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Effects of Extracts of Feed Additives Including Rosemary (*Rosmarinus officinalis*) and Aloe Vera (*Aloe barbadensis*) on the Growth Performance and Feed Utility of Nile Tilapia (*Oreochromis niloticus*)

Ebru Yilmaz[1,a,*], Deniz Çoban[2,b], Birsen Kirim[2,c], Mehmet Güler[2,d]

[1]*Bozdoğan Vocational School, Aydın Adnan Menderes University, 09760 Bozdoğan/Aydın, Turkey*
[2]*Department of Aquaculture, Faculty of Aqriculture, Aydın Adnan Menderes University, 09010 Efeler/Aydın, Turkey*
[*]*Corresponding author*

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ABSTRACT

This study was performed to determine the effects of the extracts of two alternative antimicrobial agents that do not harm the ecosystem (rosemary, *Rosmarinus officinalis* and aloe vera, *Aloe barbadensis*) when added to the feed of tilapia (*Oreochromis niloticus*) on the growth performance, proximate composition, and biometric characteristics. Rosemary extract was added to the experimental diet in ratios of 0.1%, 0.25%, and 0.5% while aloe vera extract was added to the experimental diet in ratios of 0.5%, 1%, and 2.5%. After 90 days of the feeding trial, no change was seen in the weight increase, specific growth rate, feed conversion ratio, condition factor, hepatosomatic index, or viscerosomatic index of the tilapia. The fact that proximate analyses did not differ between groups showed that the plant extracts did not adversely affect the health status of tilapia in the ratios used in this study. As a result, the addition of 0.1%, 0.25%, and 0.5% rosemary extract and the addition of 0.5%, 1%, and 2.5% aloe vera extract to tilapia feeds did not cause changes in the growth performance, biometric indexes, or chemical composition findings of the fish meat.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi 7(6): 866-870, 2019

Biberiye (*Rosmarinus officinalis*) ve Aloe Vera (*Aloe barbadensis*) Ekstraktları Katkılı Yemlerin Nil Tilapyası (*Oreochromis niloticus*) Balıklarının Büyüme Performansı ve Yem Kullanımı Üzerine Etkilerinin Araştırılması

MAKALE BİLGİSİ

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ÖZ

Bu çalışmada, ekosisteme zarar vermeyen iki antimikrobiyal alternatif ajanın ekstraktlarının (biberiye, *Rosmarinus officinalis* ve aloe vera, *Aloe barbadensis*) tilapia (*Oreochromis niloticus*) balığı yemlerine eklenmesinin büyüme performansı, kimyasal besin madde analizleri ve biyometrik özelliklere etkisi araştırılmıştır. Biberiye ekstraktı %0,1 0,25 ve 0,5 oranlarında ve aloe vera ekstraktı %0,5, 1 ve 2,5 oranlarında deneme yemine ilave edilmiştir. Deneme sonunda biberiye ve aloe vera ekstraktı içerikli yemlerle beslenen balıkların ağırlık artışı, spesifik büyüme oranı, yem dönüşüm oranı, kondüsyon faktörü, hepatosomatik indeks ve visserosomatik indeks miktarlarında değişim görülmemektedir. Kimyasal besin madde analizlerinin gruplar arasında farklılık göstermemesi bitki ekstraktlarının tilapyanın sağlık durumunu araştırmada kullanılan oranlarda olumsuz etkilemediğini ortaya koymuştur. Sonuç olarak tilapya yemlerine %0,1, 0,25 ve 0,5 oranlarında biberiye ekstraktı ve %0,5, 1 ve 2,5 oranlarında aloe vera ekstraktı eklenmesinin büyüme performansı, biyometrik analizler ve balık etinin kimyasal besin kompozisyon bulgularında değişime neden olmadığı gözlenmiştir.

[a] *ebruyilmaz@adu.edu.tr* *https://orcid.org/0000-0003-1905-1265*
[b] *deniz.coban@adu.edu.tr* *https://orcid.org/0000-0001-7058-3123*
[c] *birsen.kirim@adu.edu.tr* *https://orcid.org/0000-0003-3292-1368*
[d] *m.izmir@gmail.com* *http://orcid.org/0000-0001-8257-1138*

Introduction

The annual quantity of total tilapia and other cichlids obtained by aquaculture worldwide is 5,576,800 tons, accounting for 6.7% of the cultured animal-based aqua products with an economic value of USD 7,656,257,000 (FAO, 2015). The gradual increase in production through aquaculture in the world and intense production cause stress and diseases resulting in economic losses in addition to the increase of antibiotics and other chemicals that are not desired for the environment and consumers (Yıldırım and Okumuş, 2004).

Antibiotics have been commonly used in the feeds of cultured fish for many years for the treatment of disease and for the increase of growth performance (Serrano, 2005). However, the use of antibiotics in the farming of fish and other animals is prohibited in many countries today (Ng and Koh, 2016).

Currently, it is necessary to evaluate alternative feed additives due to the restrictions on the use of antibiotics. Even for therapeutic purposes, the use of antibiotics increases the antibiotic resistance of bacteria. At the same time, problems arising from fish and the applications that fish are subjected to in different periods of farming have direct effects on production and operation economy (Ellis et al., 2002; North et al., 2006). Therefore, the addition of various additives as alternatives to synthetic products is currently being studied and this is a subject that requires further research. The addition of medicinal plants to fish feeds is a topic that has been recently studied in fish farming (Olusola, 2013).

There have also been various studies on the use of medicinal plants in relation to feed additives in aquaculture, growth, appetite, nutritional composition, immunity, larval growth, blood serum and hematology, and resistance to diseases, stress, bacteria, fungi, viruses, and parasites (Tafi et al., 2018; Stoyanova et al., 2018; Rahman et al., 2018; Panase et al., 2018; Panase and Tipdacho, 2018).

It is understood that medicinal plants are not sufficiently used in aquaculture in Turkey and that the suitable doses to be added to feeds have not been determined yet. In addition, it is currently necessary to determine in vivo effective doses of natural and reliable alternative antimicrobial agents that do not harm the ecosystem and to incorporate them into the sector. Rosemary (*Rosmarinus officinalis*) has antibacterial, antidiabetic, anticarcinogenic, antimicrobial, antiedema, antiviral, and antioxidant effects while aloe vera (*Aloe barbadensis*) has antiseptic and antioxidant effects (Ahmad et al., 2006; Poorfarid et al., 2013; Nwaoguikpe et al., 2010). In addition, rosemary is rich in carnosic and rosmarinic acid, compounds that were reported to have significant antioxidant features (Thorsen and Hildebrandt, 2003; Erkan, 2008). Rosemary increased the survival rate of tilapia infected with *Streptococcus iniae* by 25% (Abutbul et al., 2004).

Aloe vera plants contain large amounts of active compounds. These include aloesin, vitamins, enzymes, minerals, sugar, lignin, saponin, salicylic acid, and amino acids (Ajose, 2007). Aloe vera and rosemary plants have been studied in various fish species (Gabriel et al., 2015; Zilberg et al., 2010; Mahdavi et al., 2013; Tafi et al., 2018). However, there is no current study on the extracts of these plants and tilapia. Therefore, the present study has been conducted to examine the effects of extracts of aloe vera and rosemary plants on the growth performance and feed use of tilapia.

Materials and Methods

Fish and Experimental Design

A total of 840 juvenile Nile tilapia fish (*Oreochromis niloticus*) were stocked with a stocking ratio of 40 fish/tank in 21 experimental aquariums (40×50×100), the fish being of similar weights. The juvenile tilapia fish (average weight: 7.516±0.05 g) used in this study were obtained from the Fisheries Faculty of Çukurova University and were not subject to any prior diseases. They were brought to the Aydın Adnan Menderes University Faculty of Aquaculture's Research and Application Unit, and relevant analyses were conducted in the TARBİYOMER Laboratory of the Aydın Adnan Menderes University Faculty of Agriculture. During the experimental period the water quality remained as follows: temperature, 24.01±0.11°C; pH, 7.09±0.39; salinity, 0.5 ppt; total dissolved solids (TDS), 11.95 mg/L; and dissolved oxygen, 8.01±0.37 mg/L.

Experimental Diet

Rosemary extract and aloe vera extract were obtained from Talya Herbal Company, Turkey, and supplemented in tilapia diets at rates of 0.10%, 0.25%, and 0.5% and 0.5%, 1%, and 2.5%. These treatments were designated as R01, R025, R05, A05, A1, and A25 respectively. Previous studies were taken into consideration when selecting the usage rates of plant extracts (Gabriel et al., 2015; Mahdavi et al., 2013; Turan and Yiğitaslan, 2016). In the experiment, the rosemary and aloe vera extracts were added by spraying method after being solubilized in alcohol at ratios of 0.10%, 0.25%, and 0.5% for rosemary and ratios of 0.5%, 1%, and 2.5% for aloe vera. No plant extracts were added for the control group. Before the experiment, juvenile tilapia fish were divided into groups after 15 days of adaptation. The fish were fed twice a day at 08:00 and 16:00 hours for 90 days at 2% of their weight. The commercial diet (pellet size: 1 mm) was obtained from Gümüşdoğa Feed Company (Table 1).

Table 1 Proximate analysis of the commercial diet (pellet size: 1 mm) and extracts.

Nutrient	Diet %
Crude protein (%)	53
Crude lipid (%)	15
Crude cellulose (%)	0.9
Crude ash (%)	11
Compound	Rosemary extract %
Rosmarinic acid	5.32
Compound	Aloe vera extract %
Aloesin	3.88

Raw materials used in fish feed are fish meal, soybean meal, fish fat, wheat and byproducts, minerals, and vitamins. The ratios of phenolic compounds in the extracts of aloe vera and rosemary were obtained from Talya Herbal Company.

Growth Performance and Biometric Indices

Calculations were conducted as follows for weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI):

$$WG(g)=FW(g)-IW(g) \quad (1)$$

$$SGR(\%\ day^{-1})=\frac{(FW(g)-IW(g))}{Days}\times 100 \quad (2)$$

$$FCR=\text{Feed intake (g)}\ /\text{Weight gain (g)} \quad (3)$$

$$CF=\text{Body weight (g)}\ /\text{Total length}^3\ \times 100 \quad (4)$$

$$HSI=\frac{WWL}{(WBW-WWL)}\times 100 \quad (5)$$

WWL: Wet weight of liver (g)
WBW: Wet body weight (g)

$$VSI=\frac{WWVF}{(WBW-WWVF)}\times 100 \quad (6)$$

WWVF: wet weight of viscera and associated fat (g)

Proximate Composition

Proximate analyses were conducted using standard methods. Moisture analyses were conducted by drying the samples in order to fix a stable weight at 105°C for 24 h in an oven, crude protein was analyzed by the Kjeldahl method, and crude ash was dried by being burned at 525°C in a muffle furnace for 12 h (AOAC, 1998). The methanol chloroform extraction method was used to measure crude fat levels of the samples (Folch et al., 1957).

Statistical Analysis

The analyses of the data obtained in the experiment were done using the SPSS 17 statistics program. One-way analysis of variance (ANOVA) was applied to the data, which were then subjected to Tukey's multiple comparison test. The differences between groups were evaluated to be $P<0.05$ (Logan, 2010).

Ethics Statement

Fish experiments were performed in accordance with the guidelines for fish research of the animal ethics committee at Aydın Adnan Menderes University (Project Number ADÜ BAP ZRF-17054).

Results and Discussion

Growth results are presented in Table 2. No particular difference was found in the final weight gain, FCR, SGR, CF, HSI, or VSI ($P>0.05$).

Similarly, in the work of Golestan et al. (2015), the average initial weight of aloe vera extract did not affect the growth performance of 9.5 g of rainbow trout. In a different study, in rainbow trout with an average weight of 50.3 g, it was determined that aloe vera added to feed in ratios of 0.1% and 1% increased SGR and reduced FCR (Heidarieh et al., 2013). It was observed in another study that SGR was increased and FCR was decreased by adding aloe vera extract in ratios of 0.1%, 0.5%, and 2.5% to the feed of carp (*Cyprinus carpio*) with an average weight of 29 g (Mahdavi et al., 2013). Turan and Yiğitaslan (2016) reported that addition of rosemary extract in ratios of 0.25% and 0.5% to the feed of carp (*Cyprinus carpio*) resulted in a weight increase and decreased FCR. This difference may be related to the variance in the diets of carnivorous and omnivorous fish.

Excessive increase in condition factor is considered to be obesity while decrease is considered to be leanness (Timur, 2006). In other studies conducted with fish, it was seen that plants generally do not change the condition factor (Dügenci et al., 2003; Aly et al., 2008; Dakar et al., 2008). When green tea byproduct and a high ratio of black cumin (2%) were used, the condition factor decreased (Cho et al., 2007; Diab et al., 2008). In these studies, there was no change in the condition factor with other forms of green tea use or the use of black cumin at a ratio of 1%. These results show that the form and the ratio of some plants may adversely affect the condition factor. A mixture of different plants increased the condition factor in common sea bream (Ji et al., 2007).

The invariance in the VSI and HSI scores while the condition factor increased can be associated with the increase in meat mass. Similarly, Gabriel et al. (2015) found that there was no change in condition factor when tilapia was fed with aloe vera.

It is generally known that the liver of fish produced in aquaculture is too fatty and their color becomes lighter (Roberts, 2001). The excess fat from the feed is not used as energy by fish; rather, it is stored in the organs and tissues, which negatively affects the health of the fish and reduces the utilization of feed. As the amount of fat stored in the liver increases, the weight of the liver also increases, which can be detected by the HSI.

Table 2 Fish performance and feed utilization for tilapia fed diets containing different herbal extract supplements for 90 days.

	Control	R01	R025	R05	A05	A1	A25
IFW	7.58±0.05	7.51±0.05	7.51±0.01	7.48±0.06	7.51±0.03	7.48±0.02	7.50±0.11
FFW	23.57±0.05	23.91±0.19	23.87±0.40	23.62±0.15	23.66±0.10	23.68±0.19	23.88±0.03
WG	15.98±0.08	16.39±0.24	16.35±0.40	16.13±0.08	16.14±0.07	16.19±0.20	16.37±0.12
CF	1.28±0.00	1.26±0.00	1.28±0.03	1.31±0.04	1.33±0.00	1.28±0.01	1.30±0.01
FCR	1.43±0.02	1.42±0.00	1.39±0.08	1.37±0.09	1.41±0.06	1.40±0.08	1.38±0.05
SGR (%/day)	1.02±0.00	1.04±0.01	1.04±0.01	1.03±0.00	1.03±0.00	1.03±0.00	1.04±0.01
HSI	2.03±0.30	1.71±0.19	2.00±0.21	1.88±0.93	2.08±0.23	2.04±0.22	2.37±0.48
VSI	12.01±1.42	11.14±0.20	12.39±1.63	11.39±0.35	12.38±1.63	10.73±0.97	11.18±0.06

IFW: Initial fish weight (g), F: Final fish weight (g), WG: Weight gain (g), Values are mean±SE (n=6).

In our study, it was determined that the herbal sources used did not affect the liver fat in the fish. Similarly, in a study by Cho (2011), fig extract, onion extract, and Indian fig were reported to be ineffective on liver fat amount and HSI. However, in a study with channel catfish, oregano (*Origanum heracleoticum* L.) was reported to reduce the values of HSI and VSI (Zheng et al., 2009). In the same study, the decrease in the rate of HSI in parallel to VSI may have been due to the reducing effect of oregano on liver fat. A reduction in HSI scores was achieved by the use of *Quillaja saponin*, *Astragalus radix + Lonicera japonica*, and green tea plants in different studies on fish (Francis et al., 2002; Zakes et al., 2008; Cho et al., 2007). In a similar study, increased HSI was observed when genetically improved farmed tilapia juveniles with an average weight of 4 g were fed with feed including 31.7% protein and 7.3% lipid and feed with aloe vera additives in ratios of 0.5%, 1%, 2%, and 4% per kilogram while VSI was not changed (Gabriel et al., 2015). VSI, which includes all the internal organs, generally gives information about weight gain or loss. In our study, it was determined that aloe vera and rosemary extracts did not change the values of VSI in tilapia. Similarly, when 0.5-4 g/100 g of *Rheum officinale* extract was added to the feed of *Cyprinus carpio* with an average weight of 5.39 g, HSI and VSI scores did not change. No significant change was observed in whole-body proximate composition in the control group or the experimental groups (Xie et al., 2008).

The whole-body proximate compositions of fish at the end of the our experiment are presented in Table 3. There was no significant difference related to protein, lipid, ash, or moisture between the experimental groups and the control group.

Table 3 Whole-body proximate composition (%) of tilapia fed diets with different levels of herbal extract supplements for 90 day.

Composition (%)	Control	R01	R025	R05	A05	A1	A25
Crude protein	17.13±2.06	17.28±0.46	16.93±0.37	17.82±1.23	17.94±0.62	16.79±0.31	16.19±0.24
Crude lipid	2.84±0.89	2.01±0.90	2.48±0.90	2.74±1.63	2.62±0.28	2.34±1.38	3.81±0.19
Crude ash	3.13±0.81	3.64±0.70	3.22±0.77	3.10±0.74	4.08±0.54	3.33±0.71	3.64±0.62
Moisture	72.51±1.23	73.42±1.69	72.71±0.43	71.37±0.93	71.19±1.17	71.63±1.07	70.77±0.16

Values are mean±SE (n=6).

Similarly, when the rosemary was added to the feed of sea bass with an average weight of 20.43 g, no significant change was observed in the whole-body proximate composition ($p>0.05$) (Yılmaz et al., 2012). Gabriel et al. (2017) found in their study that aloe vera plant did not change the amount of moisture, ash, or lipid in tilapia juveniles, but it reduced the amount of protein. In light of all these studies, it is seen that plant sources, with the components they contain, make positive or negative changes in fish meat nutritional values. Therefore, the type of plant to be used is of great importance. It should be kept in mind that the same plant species may have different effects in different fish due to differences in fish species.

In conclusion, the present study indicates that after feeding Nile tilapia with a diet containing herbal extracts over a period of 90 days, it was found that rosemary and aloe vera extracts did not have any negative effect on the metabolism of the tilapia.

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