaturated Fatty Acids</i>	45.42
Conjugated Linoleic acid	5.08
<i>Total Trans Fatty Acids</i>	5.08

Although quite limited, there is some research on the use of hypericum in broiler nutrition. A study has shown that dry extract of hypericum supplementation in broiler feeds induces feed conversion ratio improvement and reduces mortality rate, also increasing New Castle antibody titer in broilers (Feizi and Nazari, 2011). These results were confirmed by measurements taken on days 10, 25, 34 and 42. Besides, Chen et al. (2019) demonstrated that hypericum extract had significant anti-IBV effects in vitro and in vivo, respectively.

In their study Landy et al. (2012), examined the effects of the adding dried hypericum (5 and 10 g/kg) in diets on broilers and compared it with the impact of antibiotics. They found the highest avian influenza antibody titers in 10g/kg hypericum group at day 42. Serum protein, albumin, triglyceride and low-density lipoprotein levels

were not affected. However, hypericum significantly decreased total cholesterol (5 g/kg) and high density lipoprotein levels (10 g/kg). In addition, carcass traits were not significantly influenced by dietary treatments except for percentage of liver and heart that increased antibiotic and control group. According to these results, hypericum improved immunity and has beneficial effects on lipid metabolism.

Some researchers state that adding alcoholic extracts of hypericum in drinking water (2 ml/l) of broiler has some positive effect on physiological response to stress and welfare (Skomorucha and Sosnowka-Czajka, 2013). It has contributed to a decrease in cholesterol levels and an increase in the level of the immunoglobulins in the blood. Furthermore, hypericum extract increased body weight and reduced mortality.

Davoodi et al. (2014) investigated that the effect of adding hypericum to drinking water in comparison to virginiamycin on performance and welfare of broilers. They found that feed intake and final body weight have increased with all hypericum doses (150, 200 and 250 ml/l) and virginiamycin. Liver, heart and gizzard percentages were significantly lower in all treatment groups. Triglyceride, cholesterol and low density lipoprotein level decreased and high density lipoprotein levels increased significantly in all hypericum and virginiamycin groups. It has been determined that adding hypericum (all levels) and virginiamycin in the water increases New Castle antibodies, also data showed that *E. coli* population reduce and *Lactobacillus* population increased significantly in all treatments groups. This research data shows that hypericum has important positive effect on broiler health.

In a study, Stress-Bio-Max (SBM) mixture (betaine, vitamin C, lavender, *Melissa officinalis* and hypericum) that the main component is hypericum was used against heat stress on broilers (Behboodi et al. 2021). This mixture has been added to drinking water at certain levels (0, 0.25, 0.50 and 1 ml/l). The effects of the mixture against heat stress are quite visible in this study. Especially at 0.25 and 0.50 ml/l levels, it has been observed that live weight gain is increased. Nonetheless, corticosterone levels were found lower in these groups on the day 32 and 42, compared to the control group. Glutathione peroxidase and superoxide dismutase activities were decreased, and total antioxidant capacities were increased in all treatment groups. Malondialdehyde, aspartate transaminase and alanine aminotransferase levels also were decreased to 0.25 and 0.50 ml/L. Researchers have stated that SBM can alleviate the negative effects of temperature stress in broilers.

Conclusion

It is important to protect animal health for a sustainable poultry meat production. Hypericum has been used in traditional medicine, but it has also attracted attention in modern medicine due to the bioactive substances it contains. This plant, which grows in Türkiye and in many regions of the world, has been used as a natural health protection agent. It is given to broilers in different forms and levels within feed or water. A limited number of studies show that hypericum strengthens immunity against viral diseases such as New Castle and Avian Influenza. It has a repressive effect against pathogenic microorganisms

in the intestine, while supporting beneficial bacteria. It can positively affect blood biochemical parameters especially lipid profile. Due to all these effects, it has also shown improvement in performance parameters.

In conclusion, it is thought that hypericum may be a health-protective, performance-improving natural feed additive in the diets of broilers. According a few studies show that St John's wort can be given to broilers in different forms, mixtures and methods. 50 ml/l or a different form can be added up to 250 ml/l as an alcohol extract in water. The addition of 10 g/kg to the feed provided positive improvements. In addition, more comprehensive studies are needed to determine the way it is given (feed or drinking water), the form (as extract, dried or only active ingredients) and levels.

Declarations

Author Contribution Statement

Please indicate how each author contributed to this work and at what stage. For example:

Özgün Işık: Investigation, supervision, writing, editing.

Helin Atan: Investigation, writing.

Figen Kırkpınar: Investigation, supervision.

Ayşe Betül Avcı: Investigation, writing.

Conflict of Interest

The authors declare no conflict of interest.

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Aflatoxin M1 Levels in Cheeses in Türkiye: A Review

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ARTICLE INFO

Review Article

Received : 14.08.2024

Accepted : 10.09.2024

Keywords:

Mycotoxins

Aflatoxin M1

Cheese

Milk and Dairy Products

Legal Regulations

ABSTRACT

Milk and dairy products mostly contain mycotoxins such as aflatoxin M₁, aflatoxin M₂, ochratoxin, cyclopiazonic acid, trichothecene, zearalenone, patulin. Mycotoxins in cheese are produced by certain types of fungi. These either directly contaminate the cheese or feed, or indirectly contaminate the milk used in cheese production. Aflatoxin, causes serious impacts on human and animal health, thus costs the world economy billions of dollars. The first legal regulation regarding aflatoxin M₁ in Türkiye came into force in 1990. Subsequently, various legislations were published in 1997, 2002, 2008, 2011 and 2023. In this study, 60 publications were examined that investigate the presence of aflatoxin M₁ in various cheeses in Türkiye in the last 50 years (1973-2023). In the studies, aflatoxin M₁ was detected in almost all cheeses, and only a few were found in rates exceeding the Turkish Legal Limits. Researchers stated different limits for aflatoxin M₁ in cheese. To minimize the presence of aflatoxin M₁, the following recommendations should be considered. Developing new detection methods, enacting new legal regulations, increasing the frequency of legal inspections, improving the production, transportation and storage conditions of milk and dairy products, improving feed quality, increasing awareness of the health effects of aflatoxin M₁.

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Introduction

Uncontrolled mold growth or some molds are harmful to human health and pose a serious threat to food chains. Molds can multiply in most acidic products (like fruits or juices) and in foods with moderate moisture content (like breads and baked goods) (Jubayer et al., 2021). Molds are found in large quantities in the environment and cause many problems in agriculture, medicine, food, pharmaceutical and cosmetic industries, etc. (Lecellier et al., 2015). The food groups that cause mold multiply are rich in carbohydrates, protein, fat and moisture (Garnier et al., 2017). Molds produce chemicals called mycotoxins, which can be carcinogenic, mutagenic, teratogenic and toxic to humans and animals (Moss, 1990).

Mycotoxins are secondary metabolic products of fungi of *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizoctonia*, *Claviceps* and *Stachybotrys* (Leś et al., 2023). Aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A are the main mycotoxins (Abd-Elsalam & Rai, 2019). Aflatoxin, one of the mycotoxins, is mostly multiplied by *Aspergillus parasiticus* and *flavus*. Aflatoxin can occur in dry oily fruits (hazelnuts, peanuts, etc.), dried fruits (dried plums, dried mulberries, etc.), oilseeds, cereals and cereal products, and animal foods (milk, meat, cheese). It causes serious impacts on human and animal health, thus costs the world economy billions of dollars (Shabeer et al., 2022).

Aflatoxins were found in 1960 with the association of “Turkey X disease”. Since then, it has been understood that low levels of mold metabolites in foods and feeds can cause disease in humans and animals (Leś et al., 2023). Aflatoxins presence in foods and feeds and are toxic metabolites (Garrido et al., 2003). Studies have determined that aflatoxins are seriously carcinogenic (Kimanya et al., 2021; Koshiol et al., 2017; Marchese et al., 2018; Saha Turna & Wu, 2019; Wang & Tang, 2004).

When ruminants consume foods containing aflatoxin B₁ and aflatoxin B₂, these toxins are metabolized and excreted through milk as aflatoxin M₁ and aflatoxin M₂ (Garrido et al., 2003). About 0.3-6.2 % of consumed aflatoxin B₁ is changed to aflatoxin M₁ (AFM1) in the liver (lactating animals) via cytochrome P 450 and excreted into milk (Iqbal et al., 2015; Ketney et al., 2017).

AFM1 can be presence in dairy products due to the binding to milk proteins, especially casein (Vaz et al., 2020). Milk and dairy products mostly contain mycotoxins like AFM1, AFM2, ochratoxin, cyclopiazonic acid, trichothecene, zearalenone, patulin (Oruç, 2014). The International Agency for Research on Cancer (IARC) determines AFM1 as a Group 2B agent (possibly carcinogenic to humans) (IARC, 1993).

Cheese is an ideal substrate for mold growth but a poor substrate for mycotoxin production (Bullerman, 1981). Mold formation in cheese causes problems (about food safety and food quality) and significant economic losses (Kure & Skaar, 2019). There are approximately 4000 types of cheese in World and Türkiye is rich in cheese diversity (Çakmakçı & Salık, 2021). About 200 different types of cheese are produced in Türkiye, excluding traditional cheeses produced in limited areas (Öründü & Tarakçı, 2021). Mycotoxins in cheese are produced by certain types of fungi. These either directly contaminate the cheese or feed, or indirectly contaminate the milk used in cheese production (Hymery et al., 2014). Mycotoxins isolated from cheeses are AFM1, cyclopiazonic acid, isofumigaclavine A, mycophenolic acid, patulin, penitrem A, PR imine, roquefort C, sterigmatocystin, penicillic acid and ochratoxin A (Richard & Arp, 1979; Scott et al., 1977; Sengun et al., 2008).

The levels of mycotoxins reported in cheese are quite low. Some studies indicate that they are not risky for human health (Dobson, 2017). Sengun et al. (2008) stated that cheese does not optimum for toxin production because of low carbohydrate level and has low temperature during ripening.

Legal Regulations Regarding Aflatoxin M₁ Levels in Cheeses in Türkiye

The first legal regulation regarding AFM1 in Türkiye came into force in 1990. Subsequently, various legislations were published in 1997, 2002, 2008, 2011 and 2023. The legislations published regarding AFM1 are shown in chronological order in Table 1.

In the Communiqué on Aflatoxin Control in Türkiye in 1990, aflatoxin M₁ and B₁ were evaluated together and the maximum limit was determined as 0.50 ppb (500 ng/kg) for milk and dairy products (Resmi, 1990). Later, in the Turkish Food Codex Regulation in 1997, AFM1 was evaluated alone for cheese for the first time. In this regulation, the maximum limits are 0.00025 mg/kg (250 ng/kg) for cheese and 0.00005 mg/kg (50 ng/kg) for milk and dairy products (Resmi, 1997). The first limitation of

AFM1 for cheese in Turkish Legislation was determined by this regulation.

Afterwards, the Turkish Food Codex Communiqué on Contaminants was published in 2002. In this communiqué, the maximum limit of AFM1 for cheese was evaluated as 0.25 µg/kg (250 ng/kg) (Resmi, 2002). The European Union Commission Regulations began to be cited as a basis for legislation published in 2008 and later. In 2008 and later, no specific limit was specified for cheese; the limits were determined as “raw milk, heat-treated milk and milk used in the production of MBPs (milk-based products)”. In the Turkish Food Codex’s published in 2008, 2011 and 2023, the maximum AFM1 level for “raw milk, heat-treated milk and milk used in the production of MBPs” was determined as 0.050 µg/kg (50 ng/kg) (Resmi, 2008, 2011, 2023). Over the years, with the development of technology and industry, new methods and devices have been developed for the determination of contaminants. The levels of AFM1 detected in cheeses have decreased and the legal limits have been lowered accordingly. The reasons for this can be listed as follows;

- The lower detection limits of new devices
- More hygienic production of cheeses
- Reducing the risk of contamination
- Improving storage conditions
- More attentive preparation of animal feeds

Studies on Aflatoxin M₁ Levels in Cheeses in Türkiye

In this study, 60 publications investigating the presence of AFM1 in various cheeses in Türkiye in the last 50 years were examined (1973-2023) (Table 2a, 2b). Doctoral and master’s theses were excluded from the study. Researchers examined cheeses such as white, tulum, kashar, processed, blue mold, herby, cecil, lor, cream, pickled, surk, plaited, bovine, ovine, caprine, urfa, mihalic, organic, string, halloumi, cokelek, herby lavash, ezine, gruyere and cube. While some researchers examined only one type of cheese regarding AFM1 levels, some of them examined more. Rates exceeding TLL (Turkish Legal Limit) were written as specified by the researchers, and unspecified rates were calculated.

Table 1 Legislations published in Türkiye regarding AFM1

Official Gazette (References)	Legislation Name	Section	Maximum Limits for AFM1
2 May 1990 – 20506 (Resmi, 1990)	Communiqué on Aflatoxin Control (KKGGM 90/1)	Article 1	0.5 ppb (Aflatoxin B ₁ +M ₁) (Milk And Dairy Products)
16 November 1997 – 23173 (Resmi, 1997)	Turkish Food Codex Regulation	Annex-14 Microbial Toxins - Aflatoxin M ₁	0.00025 mg/kg (Cheese) 0.00005 mg/kg (Milk And Dairy Products)
23 September 2002 – 24885 (Resmi, 2002)	Turkish Food Codex Communiqué on Maximum Limits of Certain Contaminants in Foodstuffs (Communiqué No: 2002/63)	Annex-1 Microbial Toxins - Aflatoxin M ₁ (Table 1)	0.25 µg/kg (Cheese)
17 May 2008 – 26879 (Resmi, 2008)	Turkish Food Codex Communiqué on Maximum Limits of Contaminants in Foodstuffs (Communiqué No: 2008/26)	Annex-2 Mycotoxins-Aflatoxin M ₁ (2.1.5) (2.1.10)	0.050 µg/kg (Raw Milk, Heat-treated Milk and Milk Used in the Production of MBPs); 0.5 µg/kg (Other Foodstuffs (Potentially Risky Foods))
29 December 2011 – 28157 (Resmi, 2011)	Turkish Food Codex Regulation on Contaminants	Annex-1 Mycotoxins-Aflatoxin M ₁ (2.1.12)	0.050 µg/kg (Raw Milk, Heat-treated Milk and Milk Used in the Production of MBPs)
5 November 2023 – 32360 (Resmi, 2023)	Turkish Food Codex Regulation on Contaminants	Annex-1 Mycotoxins-Aflatoxin M ₁ (1.1.16)	0.050 µg/kg (Raw Milk, Heat-treated Milk and Milk Used in the Production of MBPs)

Table 2a. Incidence of AFM1 in cheeses in Türkiye (1973-2023)

Cheese Type	AFM1 Occurrence Rates, %	AFM1 Occurrence Rates Exceed TLL, %	References
White	0.00 %	Undefined	(Demirer, 1973)
Tulum	0.00 %		
Kashar	0.00 %		
Processed	0.00 %		
Blue Mold	0.00 %	Undefined	(Demirer, 1974)
Tulum	0.00 %		
Herby	0.00 %		
White	0.00 %	Undefined	(Demirer et al., 1989)
Tulum	0.00 %		
Herby	0.00 %	Undefined	(Kıvanç, 1990)
White	0.00 %		
Kashar	0.00 %	Undefined	(Kıvanç, 1992)
Mixed	45.20 %	1.33 %*	(Dağoğlu et al., 1995)
Kashar	0.00 %	Undefined	(Gürbüz et al., 1999)
Tulum	0.00 %		
White	0.00 %		
White	89.47 %	12.28 %*	(Oruc & Sonal, 2001)
White	65.00 %	Undefined	(Aycicek et al., 2002)
Kashar	60.00 %	32.55 %*	(Günşen & Büyükyörük, 2003)
White	82.00 %	27.00 %*	(Sarimehmetoglu et al., 2004)
Tulum	81.00 %	24.00 %*	
Kashar	85.00 %	34.00 %*	
Processed	79.00 %	25.00 %*	
White	39.13 %	0.00 %*	(Gürses et al., 2004)
Kashar	42.86 %	0.00 %*	
Tulum	63.64 %	0.00 %*	
Cecil	44.44 %	0.00 %*	
Lor	33.33 %	0.00 %*	
White	5.00 %	1.00 %*	(Yaroglu et al., 2005)
Kashar	6.00 %	1.00 %*	
Processed	4.00 %	1.00 %*	
Kashar	56.00 %	4.00 %*	(Çetin et al., 2005)
Herby	86.70 %	80.00 %*	(Tekinşen & Tekinşen, 2005)
White	62.00 %	40.00 %*	
Mixed	90.58 %	8.52 %*	(Aycicek et al., 2005)
Cecil	20.00 %	0.00 %*	(Kamber, 2005)
Kashar	13.30 %	0.00 %*	
Mixed	93.66 %	22.04 %*	(Başkaya et al., 2006)
White	100.00 %	2.00 %*	(Alkan & Gönülalan, 2006)
Mixed	28.21 %	0.00 %*	(Gürbay et al., 2006)
White	95.00 %	20.00 %*	(Kireççi et al., 2007)
Kashar	80.00 %	30.00 %*	
Processed	75.00 %	10.00 %*	
Mixed	71.42 %	38.08 %*	(Yapar et al., 2008)
White	6.25 %	0.00 %*	(Ardic et al., 2008)
Cream	99.00 %	18.00 %*	(Tekinşen & Uçar, 2008)
Kashar	82.60 %	27.30 %*	(Tekinşen & Eken, 2008)
Pickled	82.40 %	26.40 %*	(Ardic et al., 2009)
Surk	60.00 %	13.30 %*	(Aygün et al., 2009)
Plaited	46.67 %	14.44 %*	(Erkan et al., 2009)
White	80.00 %	5.00 %*	(Var & Kabak, 2009)
Kashar	50.00 %	5.00 %*	
White	60.00 %	13.30 %**	(Hampikyan et al., 2010)
Kashar	40.00 %	6.70 %**	
Tulum	55.00 %	10.00 %**	
Bovine	81.82 %	9.09 %*	(Turgay et al., 2010)
Ovine	0.00 %	0.00 %*	
Caprine	77.78 %	0.00 %*	
Mixed	28.00 %	10.00 %*	(Filazi et al., 2010)
White	12.00 %	0.00 %***	(Aksoy et al., 2010)
Kashar	80.00 %	0.00 %***	
White	82.40 %	16.50 %***	(Aydemir Atasever et al., 2010)
Kashar	80.00 %	14.70 %***	
Cecil	19.40 %	00.00 %***	
Cream	84.20 %	6.10 %***	

Table 2b. Incidence of AFM1 in cheeses in Turkiye (1973-2023)

White	7.14%	0.00 %**	(Er et al., 2010)
White	66.70 %	5.60 %*	(Gücükoğlu et al., 2010)
Kashar	76.50 %	29.40 %*	
Tulum	0.00 %	0.00 %*	
Processed	20.00 %	0.00 %*	
String	88.90 %	55.50 %*	
White	70.00 %	0.00 %*	(Ertas et al., 2011)
Kashar	40.00 %	5.00 %*	
Tulum	80.00 %	10.00 %*	
White	28.30 %	10.20 %*	(Kav et al., 2011)
Tulum	60.00 %	0.00 %***	(İşleyici et al., 2011)
White	88.90 %	6.67 %***	(Kocasari et al., 2012)
Urfa	50.00 %	0.00 %**	(Dinçel et al., 2012)
Cecil	0.00 %	0.00 %**	
Mihalic	0.00 %	0.00 %**	
Herby	0.00 %	0.00 %**	
Kashar	0.00 %	0.00 %**	
White	73.50 %	15.69 %*	(Dinçoğlu et al., 2012)
Kashar	57.80 %	19.28 %*	
Blue Mold	0.00 %	Undefined	(Güley et al., 2013)
Organic	43.00 %	7.70 %*	(Tosun & Ayyildiz, 2013)
Kashar	97.96 %	10.88 %***	(Gul & Dervisoglu, 2014)
White	20.00 %	0.00 %*	(Temamogullari & Kanici, 2014)
Kashar	50.00 %	2.50 %***	(Bakirdere et al., 2014)
Tulum	18.75 %	0.00 %***	
String	13.64 %	0.00 %***	
Cream	38.10 %	0.00 %***	
White	53.73 %	7.46 %***	
Halloumi	28.80 %	0.00 %***	(Öztürk et al., 2014)
(Industrial)	21.70 %	0.00 %***	
Halloumi			
(Traditional)			
Blue Mold	41.70 %	0.00 %*	(Kolucaık et al., 2015)
White	92.60 %	0.00 %*	(Sarica et al., 2015)
Blue Mold	52.00 %	17.00 %*	(Özgören & Seçkin, 2016)
White	79.39 %	0.96 %***	(Yeşil et al., 2019)
Cecil	100.00 %	0.00 %***	
Cokelek	66.67 %	0.00 %***	
Cream	100.00 %	0.00 %***	
Lor	83.33 %	0.00 %***	
Herby	62.79 %	3.70 %***	
Herby Lavash	38.46 %	0.00 %***	
White	40.00 %	0.00 %**	(Öztürk Yılmaz & Altinci, 2019)
Kashar	65.40 %	0.00 %**	
White	60.00 %	0.00 %**	(Acaroz, 2019)
Tulum	67.50 %	0.00 %**	
Ezine	50.00 %	0.00 %**	(Eker et al., 2019)
Blue Mold	16.00 %	0.00 %***	(Aksoy & Sezer, 2019)
Kashar	36.00 %	0.00 %***	
Gruyere	68.00 %	0.00 %***	
Cube	100.00 %	1.11 %**	(Ağaoğlu et al., 2020)
White	100.00 %	100.00 %**	(Demir et al., 2021)
Processed	100.00 %	1.20 %**	(Mortaş et al., 2022)
White	35.71 %	2.38 %***	(Ergin et al., 2023)
Tulum	45.23 %	0.00 %***	

AFM1 Occurrence Rates: Shows how many of 100 samples contain AFM1 (as a percentage); TLL: Turkish Legal Limit; * TLL: 250 ng/kg, 0.25 µg/kg, 0.00025 mg/kg, 250 ppt, 0.25 ppb, 0.00025 ppm; ** TLL: 50 ng/kg, 0.05 µg/kg, 0.00005 mg/kg, 500 ppt, 0.050 ppb, 0.00005 ppm; *** TLL: 500 ng/kg, 0.50 µg/kg, 0.0005 mg/kg, 500 ppt, 0.50 ppb, 0.0005 ppm

In studies until 1995, AFM1 could not be detected in cheeses, so rates exceeding the TLL were not mentioned. In 1995, Dağoğlu et al. (1995) indicated the AFM1 in cheese as 45.20 % and the rate of exceeding TLL as 1.33 % (out of 100 samples). In almost all studies in 2001 and later, AFM1 was detected in varying rates in cheeses.

In the communiqués published in 1997 and 2002, the term “cheese” was clearly stated and the legal limit was

250 ng/kg. In the legal regulations of the following years (2008, 2011 and 2023), the term “cheese” was not clearly stated, and instead the maximum AFM1 limit for “raw milk, heat-treated milk and milk used in the production of MBPs” was mentioned as 50 ng/kg. In the communiqués published in 2008, the maximum AFM1 limit for “potentially risky foods” (other foodstuffs) was mentioned as 500 ng/kg.

In many studies, researchers have noted both the presence of AFM1 and rates exceeding TLL in cheese. Although there are differences according to the years in which the legal regulations were published;

- Some studies cited the expression “cheese” as reference (250 ng/kg),
- Some studies cited the expression “raw milk, heat-treated milk and milk used in the production of MBPs” as reference (50 ng/kg),
- Some studies cited the expression “potentially risky foods” as reference (500 ng/kg).

TLLs cited by researchers are indicated with * in Table 2.

Results

Aflatoxins are toxic metabolites for human and animals. It is necessary to determine the maximum limits of presence in foods for these metabolites that have been proven to be toxic to humans and animals. So, legal limits have been determined in many countries and various international committees. Many regulations have been enacted for AFM1 in Türkiye over the years and researchers have referred the limits in these regulations in studies.

In this review, the studies on AFM1 in cheese was examined and it was noticed that researchers cited different limits. In the studies, AFM1 was detected in almost all cheeses, and only a few were found in rates exceeding the TLL.

Discussion

Oruç (2003) examined the levels of Aflatoxin M1 (AFM1) in milk and dairy products in Türkiye. The study stated that the rate of AFM1 in milk and dairy products in Türkiye is high and that milk and milk products may contain AFM1 at levels that could threaten public health. This review is parallel to the mentioned study, includes more current studies and is only specific to “cheese”.

Studies published in Türkiye are mostly related to “milk and dairy products”. There are many studies on topics such as the possible risks of aflatoxins in milk and dairy products (Ay & Şanlı, 2018; Özdemir & Demirer, 2021; Şimşek & Ağaoğlu, 2023), residue risks (Çoşkun & Şanlı, 2016; İnce & Karatekeli, 2020; Mortaş et al., 2022), the presence of peptides (Ay & Şanlı, 2018; Özdemir et al., 2021), the presence of microplastics (Halıcı Demir et al., 2024) and the presence of some pathogens (Ekici et al., 2004; Mohamed & Alçay, 2020; Sipahi & Çelik Doğan, 2023). The wide variety of cheeses in Türkiye and the high level of traditional production as well as industrial production have necessitated the examination of cheese separately from other dairy products.

In studies published between 1973 and 1992, researchers did not detect AFM1 in cheese varieties. The reasons for this may be technological deficiencies or high detection limits of the devices. The existence of AFM1 was mentioned in almost all of the studies published in subsequent years. It is thought that the methods used by researchers affect the results both qualitatively and quantitatively. In the studies, researchers generally preferred Capillary Electrophoresis, HPLC, ELISA, GC and GC-MS methods. The development of these methods

has made the analyses more sensitive over the years. Until 2010, researchers cited 250 ng/kg as the reference amount exceeding the TLL. As explained below, different limits were cited as reference even in studies conducted in the same year. For example, in 2010, it was observed that researchers cited 250 ng/kg (Filazi et al., 2010; Gücükoğlu et al., 2010; Turgay et al., 2010), 50 ng/kg (Er et al., 2010; Hampikyan et al., 2010) and 500 ng/kg as reference values (Aydemir Atasever et al., 2010).

Conclusion

In the latest legal regulations, no maximum legal limit has been specified for AFM1 in “cheese”. It should be defined with the new regulations. Developed countries are making special efforts to reduce AFM1 in milk and dairy products. To minimize the presence of AFM1, the following recommendations should be considered.

- Developing new detection methods
 - Enacting new legal regulations
 - Increasing the frequency of legal inspections
 - Improving the production, transportation and storage conditions of milk and dairy products
 - Improving feed quality
- Increasing awareness of the health effects of AFM1

Declarations

Author Contribution Statement

B.O: Data collection, writing the original draft, investigation, review and editing.

Conflict of Interest

“The author declares no conflict of interest.”

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Building Trust from Farm to Fork in Organic Agriculture: A Closer Look at Inspection and Certification Systems

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ARTICLE INFO

ABSTRACT

Review Article

Received : 15.08.2024

Accepted : 15.10.2024

Keywords:

Organic agriculture

Inspection

Certification

Standards

Trust

As the organic agriculture sector grows, the need for standards, inspection, and certification systems to ensure trust in organic products increases. Organic agriculture revolves around standards that determine the practices that farmers must follow. These standards also create a plan for inspection and certification systems. These systems are the cornerstone of ensuring reliability in the organic agriculture sector. This study has been prepared to evaluate the inspection and certification systems within the framework of the sense of trust, which is a critical issue in the organic agriculture sector from farm to fork. The focus of this framework is to ensure that inspection and certification systems keep the principles of organic agriculture intact from farm to fork. This is because these systems are necessary to build trust, access markets, increase consumer confidence and support fair and ethical practices in the sector. However, it is suggested that areas such as facilitating accessible inspection and certification for small-scale farmers, improving consumer education and promoting cooperation for global standards should be studied as potential areas.

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Introduction

Over the past three decades, organic agriculture has seen significant growth, with demand for organic products rising by 16% annually over the last decade (Reddy et al., 2022). Originating in the early 20th century, it emerged as a response to issues created by industrial agriculture, such as resource depletion, declining food quality, and the economic challenges facing rural areas (Brzezina et al., 2017). Organic agriculture is guided by four core principles: “health, ecology, fairness, and care.” These principles support environmental preservation, equitable access to resources for farmers and workers with fair compensation, and consumer access to trustworthy, high-quality, affordable food (Sligh & Cierpka, 2007).

Increasing costs of chemical inputs, like fertilizers and pesticides, along with a growing demand for Non-Genetically Modified Organisms (Non-GMO) foods, also drive farmers toward organic and environmentally friendly Agriculture (Reddy et al., 2022). This rapid development process in organic agriculture has triggered important transformations in producer and consumer behaviors (Seidel et al., 2019).

The primary concern associated with certification revolves around its efficiency. Numerous studies have consistently demonstrated that certified products are more sustainable than their non-certified counterparts. For instance, in the Ecuadorian banana agri-system, organic production results in better outcomes both environmentally and in terms of producer revenues (Bonisoli et al., 2019). Another study is related to farm income. Certified organic coffee production has been reported to contribute to higher farm revenues in Uganda (Ssebunyaa et al., 2019). In another study, the issue of organic agriculture and sustainability was emphasized. Organic and other agricultural certification schemes usually attempt to increase sustainability by following specific regulations such as those prohibiting the use of chemically treated planting material, genetically modified organisms (GMOs), synthetic fertilizers and pesticides as well as non-organically produced feed and prophylactic use of antibiotics for livestock. On the other hand, there are also statements that the sustainability of organic agriculture is contested. For instance, organic agriculture is criticized for generating lower yields, which can translate into lower profitability and inadequate food

production compared to conventional agriculture (Kamau et al., 2022). However, it is worth noting that, in certain instances, as highlighted by Veldstra in 2014, some farmers who acknowledge the benefits of certification have expressed concerns about the complexity of the certification process, such as application, inspection procedures, pricing, etc. They have also identified challenges in their interactions with the certification bodies, which they consider a significant obstacle.

While there is a wealth of literature delving into various aspects of organic products, foreign trade, and technical aspects, this article focuses on inspection and certification systems and the trust among the stakeholders. Our article contributes to the academic literature by evaluating global inspection and certification systems in organic agriculture. While numerous studies have explored certified organic products' economic, social, and environmental sustainability, there remains a gap in the literature regarding inspection and certification systems and the trust factor. For example, in the study conducted by Jawtusich et al. 2011 it was stated that most impact studies are concerned with the environmental impacts of organic farming. Bellassen et al. 2022 stated that existing impact analyses for organic agriculture are more comprehensive and cover both the economic and environmental pillars of sustainability and even some aspects of social sustainability. Canwat and Onakuse (2022) stated that organic markets are a type of market with social, economic, environmental, cultural, regional and other impacts. Organic agriculture is frequently associated with or subsumed under the rubric of "sustainable agriculture" with many using the terms interchangeably. In theory, sustainable agriculture refers to a system that integrates environmental health, economic profitability, and social and economic equity. Shreck et al. 2006 noted that organic farming is often associated with or subsumed under the concept of "sustainable agriculture" and that many use the two terms interchangeably. Furthermore, in this study, it was theoretically stated that sustainable agriculture refers to a system that integrates environmental health, economic profitability and social and economic equity. Taking into account these studies our contribution to the literature has focused on the role of inspection and certification systems in building trust, a relatively underexplored aspect in the existing literature.

This paper aims to fill the research gaps in this field by examining the procedures and requirements for organic inspection and certification, investigating global inspection and certification systems, understanding how organic farming affects inspection and certification systems, and exploring the links between organic farming and trust building. Through these objectives, we aim to comprehensively analyze organic inspection and certification systems, focusing on their impact and the critical role of trust in organic agriculture.

Few interdisciplinary studies, especially in the social sciences, refer to organic inspection and certification systems (Guevara-Hernández et al., 2014). In this context, our article can potentially make a valuable contribution by addressing this gap in the field.

Organic Concept and Definition of Organic Agriculture

Conventional agriculture has seen a substantial increase in productivity in recent decades, driven by the introduction of modern technologies, including monoculture practices and the widespread use of chemical inputs, commonly referred to as agrochemicals. Nevertheless, although these factors have contributed to higher agricultural productivity, there is a drawback to consider. Excessive reliance on toxic chemical inputs has been linked to several environmental issues. These include erosion, deforestation, pollution of both water and soil, and a decline in biodiversity. These adverse consequences pose significant risks to the sustainability of natural resources (Esguícero et al., 2019).

The extensive and unregulated use of harmful substances in conventional agriculture worldwide has significantly affected the health and well-being of humans, animals, and the environment. Farmers' endeavors to cultivate wholesome, chemical-free food have gained recognition and legitimacy in response to these concerns. This recognition has been formalized by establishing organic standards, certification processes, and labeling mechanisms (Guevara-Hernández et al., 2014). These measures assure consumers of organic produce's quality and safety, addressing the growing demand for healthier and more environmentally responsible food choices.

IFOAM (International Federation of Organic Agriculture Movements) defines organic agriculture as "a production system that sustains the health of soils, ecosystems, and people." This approach places reliance on ecological processes, biodiversity, and locally adapted cycles, as opposed to the utilization of inputs that can have negative impacts (IFOAM, 2019).

The term "*organic*" encompasses two key dimensions: measurable product standards and process standards. In the first case, specific attributes define organic products, notably the absence of detectable pesticides. However, the latter definition of organic pertains more to the principles governing production and processing than distinguishable qualities in the final product (McCluskey, 2000).

The imperative drove the emergence of organic agriculture to mitigate the detrimental effects of chemical fertilizers, crop protection agents, and livestock practices on the ecosystem. This shift towards organic methods was motivated by recognizing the social and environmental benefits it offered in contrast to conventional intensive agricultural practices (Leksina et al., 2020).

Organic agriculture is a form of inspected and certified agricultural production at every stage, from production to consumption. All stages of this process are carried out according to national and international validity rules, and independent inspection and certification bodies check and certify compliance with these standards. This comprehensive oversight strengthens consumer trust in organic products, assuring the genuineness and commitment to organic principles (Demiryürek & Bozoğlu, 2007).

Materials and Methods

This paper reviews the literature review complemented by the author's firsthand experience as inspectors and certifiers working with inspection and certification bodies in Türkiye, Europe, and various global regions. We have specifically examined relevant scholarly literature through comprehensive bibliographic research.

This study encompasses research from many sources worldwide, including studies conducted by private and governmental institutions on global organic inspection and certification systems. In addition to academic sources, internet resources, books, and articles on these subjects have been consulted. The research methodology includes interpreting existing studies, comprehensively evaluating and synthesizing these sources, and thoroughly examining organic inspection and certification systems with a focus on trust. We aim to clarify the landscape of inspection and certification systems, related policies, and a sense of trust within organic agriculture.

Research Findings

The Standards, Trust, and Organic Inspection and Certification

For a long time, organic farming was understood as a more natural form of agriculture, primarily characterized by the avoidance of chemicals and synthetic inputs. However, this initially narrow view evolved when organic farming was systematically defined within private standards and later formalized in regulations. As the relationship between farmers and consumers became more impersonal, centralized, and globalized, the need for standards and an inspection system became apparent. These measures were necessary to protect producers from unfair competition and consumers from fraud. This need was especially clear when adherence to these standards became a requirement for receiving direct support payments (Schmid, 2007).

Initially, organizations in the realm of organic agriculture developed their production standards. However, in numerous European countries, the practice of organic agriculture has undergone institutionalization and regulation through national and international legislation. Consequently, the responsibility for establishing standards and defining what qualifies as organic has shifted from the purview of private organizations to the domain of public policy (Seppänen & Helenius, 2004).

As they are employed in various systems, standards essentially formalize diverse quality norms and established perceptions of what constitutes high-quality food. This includes exploring sustainability standards, practices for setting standards, and quality agreements within various product chains, production networks, and value chains (Kurtz et al., 2020). Globally, the organic certification sector is mainly regulated by three major government-led systems: the Council Regulation (E.C.) No. 834/2007 in Europe, the United States Government's National Organic Program (NOP), and Japan's Japanese Agricultural Standard (JAS), with the European system being the oldest (Zorn et al. 2012).

In terms of regulations, the most recent data gathered by IFOAM-Organics International in 2022 reveals that

seventy-four countries had successfully implemented comprehensive regulations governing organic agriculture. Additionally, twenty-one countries had formulated organic regulations that were in the process of being fully implemented, while fifteen countries were drafting legislation in this regard. Notably, substantial regulatory changes were observed in regions such as the European Union, North America, and the Pacific Region (Willer et al., 2023).

Building Trust, Labeling, and the Essential Role of Inspection and Certification

Food consumption trends in recent years emphasize consumers' preference for quality, healthy, and environmentally friendly products, as well as an increasing interest in food safety (Bernabéu et al., 2008). For example, a study by Baird in Thailand found that consumers increasingly demand rice grown without chemicals (Baird, 2024).

Regulating the organic production sector is essential to ensure consumer protection and the reliability of certified organic products (Willer & Lernoud, 2018). Indeed, trust is built on the foundation of transparent information. Consequently, the certification process plays a vital role in building trust and strengthening the overall marketing of organic products (Khanna & Tripathee, 2018).

Organic agriculture has four pillars: trust, honesty, transparency, and respect for commitment. Trust should not be perceived solely as an outcome of personal relationships and local economies or merely a result of certification processes. Instead, organic certification practices actively fostered and upheld trust (Galvin, 2018).

The trust of the consumer that organic products are included in the follow-up system from the production stage to the safety and quality control, packaging, labeling, presentation of the origin information, transportation, marketing, and standardization of the methods to be followed, and placing them on a legal basis (Siderer et al., 2005). Sønderkov and Daugbjerg (2011) emphasized that methods aimed at bolstering consumer confidence, particularly those involving government oversight in the certification and labeling processes of organic agriculture production, positively impact consumer confidence levels.

Inspection serves as a bridge connecting transparency and trust, operating through mechanisms that prioritize visibility and clarity, with an emphasis on documentation and rigorous inspections. Its primary objective is to yield normative outcomes, including cultivating public trust and accountability (Galvin, 2018). Although inspection culture is often seen as a response to a "general decline of trust" (Brown, 2010), inspections paradoxically require trust in their procedures and conclusions. Thus, inspections are not merely systems of "trust-making" but are designed to foster widespread public trust and fundamentally depend on internal trust (Galvin, 2018).

Certification offers compelling evidence of various social, economic, and environmental advantages. It serves to enhance practices and accountability within transnational supply chains. Certification can play a pivotal role in preserving and safeguarding natural habitats and promoting compliance with existing laws that may be in place but are inadequately enforced (Tayleur et al., 2017).

Organic certification carries dual significance for consumers: it represents a commitment to personal health and environmental preservation. Consumers frequently worry about agrochemicals and artificial additives in fruits and vegetables, and they generally prefer organic food, which usually contains only about one-third of the pesticides found in conventional options. The safety, nutritional benefits, and health considerations associated with organic certification justify consumers' willingness to pay a premium for these products (Liberatore et al., 2018).

Organic certification provides a less intensive alternative that requires greater traceability and offers economic benefits. Certification proves financially beneficial for businesses as certified products often command higher prices than their non-certified counterparts (Johnson et al., 2019).

Consumers have varying levels of trust in organic-certified versus non-certified labels, and they believe that organic-certified natural foods are worth the higher price. The degree of trust in these labels affects willingness to pay, with trust in organic-certified labels having a significantly greater impact than trust in non-certified labels (Lang et al., 2022). Lang et al. show that natural foods with an organic-certified label justify a higher price compared to those without certification, with consumers willing to pay a 10% premium for organic-certified natural foods over their non-certified counterparts (Lang et al., 2022). In summary, organic certification is crucial in distinguishing organic products from their non-organic counterparts and promoting them in the market, often commanding a premium price (Thimmaiah, 2014).

The standards-based regulation asserts that it primarily functions as a mechanism for quelling the oppositional social aspects of organic agriculture while aligning its economic dimensions with agri-business interests, including farm input suppliers, large-scale commercial farms, produce traders, food processors, and retailers (Gibbon, 2008).

To establish consumer trust, the alignment of a producer's practices with organic standards is verified through independent inspection and certification bodies. Additionally, these bodies must obtain accreditation from an external authority to ensure their inspection competence (Dabbert et al., 2014).

One of the critical issues regarding the relationship between inspection and certification systems and trust is the labels on the products. The organic certification label represents consumer trust in organic foods (Watkins, 2016). Generally, labels are used to develop markets and promote production practices by informing consumers and influencing their purchasing decisions. When consumers see an organic label, they associate it with environmental stewardship, animal welfare, and a commitment to healthier, more nutritious food (Zander et al., 2015). Trust in organic standards transcends the label itself, assuring inspection and certification bodies have rigorously assessed and verified adherence to these standards.

For a product to be labeled "organic" it must be produced according to specific organic methods or processes and use only permitted inputs (Giovannucci, 2006). Initially, the product is grown in an organic production system that emphasizes plant and animal health, preventive pest management, and the careful use of

approved materials. It is then monitored and protected against contamination throughout its journey from the field to the final point of sale, whether as a raw agricultural commodity or a processed product with multiple ingredients. The label may indicate "100 percent organic," "organic" (95% to 100%), or "made with organic ingredients" (at least 70% organic ingredients) (Khanna & Tripathee, 2018).

Labeling will be effective in building high consumer trust when it sets and enforces reasonable standards. For labeling to be truly impactful, it relies on consumers' trust in the certification system and their confidence that the standards align with their expectations. This highlights the importance of labels being based on standards validated by both competitors and consumers and supported by independent third-party certification systems (Zander et al., 2015). There is a general feeling of trust towards the inspection system behind the standards. Trust and credibility, as well as the perception of the inspection system behind the label, are similarly important (Stolz et al., 2013).

Certification labels influence consumers' decisions regarding their demand for organic products (Esguicero et al., 2019). These organic labels certify that products have various attributes, including being pesticide-free, free from genetically modified seeds, environmentally friendly, protective of worker safety, associated with small-scale production, part of a local production system, adhering to sustainable agricultural practices without chemical fertilizers, and promoting health-beneficial foods (Rodrigues et al., 2016).

The logo is another crucial element in creating a sense of trust in the system. Organic logos are pivotal in communicating to consumers that a product is "organic" (Dabbert et al., 2014). Various logos signify that a specific product adheres to organic standards. For example, all products sold as organic in Europe must display an E.U. logo. This logo signifies that a product meets the major regulations for organic standards. To use any private organic logos, producers must undergo an additional inspection and certification process. Consequently, these logos offer consumers an extra level of assurance.

Logos can impact both producers and consumers in various ways. They not only help producers secure premium prices but also shape consumer perceptions on a variety of issues. This can lead to brands attracting more customers and increasing consumer trust. Organic product certificates help health-conscious consumers to prefer these products and help producers to gain a competitive advantage. Many studies have been conducted to support these results. For example; Yemez and Akca (2024) found that organic food labelling positively affects consumption and utilitarian consumption behavior also positively affects purchasing behavior. Gerrard et al. (2013) found that United Kingdom consumers' trust in the logo depends on the standards and inspection system that they think underlie the logo.

Nevertheless, consumer awareness of the European Union organic logo remains rather low, which suggests a need for more effective information campaigns and marketing actions. However, the study by Van Loo et al. (2013) points out that consumer awareness of the European Union organic logo is quite low and that more effective

information campaigns and marketing activities are needed. In the study conducted by Larceneux et al. (2011), it was stated that the organic label makes the environmentally friendly feature of the product prominent and this has a positive effect on perceived quality. These references provide a comprehensive framework for examining the effects of logos and certification systems on the behavior of both producers and consumers.

The Inspection Process in Organic Agriculture

The inspection process holds significant importance in organic agriculture for verification of compliance, quality assurance, consumer trust, market access, environmental protection, fair trade, global consistency, and continuous improvement.

Organic agriculture inspections are conducted by independent inspection and certification bodies that adhere to standards set by external organizations. This process starts with the movement of products among farmers, processors, and consumers. The supplier provides an organic certificate, which acts as a quality indicator, issued by an impartial certifying entity according to the quality and certification criteria set by the public sector, such as E.U. regulations (Jahn et al., 2005).

In organic agriculture, a highly interdependent relationship exists between standards and inspection. Organic agriculture standards provide comprehensive regulations that govern the entire process of organic farm production. Producers follow these rules, and their adherence is verified by an impartial third-party organization known as the inspection body (Lippert et al., 2016).

The inspection process for organic farms consists of two primary components. Initially, a farm visit is conducted where the inspector, accompanied by the farmer, assesses all fields and storage areas on the farm. The inspector completes paperwork, primarily inside the farmhouse. During this process, the inspector engages in discussions, asks questions, offers advice, makes observations, and gathers information from the farmer, creating a comprehensive picture of the farm's practices.

Following the farm visit, inspection documents are compiled inside the farmhouse. These documents encompass crucial information about the farm, including its contact details, the primary fertilizers, other inputs utilized, and details about the farm's livestock. Additionally, the inspection report records information such as acreage, crop types, crop rotation practices, sources of plant propagation materials, field locations (including maps), estimated crop yields, historical field usage or land use records, input procurement and sourcing records, certification documentation, labeling details, and information about plant protection methods and crop varieties for each field. Following the conclusion of these inspections, the farmer endorses the inspection report with their signature, and the inspector provides them with a copy for their records.

As shown in Figure 1, the inspection process consists of four distinct procedures. One of these procedures involves the inspection and certification body overseeing producer-farmers, agricultural holdings, and the companies' producing inputs. Another aspect of the inspection process entails the accreditation body

monitoring the inspection and certification body. Additionally, the inspection and certification bodies are subject to oversight by the public authority (for example, the Ministry of Agriculture and Forestry in Türkiye). Furthermore, public authorities can also inspect organic farming producers, as shown by the dashed arrow line. All actors within the system play a crucial role in ensuring effective inspections.

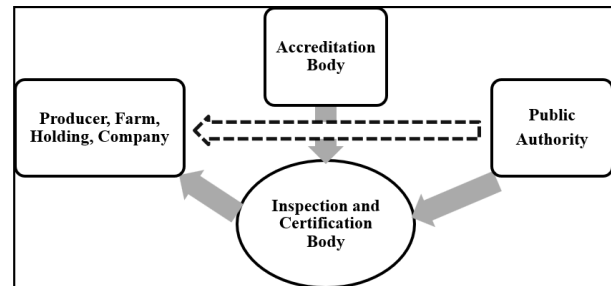


Figure 1. Inspection process between the main actors in the system

Source: Developed by the authors

The Certification Process in Organic Agriculture

The certification process plays a vital role in organic agriculture for the following key reasons: reliability and trust, quality assurance, market access, environmental management, consumer confidence, global trade, continuous improvement, fair trade, and ethical practices.

Certification ensures to the consumer that a product or service adheres to specified standards and maintains a certain level of quality (Thimmaiah, 2014). Certification systems are established to protect consumers by providing quality labels that enhance market transparency (Jahn et al., 2005).

The primary role of certification is to ensure that actors adhere to established standards. The common certification model, known as "third-party certification," involves independent private entities serving as certifiers (Fouilleux & Loconto, 2015). Additionally, certification is a process in which a third party provides written assurance that a product, process, or service meets specific standards. Certification typically has three basic requirements (Shahane & Behera, 2022):

- The methods and materials used in production must meet organic standards,
- There must be clear and ongoing documentation of these methods and materials, and management system procedures to be in place,
- There must be a paper trail tracing a product back to its production site to verify the methods and materials used in production.
- An internal audit should be conducted before an external audit.

Certification primarily aims to regulate and facilitate the sale of organic products to consumers, assuring them that organic production maintains food integrity from seed to sale (FAO, 2007). It also ensures that production and processing are managed with a comprehensive approach that promotes ecosystem health (Santacoloma, 2007).

Regional variations in standards and certification processes for organic agriculture can be advantageous, considering the diverse geographical, agronomic, cultural, and developmental aspects worldwide. However, these differences present challenges for Certification Bodies in recognizing and certifying organic products. Consequently, organic producers may also find it difficult to have their certified products recognized across various markets (FAO/IFOAM/UNCTAD, 2012). For example, a study conducted in Thailand stated that serious obstacles were encountered in the project implemented because organic certification standards were incompatible with international standards. This has adversely affected the support structures for organic farming by setting unrealistic expectations for farmers about what is required to produce organic rice for the international market (Baird, 2024).

The certifying process of a farm can be lengthy, often spanning up to three years, coupled with thorough planning and costly procedures. Organic certification creates an even higher cost for large-scale organic operations that cultivate thousands of acres and sell to commercial buyers such as grocery stores. This leads to selling organic products at a premium compared to conventional ones. This price difference arises from the rigorous regulatory requirements farms must adhere to and the increased cost associated with organic production methods (Watkins, 2016).

The decision to certify organic depends on each country's specific legal regulations governing certified organic production and marketing. Organic certification requires producers to manage their land using organic practices for three years. During this transition period,

producers cannot sell their produce as organic, although they may charge a higher price for being in the “transitional” phase (Veldstra, 2014); producers can sell their products with the phrase that it is a transition process product, except in the case of fibers according to the Private Organic Textile Standards.

Upon successful completion of the inspection phase, the organic certification process proceeds. The first step of organic certification is the selection of a certifier, which can be the International Federation of Organic Agricultural Movements (IFOAM), the European Union (E.U.), Japanese Agricultural Standards (JAS), National Organic Program (NOP), or national inspection and certification bodies in the producer's home country. Subsequently, the second step entails submitting an organic systems plan, including the application process and a review of the plan by the certifier, culminating in an organic inspection (Khanna & Tripathee, 2018).

Applicants must contact an organic certifying body to obtain application forms, which usually involve a fee. After reviewing the application, a qualified inspector will perform an on-site inspection for a fee, submit a report to the certifying body, and, if successful, the certification body will issue a certification certificate. The initial certification process can take several months, depending on the certifying body's schedule and the complexity of the application. However, if organic practices are already in place and documented during the initial visit, certification may be granted shortly thereafter (Ferguson, 2004). Detailed information on the certification process can be seen in Figure 2 below.

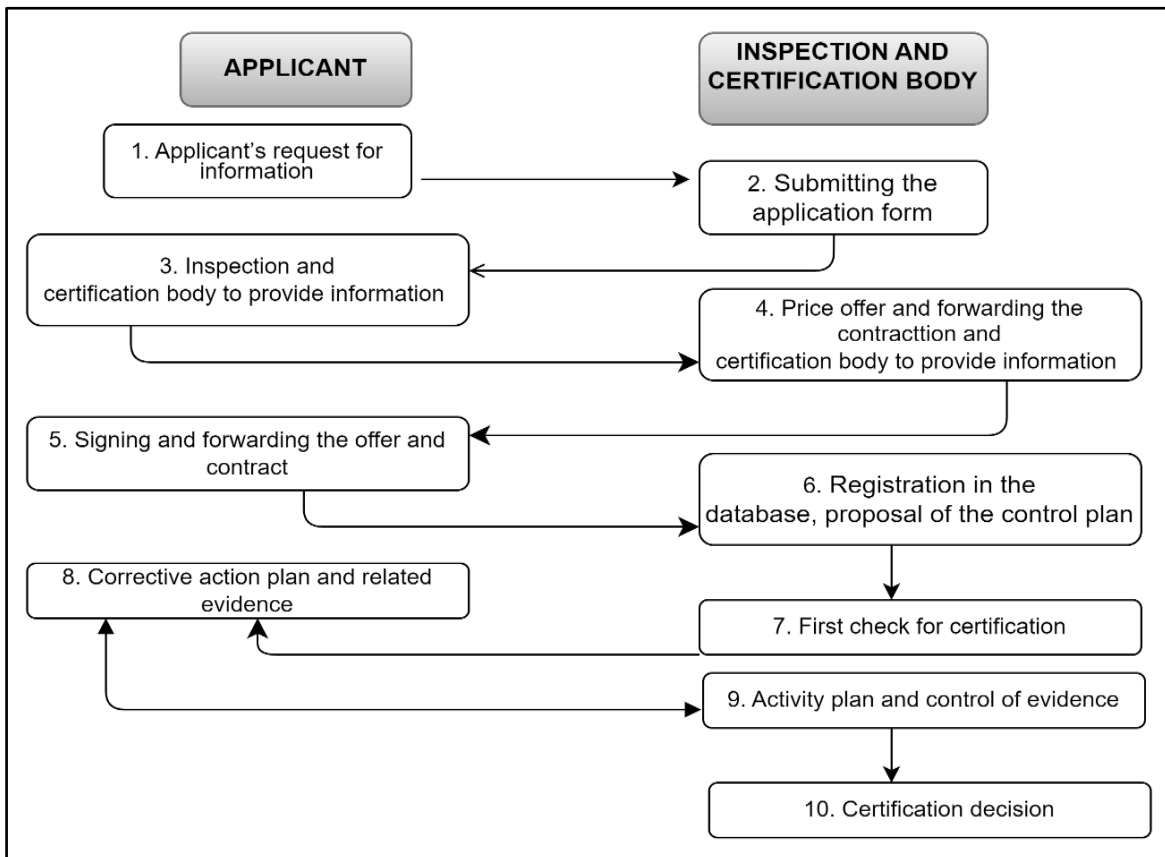


Figure 2. Certification Process (Source: Developed by the authors)

One of the critical issues concerning the certification process is the cost of certification. Certification costs vary depending on the fees set by the inspection and certification bodies. Each inspection and certification body is free to establish its pricing policy. Whether the application is made individually or as a group is crucial in determining the costs. The cost of individual applications is higher than that of group applications, as shown in Solfanelli et al. 2021.

Certification is essential for both national and international markets, as all major markets require it for organically marketed products. Domestic bodies typically concentrate on certification for local markets, while international bodies focus more on the export sector (UNCTAD/UNEP, 2008). However, there are also cases where the opposite happens; for example, many companies in Türkiye trade organic products domestically and internationally.

Another different issue is the recognition of organic products between countries. For instance, organic products approved in one country may not be recognized in another. To export organic products internationally, operators must comply with different export market standards. In such cases, obtaining multiple certifications might be the best approach. However, the bureaucratic complexities within the organic certification sector often create barriers to imports and exports, leading to increased costs. This issue is particularly prominent when a certification body conducts a single inspection against multiple organic standards (Xie et al., 2009).

The Role of Inspection and Certification Bodies

An inspection and certification body refers to individuals or organizations authorized by the public authority to oversee and certify every organic product or input phase, from production to the end consumer.

In organic food marketing, inspection measures are essential for maintaining food quality. Responsibility for ensuring quality involves proper management monitoring, inspection, and the implementation of an effective quality assurance system. Third-party inspection and certification bodies can effectively perform this role by providing transparent and independent procedures. They assure all stakeholders that inspection and monitoring are effectively integrated into the producer's quality assurance system. These third-party bodies are committed to providing certifications as quality guarantees, bridging the information gap between producers and consumers (Canavari et al., 2010).

Certification serves as a guarantee for production processes and the quality attributes of goods, and it can be a crucial market tool for product differentiation. However, the effectiveness of this mechanism, which aims to eliminate distortions caused by asymmetric information, may vary according to the ethical behavior of third-party audit and certification bodies (Giannakas, 2002).

In the organic sector, it is essential for certification systems to address specific issues that affect the confidence of both market operators and consumers. This confidence is crucial for ensuring that organic products meet market needs and expectations. The relatively slow global growth of the organic market, ongoing debates about the definition of organic, and skepticism surrounding organic food

underscore the need for a comprehensive examination and careful design of the third-party inspection and certification system in the organic food sector (Canavari et al., 2010).

Inspection and certification bodies are critical to verifying compliance and building trust in the standards system. Certification involves auditors interpreting standards, leading to considerable variation in how Certification Bodies operate and what they accept as valid evidence of compliance. This can result in consumer confusion or potential fraud within the system (Fouilleux & Loconto, 2017).

One of the issues related to the field of inspection and certification bodies is the fee they receive in return for the services they provide. Each inspection and certification body has its fee policy. From the perspective of these bodies, providing affordable certification can be a key competitive advantage in the certification market. However, implementing low-cost strategies may significantly affect the quality of inspections. Consequently, the underlying institutional structure can greatly impact the overall effectiveness and reliability of the certification system (Jahn et al., 2005).

There is an inextricable link between inspection and certification bodies and the accreditation system. Accreditation plays a vital role in the operation of inspection and certification bodies, as it is a fundamental requirement for their viability. Without accreditation, these bodies cannot effectively function. Accreditation serves as a mechanism to ensure the credibility of third-party inspection and certification bodies. This entails complying with the ISO 17065 standard, which pertains to conformity assessment bodies with organic standard specifications (Fouilleux & Loconto, 2017).

The accreditation program for overseeing organic certification bodies adheres to the guidelines outlined in the International Organization for Standardization (ISO) Guide 17065 (It was previously known as ISO 65). For example, according to ISO 17065, the certification body shall not advise or provide consultancy services to the applicant (Seppänen & Helenius, 2004). According to ISO 17065, these and many similar issues are recorded, and these rules are systematically implemented.

When examining the accreditation system in the realm of organic agriculture, two primary accreditation systems emerge. One is integrated into national and supranational legal frameworks and is overseen by national Accreditation Bodies (A.B.s) affiliated with the International Accreditation Forum (IAF). The other system is strictly private and carried out by the specialized organization, the International Organic Accreditation Service (IOAS). In contrast, the first system is fully controlled by the State. The checklist system functions as a "shadow accreditation" system overseen by the EC, a central authority monitoring certification activities in third countries. For example, on the website of ETKO, an inspection and certification body operating in Türkiye and abroad presents their E.C. approval as an E.U. accreditation. A private transnational A.B. performs the second system of organic accreditation. IOAS, a US-based non-profit organization, offers ISO/IEC 17065 accreditation to third-party Certification Bodies based on the 2010 IFOAM auditable standard (Fouilleux & Loconto, 2017).

Discussion

This paper reviews the literature and seeks to answer whether inspection and certification systems encourage building a sense of trust in the organic farming sector. While consumers may seem to be the system's focus at first glance, it is clear that many stakeholders, including inspection and certification bodies, their employees, and public authorities, significantly impact building trust in the system.

Inspection and certification systems have the potential to reassure consumers. However, the degree of trust can vary due to several factors. These systems are primarily designed to ensure that products or services comply with specific standards related to quality, safety, environmental impact, or ethical considerations. When consumers come across a certification label on a product, it typically leads them to trust that the item has undergone an impartial verification process and adheres to the stipulated criteria. However, several factors influence consumers' trust in inspection and certification systems. These factors include trust in the inspection and certification body, transparency, rigor, consumer awareness, legal and regulatory framework, consistency, independent reviews, and alignment with consumer values.

Organic farming and food production adhere to specific standards and practices designed to reduce synthetic chemicals, promote sustainable farming methods, and ensure transparency throughout production. Robust inspection and certification systems are indispensable to guarantee that consumers receive genuinely organic products and that farmers remain committed to these principles. These systems are established to verify and authenticate compliance with these organic standards.

Below, we explain how inspection and certification systems build trust in the organic agriculture sector.

- *Building Consumer Trust:* Organic certification labels are recognizable symbols of organic authenticity. Consumers tend to place greater trust in products featuring official organic certification labels because these labels signify adherence to specific organic standards.
- *Regulatory Compliance:* Organic certification often aligns with government regulations and standards for organic farming. Adherence to these standards assures consumers that organic products meet established legal requirements.
- *Continuous Monitoring:* Organic certification is not a one-time procedure; it includes ongoing monitoring and periodic inspections to ensure sustained compliance with organic standards. State authorities oversee inspection and certification bodies, while accreditation bodies monitor these organizations. This ongoing oversight helps maintain trust in the sector over time.
- *Transparency:* Certification systems mandate record-keeping and documentation of farming practices and product handling, which are essential aspects of inspection and certification systems in organic agriculture. This transparency enables consumers to trace organic products' origin and production process, bolstering trust in their authenticity.

- *Quality Assurance:* Inspection and certification systems ensure that organic farms and food producers adhere to rigorous quality standards. This verification process plays a crucial role in guaranteeing the quality of organic products, a critical factor in building consumer trust.
- *Third-Party Verification:* Many organic certification programs involve third-party organizations or certification bodies that operate independently of the producers. This independence eliminates conflicts of interest and ensures impartial evaluations, enhancing confidence in the certification process.
- *Market Access:* Organic certification is often a prerequisite for access to organic markets. Farmers aiming to sell their products as organic must adhere to certification requirements, enabling them to access premium markets and command higher prices.
- *Environmental and Ethical Considerations:* Organic certification often includes criteria related to environmental sustainability and ethical farming practices. Consumers who value these principles are more likely to trust certified organic products.
- *Global Recognition:* Many organic certification systems are internationally recognized, meaning organic products certified in one country are accepted in others. This global recognition enhances trust in organic products on an international scale.
- *Market Growth:* As the demand for organic products grows, inspection and certification systems are evolving to meet consumer expectations. This adaptability and responsiveness to consumer needs contribute to trust in the sector's commitment to organic principles.

Conclusions and Recommendations

This study assesses the inspection and certification systems within the organic agriculture sector and examines the key factors influencing trust among the actors involved.

In conclusion, inspection and certification systems are essential for ensuring trust, authenticity, and sustainability in the organic agriculture sector. By applying rigorous verification processes, third-party oversight, and adherence to established organic standards, these systems give consumers and other actors in the system confidence that organic products meet quality and ethical standards.

Although inspection and certification systems have significantly benefited the organic agriculture sector, there are still opportunities for further improvement and research.

Many producers involved in organic agriculture operate small farms. Simplifying and streamlining the certification processes for these small-scale, resource-constrained organic farmers can make certification more accessible. Additionally, establishing various support mechanisms for small-scale farmers to participate in inspection and certification systems is crucial. Creating incentives in this regard is necessary to ensure the inclusion of these producers in the organic product supply chain.

Education and consumer awareness are pivotal in establishing trust in the system. To achieve this, it must invest in educational programs and outreach initiatives that enhance consumer awareness and understanding of organic product labels and their role in fostering trust.

There are numerous inspection and certification bodies in organic agriculture worldwide. There is a need to promote greater consistency and standardization in global organic certification criteria to minimize confusion and inconsistencies among different inspection and certification bodies.

Traceability of organic products is paramount for building trust in the system. Utilizing advanced tracking systems to improve organic products' traceability and provide consumers with information about the product's origin and processing will benefit the system.

Declarations

Author Contribution Statement

Yener Ataseven: Literature review, investigation and writing the original draft

Alper Demirdöğen: Literature review, commenting and editing

Mustafa Akyüz: Consulting, commenting and investigation

Conflict of Interest

The authors declare no conflict of interest.

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