



The Effect of Clove Oil Supplementation in Laying Hen Diets on Performance, Egg Quality, Some Blood Parameters, and Yolk TBARS

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ARTICLE INFO

Research Article

Received : 29/05/2021

Accepted : 08/11/2021

Keywords:

Laying hen

Clove oil

Performance

Egg quality

Blood parameters

ABSTRACT

In this study, it was investigated the effect of clove oil supplementation at increasing levels into laying hens' diets on performance, egg quality traits, some blood parameters and yolk TBARS (Thiobarbituric Acid Reagent) values. For this purpose 96 Lohman white laying hens, 28 weeks of age, were divided into four treatment groups. Control group was fed with basal diet (C) and treatment groups were fed with diets formed by addition of clove oil at 50 ppm (CO1), 100 ppm (CO2) and 150 ppm (CO3), respectively. During the trial, feed and water were given as *ad-libitum*, and poultry house was illuminated for 17 hours. Experiment lasted for 13 weeks. Addition clove oil at increasing rates into diet did not affect the live weight. The data analysed as polynomial showed that supplementation of clove oil into layer diet linearly improved feed conversion ratio and increased the egg production. But, daily feed consumption, egg weight, damaged egg ratio and egg quality parameters were not affected by treatments. Serum parameters such as triglyceride, glucose, aspartate aminotransferase, alanine aminotransferase and calcium were not affected by the clove oil supplementation. TBARS values in C, CO1, and CO2 were found higher than the CO3 group fed with diet including 150 ppm clove oil. In conclusion, clove oil at 150 ppm level in diets of laying hens could be used due to extend the egg shelf life and to decline serum cholesterol content.

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Introduction

Antibiotics have been used in poultry feeds over the years to improve growth performance, prevent some specific pathogenic microorganism, and increase some beneficial microorganisms in intestinal microflora (Mohammadi et al., 2014). However, with the public becoming increasingly concerned about the risk of a rise in antibiotic resistant bacteria, producers have faced objections to the presence of such antibiotics in livestock feeds (Arpášová et al., 2017). After the EU banned antibiotics from animal feed in 2006, the search was on to find substitutes, particularly in respect to egg quality and egg production (Kaya et al., 2015). In recent years, products containing essential oils derived from a number of spices and herbs are used as growth promoting feed additives in animal nutrition, but more studies are needed to optimize their use (Mohammadi et al., 2014).

Recently, herbs have come to be viewed as possible substitutes for antibiotics and one of the most efficient is clove (*Syzygium aromaticum*), belonging to the family *Myrtaceae*. The ingredients in clove oil (CO) including eugenol, isoeugenol, caryophyllene, α -humulene, and

eugenyl acetate are viewed as particularly valuable. Eugenol is the most biologically active compound in cloves and makes up 70-80% of clove oil (Al-Mufarrej et al., 2019). Eugenol is a substance found in clove oil that has anti-microbial and anti-inflammatory properties and flavonoids that boost its anti-inflammatory abilities (Mukhtar, 2011).

It has been stated that the components of clove have strong antibacterial, antiseptic, appetite and digestion stimulation, antimicrobial and antifungal, analgesic and anti-inflammatory and anticarcinogenic, antiparasitic and antioxidant properties (Mukhtar, 2011). Ingredients with good nutritional value including natural antioxidants, essential fatty acids, and lipid-soluble bioactive molecules are found in cold pressed clove oil (Hussein et al., 2019). Thus, in this study, research was carried out to determine the effect of increasing the dietary levels (50, 100, 150 ppm) of clove oil to examine its natural antioxidant effects and its effect on performance, egg quality traits, some blood parameters, and yolk TBARS (Thiobarbituric Acid Reagent) values for laying hens.

Materials and Methods

Animals, Diet, and Management

The Research Animal Ethics Committee of Atatürk University approved this experimental protocol. The animal subjects of the study were ninety-six Lohmann white laying hens. They were divided into four groups randomly. Six subgroups of four hens each were created from each of the above groups and housed in identical cages (50× 46×46 cm). After a one-week period to allow the hens to adapt, they were fed for 12 weeks with a diet containing varying amounts of clove oil (CO) or no additives. Experimental groups were fed standard commercial layer diet (control), without clove oil addition, or control with the inclusion of clove oil at 50 ppm (CO1), 100 ppm (CO2), and 150 ppm (CO3). The CO added in different amounts to the basal was obtained from Aksu Vital Doğal Ürünler Gıda San. & Tic. Lt. The basal diet was formulated to meet nutrient requirements proposed by NRC (1994). AOAC (2000) methods were used to analyse the experimental diets. Chemical compositions of the basal diet, the CO as well as their ingredients, may be seen in Table 1. During the 12 weeks of the experiment, the hens were fed at 08:30 once per day, had water available all the time, and were exposed to light for 17 h per day.

Sample Collection and Analytical Procedure

The hens were weighed at the beginning and the end of the experiment. The daily feed consumption and number of laid eggs was recorded. The feed conversion ratio (FCR) calculated by one kilogram of feed consumed per kilogram of eggs produced. Once per month, the eggs laid by the hens were gathered (n=12 per group); they were kept for 24 h at room temperature in order to evaluate egg quality parameters including shape index (SI), shell strength (SS) (determined by using a machine with a spiral pressure system), shell thickness (ST) (determined in three different parts by using a micrometer), albumen index (AI), yolk index (YI), yolk color (YC) (the Yolk Color Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit (HU). The Kaya and Macit method (2012) was used in the assessment.

Bloods were collected in a sample amount of 5.0 mL from the axillary vein of 24 hens (six from each group) at the end of the experiment period. These were placed into non-heparinized tubes using sterilized needles. Incubation of these samples took place at 37°C for 2 h, and then they were centrifuged at 3 000 rpm for five min. While the serum parameters were being determined, the samples were stored in 1.5 mL Eppendorf tubes at -80 °C. These serum parameters including cholesterol, triglycerides, glucose, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) Ca, P, and Mg were measured using commercial kits (DDS® Spectrophotometric Kits, Diasis Diagnostic Systems Co., İstanbul, Turkey) with the Autoanalyzer Mindray Perfect Plus 400.

An index of lipid peroxidation provided the basis for the evaluation of the malondialdehyde (MDA) formed during refrigerated storage (Kaya et al., 2014). 24 eggs from each group were selected, shelled, and stored for 0, 7, 14, 28, and 56 days at + 4°C to determine the MDA in the yolk. At this point, the method of Placer et al. (1966) was

used with a Biotek ELISA Reader (Bio Tek µQuant MQX200 Elisa Reader, USA) to analyze the samples. According to the method, the egg homogenate was prepared by taking 3-5 grams of egg yolk and diluting it with 0.9% NaCl at a ratio of 1/9. 2.25 ml of color reagent was added on 250 µl egg homogenate. This mixture was kept in a boiling water bath for 20 min, then cooled and centrifuged at 2,000 rpm for 5 min, and the absorbance was measured at 532 nm, and thiobarbituric acid reagent (TBARS) values were determined as micrograms of MDA per gram of yolk. The egg yolk TBARS value was calculated using the formula below:

$$(TBARS) \text{ (ng/mg)} = 356.1 \times \text{absorbance value}$$

Statistical Analysis

The ANOVA by means of the GLM procedure of SPSS 10.0 software (2011) was used on the data. To determine the effect of the feeding level of clove oil in the diets, a polynomial contrast was created. A significance level at P<0.05 for the effects of the dietary treatments on response variables was established.

Table 1. Analysed Chemical composition of the experimental diets

Ingredient, (%)	
Corn	59.63
Soybean meal (46% CP)	19.50
Sunflower seed meal (%36 CP)	7.40
Soybean oil	1.49
Meat-bone meal	1.50
Monocalcium phosphate	0.07
Marble	9.50
Vitamin-mineral premix ¹	0.30
Salt (NaCl)	0.20
Sodium bicarbonate	0.15
Ekobond (Toxin binder)	0.10
Salmonil LCT (Organic Acid Mixture)	0.10
DL-Methionine	0.06
(on a DM basis)	
Dry matter (%)	88.36
Crude protein (%)	17.58
Crude fiber (%)	3.19
Ether extract (%)	13.75
Crude Ash (%)	3.77
Calcium (%)	3.80
Phosphorus (%)	0.47
ME ² (kcal/kg)	2724

¹The premix provided per 1 kg of diet: 4.000.000 IU Vitamin A; 800.000 IU Vitamin D3, 10.000 mg Vitamin E; 100.000 mg Choline chloride; 26.667 mg Manganese oxide; 20.000 mg Zinc oxide; 20.000 mg Iron sulfate; 1.667 mg Copper sulfate; 333 mg Calcium iodate; 50 mg Sodium selenite; 300 mg Hydroxy methionine. ²ME: Metabolizable energy value calculated according to TSE (1991)

Results and Discussion

Laying Performance

The effects of addition different levels (0, 50, 100, 150 ppm) of clove oil in diet, as a natural antioxidants, on the Lohmann white layers performance parameters are presented in Table 2.

Table 2. Performance parameters of the control and clove oil groups

Treatment	Feed Consumption (g/d)	Egg Production (%)	Egg Weight (g)	FCR (kg feed/kg egg)	Body weight (g)		
					Initial	Final	Change
Control	119.01	91.02	66.31	1.97	1615.62	1716.57	100.93
CO1	117.39	93.99	65.95	1.89	1611.67	1697.07	85.84
CO2	117.16	94.84	65.83	1.88	1621.7	1704.88	83.21
CO3	116.53	94.94	66.11	1.86	1612.7	1739.37	126.67
SEM	1.74	0.76	0.80	0.04	25.86	31.56	20.20
Polynomial Analyses							
L	0.480	0.007	0.841	0.009	0.991	0.595	0.416
Q	0.494	0.066	0.692	0.580	0.924	0.403	0.160
C	0.767	0.080	0.963	0.661	0.779	0.996	0.725

CO1= 50 ppm Clove Oil, CO2= 100 ppm Clove oil, CO3= 150 ppm Clove Oil, L=Linear, Q=Quadratic, C=Cubic
SEM = Standart error mean, FCR: Feed Conversion Ratio

Table 3. Egg quality parameters of the control and clove oil groups

Groups	Egg Weight (g)	Shape Index (%)	Shell Strength (kg/cm ²)	Shell Thickness (cm×10 ⁻²)	Shell Weight (g)	Yolk Colour	Yolk Index (%)	Albumen Index (%)	Haugh Unit
Control	66.25	74.11	3.43	0.396	8.84	12.02	42.42	9.96	89.08
CO1	66.34	72.72	3.27	0.393	8.56	11.70	43.56	10.22	88.90
CO2	67.00	73.58	2.89	0.384	8.54	11.83	42.63	10.27	88.59
CO3	64.64	72.75	3.13	0.379	8.20	11.63	43.49	10.08	89.59
SEM	1.46	0.59	0.23	0.01	0.17	0.15	0.80	0.39	1.53
Polynomial Analyses									
L	0.528	0.233	0.216	0.056	0.084	0.125	0.528	0.811	0.858
Q	0.410	0.640	0.380	0.859	0.896	0.668	0.866	0.574	0.702
C	0.589	0.147	0.418	0.626	0.602	0.240	0.290	0.985	0.837

CO1= 50 ppm Clove Oil, CO2= 100 ppm Clove oil, CO3= 150 ppm Clove Oil, L=Linear, Q=Quadratic, C=Cubic, SEM = Standart error mean.

According to the results of polynomial analysis, it was observed that there was no effect in feed consumption in parallel with the increase of clove oil added to the ration. Mukhtar (2011) showed an increase in the feed intake by broiler chicks fed with 600 mg clove oil/kg as compared with those from the control, 200 mg/kg and 400 mg/kg clove oil groups, but this increase was not significant ($P>0.05$). In another experiment, Hussein et al. (2019) reported that increasing clove oil levels (0, 0.75, and 1.5 mL/kg) gradually increased feed intake in Japanese quails. Similar to the findings from the current study, Gandomani et al. (2014) reported that adding clove buds to different levels (0.2%, and 0.4%) of laying hens and broiler does not affect daily feed consumption.

Hens fed the control diet produced less egg than birds fed the clove oil supplemented diets in this study. In response to increasing level of clove oil supplementation, egg production linearly increased. This result is in contrast with those Arpašová et al. (2017) who introduced that the addition of 0.3 ml kg⁻¹, 0.6 ml kg⁻¹, and 1 ml kg⁻¹ clove oils into hens' diet had no significantly different to the egg production. Similar to the present study, Gandomani et al. (2014) found that the addition of different levels (0.2 and 0.4%) of clove bud to laying hen feeds increased egg production. Essential oils' functions is based on organoleptic effect and stimulation of organism to the production of digestive juices due to consisted compounds. As a result, higher nutrient digestibility and absorption were observed (Arpašová et al., 2017).

As shown in Table 2, clove oil supplementation at increasing levels into diet of laying hens had a statistically significant effect on FCR, which could be attributed to enhanced nutrient utilization. In response to increasing level of clove oil supplementation, FCR linearly decreased ($P<0.05$). Feed utilization rate was more important performance parameter than feed intake.

This may be due to the stimulation mechanisms of digestive enzyme secretion and the ecosystem stabilization of the gut microflora, leading to improved feed utilization (Zhai et al., 2018). In addition, many studies have confirmed the positive effect of clove oil on FCR (Mukhtar, 2011; Hussein et al., 2019).

Egg Quality Parameters

As can be seen from Table 3, according to the results of polynomial analysis, none of the egg quality parameters including SI, SS, ST, AI, YI, YC and HU was affected by increasing level of clove oil (50, 100, 150 ppm) supplementation. It expressed similarly with this study that the addition of clove essential oil supplementation (0.3, 0.6 and 1 mg kg⁻¹) to diets had not a significant effect on yolk index, yolk colour but unlike this study the albumen index significantly ($P<0.05$) influenced by the addition of 1 ml kg⁻¹ clove oil. Also, Haugh units were significantly influenced ($P<0.05$) by the addition of 0.6 ml kg⁻¹ and 1 mg/kg clove oil (Arpašová et al., 2017). These results agree with those of Gandomani et al. (2014), who reported that SI, SS, ST, AI, YI, YC and HU were not influenced by increasing dietary clove bud content. Also the same

researchers stated that increasing the clove bud content improved eggshell strength during the trial and it differs from this study.

Some Serum Parameters

The effects of supplemental CO (50, 100, and 150 ppm) on some serum parameters are presented in Table 4. As shown in Table, triglycerides, glucose, AST and ALT did not differ across the experimental diets. Serum cholesterol, Alb, Ca and P concentration linearly decreased by CO supplementation. These findings were agree with those of Hussein et al. (2019) who indicated that serum levels of ALT, AST did not show significant difference in Japanese quails' diets supplemented with different levels of CO (0.75 ml and 1.5 ml oil kg⁻¹ diet).

Different from the current work, some researchers found that the addition of clove oil to broiler diets did not affect serum cholesterol levels (Najafi and Torki, 2010; Mohammadi et al., 2014). Also it is stated that serum glucose level decreased with the increase of CO levels in the ration (Mohammadi et al., 2014). Chowdhury et al. (2018) stated that used dietary clove bud oil at 0.6% did not a significant influence on serum total protein in broiler chickens. Increase in serum AST levels may result from necrosis or changes in cell membrane permeability or hepatocyte damage attributed to liver dysfunction (Traesel et al., 2011). In the present study, it was determined that the addition of clove oil to the ration did not affect the AST

values.TBARS (Egg Yolk Thiobarbituric Acid Reagent) Values

The rancidity of lipids, one of the most important factors affecting egg shelf life, is determined by Thiobarbituric acid reactive substance (TBARS). In this study, it was determined that the effect of different levels (0, 50, 100 and 150 ppm) of clove oil (CO) supplementation on egg shelf life. TBARS values of groups are summarized in Table 5. It was observed that there was no significant difference between the groups in TBARS values in egg samples stored for 0,7,14 and 56 days. In the polynomial analysis, In response to increasing level of clove oil supplementation linearly decreased the TBARS values for the eggs stored for 28 days. Studies have shown that clove oil has a strong antioxidant effect due to its high content of eugenol (Wang et al., 2010; Gülçin et al., 2012). It is reported that this effect is achieved by scavenging free radicals or binding metal ion (Gandomani et al., 2014).

The effect of storage time on TBARS values was found to be significant and it was observed that TBARS values increased as time progressed (P<0.01). As can be seen from Figure 1, it was observed that increasing clove levels slowed the formation of TBARS, especially in the CO3 group and TBARS values increased over time. The antioxidant capacities of plant essential oils are derived from phenolic compounds, which are present in large quantities in their compositions.

Table 4. Some serum parameters of the control and clove oil groups

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)	Albumin (g/dL)	AST (unit/L)	ALP (unit/L)	ALT (unit/L)	Calcium (mg/dL)	Phosphorus (mg/dL)
Control	139.40	1142.0	225.80	1.70	217.0	404.40	1.40	28.34	4.70
CO1	134.40	1053.8	230.00	1.56	181.6	730.60	1.60	25.04	3.96
CO2	136.40	1101.2	224.80	1.50	213.0	1501.20	1.80	24.28	3.64
CO3	102.20	1135.6	225.40	1.50	183.0	776.20	0.80	25.08	3.60
SEM	9.23	95.99	9.21	0.05	16.7	240.59	0.38	1.32	0.23
Polinomial Analyses									
L	0.017	0.948	0.878	0.022	0.360	0.817	0.369	0.050	0.039
Q	0.133	0.532	0.847	0.087	0.874	0.030	0.141	0.140	0.103
C	0.311	0.734	0.717	0.552	0.107	0.034	0.498	0.870	0.060

CO1= 50 ppm Clove Oil, CO2= 100 ppm Clove oil, CO3= 150 ppm Clove Oil, L=Linear, Q=Quadratic, C=Cubic, AST: aspartate aminotransferase, ALP: alkaline phosphatase, ALT: alanine aminotransferase, SEM = Standart error mean.

Table 5. The effects of feeding different levels CO on TBARS values of egg samples stored for 0, 7, 14, 28 and 56 days (MDA ng/g)

Groups	TBARS					
	Day 0	Day 7	Day 14	Day 28	Day 56	
Control	5.07	5.19	5.75	10.15	12.38	
CO1	4.25	5.10	5.61	10.37	12.47	
CO2	4.78	5.64	6.49	8.53	13.09	
CO3	4.82	5.27	6.37	6.83	12.10	
SEM	0.27	0.22	0.36	0.35	0.51	
Polinomial Analyses						
L	0.853	0.434	0.100	0.002	0.920	
Q	0.124	0.530	0.971	0.011	0.296	
C	0.131	0.134	0.2223	0.174	0.348	
Overall Effects						
Group Effect	C	CO1	CO2	CO3	SEM	P
	7.71 ^a	7.56 ^a	7.71 ^a	7.07 ^b	0.16	0.017
Time Effect	Day 0	Day 7	Day 14	Day 28	Day 56	P
	4.73 ^e	5.30 ^d	6.06 ^c	8.97 ^b	12.51 ^a	0.000

CO1= 50 ppm Clove Oil, CO2= 100 ppm Clove oil, CO3= 150 ppm Clove Oil, L=Linear, Q=Quadratic, C=Cubic, a,b,c,d,e: Means within lines with different superscripts differ at P<0.05, SEM = Standart error mean.

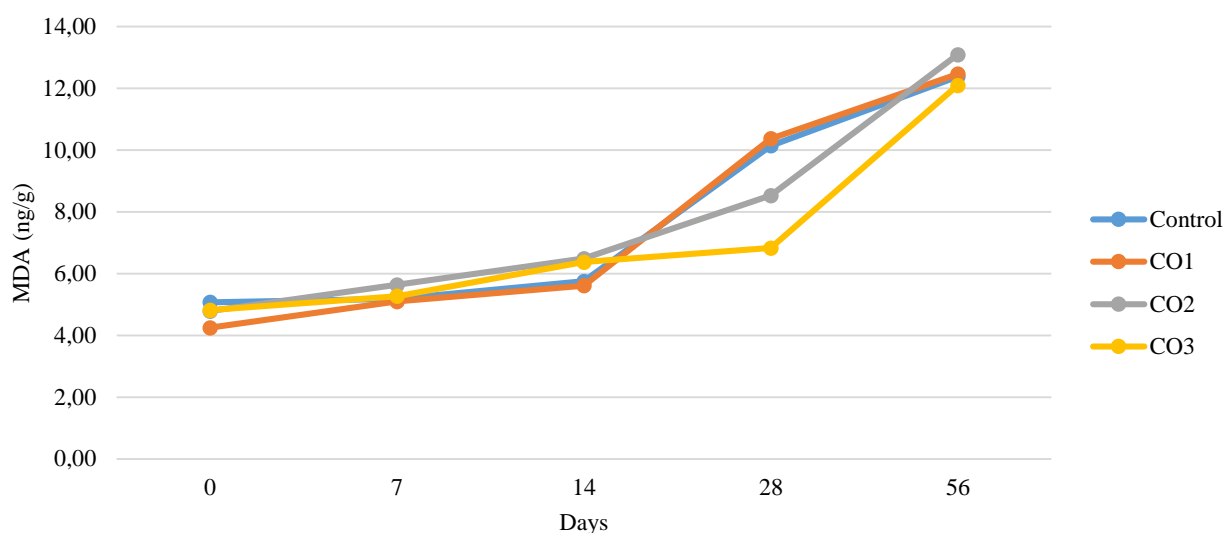


Figure 1. The effect of storage time and level on TBARS value

Phenolic compounds, due to phenol structures or phenolic sequences in molecular structures, give rise to hydrogen from phenolic hydroxyl groups and prevent oxidation by inhibiting the formation of free fatty acid radicals at the beginning (Saricaoglu and Turhan, 2018). It is considered that the antioxidant capacity of CO is highly related to eugenol content (Saricaoglu and Turhan, 2018). CO contain eugenol (90 to 95% of oil content), a potent polyphenolic compound and is known to exhibit pharmacological properties such as antioxidant (Hussein et al., 2019).

Also it reported that using the highest level of clove bud (levels of clove bud 0.0, 2.0, and 4.0 g/kg) led to decline the MDA content of egg yolk in eggs kept for 30 d in the refrigerator and for 15 d in storage conditions (Alizadeh et al., 2015). In another study, it stated that oxidative stability of hen liver was enhanced by essential oil mixture, thereby increasing hepatic antioxidant enzymes (i.e., superoxide dismutase and glutathione peroxidase) compared to those of the control group (Bozkurt et al., 2012). Antioxidant is defense system that provide a strong defense against the destructive effects of free radicals which are affect many compounds and molecules present on the cell membranes. Lipid peroxidation is the chain of reactions of oxidative degradation of lipids. Malondialdehyde (MDA) is one of the most known secondary products of lipid peroxidation (Iskender et al., 2016).

Conclusion

In recent years, researchers have been focused on natural antioxidant additives such as essential oils. This study reveals that clove oil exhibit remarkable antioxidant capacity. In conclusion, clove oil at 150 ppm level in diets of laying hens might be used due to extend the egg shelf life and to decline serum cholesterol content.

Acknowledgements

This study was taken from the part of Melek Şehitoğlu's master thesis.

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