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RESEARCH ARTICLE

The Effects of Black Tea Factory Waste Supplementation into Laying Hen Diets on Performance, Egg Quality, Yolk Peroxidation, and Blood Parameters

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Summary

The experiment was carried out to evaluate the effects of supplementing laying hen diets with different percentages of black tea factory waste (BTFW), evaluating performance, egg quality, yolk oxidation, and blood parameters. Twenty-four-week-old Lohmann layers (n=144) were divided into six dietary treatment groups (24 hens each), which were fed standard commercial diets supplemented with 0% (control), 2%, 4%, 6%, 8%, and 10% BTFW for 12 weeks. In this study, increasing BTFW levels were associated with impaired linearly feed consumption, final body weight, shell strength, shell weight, shell thickness, and increased cracked egg yield. The results showed a quadratic effect on albumen index, haugh unit score, and a cubic effect on feed conversion ratio, egg production, egg weight, yolk color due to the BTFW supplementation into layer diets. Whereas, shape index and yolk index were not affected by BTFW. In response to increasing BTFW percentage, yolk MDA values were decreased following storage for 14 and 28 days, but not 56 days. Plasma cholesterol, HDL, and aspartate aminotransferase (AST) were not influenced by BTFW. Increasing BTFW percentages led to linear increases in serum albumin and total protein, quadratic increases in triglyceride and alanine aminotransferase, and decreases in glucose (quadratic) and alkaline phosphatase (cubic). Results from present study showed that supplementing laying hen diets with 2% and 4% BTFW resulted in strong antioxidative activity without adverse effect on laying performance, quality characteristics, and blood parameters. In addition, more than 4% BTFW had deleterious effects on performance and egg quality traits, due to high tannic acid content.

Keywords: *Antioxidant, Black tea factory waste, Egg quality, Laying hen, Performance*

Yumurtacı Tavuk Rasyonlarına Siyah Çay Fabrika Atığı İlavesinin Performans, Yumurta Kalitesi, Yumurta Sarısında Lipid Peroksidasyonu ve Bazı Kan Parametreleri Üzerine Etkisi

Özet

Bu çalışma fabrika siyah çay atığının (SÇFA), farklı seviyelerde yumurta tavuğu yemlerine ilavesinin performans, yumurta kalitesi, yumurta sarısı oksidasyonu ve bazı kan parametreleri üzerine etkilerini incelemek amacıyla yapılmıştır. Bu amaçla, 24 haftalık yaşta 144 adet beyaz Lohmann yumurta tavuğu her grupta 24 hayvan olacak şekilde 6 gruba ayrılarak, ticari yumurta tavuğu yemine; %0 (Kontrol), %2, %4, %6, %8 ve %10 düzeylerinde fabrika siyah çay atığı ilave edilerek oluşturulan rasyonlarla 12 hafta süre ile beslenmişlerdir. Çalışmada, SÇFA'nın artan seviyesiyle birlikte, yem tüketimi, deneme sonu canlı ağırlığı, kabuk ağırlığı, kabuk kalınlığı, kırılma mukavemeti linear olarak azalmış, hasarlı yumurta oranı linear olarak artmıştır. Rasyona SÇFA ilavesinin haugh birimi ve ak indeksi üzerine kuadratik, yumurta verimi, yumurta ağırlığı ve yumurta sarı rengini üzerine kübik etkiye sahip olduğu belirlenmiştir. Buna karşın, şekil indeksi ve sarı indeksi değerleri ise rasyona SÇFA ilavesinden etkilenmemiştir. 14 ve 28 gün depolanan yumurtaların MDA değerleri rasyona artan seviyede SÇFA ilavesiyle birlikte azalırken, 56 gün depolanan yumurtaların MDA değerleri etkilenmemiştir. Diyetsel muamelenin plazma kolesterolü, HDL ve aspartat aminotransferaz (AST) üzerine etkisi önemsiz olmuştur. Artan SÇFA'nın seviyesiyle birlikte serum albumini ve toplam protein linear olarak artmış, trigliserid, alanine aminotransferaz (ALT) ve glukoz kuadratik, alkalin fosfataz (ALP) ise kübik olarak etkilenmiştir. Çalışmadan elde edilen sonuçlar yumurtacı tavuk rasyonlarına %2 ve %4 düzeylerinde SÇFA ilavesinin performans, yumurta kalite kriterleri ve kan parametreleri üzerine olumsuz bir etki yapmaksızın antioksidan aktiviteye sahip olduğunu göstermiştir. Ayrıca, SÇFA'nın yüksek tannik asit içeriğinden dolayı rasyona %4'ten daha fazla seviyede ilavesi performans ve kalite kriterlerini olumsuz etkilemiştir.

Anahtar sözcükler: *Antioksidan, Fabrika siyah çay atığı, Performans, Yumurta Kalitesi, Yumurtacı tavuk*

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INTRODUCTION

Tea made from the plant *Camellia sinensis* is one of the cheapest and most popular beverages worldwide [1]. *C. sinensis* is grown in about 30 countries around the world, and freshly harvested tea leaves are processed differently in different parts of the world to produce oolong tea (2%), green tea (20%), or black tea (78%) [2]. Fresh tea leaves comprise 36% polyphenolic compounds, 25% carbohydrates, 15% protein, 6.5% lignin, 5% ash, 4% amino acids, 2% lipids, 1.5% organic acids, 0.5% chlorophyll, and less than 0.1% carotenoids and other volatile substances [3]. However, the exact chemical composition of tea leaves varies depending on several factors, including tea type, species, shrub or tree, age of leaves, collection season, climate, drying conditions and technological processing during tea production, soil condition, cultivation method, and environmental pollution [4]. Flavanols are important and characteristic tea polyphenols that predominantly include catechins, such as epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, and catechin [5]. Black teas also contain the flavonols kaempferol, quercetin, myricetin [6], and the two major pigments theaflavins and thearubigins . Many studies have already described the positive effects of tea, tea catechins, tea extract, and tea powder on animal performance and product quality [8-15]. However, many of these materials are too expensive to use in the livestock industry [16]. Green tea by-product obtained through tea beverage production is reasonably cheap and effective, and has therefore been used as a feed supplement [16]. Yang et al.[17] reported significantly lower TBA values in broiler meat of broilers that are fed diets containing 0.5% to 2.0% green tea by-product supplementation compared to those fed a diet containing antibiotics.

Livestock sector plays a significant role in Turkey and is essential for the food security of rural population. However, inadequacy of animal feed resources in both quantitatively and qualitatively is most often the limiting factor of the development of livestock production in Turkey [18]. Many agricultural and agro-industrial by product have the potential as animal feeds. Black tea factory waste (BTFW), being one of these by products, obtained from used tea leaves in factories. BTFW is typically disposed of as compost, dumped into landfills, or burned, which causes both economical and environmental problems [19]. In Turkey, the total tea plantation area is 70.000 hectare, and tea leaf production is about 1.2 billion tons [20]. The amount of factory tea waste produced depends on the manufacturing techniques and physical properties of the raw tea leaf; it varies from 7% to 15% of the total dried tea leaf amount, which is about 30.000 tons annually from state-owned companies [21]. When BTFW was used correctly in the least cost feed formulation diets, they will allow for replacement of wheat bran due to the same ingredient.

No published information is available on the quality of feed provided by BTFW or on how it could be evaluated as feed ingredient in laying hen diets. Therefore, the objectives of this study were to investigate the possibility of BTFW into the diets of laying hens and to determine the effects of different percentages of BTFW (2%, 4%, 6%, 8%, and 10%) on performance, egg quality, yolk oxidation degree, and some blood parameters.

MATERIAL and METHODS

Animals, Diet, and Management

This study was conducted by the researchers based on protocols by Atatürk University Ethical Commission Report (No: 2013 /4/111).

One hundred and fourty four 24-week-old Lohmann layers were blocked according to the location of the battery type cages (50 × 46 × 46 cm, width × depth × height), and then randomly assigned to receive one of six dietary treatments with 6 replicates of four hens in each replicate for 12 weeks. We obtained BTFW from a tea factory in Rize (northern Turkey), and substituted it for wheat bran in the diet at 2%, 4%, 6%, 8%, and 10% based on weight. The experimental diets were formulated to meet NRC recommendations [22] and analyzed using AOAC methods [23]; Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were determined according to Goering and van Soest [24], Metabolizable energy contents of the experimental diets were calculated from tabular values of feedstuffs reported for chickens [22]. *Table 1* lists their ingredients and analyzed compositions. *Table 2* presents the mineral and chemical composition of the BTFW used in this study, which was analyzed for tannin using the method of Kondo et al.[25]. Macro and micro elements content of BTFW were determined Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) [26]. From 24 to 36 weeks of age, the hens were fed either a standard commercial laying hen diet with no BTFW (Control) or the basal diet plus 2%, 4%, 6%, 8%, or 10% BTFW. Hens were fed *ad libitum* once daily at 08:30 hours, and water was available at all times. Lighting was 17 h light/7 h dark throughout the experimental period.

Sample Collection and Analytical Procedure

Feed intake and egg production were recorded daily, egg weight was measured bi-weekly using egg wights taken as daily from each treatment groups, and body weights were measured at the beginning and end of the experiment as to cage basis in all of the treatment groups. Feed conversion ratio (FCR) was recorded as kilogram of feed consumed per kilogram of eggs produced. Before determination of egg weight, 12 eggs from each experimental

Table 1. *Ingredients and chemical composition of the experimental basal diets*
Tablo 1. *Denemede rasyonlarının bileşimi ve kimyasal komposizyonu*

Ingredient (%)	Experimental Diets (% Black Tea Factory Waste)					
	0%*	2%	4%	6%	8%	10%
Corn	35	35	35	35	35	35
Soybean meal (48% CP)	20	20	20	20	20	20
Tea waste	-	2	4	6	8	10
Wheat bran	10	8	6	4	2	-
Wheat	15	15	15	15	15	15
Sunflower seed meal	5	5	5	5	5	5
Fish meal	2.5	2.5	2.5	2.5	2.5	2.5
Vegetable fat	2	2	2	2	2	2
Dicalcium phosphate [1]	2.5	2.5	2.5	2.5	2.5	2.5
Marble	7.3	7.3	7.3	7.3	7.3	7.3
Vitamin-mineral premix [2]	0.22	0.22	0.22	0.22	0.22	0.22
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Methionine [3]	0.12	0.12	0.12	0.12	0.12	0.12
Lysine [4]	0.06	0.06	0.06	0.06	0.06	0.06
Chemical composition (analyzed on dry matter basis)						
Dry matter (%)	90.1	89.9	89.7	89.9	90.7	90.4
Crude protein (%)	17.3	17.4	17.6	17.5	17.8	17.8
Crude fiber (%)	3.6	4.5	4.6	5.3	5.6	6.3
Ether extract (%)	2.8	2.8	2.7	2.9	2.4	2.8
Crude ash (%)	11.4	11.6	11.0	11.8	11.3	11.6
Metabolizable energy (kcal/kg) [5]	2649	2626	2619	2610	2621	2606

*[1] Each kilogram contained: 24% Ca and 17.5% P, [2] The premix provided per 1 kg of diet: vitamin A, 15.000 IU; cholecalciferol, 1500 ICU; DL-a-tocopheryl acetate, 30 IU; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 µg; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg, [3] DL-methionine, [4] L-lysine hydrochloride, [5] This value was found to with calculation from tabular values of feedstuffs reported for chickens NRC [22], * Basal diet contained: 4.11% Ca and 0.59% total P*

Table 2. *Mineral and chemical composition of black tea factory waste*
Tablo 2. *Siyah çay fabrika atığının mineral madde ve kimyasal komposizyonu*

Mineral	mg/kg	Chemical Composition	On A DM Basis (%)
Na	0.01	DM	92.1
K	2.10	Crude protein	18.2
Ca	0.59	Crude fiber	19.8
Mg	0.24	Ether extract	1.6
P	0.19	Crude ash	5.8
S	0.63	ADF	35.4
Fe	0.05	NDF	46.4
Zn	0.01	Metabolizable energy *	2442 (kcal/kg)
Al	0.20	Tannin	5.7
Mn	0.14		

** This value was found to with calculation, TSE [49]*

group were stored for 24 h at room temperature. Twelve eggs were randomly selected from each experimental group every month to evaluate egg quality parameters, including shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Haugh unit. Eggs were assessed according to the method of Kaya et al.[27].

At the end of the experiment, blood samples taken from the subcutaneous vena ulnaris were collected from two hens from each cage and stored in additive-free vacutainers. Serum was obtained following centrifugation at 3.000 g for 10 min at 20°C, and kept at -20°C until laboratory analyses. Serum parameters-albumin, triglyceride, cholesterol, high density lipoprotein (HDL-C), total protein, glucose, ALP, AST, and ALT were measured using commercial kits (DDS® Spectrophotometric Kits, Diasis Diagnostic Systems Co., İstanbul, Turkey) with an autoanalyzer Vitros 5.1FS.

The malondialdehyde (MDA) formed during refrigerated storage was evaluated as an index of lipid peroxidation[28]. To determine the MDA in yolk, 24 eggs were taken from each group at the end of the experiment, and were stored for 0, 14, 28, and 56 days at +4°C. Six egg samples from each group were then analyzed following the method of Placer et al.[29] using a Biotek ELISA Reader.

Statistical Analysis

Data from the present experiment were statistically analyzed with ANOVA using the GLM procedure of SPSS software [30]. Linear, quadratic, and cubic polynomial contrasts were used to evaluate treatment effects. The effects of the dietary treatments on response variables were considered significant at P<0.05.

RESULTS

Table 3 summarizes the effects of the experimental diets on laying performance parameters. Increasing levels of BTFW supplementation were associated with a linear decrease in feed intake (P<0.05). There was a cubic effect of BTFW levels on egg production values, with the 2% and 4% BTFW groups showing higher production than the control and 6%, 8%, and 10% BTFW groups; the lowest egg production was seen in the 8% BTFW group (P<0.001). BTFW levels had a similar cubic effect on the feed conversion ratio (P<0.001); with increasing levels of BTFW supplementation, FCR first decreased (2% and 4%), then increased (6% and 8%) and then decreased again (10%). Increasing BTFW supplementation was associated with linear increases in cracked egg yield (P<0.01) and linear decreases in egg weight (P<0.001).

Table 4 summarizes the effects of the experimental diets on egg quality parameters. BTFW supplementation did not affect the shape index or yolk index. Shell strength (P<0.001), shell thickness (P<0.001), and shell weight (P<0.01) decreased linearly with increases in BTFW supplementation level. There was a cubic effect of BTFW levels on yolk color (P<0.05), as increasing level of BTFW supplementation caused yolk color to first decrease (2%, 4%, and 6%), then increase (8%), and then again decrease (10%). Albumen index and Haugh unit increased quadratically with the increasing proportion of BTFW in the diet (P<0.01 and P<0.01, respectively).

Table 5 summarizes the yolk lipid oxidation effects of dietary treatments measured at different time periods. The extent of lipid oxidation was measured based on yolk MDA content, which did not differ among dietary treatments after 56 days of storage. However, increasing supplemental BTFW level was associated with quadratic decreases in MDA values in the yolks of eggs stored for 14 and 28 days (P<0.01). Compared to the control treatment, the egg yolk MDA values tended to be lower with increasing levels of BTFW.

Table 6 presents the effects of different levels of BTFW supplementation on some blood parameters. Plasma cholesterol, HDL, and AST (aspartate aminotransferase) were not influenced by the increasing levels of BTFW. Increasing proportions of BTFW in the diet were associated with linearly increased serum albumin and total protein concentrations, quadratically increased triglyceride and alanine aminotransferase (ALT) concentrations, and quadratically decreased glucose concentration. BTFW levels had a cubic effect on serum alkaline phosphatase (ALP) (P<0.01).

DISCUSSION

To our knowledge, no previous publication has used the same animals and type tea waste as in our study; therefore, the present findings have been mostly compared

Table 3. *Effects of black tea factory waste supplementation on laying performance parameters*
Tablo 3. *Siyah çay fabrika atığının performans parametreleri üzerine etkisi*

% BTFW	Perfromance Parameters							
	Feed Consumption (g/d)	Egg Production (%)	Cracked Egg Yield (%)	Egg Weight (g)	FCR¹	Body weight (g)		
						Initial	Final	Gain
0	123.62	88.19	0.74	59.85	2.36	1500.3	1576.0	75.7
2	113.04	91.67	0.54	59.17	2.17	1512.3	1598.8	86.5
4	115.43	89.03	2.28	56.39	2.29	1465.8	1517.0	51.2
6	118.93	76.38	3.40	54.96	2.83	1500.3	1505.7	5.4
8	111.98	64.64	3.87	52.94	3.28	1474.0	1442.8	–31.2
10	110.88	72.21	2.67	53.23	2.89	1487.7	1413.3	–74.4
SEM	3.74	2.59	0.78	0.52	0.11	24.61	24.82	22.63
Probabilities								
Polynomial contrasts								
Linear	0.045	0.000	0.002	0.000	0.000	0.488	0.000	0.000
Quadratic	0.770	0.504	0.160	0.097	0.522	0.698	0.419	0.195
Cubic	0.164	0.000	0.86	0.021	0.000	0.840	0.338	0.403

¹ ***FCR:*** *feed conversion ratio (kg feed intake:kg egg yield)*

***Table 4.** Effects of black tea factory waste supplementation on egg quality parameters*
***Tablo 4.** Siyah çay fabrika atığının yumurta kalite kriterleri üzerine etkisi*

% BTFW	Parameter							
	Shape Index (%)	Shell Strength (kg/cm²)	Shell Thickness (mm × 10⁻²)	Shell Weight (g)	Yolk Color	Yolk Index (%)	Albumen Index (%)	Haugh Unit[1]
0	76.36	2.18	0.35	7.11	11.72	44.92	8.62	82.15
2	75.47	2.02	0.33	6.99	11.22	44.58	10.12	88.16
4	75.33	1.72	0.32	6.34	11.00	44.37	10.36	87.9
6	74.97	1.74	0.32	6.70	10.89	44.47	11.60	92.39
8	75.41	1.45	0.31	6.22	11.73	44.06	11.40	91.28
10	75.53	1.35	0.30	6.68	11.33	44.68	11.51	91.48
SEM	0.93	0.14	0.01	0.20	0.16	0.70	0.34	1.18
Probabilities								
Polynomial contrasts								
Linear	0.538	0.000	0.000	0.005	0.679	0.632	0.000	0.000
Quadratic	0.212	0.767	0.100	0.149	0.001	0.506	0.008	0.003
Cubic	0.454	0.876	0.453	0.226	0.014	0.816	0.898	0.650

[1] ***Haugh unit:*** *100 log (H + 7.57 – 1.7 $W^{0.37}$);* ***H:*** *albumen height in mm;* ***W:*** *egg weight in g*

***Table 5.** Effects of black tea factory waste supplementation on MDA values (ng/g) of egg samples stored for 14, 28, and 56 days*
***Tablo 5.** Siyah çay fabrika atığının 14, 28 ve 56 gün depolanan yumurta örneklerindeki MDA düzeylerine (ng/g) etkisi*

% BTFW	Days		
	14	28	56
0	1.81	2.25	2.53
2	1.74	1.89	2.21
4	1.72	1.84	2.38
6	1.77	1.89	2.26
8	1.21	1.82	2.23
10	1.16	1.78	2.14
SEM	0.07	0.07	0.15
Probabilities			
Polynomial contrasts			
Linear	0.000	0.000	0.125
Quadratic	0.002	0.013	0.801
Cubic	0.776	0.26	0.436

to the results of other studies conducted with hens fed green tea or green tea by-product. The present results showed that inclusion of different percentages of BTFW into the diet of laying hens affected all laying performance variables *(Table 3)*. Increasing levels of BTFW decreased feed consumption. In contrast, Uuganbayar et al.[12] previously reported increased feed intake following addition of green tea powder (0.5%, 1.0%, and 1.5%) into laying hen diets. The present study also showed that BTFW supplementation at 2% and 4% led to a trend of increasing egg production; however, inclusion of 6%, 8%, and 10% BTFW in the basal diet caused reduced egg production.

The high egg production observed in groups fed 2% and 4% BTFW could occur due to absorption of the flavonoid and catechin contents of BTFW through the intestinal wall, which may positively affect both digestive function and the egg formation process of laying hens. However, high BTFW levels would substantially increase the fiber content of the diet, potentially preventing these benefits. These findings support the results of Kojima and Yoshida [31], which showed that egg production decreased when hen diets were supplemented with 5% and 10% green tea powder, but revealed no significant differences in egg production between two layer groups that were fed diets containing 1% green tea powder or control. Yang et al.[32] reported significantly increased egg production rates in hens fed diets containing 4.0% and 6.0% green tea by-product. The incorporation of 4% BTFW into the basal diet was associated with increases in cracked egg yield and FCR, and decreases in egg weight, final body weight, and body weight change *(Table 3)*. Azeke and Ekpo [33] reported that inclusion of 1% and 2% black tea into laying hen diets had no effect on egg weight. It has also been noted that egg weight was not significantly reduced when layers were fed diets containing 0.6% green tea supplementation [34]. Uuganbayar et al.[12] reported no significant differences in egg weight between two layer groups fed diets containing 1.0% or 1.5% green tea powder; however, egg weight was significantly decreased in the layers fed a 0.5% green tea diet compared to that of the control. Xu et al.[13] found that feed conversion ratio was positively affected by laying hen diets containing 0.5%, 1.5%, and 2.5% black tea powder. The differences between the present results and those of other studies may be due to differences in the varieties or levels of tea and tea waste, and in the types of animals studied. In general, the body weight gain tended to decrease with

Table 6. *Effects of black tea factory waste supplementation on metabolic profile in hens serum*
Tablo 6. *Siyah çay fabrika atığının serum metabolik profili üzerine etkisi*

% BTFW	Response Variables[1]								
	Alb	**TG**	**Chol**	**HDL**	**TP**	**Glu**	**ALP**	**AST**	**ALT**
0	1.58	786.4	116.5	26.3	4.2	206.0	218.3	173.3	16.5
2	1.56	852.5	109.3	26.0	4.5	192.0	288.5	165.5	16.0
4	1.90	847.0	104.8	26.5	4.1	178.5	189.0	164.0	18.5
6	2.00	969.0	111.0	25.5	5.0	177.0	181.5	171.0	17.0
8	1.98	808.3	112.0	28.3	4.9	179.5	108.0	176.5	33.0
10	1.90	839.5	112.0	26.5	5.2	203.0	139.0	182.0	40.0
SEM	0.13	40.86	17.64	2.35	0.20	6.33	22.45	9.79	1.89
Probabilities									
Polynomial contrasts									
Linear	0.015	0.464	0.379	0.726	0.000	0.322	0.000	0.320	0.000
Quadratic	0.170	0.048	0.688	0.945	0.505	0.000	0.659	0.307	0.000
Cubic	0.342	0.874	0.729	0.743	0.570	0.368	0.008	0.647	0.861

[1] ***Alb:*** *albumin (mg/dL),* ***TG:*** *triglyceride (mg/dL);* ***Chol:*** *cholesterol (mg/dL),* ***HDL:*** *high-density lipoprotein (mg/dL),* ***TP:*** *total protein (g/dL),* ***Glu:*** *glucose (mg/dL);* ***ALP:*** *alkaline phosphatase (unit/L),* ***AST:*** *aspartate aminotransferase (unit/L),* ***ALT:*** *alanine aminotransferase (unit/L)*

increasing levels of BTFW. This is probably due to the high fiber content of the BTFW used in our experiment, and the high tannin levels *(Table 2)* that may interfere with protein metabolism since the tannins can presumably form tannin-protein complexes. Excessive tannin consumption is known to result in depressed growth rate, poor feed efficiency, and decreased nutrient digestibility, with the maximum tannin tolerance reportedly 1% for chickens [35]. Kondo et al.[25] reported that tea leaves are rich in nitrogen compounds, amino acids, tannins, polyphenols, and vitamins, and they observed reduced feed intake and feed digestibility in animals fed tannin-rich diets. In the present study, although BTFW added to the basal diet at 2%, 4%, 6%, 8%, and 10% did not (P>0.05) affect feed consumption, this property decreased numerically with increasing levels of BTFW.

In present study, it was observed that supplementation of the basal diet with different levels BTFW adversely affected eggshell thickness, shell weight and strength. This finding was in agreement with data from the existing literature. Uuganbayar et al.[12] found that eggshell thickness was reduced significantly (P<0.05) in layer group fed diets containing green tea powder, regardless of the percentage (0.5%, 1.0%, 1.5%, or 2.0%). Similarly, Kojima and Yoshida [31] reported that weaker and thinner egg shells were produced in layers that were fed increasing levels of green tea powder (0%, 1%, 5%, and 10%). Yang et al.[32] also found that eggshell thickness was reduced when layers were fed diets containing 2% to 6% green tea by-product supplementation. The results regarding shell strength and shell thickness in the present study supported those of Zhang and Xu [14], who found that black tea powder (0.5%, 1.5%, and 2.5% of laying hen diets) reduced shell thickness and shell strength. Decreased shell thickness and strength may lead to lower nutrient retention and nutrient availability, especially of calcium through the intestines during shell formation due to the use of BTFW containing tannin. The differences between the present results and those of other studies may be due to differences in the varieties or levels of tea and tea waste.

Dietary BTFW had significant effect on yolk color in this study. In contrast, Kojima and Yoshida [31] reported no significant differences in the yolk color fan score among groups of hens fed diets with 0%, 1%, 5%, and 10% green tea powder. However, Abdo et al.[36] found that in Inshas hens fed diets containing 1%, 3%, and 5% green tea leaves the yolk color scores increased gradually with increasing levels of green tea leaves.

In accordance with the present findings, Biswas et al.[32] reported that the Haugh unit score was improved for eggs from layers fed a diet containing 0.6% green tea. However, Uuganbayar et al.[37] found that the Haugh unit scores did not differ between eggs from layers fed diets containing 1% and 2% green tea or control diets. The general nutrients in layer feed did not appear to have any beneficial effect on Haugh unit, but it has been suggested that certain natural antioxidants, such as vitamin C, vitamin E, and selenium, may be beneficial to albumen quality due to their antioxidant properties [38]. Farhoosh et al.[39] reported that BTFW has antioxidant activities and can be used as a potent natural antioxidative source.

In this experiment, it was found that the egg yolk lipid peroxidation (measured as MDA formation in yolk) was significantly altered by the inclusion of BTFW in the basal diet after 14 and 28 days of refrigerated storage, but not after 56 days. This finding indicates that supplementation of the laying hen diets with BTFW retarded oxidation in egg yolks at least up until the 28th day of storage. It is possible that transfer of the antioxidant constituents (catechins and theaflavins) of BTFW into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk and reducing the MDA level in the yolk. Ishikawa et al.[40] reported that flavonoids from green and black tea, when added directly to isolated LDL, protect against the lipid peroxidation induced by free radicals, copper ions, and cells. These results are in accordance with those found by Biswas et al.[34] and Uuganbayar et al.[37], who reported that different levels of green tea addition to laying hen diets reduced the TBA values of egg yolk. Şahin et al.[41] also reported that 200 or 400 mg of epigallocatechin-3-gallate (EGCG), a polyphenol derived from green tea, exerted antioxidant effects and decreased the hepatic MDA level in quails.

The polyphenols found in black tea are also very strong antioxidants [3]. Leung et al .[42] reported that the theaflavins present in black tea possess at least the same antioxidant potency as catechins present in green tea, and that the conversion of catechins to theaflavins during fermentation while making black tea does not alter their free radical-scavenging activity.

Supplementation of the basal hen diet with BTFW had a significant effect on the investigated serum parameters in this study. Ahmad et al.[43] indicated that ALT, AST, and ALP in liver cells are liver function indicators, with functional and structural alterations of the liver leading to increased levels of these enzymes in circulation. All of these enzymes are intracellular and located in the mitochondria, cytoplasm, or both; when the cell's function is altered, damaged, or destroyed, the enzymes escape into the blood [44]. Tannin interferes with protein metabolism, and compromises starch digestibility and activities of pancreatic and intestinal enzymes, and consequently may adversely affect the metabolic profile [35]. Our present findings are in agreement with the results of El-Deek and Al-Harthi [45], who reported that addition of green tea at level of 0.5% into the broiler diet had no significant effect on plasma albumin, cholesterol, and AST. Similarly, Xu et al.[46] found that supplementation with fuzhuan tea (a Chinese dark tea produced by fermentation) did not affect levels of total serum cholesterol and high density lipoproteins (HDL-C) in laying hen serum. Yang et al.[17] found that addition of 0.5% and 2% green tea by-product to broiler diets tended to increase HDL levels, but not addition of 1% green tea by-product. In contrast to the present results, Abdo et al.[34] reported that inclusion of 3% and 5% green tea leaves or between 0.5 and 2.5 L/100 kg green tea extract into the diet of Inshas hens significantly decreased blood plasma cholesterol. Tea leaves destined to become black tea are rolled and allowed to ferment (oxidize), resulting in relatively high theaflavin and thearubigin concentrations and relatively low catechin concentrations [47]. Theaflavins and thearubigins account for 10% and 50-60% of total flavonoids, respectively, and the catechin content of black tea is only 20-30% [48]. The differences between the present results and those of other studies may be alter due to differences in flavonoids of the varieties of tea and tea waste used or the types of animals studied.

As a results of the present experiment supplementation of a laying hen diet with black tea factory waste at a percentage above 4% has deleterious effects on performance and egg quality traits, due to the high tannic acid content. However, supplementation of the laying hen diet with 2% and 4% black tea factory waste showed strong antioxidative activity, without having negative effects on laying performance, quality characteristics, or blood parameters. Further studies should be performed to investigate whether suitable, easy, and cheap methodologies, such as soaking, could be used to enable the incorporation of higher amounts of BTFW into laying hen diets without deleterious effects.

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