

Growing and laying performance of two different-plumage color Japanese quail varieties supplemented with corn silk in their diet

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ABSTRACT The current study aimed to investigate the dietary supplementation effect of corn silk (CS) on performance and blood chemistry of brown and white-feathered quails during the grower and layer periods. Japanese quails of brown and white-feathered color (180 birds/variety at 2 wks old) were randomly allotted into three groups with 3 replicates each (n = 20 birds/replicate). Corn silk powder (CS) was supplemented to the basal diet at 0, 1, and 2% of the diet for each quail variety for 1-month growing period, then continued for another 6-wk laying period to assess the egg production and egg quality characteristics. CS supplementation at 1% and 2% for brown and white-feathered quails respectively improved their growth performance (body weight and weight gain), carcass yield, and intestinal villi length with increasing feed consumption but without changes in feed conversion ratio. In both quail varieties, CS addition had a hypolipidemic effect, confirmed by

lowering serum triglyceride (TG), cholesterol (CHO), and low density lipoprotein (LDL) while increased high density lipoprotein (HDL) concentrations ($P < 0.05$) with a clear response observed in white quails than the brown ones. Besides, CS supplementation increased ($P = 0.002$) hen day egg production in brown feathered quails, while reducing it in the white-feathered quails compared with the CS-free diet. The increased egg production was not significantly ($P > 0.05$) correlated with lower content of TG and CHO, while significantly increased the antioxidant content in both quail varieties ($P < 0.05$). Moreover, CS dietary supplementation significantly enhanced ($P = 0.003$) the yolk color, especially in brown-feathered quail. In conclusion, CS can be safely supplemented to the Japanese quail diet (1% and 2% for brown-feathered and white-feathered quails respectively) to improve growth performance, and egg quality characteristics.

Key words: corn silk, Japanese quail, growth, egg quality, blood biochemistry, intestinal morphology

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INTRODUCTION

The worldwide need for poultry products has continuously increased over the last years (FAO, 2013). Thus, several attempts have been incessantly made to look for new strategies to improve animals' productivity. Globally, the Japanese quail is recognized as a model species

used for several reproduction studies (Maruccio et al., 2016) but also for the production of meat and eggs, due to its nutritional and physiological characteristics (Jeke et al., 2018). They are characterized by small body size, fast growth rate, and short generation interval (Elsaidy et al., 2021). Thus, they are mainly raised for solving the lack of animal protein in human food in developing countries. The genetic mapping of some genes regulating the plumage color trait in quail resulted in the identification of plumage color mutations (Minvielle et al., 2005). Accordingly, some varieties of Japanese quail, depending on the resulting plumage color, were documented such as white, dark brown, golden, yellow, rusty, lavender, and roux which were

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correlated to quail growth performance (Minvielle et al., 2007; Inci et al., 2015; Nasr et al., 2017). Therefore, Japanese quail growth features are significantly influenced by the strain type (Kumari et al., 2008). A recent increase in the production of white-feathered Japanese quails has been observed on a commercial scale, as they are preferred for the meat production (Nasr et al., 2017) owing to their higher body weights and superior feed conversion ratios (FCR) than the brown-type (Inci et al., 2015; Bagh et al., 2016; Nasr et al., 2017). Most studies concentrated on determining how plumage variants are passed down, but only a small number of efforts were made to investigate their effects on productive performance.

As a result of the growing concern over antibiotic resistance for several microorganisms resulting from sub-therapeutic usage in animals, the use of medicinal plants for animal production and human health is expanding globally (Roy Chowdhury et al., 2014; Bilal et al., 2021; Arif et al., 2022). Numerous studies have been conducted addressing different medicinal plants, herbs, and their byproducts (extracts and powders) as novel feed supplements, functional foods, and nutraceuticals in the poultry (Gong et al., 2013; Zeng et al., 2015; Ghanima et al., 2020; Jamil et al., 2022) exerting several beneficial effects such as growth and nutrient absorption enhancers, antioxidants, immune boosters, antimicrobials, antiparasitic and health-protecting agents (Abd El-Hack et al., 2020; Attia et al., 2020; Elbaz et al., 2021; Saleh et al., 2022).

Corn silk (CS) exists as thread-like material found under the husk of corn grains and is considered a waste material from the corn cultivation (Hasanudin et al., 2012). It is regarded as an agro-industrial product, inexpensive, and widely available. It is a traditional Chinese herb with numerous medicinal characteristics which has long been used as a treatment for a variety of illnesses (Hasanudin et al., 2012). The value of CS is attributed to its content of proteins, carbohydrates, minerals (Na, Ca, K, 95 Fe, Ze, and Cl) (Rahman and Wan Rosli, 2014), vitamins (E and K), flavonoids, phytosterols, volatile oils, steroids, saponin, alkaloids, and tannins (Ren et al., 2013). Several earlier studies have reported that CS has several potential applications, used as an antioxidant and in the health care (Ebrahimzadeh et al., 2008), antibacterial, anti-inflammatory, antiviral, anti-allergic (Alam, 2011), anti-diabetic properties (Zhao et al., 2012), and anti-obesity (Chaittanan et al., 2016). In broilers, feeding on untraditional CS meal with endo-1,4-D-mannanase was associated with improved growth and immune response along with enhancing the expression of growth regulating genes (Kirrella et al., 2021). In Nile tilapia, CS showed has a potential role as an antioxidant and immunostimulant which was mediated by enhancing the phagocytosis and lysozyme level and lowering the MDA level in the CS-fed tilapia (Catap et al., 2015). In rabbits, CS methanolic extract administration resulted in lowering total cholesterol and triglyceride concentrations (Ozurouke et al., 2016). Until now, CS has not received much attention as a

natural supplement or unconventional feed ingredient in the diet of quail and it is hypothesized that the dietary inclusion of CS is expected to exert beneficial impacts on the growing and laying quail.

Therefore, the present feeding trial aimed to investigate the effect of CS dietary supplementation in Japanese quail and whether the response differs depending on the quail's varieties. So, two varieties of Japanese quail (Brown-feathered & White-feathered) were used in this study. This objective was assessed by evaluating their growth performance, blood biochemistry, and intestinal morphology during the growing period as well as the egg production and egg quality parameters during the laying period.

MATERIALS AND METHODS

Bird management procedures followed the regulations of the Animal Care and Ethics Committee at the Faculty of Agriculture, Kafrelsheikh University, Egypt.

Quail husbandry and Experimental Design

This study depended on mixed-sex, one-day-old Japanese quail chicks of 2 plumage-associated colors (brown-feathered and white-feathered) which were obtained from the poultry extension unit, faculty of agriculture, Kafrelsheikh University, Egypt. Chicks were kept together for two weeks adaptation period for controlling the survival rate of chicks (Elsaidy et al., 2021), during which chicks received the basal diet (BD). At 2 wk old, quails were individually weighed (average initial body weight $82.79 \text{ g} \pm 0.95$) and then randomly assigned into 3 groups for each variety with 3 replicates each. For each quail variety, 180 birds were allocated to 3 experimental treatments (60 birds/group). The first group (G1) was considered as control with 0.0% CS while G2 and G3 received the BD supplemented with 1 and 2% CS respectively. There were three replicates of each group, each with 20 birds. The temperature was 33 to 34°C at the start of the experiment (during the brooding stage), and it gradually dropped to 22 to 25°C at the 21 d of age, which is the normal rearing temperature.

Quail chicks were raised on a pen floor system during the growing period (continued 4 wk), then shifted to conventional cages (90 × 40 × 40 cm) during the laying period (started at 5 wk old and continued for 6 successive weeks). All birds were kept under the same management conditions, with free access to the water and feed throughout the experiment. To fulfill the nutrient requirement of quails, basal diets utilized during the growing and laying period were formulated according to the international standards (NRC, 1994). Table 1 illustrates the BD's ingredient composition.

The fresh corn silk was collected from planted corn and shade-dried for 5 d in accordance with the procedure described by (Kirrella et al., 2021) after that the dried CS was ground into powder and stored in plastic bags at 4°C until needed. The chemical composition of CS was

Table 1. Ingredient composition of the used basal diet.

Ingredients (%)	Growing diet	Laying diet
Yellow corn	51.27	57.30
Corn Gluten Meal	6.90	4.00
Soybean meal (47%)	36.00	28.30
Soybean Oil	2.00	1.50
DCP ¹	1.40	1.70
Limestone ²	1.60	6.40
Premix ³	0.30	0.00
Premix ⁴	0.00	0.20
Common Salt	0.30	0.25
DL-methionine ⁵	0.10	0.20
Lysine HCl ⁶	0.03	0.05
Choline chloride	0.05	0.05
Mycotoxin adsorbent	0.05	0.05
Calculated Composition (%)		
Crude protein	23.77	19.11
Calcium	0.97	2.70
Available Phosphorus	0.31	0.31
Lysine	1.26	1.04
Methionine	0.51	0.54
ME (Kcal/kg) ⁷	2981.50	2865.30

¹DCP = Dicalcium phosphate (17% Phosphorus and 21% Calcium).

²Limestone (contain 35 % calcium).

³Growing Premix: each 3 kg vitamin and mineral mixture contain: vitamin A 12,000,000 IU; vitamin D3 2,500,000 IU; vitamin E 10,000 mg; vitamin K3 2000 mg; vitamin B11000 mg; vitamin B2 5,000 mg; vitamin B6 1,500 mg; vitamin B12 10 mg; niacin 30,000 mg; biotin 50 mg; folic acid 1,000 mg; pantothenic acid 10,000 mg; manganese 60,000 mg; zinc 50,000 mg; iron 30,000 mg; copper 4,000 mg; iodine 300 mg; selenium 100 mg; and cobalt 100 mg).

⁴Laying Premix added (0.1% vitamin premix + 0.1% mineral premix produced by Devenish nutrition Company, UK; each containing 1kg of vitamin premix composed of: Vit A (24000000 IU), Vit D (8000000IU), Vit E (80,000 mg), Vit K₃ (6,000 mg), Vit B₁ (4,000 mg), Vit B₂ (12,000 mg), vitB₆ (10,000 mg), Vit B₁₂ (10gm), nicotinic acid (90,000 mg), pantothenic acid (24,000 mg), folic acid (3,000 mg), biotin (200 mg), BHA/BHT% (10,000 mg), and carrier (calcium carbonate) up to 1 kg; each 1 kg contains of mineral premix contains: iron (60,000 mg), copper (10,000 mg), zinc (120,000 mg), manganese (20,000 mg), iodine (1,000 mg), selenium (400 mg), cobalt (200 mg) and carrier up (calcium carbonate) to 1kg.

⁵DL-Methionine (Produced by Evonik Co. and containing 99% methionine)

⁶Lysine = lysine hydrochloride (containing 98% Lysine).

⁷Calculated composition according to NRC (NRC, 1994).

previously analyzed by Kirrella et al. (2021) and found to include 95.9% dry matter, 12.51% crude protein, 9.5% crude fiber, 2,550 Kcal/Kg metabolizable energy (ME), 0.31% Ca, 0.24% available phosphorus, 0.62% digestible lysine and 0.23% digestible methionine. Birds received vaccination against the Newcastle disease virus on d 25th of age.

Growth Performance and Carcass Traits

The growth performance of quail in each variety was assessed by recording the quail body weight (BW) and feed intake (FI) weekly. Based on BW and FI, the body weight gain (WG) and feed conversion ratio (FCR, g feed/g gain) were calculated. At the end of the growing period (42nd days of bird age), 15 male quail/group (n = 5/replicate) were randomly selected and used for sample collection. Collected birds were weighed and slaughtered after fasting for 6 hr. Birds were then eviscerated and the carcass weight was recorded. The carcass yield was calculated as follows; carcass weight (g)/live body weight (g) × 100. Internal organs (heart, liver, and gizzard) and abdominal fat weights were also recorded.

Blood Biochemical Parameters

Blood samples were collected from birds (at the end of the growing and laying period) in clean vials for serum separation. Coagulated blood was centrifugated at 3,000 rpm for 10 min. Serum samples were stored at -20°C for subsequent analysis. Blood biochemical parameters measured at the end of the growing period included: total protein, albumin, lipid profile (cholesterol, triglycerides, low and high-density lipoprotein (LDL and HDL), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), and creatinine. Following the manufacturer's instructions, these parameters were determined by spectrophotometer using standard commercial kits (Biodiagnostic Co, Egypt). Only the serum lipid profile parameters were examined at the end of the laying phase.

Intestinal Morphology

Duodenum of the slaughtered birds was collected (n = 15 male quail/group) for morphological examination. Approximately 2 cm of the duodenum was excised, washed with physiological saline, and fixed in formalin 10% for 24 hr. Slides were prepared and stained with hematoxylin and eosin (H&E) and examined under a light microscope Bancroft et al. (2013). The morphometric study was assessed by examination of villus length, width, and crypt depth. The measurements were done using a computerized image analysis system (Image J software, Bethesda, MD).

Egg Production and egg Quality Parameters

For 6 wk laying period, 27 females/group (9 females/replicate) were assessed for their productive performance in terms of egg production and egg quality characteristics. Eggs were collected every day and the hen day egg production % (HDEP) was calculated by applying the following equation (number of eggs produced per treatment/number of laying females on that day × 100). Thirty eggs/group were collected and weighed individually using a digital scale. At the end of the laying period, egg quality parameters, including external egg quality (shell relative weight and thickness, egg shape index) and internal egg quality [yolk and albumen relative weight, yolk, and albumen index, Haugh unit (HU); and yolk color], were determined. Egg length and width were measured using an electronic digital caliper.

Egg shape index (ESI) was calculated = (egg width/egg length) × 100. Each egg was broken in a petri dish and albumen and yolk heights were determined using a micrometer. Each eggshell was air-dried and then weighed. Eggshell thickness was estimated by determining the thickness mean values taken at 3 locations on the egg (air cell, equator, and sharp end) utilizing a (Mitutoyo, 0.01–20 mm, Tokyo, Japan). The egg yolks were separated from the albumen and each one was separately weighed and calculated relative to egg weight. Albumen index (%) = Albumen height, mm/(Albumen

length, mm + Albumen width, mm²) × 100. Yolk index (%) = (yolk height/yolk diameter) × 100. Haugh unit (HU) was calculated using the egg weight and albumen height. The yolk color score was measured utilizing the Roche yolk color fan method (DSM Yolk Color Fan, Basel, Switzerland). Egg yolk samples were analyzed for total cholesterol content, triglyceride content, and total antioxidant activity (Rotenberg and Christensen, 1976). Additionally, the ovarian follicle number was counted. The follicles were grouped according to the size of the follicle then follicles of nearly the same size were counted.

Statistical Analysis

The obtained results were analyzed by the SPSS package (IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM). Two-way ANOVA was performed to investigate the effect of corn silk supplementation, quail variety, and the interaction between them, followed by Tukey's multiple comparison test. Data were considered significantly different at $P < 0.05$. The results were shown as the mean ± standard error of the mean (SEM) and the figures were created using GraphPad Prism 6 (GraphPrism Software, La Jolla, CA).

RESULTS

Quail Growth Performance and Carcass Traits

Table 2 shows the effect of CS supplementation, quail variety, and their effect on quail growth performance. These factors significantly modified quail growth performance ($P < 0.05$). In brown feathered quail, 1% CS supplementation significantly increased the final BW and WG when compared with the control or the high level of CS (2%). On the other side, the white-feathered quail showed the highest BW and BG when they were supplemented with 2% of CS (2%) compared with their reference group or the 1% CS-supplemented group. Regarding the amount of feed consumed, it was significantly affected by CS supplementation and the interaction with quail variety ($P < 0.05$). FI showed a significant increase ($P < 0.05$) in brown-feathered birds that received 1% CS while was reduced with a higher level of CS (2%). Conversely, the highest feed consumed was observed in the 2% CS-fed white-feathered ($P < 0.05$). Based on the obtained WG and feed consumed, FCR was slightly improved in the CS-fed groups in brown feathered quail while remaining unaffected in the CS-supplemented, white-feathered quail compared with the control. The effect of CS supplementation on improving growth performance was reflected in carcass yield. The carcass yield was significantly altered by CS supplementation and quail variety ($P < 0.05$), while the interaction between these factors had no effect. The highest carcass yield was found in the case of 1% CS-

Table 2. Effect of corn silk (CS) dietary supplementation on growth performance of two different varieties of Japanese quail (Brown-feathered & White-feathered).

Groups	Initial Wt. (2nd-week wt.) (g)	Final Wt. (6th week Wt.) (g)	Total gain (g)	Total FI (g/ bird)	Average final FCR ¹	Carcass yield (%)	Liver Wt. (g)	Heart Wt. (g)	Gizzard Wt. (g)	Abdominal fat (g)
Brown-feathered										
Control	81.50 ± 3.19	236.07 ± 8.60 ^{aA}	154.57 ± 7.60 ^{aA}	649.19 ± 49.22 ^{aA}	4.20 ± 0.139	69.77 ± 1.76 ^{ab}	3.52 ± 0.26	2.16 ± 0.09	4.32 ± 0.13	2.51 ± 0.36
1% corn silk	78.25 ± 4.21	277.92 ± 10.08 ^{aA}	199.64 ± 8.74 ^{aA}	776.60 ± 18.48 ^{aA}	3.89 ± 0.212	79.80 ± 1.64 ^{aA}	3.63 ± 0.18	2.07 ± 0.13	3.53 ± 0.26	2.62 ± 0.60
2% corn silk	79.75 ± 3.98	242.50 ± 10.45 ^{bA}	162.75 ± 10.07 ^{ab}	647.75 ± 3.74 ^{ab}	3.98 ± 0.177	60.63 ± 3.06 ^{bB}	3.46 ± 0.17	2.11 ± 0.24	3.93 ± 0.24	2.04 ± 0.15
White-feathered										
Control	86.00 ± 3.28	242.73 ± 15.09 ^{abA}	156.73 ± 6.00 ^{abA}	620.65 ± 28.41 ^{aA}	3.96 ± 0.162	67.71 ± 2.30 ^{ab}	3.44 ± 0.33	2.28 ± 0.11	4.56 ± 0.59	2.76 ± 0.44
1% corn silk	87.25 ± 2.93	228.85 ± 7.64 ^{ab}	141.60 ± 7.04 ^b	579.14 ± 7.79 ^{ab}	4.09 ± 0.86	66.64 ± 3.31 ^{ab}	3.48 ± 0.38	1.98 ± 0.07	4.14 ± 0.25	2.53 ± 0.56
2% corn silk	84.01 ± 4.25	261.92 ± 8.87 ^{aA}	177.91 ± 6.02 ^{aA}	704.52 ± 1.44 ^{aA}	3.96 ± 1.62	80.31 ± 2.81 ^{aA}	3.71 ± 0.26	2.07 ± 0.08	4.11 ± 0.29	3.35 ± 0.51
Two-Way ANOVA										
<i>P</i> -values										
Corn silk	0.582	0.041	0.082	0.004	0.603	0.002	0.953	0.625	0.849	0.352
Quail variety	0.231	0.022	0.015	0.375	0.969	0.013	0.980	0.963	0.250	0.221
Interaction	0.412	0.003	0.004	0.007	0.513	0.523	0.712	0.366	0.225	0.536

Data are presented as means ± SEM (n = 20 quail of mixed sex/ replicate for growth parameters; n = 5 male quail/replicate for carcass traits). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

¹FCR = total FI/Total gain.

Table 3. Effect of corn silk (CS) dietary supplementation on some serum biochemical parameters of two different varieties of Japanese quail (Brown-feathered & White-feathered).

Group	Total protein gm/dl	Albumin gm/dl	Globulin ¹ gm/dl	AST ² U/L	ALT ³ U/L	Creatinine mg/dl
Brown-feathered						
Control	2.83 ± 0.33	1.57 ± 0.03 ^{ab}	1.27 ± 0.32	253.33 ± 4.91 ^{abA}	53.00 ± 6.25	0.52 ± 0.02 ^a
1% CS	2.67 ± 0.09	1.63 ± 0.03 ^{ab}	1.03 ± 0.07	241.33 ± 6.69 ^{bAB}	48.00 ± 5.51	0.47 ± 0.01 ^a
2% CS	2.70 ± 0.06	1.73 ± 0.09 ^{ab}	0.97 ± 0.07	263.67 ± 4.08 ^{aA}	51.00 ± 4.93	0.37 ± 0.09 ^a
White-feathered						
Control	2.70 ± 0.23	1.70 ± 0.06 ^{bB}	1.00 ± 0.29	236.33 ± 8.83 ^{abB}	48.66 ± 5.78	0.47 ± 0.05 ^a
1% CS	2.80 ± 0.21	2.00 ± 0.06 ^{aA}	0.80 ± 0.15	254.00 ± 7.00 ^{aA}	52.00 ± 2.08	0.53 ± 0.03 ^a
2% CS	2.80 ± 0.06	1.90 ± 0.12 ^{aAB}	0.90 ± 0.06	237.33 ± 4.67 ^{abB}	53.67 ± 5.21	0.24 ± 0.01 ^b
Two-Way ANOVA <i>P</i> values						
Corn silk	0.985	0.002	0.250	0.679	0.900	0.001
Quail variety	0.836	0.035	0.475	0.072	0.856	0.253
Interaction	0.757	0.224	0.857	0.024	0.692	0.148

Data are presented as means ± SEM (n = 5 male quail/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

¹Globulin = total protein-albumin.

²AST = Aspartate Aminotransferase.

³ALT = alanine aminotransferase.

supplemented brown-feathered and 2% CS-supplemented white-feathered quails compared to their control groups ($P < 0.05$). However, CS supplementation did not change the internal organs and abdominal fat weights.

Serum Biochemical Changes

Table 3 illustrates the serum biochemical changes of the growing brown and white Japanese quail in response to CS supplementation. Total protein and globulin did not change significantly however globulin concentration was numerically reduced in CS-supplemented birds. Serum albumin level was significantly modified by CS addition and the quail variety ($P < 0.05$). In both quail varieties, CS dietary inclusion stimulated a significant increase in albumin concentration compared to control groups fed on CS-free diets. Compared to brown feathered quail fed on 1% CS, the same group in white feathered quail recorded higher albumin contents ($P < 0.05$). Additionally, creatinine serum concentration was reduced in both varieties with CS supplementation with

the highest level of CS (2%). The liver function enzyme (AST) was significantly altered by the interaction occurring between the two factors (CS addition and quail variety) ($P < 0.05$), while ALT concentration showed no difference among treatments. In brown feathered quail, 1% CS added to the diet non-significantly reduced the serum AST, while nonsignificantly increased it compared to the control group. In the white-feathered quail, an opposite result was obtained as the supplemental CS (1%) increased AST concentrations compared to their control or the highest level of CS (2%).

Regarding the serum lipid profile presented in Table 4, CHO concentration was significantly modulated by CS supplementation ($P < 0.05$). A significant reduction of serum CHO was observed ($P < 0.05$) in the CS-supplemented, white-feathered birds compared with those fed a CS-free diet. In the same direction, CS supplementation induced a significant reduction in serum TG of the white-feathered quail compared with their reference birds ($P < 0.05$). Serum TG concentration was lower in white-feathered fed on BD supplemented with CS compared with their corresponding, brown-feathered ones.

Table 4. Effect of corn silk (CS) dietary supplementation on serum lipid profile of two different varieties of Japanese quail (Brown-feathered & White-feathered).

Group	Cholesterol mg/dl	Triglyceride mg/dl	HDL ¹ mg/dl	LDL ² mg/dl
Brown-feathered				
Control	188.33 ± 8.41 ^a	125.67 ± 2.31 ^{ab}	39.29 ± 4.19 ^{bB}	95.64 ± 2.89 ^a
1% Corn Silk	185.00 ± 7.81 ^a	124.77 ± 3.54 ^{ab}	57.65 ± 4.79 ^{aAB}	78.44 ± 8.28 ^{ab}
2% Corn Silk	179.00 ± 6.03 ^a	122.27 ± 3.43 ^{ab}	53.30 ± 2.24 ^{aAB}	68.52 ± 8.05 ^b
White-feathered				
Control	190.00 ± 2.89 ^a	161.77 ± 3.50 ^{aA}	43.72 ± 4.50 ^{bB}	98.09 ± 5.05 ^a
1% Corn Silk	174.67 ± 8.74 ^a	104.17 ± 3.11 ^{bC}	65.83 ± 6.71 ^{aA}	73.51 ± 5.90 ^b
2% Corn Silk	152.33 ± 6.96 ^b	111.53 ± 5.82 ^{bB}	66.37 ± 3.29 ^{aA}	88.03 ± 4.59 ^{ab}
Two-Way ANOVA <i>P</i> values				
Corn silk	0.019	<0.0001	0.001	0.009
Quail variety	0.065	0.616	0.038	0.276
Interaction	0.176	<0.0001	0.640	0.104

Data are presented as means ± SEM (n = 5 male quail/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

¹HDL = high-density lipoprotein

²LDL = low-density lipoprotein.

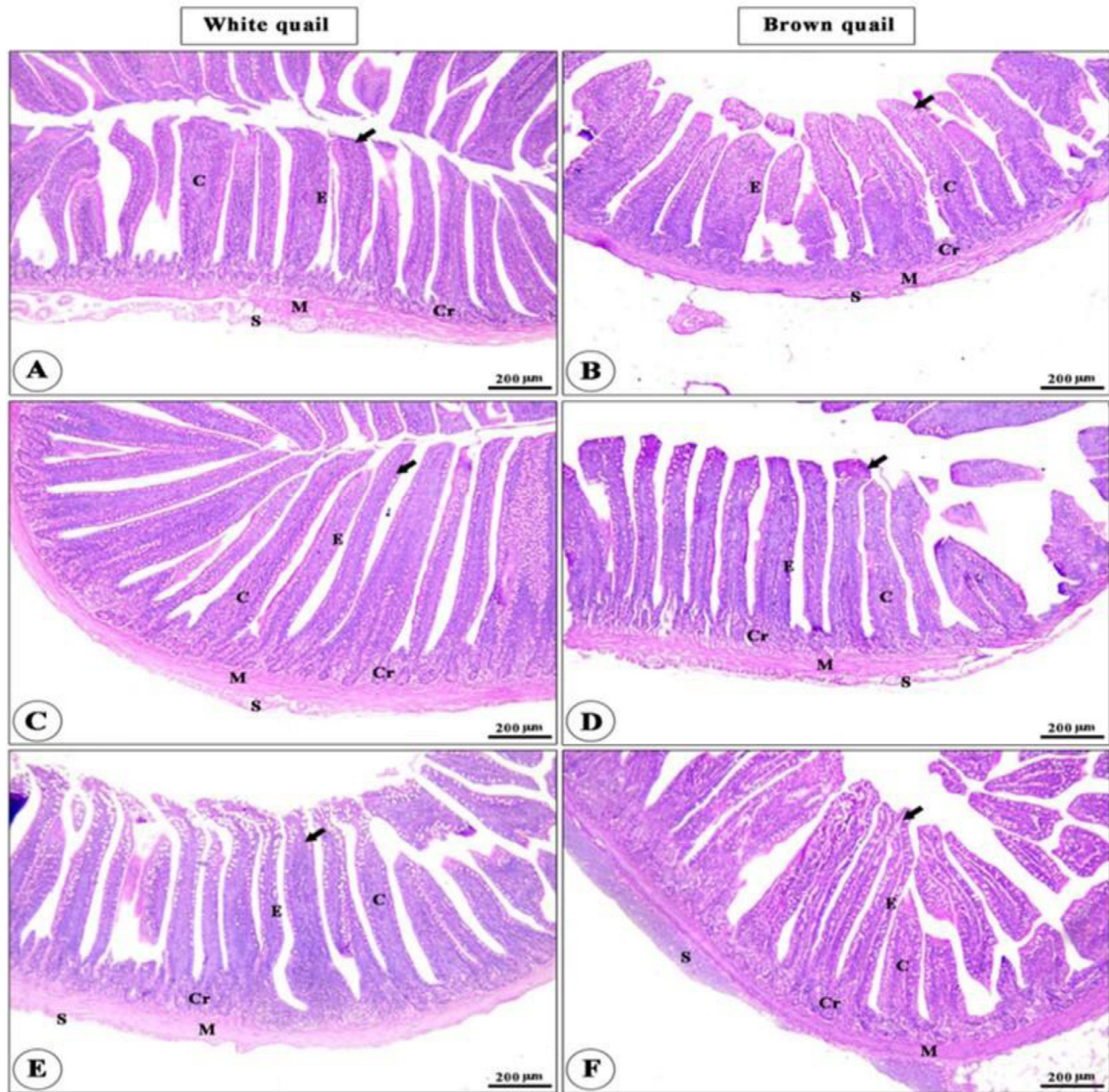


Figure 1. Photomicrographs of males' duodenum of control groups of white (A) and brown (B)-feathered quails supplemented with CS. Arrow showed intestinal villi, (E) indicates columnar absorptive cells and goblet cells in between. Cr represents intestinal crypt = crypt of Lieberkuhn = intestinal glands in the lamina propria, M = tunica muscularis, and S = tunica serosa. C and D represent treatments supplemented with 1% CS in white and brown-feathered quails. E and F are for treatments supplemented with 2% CS in white and brown-feathered quails.

Both CHO and TG were reduced in brown feathered quails. Besides, CS addition stimulated a lower LDL concentration in both quail varieties ($P < 0.05$). HDL serum concentration responded differently as it showed a significant increase with CS addition ($P < 0.05$). HDL concentration was higher in white-feathered quail compared with their comparable, brown-feathered ones.

Intestinal Morphology

Figures 1 and 2 represent quail duodenum morphology in response to CS supplementation in brown and white-feathered Japanese quail varieties at the end of the growing period. The control groups of both white- (Figure 1A) and brown- (Figure 1B) feathered quails showed normal intestinal villi (arrow), covered with

columnar absorptive cells and goblet cells in between (E), normal intestinal glands in the lamina propria (Cr), normal tunica muscularis (M) and tunica serosa (S). Intestinal glands were deep in brown quail than that in white-feathered quail. White and brown-feathered quails supplemented with 1% CS (C and D, respectively) showed a moderate increase of intestinal villi compared with control groups with a slight increase of villi in brown than white -feathered quails. Additionally, 2% CS-supplemented quails in the two studied varieties exhibited a moderate increase of intestinal villi compared with control groups (E and F). In Figure 2, in brown-feathered quail, dietary addition of 1 and 2% CS significantly improved ($P < 0.05$) the intestinal morphology in terms of intestinal villi length, width, and crypt depth when compared with quail raised on the CS-free diet. On the other hand, using 1% of CS in the

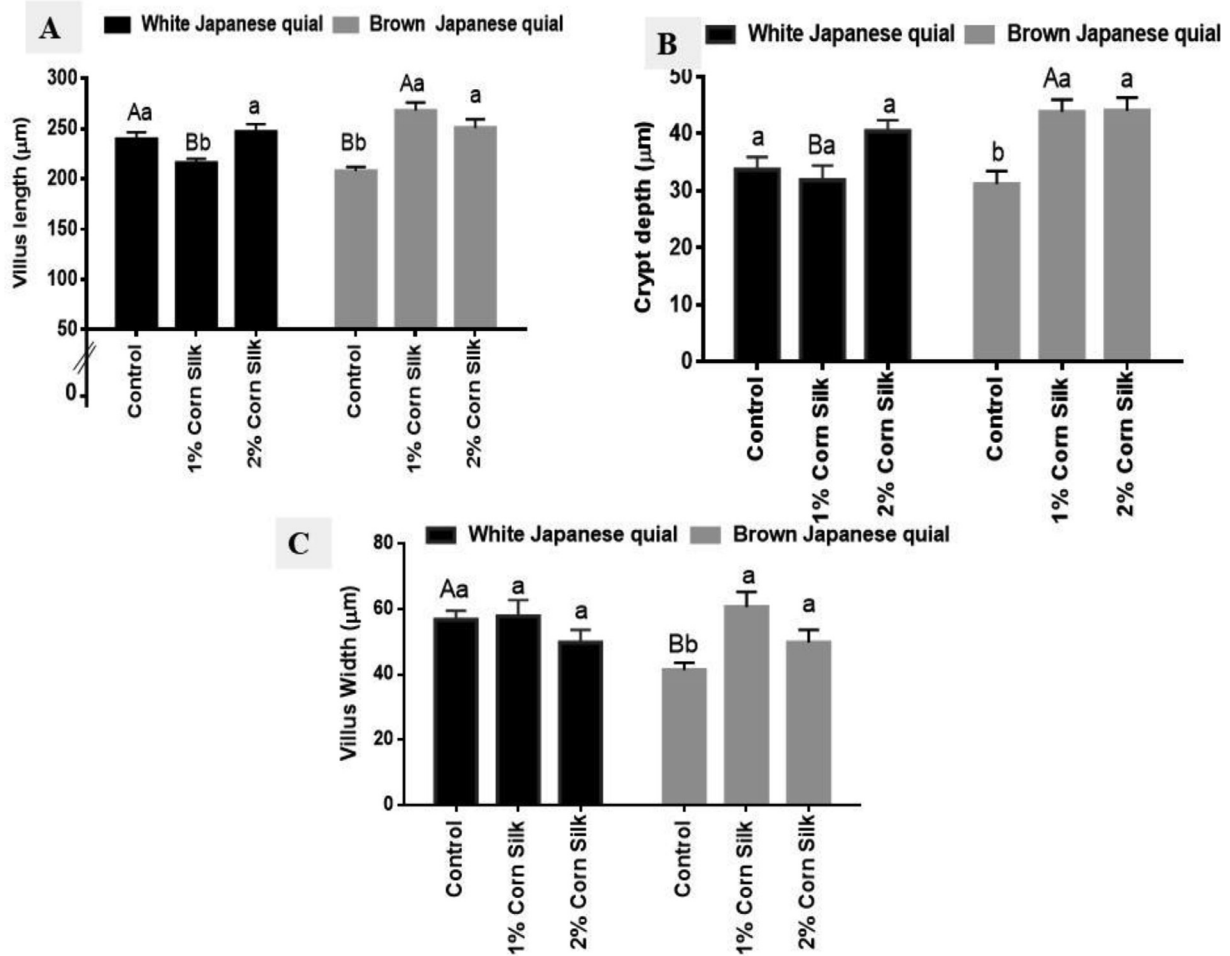


Figure 2. Effect of corn silk dietary supplementation on duodenum morphology [villus length (A), crypt depth (B), and villi width (C)] in 2 different varieties of Japanese quail (Brown-feathered & White-feathered) (n = 5 birds/replicate). Results are expressed as mean ± SEM. Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

white-feathered quail diet significantly reduced ($P < 0.05$) the villi length compared to the control group while the 2% of CS supplementation did change it. Also, villi width or crypt depth showed no changes in response to CS supplementation.

Egg Production and egg Quality

The HDEP of the two varieties of Japanese quail (Brown-feathered & White-feathered) is shown in Table 5 and Figure 3. CS supplementation increased the

Table 5. Effect of corn silk (CS) dietary supplementation on hen-day egg production (HDEP) of two different varieties of Japanese quail (Brown-feathered & White-feathered).

Group	5 th wk	7 th wk	8 th wk	9 th wk	10 th wk	11 th wk
Brown-feathered						
Control	30.56 ± 5.96 ^b	40.33 ± 6.56	65.71 ± 5.28 ^b	78.57 ± 6.70 ^{bA}	62.86 ± 8.92	52.00 ± 3.74 ^{bB}
1% corn silk	33.33 ± 5.69 ^b	54.94 ± 5.41	69.66 ± 4.38 ^a	85.94 ± 4.18 ^{aA}	78.72 ± 5.25	72.18 ± 4.64 ^{aA}
2% corn silk	50.04 ± 8.04 ^a	59.18 ± 3.73	66.07 ± 6.52 ^b	70.64 ± 3.72 ^{bA}	75.92 ± 3.86	75.00 ± 5.59 ^{aA}
White-feathered						
Control	36.67 ± 4.64 ^a	55.10 ± 7.90	65.51 ± 8.19 ^a	77.31 ± 7.54 ^{bA}	73.39 ± 4.86	68.57 ± 5.34 ^{aA}
1% corn silk	27.04 ± 4.02 ^a	46.03 ± 6.15	51.04 ± 4.90 ^b	81.57 ± 9.28 ^{aA}	69.42 ± 2.04	51.43 ± 9.69 ^{bB}
2% corn silk	30.59 ^A ± 3.42 ^a	42.78 ± 6.28	46.33 ± 8.07 ^c	61.59 ± 5.10 ^{bB}	72.26 ± 7.25	53.33 ± 4.16 ^{bB}
Two-Way ANOVA P-values						
Corn silk	0.016	0.129	0.014	0.049	0.616	0.002
Quail variety	0.578	0.090	0.864	0.003	0.721	0.036
interaction	0.406	0.124	0.348	0.666	0.421	0.751

Data are presented as means ± SEM (n = 9 females/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

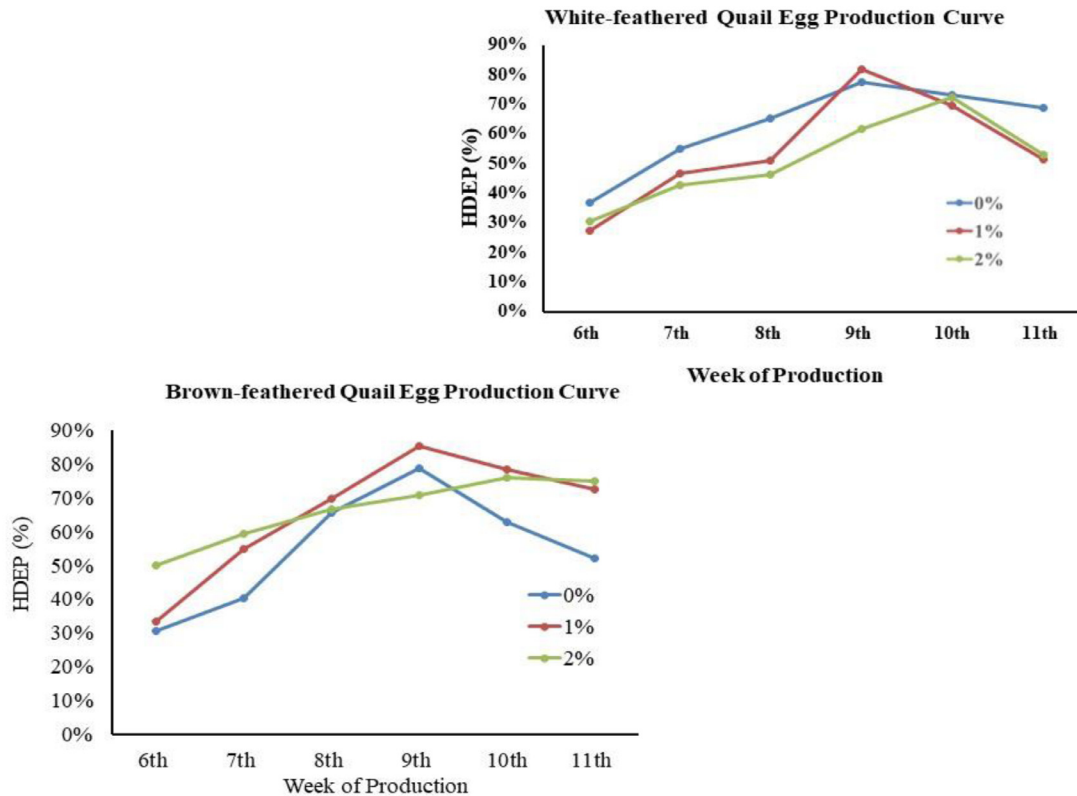


Figure 3. Egg production curve in the two different varieties of Japanese quail (Brown-feathered & White-feathered) supplemented with corn silk.

HDEP in brown-feathered quail while reducing it in the white-feathered quail compared with the CS-free diet. CS addition at 1% of the diet significantly increased ($P = 0.014$ and 0.049) the HDEP % in weeks 8th and 9th of production in both quail varieties compared with the higher level of CS (2%). Increasing the CS level to 2% of the diet non-significantly increased the HDEP % in white feathered quail during the last 2 wk of production. Regardless of the CS supplementation, quail variety affected the HDEP at the weeks 9th and 11th of production as it was higher in the brown-feathered quail than in the white-feathered quail.

As shown in Table 6, neither CS supplementation nor quail variety or their interaction affected the external egg quality (eggshell relative weight, egg shape index, and shell thickness) except for the relative shell weight which was influenced by quail variety. The relative eggshell weight of brown feathered quail that received the BD diet supplemented with 2% CS was significantly increased ($P < 0.05$) compared with the white-feathered quail that received the same level of CS. Similarly, the internal egg quality parameters in terms of yolk and albumin relative weight, yolk and albumen index, and Haugh units were not affected by the CS supplementation in the 2 studied quail varieties. However, yolk relative weight was higher in white feathered quail than the brown quail which was clear in the 2% CS supplemented birds. On the other hand, the addition of CS significantly increased ($P < 0.05$) the yolk color of the brown-feathered quail compared with the reference group. Analyzing the total antioxidant content of the egg, it was

significantly modified with CS dietary addition, quail variety, and the interaction between these factors ($P < 0.05$) (Figure 4). Compared with the control, egg antioxidant content showed a significant increase in the 2 CS-supplemented groups ($P < 0.05$) in both varieties. Regardless of the quail variety, the triglyceride and cholesterol content of eggs was not significantly reduced following CS dietary inclusion compared to the control. Furthermore, ovarian follicle numbers counted in the 2 varieties are shown in Table 7. The number of large yellow follicles (>10 mm) was increased in the brown feathered quail than in the white quail. Moreover, CS addition in the diet of both quail varieties significantly increased the number of the medium white follicles (1–3 mm) and the postovulatory follicles ($P < 0.05$) compared with their control group fed on the BD without CS. Compared to the white feathered quail, the number of the postovulatory follicles was higher in the brown feathered quail ($P < 0.05$). On the other hand, the small yellow follicle (5–10 mm) and large white follicle (3–5 mm) showed no differences among groups of both varieties.

Serum Lipid Profile in Quail Layers

Table 8 illustrates the serum lipid profile of Japanese quail layers. Both cholesterol and triglycerides were significantly altered by quail variety and CS supplementation ($P < 0.05$) as well as their interaction which affected triglyceride. These parameters were reduced in the CS-supplemented birds (brown or white feathered)

Table 6. Effect of corn silk (CS) dietary supplementation on egg quality of two different varieties of Japanese quail (Brown-feathered & White-feathered).

Groups	External egg quality				Internal egg quality				Yolk color
	Shell relative wt.	Egg shape index (ESI)	Shell thickness (μ)	Yolk relative wt.	Albumin relative wt.	Albumin index	Yolk index	Haugh unit	
Brown-feathered									
Control	26.44 ± 1.82	79.47 ± 0.81	231.33 ± 17.32	32.81 ± 1.09	40.73 ± 1.62	13.65 ± 0.64	49.59 ^A ± 0.79	94.40 ± 1.14	4.13 ^b ± 0.09
1% CS	22.91 ± 0.91	76.75 ± 0.85	213.33 ± 3.47	34.71 ± 1.56	42.36 ± 1.52	15.18 ± 0.77	49.54 ± 0.55	96.31 ± 1.22	4.80 ^a ± 0.11
2% CS	26.37 ^A ± 1.05	76.59 ± 1.12	202.00 ± 2.94	32.94 ^B ± 0.86	40.68 ± 0.98	15.19 ± 1.17	48.19 ± 0.92	98.81 ± 1.93	4.80 ^a ± 0.25
White-feathered									
Control	24.09 ± 1.16	74.75 ± 2.14	202.00 ± 6.29	34.68 ± 1.1	41.22 ± 1.41	13.13 ± 0.79	45.91 ^B ± 1.39	97.78 ± 1.14	4.30 ± 0.15
1% CS	20.63 ± 1.83	76.30 ± 0.65	197.00 ± 6.33	37.43 ± 1.79	43.27 ± 1.44	13.55 ± 0.76	48.60 ± 1.02	98.65 ± 1.57	4.30 ± 0.15
2% CS	21.43 ^B ± 1.81	76.66 ± 0.58	209.00 ± 5.47	36.65 ^A ± 1.40	41.90 ± 1.74	14.43 ± 0.49	47.02 ± 1.31	97.25 ± 0.75	4.70 ± 0.15
Two-Way ANOVA <i>P</i> values									
Corn silk	0.090	0.063	0.223	0.205	0.442	0.201	0.255	0.192	0.003
Quail variety	0.014	0.065	0.129	0.009	0.467	0.144	0.019	0.225	0.240
interaction	0.613	0.844	0.435	0.755	0.988	0.756	0.292	0.353	0.075

Data is presented as means ± SEM (n = 30 eggs, 10 eggs/replicate). Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

with a marked effect observed with 2% CS compared with control groups fed on the CS-free birds. The serum content of TG was lower in white feathered quail than brown quail, especially in groups supplemented with 2% CS ($P < 0.05$). Both HDL and LDL concentrations responded to CS dietary inclusion ($P < 0.05$). HDL showed a higher concentration ($P < 0.05$) with CS addition in both quail strains compared with their reference group. On the contrary, serum LDL was reduced in CS-supplemented birds with the lowered concentration observed with 2 % CS ($P < 0.05$).

DISCUSSION

To our knowledge, no literature reported the effects of dietary CS supplementation in Japanese quail with different plumage colors. The current study showed enhanced growth performance (BW and WG) in brown-feathered and white-feathered quails that received 1% CS and 2% CS, respectively. This improved performance with dietary CS addition could be associated with the nutritional value of the CS which is related to its high nutrients contents of protein, carbohydrates, minerals, and vitamins (Hasanudin et al., 2012) as well as the improved intestinal villi length in the duodenum which helps in enhancing birds' growth. Moreover, findings supported the present findings but in broiler chickens, they confirmed the improved growth performance with CS dietary inclusion at 4% and 8% (Kirrella et al., 2021). Additionally, the CS content of bioactive compounds (volatile oils, flavonoids, polyphenols, and organic acids) could have a role in improving birds' growth as it exerts several beneficial effects on the health (Hasanudin et al., 2012; Mesalam et al., 2021; Alagawany et al., 2022; Arain et al., 2022). Recently, Boeira et al. (2022) reported that corn stigma can be considered a source of bioactive metabolites with antioxidant and antimicrobial activities and can be used as a natural antioxidant ingredient (El-Fakhrany et al., 2021; El-Hindawy et al., 2021).

Despite the increased quail's WG with CS inclusion in both varieties (1% in brown feathered and 2% in white feathered quail), there were increased amounts of feed consumed which was reflected in no significant difference between groups in terms of FCR. This effect might be explained by the absence of the regulatory role of CS on FI regulating genes such as leptin, and CCK (Kirrella et al., 2021). Variation in the obtained response could be associated with the breed difference, experimental design in each trial, chemical composition, and ingredients of the diets used. Further studies are required to address the effect of CS supplementation on the expression of growth and FI regulating genes in Japanese quail to deeply understand its regulating role on quail's growth performance. Furthermore, different feather colors of quails used in this study participated in modifying the growth performance in terms of BW and WG and consequently the carcass yield obtained. Likewise, Inci et al. (2015) reported that different feather

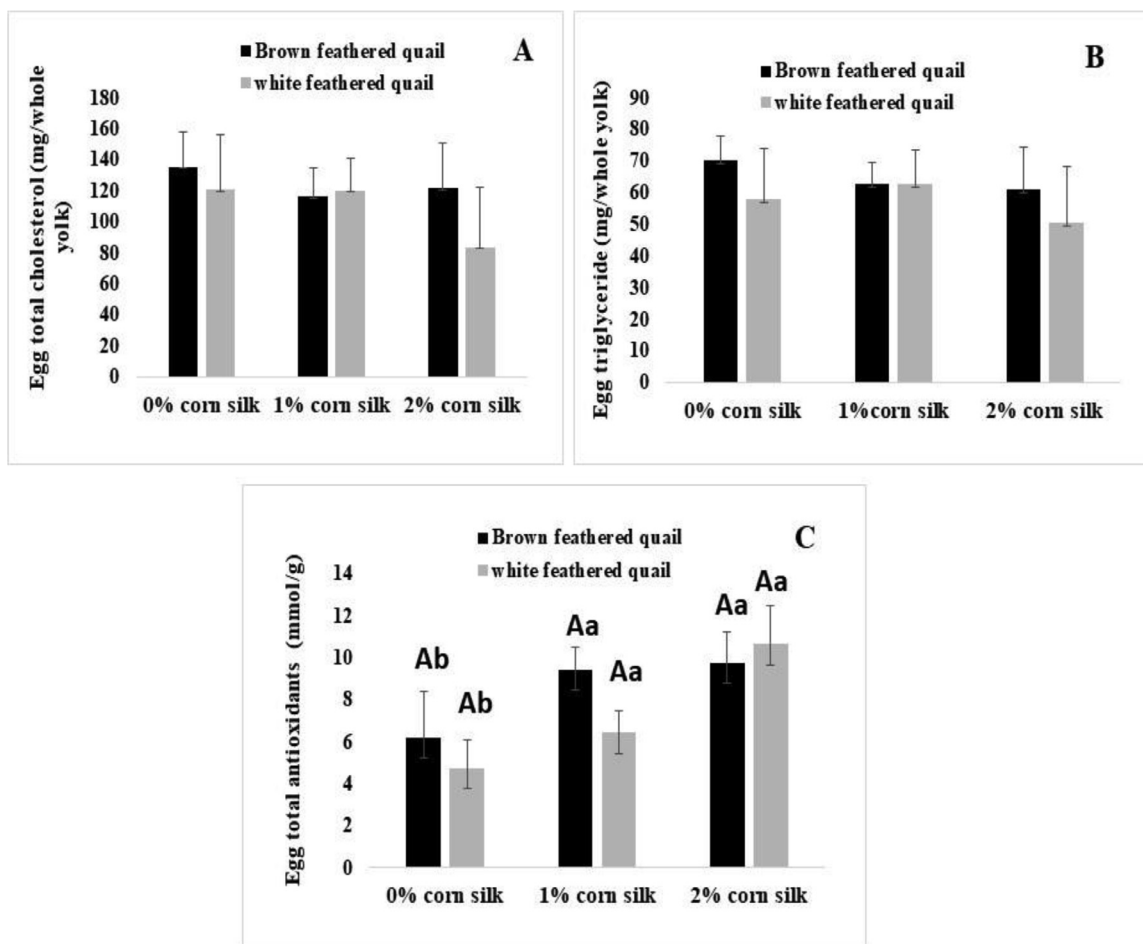


Figure 4. Effect of corn silk dietary supplementation on egg content of cholesterol (A), triglycerides (B), and total antioxidants (C) of two different varieties of Japanese quail (Brown-feathered & White-feathered). Results are expressed as mean \pm SEM. Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

colors of quail (white, dark brown, golden, and wild-type) significantly impacted live weight, FI, FCR, and carcass yield. Also, earlier studies revealed that different plumage color of quail was associated with differences obtained in the live weight (Minvielle et al., 1999; Tarhyel et al., 2012b).

In the current study, CS addition enhanced the intestinal morphology of the duodenum in both varieties by increasing the villi length which could explain the improved growth in the CS supplemented quail. To our knowledge, there is no literature available that would support our findings about the impact of corn silk

Table 7. Effect of corn silk (CS) dietary supplementation on ovarian follicle number in two different varieties of Japanese quail layers (Brown-feathered & White-feathered).

Group	Large yellow follicle (>10 mm)	Small yellow follicle (5–10 mm)	Large white follicle (3–5 mm)	Medium White follicle (1–3 mm)	Postovulatory follicle
Brown-feathered					
Control	3.33 \pm 0.21 ^A	1.50 \pm 0.22	12.16 \pm 1.97	21.57 \pm 2.61 ^b	2.83 \pm 0.65 ^{bB}
1% Corn silk	4.33 \pm 0.33 ^A	1.83 \pm 0.31	12.83 \pm 0.87	28.50 \pm 1.02 ^{ab}	5.00 \pm 0.37 ^{aA}
2% Corn silk	3.67 \pm 0.42	2.17 \pm 0.17	11.50 \pm 1.78	29.83 \pm 4.09 ^a	4.33 \pm 0.42 ^{aA}
White-feathered					
Control	2.50 \pm 0.43 ^B	1.67 \pm 0.21	13.17 \pm 1.35	18.17 \pm 1.35 ^b	2.83 \pm 0.40 ^{aB}
1% Corn silk	3.33 \pm 0.49 ^B	2.17 \pm 0.40	14.67 \pm 0.88	29.17 \pm 2.91 ^a	3.50 \pm 0.62 ^{aB}
2% Corn silk	2.83 \pm 0.40	2.00 \pm 0.37	12.71 \pm 1.32	25.83 \pm 3.69 ^a	3.00 \pm 0.37 ^{aB}
Two-Way ANOVA P-values					
Corn silk	0.076	0.202	0.598	0.007	0.023
Quail variety	0.009	0.644	0.215	0.371	0.024
interaction	0.970	0.686	0.955	0.698	0.254

Data is presented as means \pm SEM. Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

Table 8. Japanese quail layers (Brown-feathered & White-feathered) serum lipid profile in response to corn silk (CS) dietary supplementation.

Group	Cholesterol mg/dl	Triglyceride mg/dl	HDL ¹ mg/dl	LDL ² mg/dl
Brown-feathered				
Control	189.67 ± 6.01 ^{AA}	1386.0 ± 85.63 ^{AA}	42.49 ± 3.61 ^b	63.67 ± 5.78 ^a
1% Corn Silk	151.66 ± 4.40 ^{BB}	1106.7 ± 60.15 ^{AB}	69.70 ± 3.48 ^a	55.00 ± 6.66 ^{ab}
2% Corn Silk	156.33 ± 6.11 ^{BB}	944.00 ± 19.13 ^{BB}	62.1 ± 1.47 ^a	38.67 ± 2.96 ^b
White-feathered				
Control	206.00 ± 6.55 ^{AA}	1348.33 ± 42.43 ^{AA}	46.59 ± 3.91 ^b	63.33 ± 3.71 ^a
1% Corn Silk	178.67 ± 6.33 ^{ABAB}	1060.3 ± 21.86 ^{BB}	64.25 ± 1.93 ^a	50.33 ± 1.76 ^{ab}
2% Corn Silk	170.33 ± 8.11 ^{AB}	511.66 ± 54.34 ^{CC}	69.17 ± 5.43 ^a	39.34 ± 2.96 ^b
Two-Way ANOVA <i>P</i> values				
Corn silk	0.0002	<0.0001	<0.0001	0.0004
Quail variety	0.003	0.002	0.498	0.690
Interaction	0.567	0.004	0.785	0.810

Data are presented as means ± SEM. Lowercase letters represent statistical differences ($P < 0.05$) between different treatments within the same variety. The uppercase letters represent the statistical significance ($P < 0.05$) between brown and white-feathered Japanese quail.

¹HDL = high-density lipoprotein.

²LDL = low-density lipoprotein.

supplementation on the intestinal morphology of poultry. Most of the available studies investigated the different inclusion levels of other corn byproducts such as corn dried distillers' grains (DDGS), and fermented corn gluten meal. Elbaz et al. (2022) addressed the DDGS in a broiler diet and reported no significant effect on ileum villus height, crypt depth, and VH/CD ratio compared to the control group. Also, Wang et al. (2018) found that fermented corn gluten meal inclusion at 5% and 10% of the diet of 3-yellow broilers significantly increased the duodenal villus lengths and the VH/CD ratio compared to those of the control group ($P < 0.05$). Since these feed ingredients are different in their nutritional composition, phytochemical content, and antinutritional elements, therefore it would bring up the different responses to intestinal morphology which in turn would influence the growth performance of birds. Furthermore, the digestive tract section on which the morphological examination was carried on would also bring different responses.

Serum albumin concentration responded differently which showed increased values with CS supplementation, with values being higher in white feathered quail than in the brown feathered quail, especially in the 1% CS supplemented group. This result disagrees with Kirrella et al. (2021) who reported unchanged total protein and albumin concentrations with CS inclusion in the broiler chicken diet. Moreover, CS addition enhanced kidney function by reducing the serum creatinine concentration. This result is consistent with findings of Sukandar et al. (2013) who reported that corn silk extract ameliorated the elevated serum creatinine and urea but also with its nephroprotective activity against gentamicin-induced nephrotoxicity in mice (Sepehri et al., 2011).

The serum lipid profile was altered by CS dietary supplementation and quail strain. Total cholesterol, triglyceride, and LDL serum concentrations were reduced in birds fed CS, especially with the highest level of CS (2%) supplemented in both varieties of quail. The present result could suggest that increasing CS inclusion level in

the quail diet has a lowering effect on serum lipid profile parameters. These findings could be attributed to the flavonoid content of CS which has a hypolipidemic effect (Zhang et al., 2015). Similarly, Ozuruoke et al. (2016) found a significant decrease in cholesterol and TG in rabbits after one week of CS methanolic extract administration. Additionally, the increased HDL serum concentration following CS supplementation might be linked to the flavonoid contents of CS which possess an antioxidant effect and inhibit the lipid peroxidation (Ren et al., 2013). The reported lipid profile results agreed with Kirrella et al. (2021) finding who documented a reduced blood TC, TG, and LDL, and increased HDL in broiler chickens fed CS meal.

Besides, the hypolipidemic response of CS supplementation was more noticeable in the white-feathered quail than in the brown ones. This finding comes in agreement with the previous studies, which documented that white-feathered quail showed higher BW with deposition of fat in their body compared to the brown-feathered quails (Inci et al., 2015; Nasr et al., 2017). Understanding the mechanism behind the effect of CS in regulating lipid metabolism and the nature of the body gain obtained in Japanese quail varieties needs further investigation, especially at the transcriptomic level.

Throughout the laying period, CS supplementation increased the egg production in brown-feathered quail, while was reduced in the white-feathered quail. Very limited information is available on CS supplementation's effects on poultry egg production. Most of the available sources addressed other corn byproducts such as corn germ meal (Brunelli et al., 2010); dried distillers' grains (Tatiana Marques et al., 2019), and fermented corn byproducts (Yang et al., 2022) investigating the best level of inclusion for achieving optimum performance. Egg production response in these studies showed variation, which could be attributed to its nutritional composition, phytochemical content, and antinutritional factors found in these feed ingredients. Comparing the 2 different varieties in terms of egg production indicated a lower production in the white-feather quail than

in brown-feathered ones with the effect of the plumage colour being clear during the 9th and 11th wk of production. Similarly, [Bagh et al. \(2016\)](#) concluded that egg production was higher for brown followed by white and gray feathered quail. The obtained response related to the quail variety could be attributed to the growth performance of white-feathered quails as they exhibited higher growth during the growing period which consequently affected their performance during the laying period. In support, [Nestor and Bacon \(1982\)](#) found that egg production declined in heavy size and increased in low BW strains of the Japanese quail. Declined egg production in white feathered quails could also be attributed to physiological changes which resulted in the slow growth of ovarian follicles and less mature ovarian follicles ([Jatoi et al., 2013](#)).

External and internal egg quality parameters showed no difference among groups except for the yolk color which was increased with CS supplementation, especially in the brown-feathered quail. Increased yolk color score could be attributed to the carotenoid content (β -Carotene, zeaxanthin) of the CS ([Nawaz et al., 2018](#)), which are considered important pigments affecting the yolk color obtained. This result is consistent with most of the studies which investigated the effect of some corn byproducts on yolk color score ([Bittencourt et al., 2019](#)). Besides, quail variety didn't significantly affect the egg quality parameters except for the eggshell relative weight, yolk relative weight, and yolk index. These quality parameters were reduced in the white-colored quail while the yolk relative weight was increased compared to the brown feathered one. These results are in quite an agreement with [Bagh et al. \(2016\)](#) who showed no difference in external and internal egg quality among quail varieties (brown, white, and gray).

The serum lipid profile during the laying period revealed that CS dietary inclusion exhibited a hypolipidemic effect which confirmed the former obtained results during the growing period. The synthesis of adipose tissue, fat deposition, and the formation of yolk in poultry is dependent on the available serum triglycerides ([Lai et al., 2018](#)). Since the serum TG and CHO content of quail layers in both varieties were reduced in response to CS dietary addition, the egg content of these parameters mirrored the same findings. The effect of CS was extended to include the egg's antioxidant content, which was increased following the CS addition. Increasing the antioxidant content in the egg could probably be associated with the phytochemicals content of CS (flavonoids, phenolics, terpenes, and alkaloids) which has been shown to have antioxidant activity ([Liu et al., 2011](#); [Boeira et al., 2022](#)). The obtained results on egg content of TG, CHO, and total antioxidants enhance the egg quality.

CONCLUSION

Under the conditions of the current experiment, it could be concluded that dietary supplementation of corn

silk 1% and 2% for brown-feathered and white-feathered quails respectively improved their growth performance and intestinal morphology during the growing period. Corn silk dietary supplementation modified the serum lipid profile showing a hypolipidemic effect which was more pronounced in the white-feathered quail than the brown quail. During the laying period, brown feathered quails showed higher egg production than white feather quails. Corn silk supplementation during the laying period enhanced the egg quality by increasing the yolk color score and antioxidant content and reducing the total cholesterol and triglyceride providing potential benefits for human health.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study was reviewed and approved by the institutional Ethics Committee of Kafrelsheikh University, Egypt.

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DISCLOSURES

The authors declare no conflict of interest.

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