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Corn Silk Extract: A Potential Modulator for Producing Functional Low Cholesterol Chicken Eggs

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Abstract: The chicken egg is one of the most globally-consumed animal protein sources with high-quality protein value. However, there is a growing concern about the association between excessive egg consumption and the increasing risk of cardiovascular disease incidence. Meanwhile, corn silk extract (CSE) is known to have hypo-lipidemic bioactive properties, as well as antioxidant and anti-inflammatory effects. Thus, the present study was designed to investigate the effect of feeding laying hens three different CSE levels on egg cholesterol content as well as egg production performance and oxidative stress marker levels. A total of 240, 40-week-old, Hy-Line Brown laying hens were divided into 4 symmetric groups (10 hens × 6 replicates). The control group was fed a basal diet while the other three groups were given the basal diet supplemented with 100 mg, 200 mg, or 400 mg CSE per kg feed, respectively. Egg production performance was monitored for eight successive weeks. Internal and external egg quality parameters were also measured. At the end of week 48 of age, blood samples were collected to determine the plasma lipid profile, stress markers, and liver function indicators. Data revealed that supplementation of 200 mg and 400 mg CSE to laying hen diets had a positive effect on egg production performance with a significant increase in egg numbers and egg weight as well as significantly improved feed efficiency. Egg quality parameters were significantly improved with CSE supplementation. Lipid peroxidation levels and inflammation marker concentrations significantly decreased for the experimental groups that were fed 200 mg and 400 mg CSE compared with the control group. Meanwhile, blood total cholesterol decreased significantly with CSE supplementation, along with an increase in high-density and a decrease in low-density lipoprotein cholesterol content. A high positive correlation was found between liver and egg cholesterol contents ($r = 0.902$, $p < 0.0001$) which was linearly decreased with the increasing level of CSE supplementation. Egg cholesterol content significantly decreased by 9 to 19% in the CSE-supplemented groups compared with the control group. The present study demonstrated that CSE at 100 mg/kg and up to 400 mg/kg diets can be safely used to improve laying hen egg production performance with a direct effect on lowering egg cholesterol content as well as improving the redox status.

Keywords: corn silk extract; egg cholesterol content; egg quality; stress markers; lipid profile

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1. Introduction

The emerging consumer demand for safe and high-quality food is a consequence of the increasing awareness regarding the sensory quality, functionality, and nutritional value of food and food products [1]. The foods that deliver healthy, physical, and mental well-being benefits other than essential nutrition are defined as functional foods [2]. Chicken table eggs are one of the most intensively consumed protein sources of food from an animal origin worldwide. The high nutritive value and the affordable market price make eggs an economical alternative protein source compared with other expensive animal protein

sources [3]. However, there is a serious dispute between the association of increasing egg consumption and the increasing risk of cardiovascular disease incidence. Some research indicated a link between higher consumption of eggs and dietary cholesterol and the higher incident of cardiovascular diseases [4–7]. Meanwhile, other research articles indicated no association between moderate egg consumption and the occurrence of cardiovascular diseases [8–10]. Carson et al. [11] made a recommendation to promote cardiovascular health focusing on dietary patterns which include lowering cholesterol as well as saturated fatty acids consumption. In the middle of this serious debate, egg cholesterol is one of the variable egg components that is extremely influenced by laying hen diet [12,13]. Hence, the abovementioned findings imply that diet manipulation designed for reducing egg cholesterol content can be used to produce functional chicken table eggs that provide health benefits for consumers.

Under intensive commercial egg production systems, the generation of a huge oxidative stress load is inevitable. Oxidative stress could be generated by nutritional, environmental, or pathological factors that are responsible for the reported negative impacts on poultry's general performance as well as product quality [14]. Moreover, oxidative stress not only decreases laying hen performance, but it also impairs ovarian function, disturbs gut microbiota, and influences body metabolites [15]. Li et al. [16] found that heat stress increased the level of oxidative stress markers in follicular fluid as well as induced apoptosis in follicle cells by activating the FasL/Fas and TNF-α systems, which subsequently reduced follicle number and impaired egg production. Likewise, the excessive production of reactive oxygen species (ROS) was reported to induce immunosuppression, pro-inflammation responses, and imbalance redox status in laying hens challenged with avian pathogenic *E. coli* [17]. Mishra and Jha [14] suggested that exogenous supplementation of plant extracts with potent antioxidant activity might be beneficial in mitigating oxidative stress in poultry.

Corn silk (CS) is from maize (*Zea mays*) and comprises the female flower stigmas that appear as yellowish, thread-like silks that are considered an agricultural waste material [18]. It has been used in treating various illnesses and is considered an alternative natural-based treatment. Corn silk extract (CSE) is rich in several phytochemical compounds, namely: polyphenols, phenolic acids, flavonoids, alkaloids, anthocyanins, polysaccharides, glycosides, organic acids, sterols, volatile oils, carotenoids, trace elements, and multivitamins [19,20]. Such presented compounds are responsible for a series of bioactive properties of corn silk including antioxidant, anti-inflammatory, anti-diabetic, hypo-glycemic, and hypo-lipidemic effects [19,21–23]. Regarding the hypo-lipidemic properties of CSE, a meta-analysis study suggested that decoction of CSE might increase high-density lipoprotein cholesterol while reducing triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels in angina pectoris patients [21]. Research studies reported that CSE has a hypo-lipidimic effect when using a hyper-lipidemic animal experimental model [24,25]. Saheed et al. [26] suggested that CSE could be used to manage coronary heart diseases due to its hypo-lipidemic properties, with no hematotoxic effect while using rats as an animal experimental model. In broilers, Kirrella et al. [27] concluded that dietary inclusion of CS meal with non-starch polysaccharide enzyme to broiler diet can improve growth performance and decrease plasma total cholesterol as well as increase HDL-CH. Furthermore, polysaccharides found in CSE were reported to have a significant antioxidant potential both in vitro and in vivo [28]. To date, no study has been conducted on the effect of CSE on laying hen cholesterol profile, egg production, and cholesterol content. Thus, the present study aimed to investigate the effect of CSE supplementation to laying hen diet on egg cholesterol content as well as plasma and liver cholesterol levels, with special reference to oxidative stress markers, under a commercial egg production system.

2. Materials and Methods

2.1. Ethical Declaration

The experimental design of the present study followed the research ethics guidelines of King Faisal University, Saudi Arabia. Approval for the entire experimental protocol

was obtained from the Research Ethics Committee (REC) at King Faisal University, Saudi Arabia (KFU-REC/2022-02-17).

2.2. Corn Silk Extract Phenolic Acid Profile and Total Antioxidant Capacity Test

Fresh corn silk samples were collected, air dried, and ground to a fine powder. A 20 g sample of air-dried CS powder was then soaked in 500 mL ethanol solution 70% (1:4, *w*:*v*) at 40 °C with continuous stirring for one hour using a hot plate magnetic stirrer. The extract was then filtered using Whatman No. 1 filter paper. The solvent from the filtrated extract was then evaporated using rotary evaporator at 50 °C. The obtained extract weight was 3.31 g and was further used for phenolic acid quantification and total antioxidant capacity measurements.

Phenolic compounds of CSE were quantified using a high-performance liquid chromatography instrument (HPLC) (LC-10AD, Shimadzu, Japan) as described by El-Mergawi et al. [29]. The HPLC results are illustrated in Figure 1.

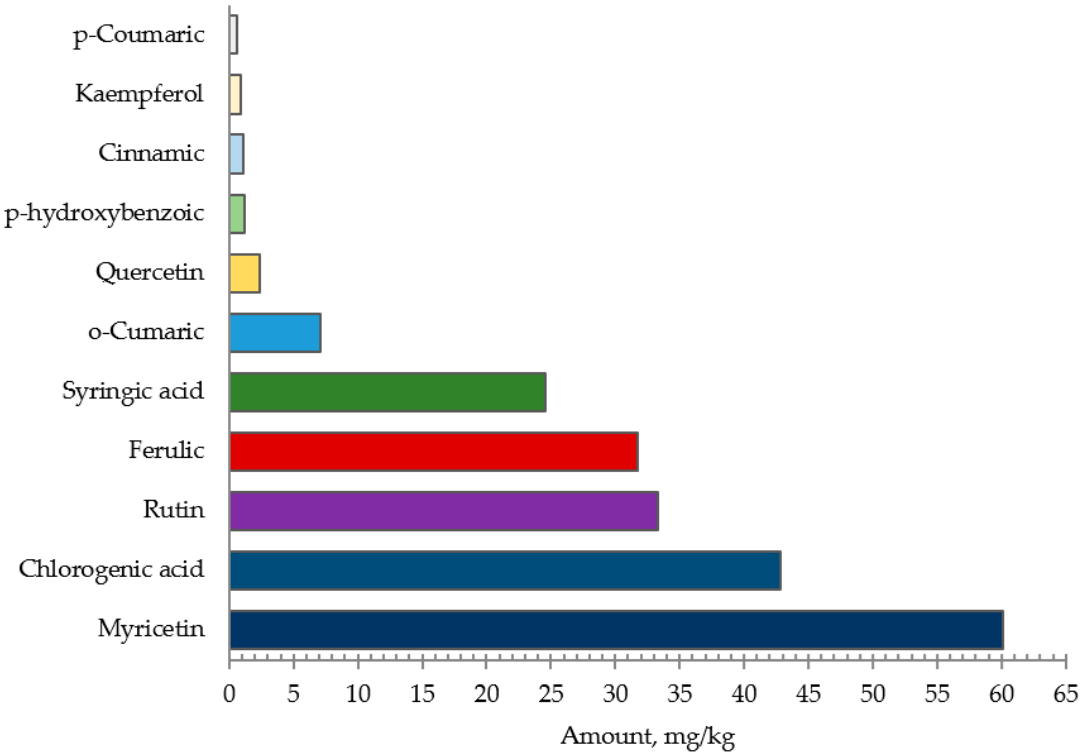

Figure 1. Phenolic acid profile of corn silk extract quantified using HPLC presented as mg of phenolic compound per each 100 g of corn silk on a dry matter basis.

2.3. Birds' Management and the Experimental Design

The experimental subjects were recruited from a commercial 40-week-old Hy-Line Brown laying hen flock. A total number of 240 laying hens were randomly distributed into four symmetrical groups (10 hens × 6 replicates) in a completely randomized design. The four experimental groups were fed a basal diet formulated to cover the nutritional demand under the guidelines of commercial Hy-Line Brown laying hens, (https://www.hyline.com/varieties/brown; accessed on 11 May 2022) (Table 1). The first group was fed the basal diet without any additional supplementation and served as the control. Meanwhile, the other three groups were given the basal diet supplemented with 100 mg, 200 mg, or 400 mg of CSE per kg of diet. The experiment lasted for eight successive weeks. The recruited laying hens were reared under a controlled environment in a closed laying house in single cages. The ambient temperature was kept at 22 ± 1 °C and 60% relative humidity with a daily lighting regime of 16 h of light and 8 h of darkness. A free access for water and feed was provided to all the birds throughout the entire experimental period.

Table 1. The basal diet ingredients and chemical analyses.

Ingredients	Content
Yellow corn, g/kg	565.5
Soybean meal (44%), g/kg	276.0
Wheat bran, g/kg	10.0
Soybean oil, g/kg	30.0
Bone meal, g/kg	30.0
Limestone, g/kg	80.0
Salt, g/kg	4.0
Premix [1], g/kg	3.0
DL-methionine, g/kg	1.5
Calculated chemical analysis	
Metabolizable energy, kcal/kg	301.15
Crude protein, g/kg	174.7
Calcium, g/kg	40.2
Available phosphorus, g/kg	5.2
Lysine, g/kg	9.5
Methionine, g/kg	4.2
Linoleic acid, g/kg	28.8
Chemical analysis (%)	
Dry matter	89.00
Crude protein	16.75
Crude fat	6.60
Crude fiber	4.70
Ash	12.90
Calcium	4.22
Phosphorus	0.42

[1] Premix supplied each kg of the basal diet with vitamins (vit) and minerals as follows: vit A, 8000 IU; vit D3, 1500 IU; vit E, 15 mg; vit K, 2 mg; riboflavin, 4 mg; niacin, 25 mg; cobalamin, 10 µg; choline, 500 mg; manganese, 60 mg; and zinc, 50 mg.

2.4. Egg Production Performance

During the course of the experimental period, egg number (EN), egg weight (EW), and feed intake (FI) per hen were recorded daily. The average EN, EW, and FI were then calculated for the entire experimental period. Total egg mass (EM) was calculated using the following formula: EM = EN (for the exact period) × average EW. Then, feed conversion ratio (FCR) was calculated as a total FI per total EM.

2.5. Egg Quality Evaluation

At the 48th week of age, 30 eggs per group (six eggs per replicate) were randomly selected to evaluate egg quality parameters. First, eggs were weighed and then broken on a flat plate. Next, the albumen and the yolk heights were measured using a tripod micrometer (Baxlo®, Barcelona, Spain). Then, the albumen and the yolk diameters were measured using a standard caliper (Total Tools, South Melbourne, Australia). The albumen index and the yolk index were then calculated as the ratio between the albumen height to the albumen diameter, and the ratio between the yolk height to the yolk diameter, respectively. After that, for the determination of shell thickness and shell strength, the obtained egg shell was rinsed and allowed to air dry. The shell thickness was then determined as the mean of the thickness measured at three different positions on the egg (sharp pole, blunt pole, and equator) using an egg shell thickness tester with 0.01 mm sensitivity (Baxlo®, Barcelona, Spain). The shell strength was also determined by applying an assisted system pressure to the egg blunt using the Egg Force Reader (ORKA Food Technology, West Bountiful, UT, USA). The yolk color score was determined using the DSM-YC Fan (ORKA Food Technology, UT, USA). Finally, the Haugh unit was calculated using the following Raymond Haugh equation [30]:

$$HU = [100 \times \log^{f_0}(AH\text{-}1.7W^{\wedge}0.37 + 7.57)]$$

where HU = Haugh units; AH = albumen height in mm; and W = egg weight in g.

2.6. Collection and Preparation of Blood and Liver Samples

After eight weeks from the start of the experiment, two hens from each group replicate were randomly chosen. Blood samples were withdrawn, after 12 h of fasting, from the brachial vein using a heparinized syringe and immediately transferred into a heparinized tube. The plasma was then separated by centrifugation at 2000× *g* for 10 min at 4 °C. The collected plasma was stored at −20 °C until further analysis. Meanwhile, six hens per experimental group (one hen per replicate) were sacrificed by cervical dislocation [31] and the whole livers were harvested and immediately stored at −20 °C until further processing.

2.7. Blood and Liver Lipid Profile and Egg Yolk Cholesterol Content

Plasma triglyceride content was quantified using enzymatic colorimetric diagnostic kits (Cat#: ab65336; Abcam, Waltham, MA, USA), while plasma total cholesterol, high-density lipoprotein cholesterol (HDL-CH), and low-density lipoprotein cholesterol (LDL-CH) were quantified using commercial kits (Cat#: ab65390; Abcam, MA, USA). Plasma cholesterol ratio was then calculated as total cholesterol divided by HDL-CH.

On the other hand, in the last week of the experimental period, six eggs per group were randomly collected (one egg per replicate). The eggs were broken to separate yolks. Total cholesterol contents in the liver and egg yolk samples were measured using a cholesterol oxidase method-based kit (Cat#: ab102515; Abcam, MA, USA) following the modified protocol described by Alzarah et al. [32]. Finally, the liver and egg total cholesterol contents were determined using a cholesterol standard solution curve.

2.8. Plasma Stress Markers and Biochemical Parameters

Plasma lipid peroxidation marker (malondialdehyde; MDA) level was determined using quantitative colorimetric assay kits (ab287797; Abcam, MA, USA). The plasma level of pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α), heat shock protein-70 (HSP-70), and corticosterone were quantified using chicken-specific ELISA kits, following the manufacturer procedures (Cat#: MBS2509660, MBS703924, MBS701668, respectively; MyBioSource, San Diego, CA, USA). Meanwhile, total antioxidant capacity (TAC) and superoxide dismutase (SOD) activities were determined in blood plasma using colorimetric assay kits (Cat#: MBS2540515 and MBS9718960, respectively; MyBioSource, CA, USA).

Meanwhile, plasma total protein, albumin, urea, and creatinine concentrations were determined using colorimetric kits (Cat#: ab102535, ab235628, ab83362, and ab65340, respectively; Abcam, MA, USA). Furthermore, the plasma activity of liver alanine amino transferase (ALT) and aspartate amino transferase (AST) enzymes were measured using commercial kits (Cat#: ab241035 and ab105135, respectively; Abcam, MA, USA).

2.9. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) with respect to the CSE supplementation levels as the independent variable, using the general linear model of SAS (SAS® 9.1.3, Service Pack 4). The mathematical model was as follows:

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

where Y_{ij} represents the *j*th observation (j = 1,2, … n_i) on the *i*th treatment levels of CSE i = 0, 100 mg/kg, 200 mg/kg, and 400 mg/kg diet is the observation. μ is the common effect for the whole experiment, τ_i represents the *i*th treatment effect, and e_{ij} represents the random error present in the *j*th observations on the *i*th treatments, assumed to be normally distributed with a mean between (0, σ^2). Furthermore, to determine the correlation between liver cholesterol and egg yolk cholesterol contents, Pearson's correlation coefficient was

performed. The results were expressed as means ± standard error, and the differences between treatment groups were determined using Tukey's Studentized Range (HSD) test with a statistical significance level of $p \leq 0.05$.

3. Results

3.1. Egg Production Performance

Egg production performance parameters of laying hens fed different levels of CSE are presented in Table 2. The produced egg number during the experimental period showed a 2.7% and 4.1% significant increase for the groups supplemented with 200 mg and 400 mg of CSE, respectively, compared with the control. Furthermore, a significant linear increase in egg weight and egg mass was observed with the increasing levels of CSE supplementation. Interestingly, a significant decrease in feed intake was observed in laying hens supplemented with 100 mg and 200 mg CSE, whereas a significant increase was noticed in the 400 mg CSE-supplemented group compared with the control. However, a significant improvement in feed conversion ratio was observed in the CSE-supplemented groups compared with the control. The best feed efficiency was observed in the 200 mg and 400 mg CSE-supplemented groups, followed by the 100 mg CSE-supplemented group.

Table 2. Egg production performance of laying hens (n = 60) fed different levels of corn silk extract (CSE: 100 mg/kg, 200 mg/kg, or 400 mg/kg diet).

Parameters	Control	CSE-100	CSE-200	CSE-400
EN, 7d	$6.38^{b} \pm 0.06$	$6.52^{b} \pm 0.07$	$6.82^{a} \pm 0.05$	$6.66^{a} \pm 0.02$
EW, g/egg	$59.13^{d} \pm 0.15$	$60.40^{c} \pm 0.15$	$61.67^{b} \pm 0.15$	$62.73^{a} \pm 0.11$
Egg mass/week	$378.12^{d} \pm 1.98$	$390.34^{c} \pm 1.79$	$404.97^{b} \pm 1.83$	$417.68^{a} \pm 1.64$
FI, g/day	$113.42^{b} \pm 0.18$	$112.52^{c} \pm 0.19$	$111.60^{d} \pm 0.15$	$115.13^{a} \pm 0.17$
FCR	$2.10^{a} \pm 0.01$	$2.02^{b} \pm 0.01$	$1.93^{c} \pm 0.01$	$1.93^{c} \pm 0.01$

Means having different superscripts in the same row significantly differ ($p \leq 0.05$). EN: egg number; EW: egg weight; FI: feed intake; and FCR: feed conversion ratio.

3.2. Egg Quality Parameters

The internal and external egg quality parameters were estimated for laying hens fed different levels of corn silk extract (CSE) and the results are presented in Table 3. Data showed a significant positive effect of CSE supplementation on the different egg quality parameters measured. Albumin index increased significantly with CSE supplementation by 3%, 4%, and 6% in the 100 mg, 200 mg, and 400 mg CSE-supplemented groups compared with the control, respectively. Moreover, yolk index also increased significantly by 2%, 4%, and 5% in the 100 mg, 200 mg, and 400 mg CSE-supplemented groups compared with the control, respectively. Furthermore, shell thickness and shell strength showed a significant linear increase with the increase in CSE-supplementation level. The increasing level of shell thickness reached 13%, 19%, and 29%, where shell strength increased by 5%, 14%, and 19% in the 100 mg, 200 mg, and 400 mg CSE-supplemented groups compared with the control, respectively. The intensity of yolk color increased significantly with the CSE supplementations. However, the Haugh unit significantly increased in the 200 mg CSE-supplemented group compared with the control.

Table 3. Egg quality parameters of laying hens (n = 30) fed different levels of corn silk extract (CSE: 100 mg/kg, 200 mg/kg, or 400 mg/kg diet).

Parameters	Control	CSE-100	CSE-200	CSE-400
Albumin index	$9.45^{c} \pm 0.06$	$9.73^{b} \pm 0.04$	$9.84^{b} \pm 0.03$	$10.01^{a} \pm 0.04$
Yolk index	$40.38^{c} \pm 0.05$	$41.10^{b} \pm 0.05$	$42.27^{a} \pm 0.35$	$42.31^{a} \pm 0.06$
Yolk color	$7.92^{d} \pm 0.05$	$8.49^{c} \pm 0.04$	9.09 ± 0.03^{b}	$9.67^{a} \pm 0.06$
Shell thickness, mm	$0.31^{d} \pm 0.001$	$0.35^{c} \pm 0.001$	$0.37^{b} \pm 0.002$	$0.40^{a} \pm 0.002$
Shell strength, kg/cm^2	$3.76^{d} \pm 0.01$	$3.95^{c} \pm 0.01$	$4.29^{b} \pm 0.03$	$4.49^{a} \pm 0.03$
Haugh unit	$81.78^{b} \pm 0.38$	$81.91^{b} \pm 0.14$	$83.38^{a} \pm 0.049$	$82.96^{ab} \pm 0.35$

Means having different superscripts in the same row significantly differ ($p \leq 0.05$).

3.3. Blood Cholesterol Profile and Egg Cholesterol Content

Data of blood and liver total cholesterol and cholesterol fractions for laying hens supplemented with different levels of CSE are presented in Table 4. The CSE supplementation of the laying hen diet significantly reduced blood total cholesterol and liver cholesterol concentration as well as altered the cholesterol fraction profile. The plasma triglyceride concentration significantly decreased in the 200 mg and 400 mg CSE-supplemented groups, compared with the control. Furthermore, plasma total cholesterol concentration decreased significantly in laying hens fed CSE at the level of 400 mg compared with the control and the 100 mg CSE-supplemented groups. However, HDL-CH, which is known as the good cholesterol, increased significantly in the laying hen groups supplemented with 200 mg and 400 mg of CSE by 12% and 30%, respectively, compared with the control. On the contrary, the LDL-CH, which is known as the bad cholesterol, showed a significant reduction in the groups fed different levels of CSE compared with the control. The decreasing level of plasma LDL-CH associated with CSE supplementation was 11%, 18%, and 28% in the 100 mg, 200 mg, and 400 mg CSE-supplemented groups, respectively. A significant reduction in the calculated plasma cholesterol ratio was detected in the 200 mg and 400 mg CSE-supplemented group compared with the control and the 100 mg CSE-supplemented groups. Meanwhile, liver cholesterol concentration showed a significant linear decrease associated with the increasing level of CSE supplementation. Likewise, egg cholesterol content followed the same trend as liver cholesterol and showed a negative linear decrease with the increasing level of CSE supplementation. A highly significant positive correlation ($r = 0.902$, $p < 0.0001$) was observed between liver cholesterol and egg yolk cholesterol contents. Meanwhile, the plasma cholesterol concentration had a moderate positive correlation with the egg yolk cholesterol content ($r = 0.629$, $p = 0.001$).

Table 4. Blood, liver, and egg cholesterol contents of laying hens ($n = 6$) fed different levels of corn silk extract (CSE: 100 mg/kg, 200 mg/kg, or 400 mg/kg diet).

Parameters	Control	CSE-100	CSE-200	CSE-400
Triglycerides, mg/dL	$216.8^{a} \pm 6.22$	$199.5^{ab} \pm 4.45$	$180.0^{b} \pm 4.92$	$158.5^{c} \pm 4.57$
Cholesterol, mg/dL	$165.7^{a} \pm 6.13$	$166.2^{a} \pm 5.31$	$148.2^{ab} \pm 3.74$	$135.5^{b} \pm 6.40$
HDL-CH, mg/dL	$42.40^{c} \pm 1.07$	$45.53^{bc} \pm 0.96$	$47.53^{b} \pm 0.73$	$55.23^{a} \pm 0.88$
LDL-CH, mg/dL	$114.5^{a} \pm 1.23$	$101.5^{b} \pm 3.80$	$94.3^{b} \pm 2.94$	$82.9^{c} \pm 1.74$
Plasma cholesterol ratio	$3.92^{a} \pm 0.15$	$3.66^{a} \pm 0.14$	$3.12^{b} \pm 0.06$	$2.45^{c} \pm 0.10$
Liver CH, mg/dL	$5.73^{a} \pm 0.14$	$4.90^{b} \pm 0.09$	$4.25^{c} \pm 0.15$	$3.42^{d} \pm 0.08$
Egg cholesterol content, mg/g	$12.82^{a} \pm 0.18$	$11.68^{b} \pm 0.14$	$11.20^{b} \pm 0.15$	$10.43^{c} \pm 0.08$

Means having different superscripts in the same row significantly differ ($p \leq 0.05$). HDL-CH: high-density lipoprotein cholesterol; LDL-CH: low-density lipoprotein cholesterol; and Liver CH: liver cholesterol.

3.4. Stress Markers and Liver Function

Oxidative stress prevalence can be monitored by a number of blood stress biomarkers. The stress markers related to lipid peroxidation, inflammation, heat shock protein, and stress hormone, as well as SOD enzyme and total antioxidant activities, were measured in the current study (Table 5). The concentration of MDA decreased significantly in CSE-supplemented groups at the levels of 200 mg and 400 mg by 1.4- and 1.6-fold, respectively, compared with the control. Moreover, TNF-α decreased significantly in the CSE-supplemented groups at the levels of 200 mg and 400 mg by 9% and 14%, respectively. Furthermore, the level of HSP-70 deceased significantly by 17%, 21%, and 24% in the groups fed 100 mg, 200 mg, and 400 mg of CSE, respectively. Meanwhile, plasma corticosterone levels showed a significant reduction of 28% in the 400 mg CSE-supplemented group compared with the control. Conversely, the activity of the antioxidant enzyme SOD increased significantly in the 100 mg, 200 mg, and 400 mg CSE-supplemented groups by 1.11-, 1.26-, and 1.38-fold, respectively, compared with the control. Furthermore, the T-AOC activity showed a significant linear increase with the increasing level of CSE supplementa-

tion. These results reflected that the CSE supplementation directly reduced stress marker levels while promoting antioxidant activity.

Table 5. Stress markers and antioxidant-related parameters of laying hens (n = 6) fed different levels of corn silk extract (CSE: 100 mg/kg, 200 mg/kg, or 400 mg/kg diet).

Parameters	Control	CSE-100	CSE-200	CSE-400
MDA, μM/mL	2.74 ^{a} ± 0.19	2.41 ^{ab} ± 0.15	1.96 ^{bc} ± 0.07	1.75 ^{c} ± 0.07
TNF-α, pg/mL	96.25 ^{a} ± 1.11	91.78 ^{ab} ± 1.15	87.57 ^{bc} ± 1.92	82.97 ^{c} ± 1.52
HSP-70, ng/mL	26.19 ^{a} ± 1.30	21.67 ^{b} ± 0.79	20.59 ^{b} ± 0.95	20.03 ^{b} ± 1.21
Corticosterone, ng/mL	6.31 ^{a} ± 0.20	6.10 ^{a} ± 0.30	5.38 ^{ab} ± 0.21	4.57 ^{b} ± 0.25
SOD, U/mL	4.73 ^{c} ± 0.13	5.04 ^{bc} ± 0.10	5.26 ^{b} ± 0.06	5.66 ^{a} ± 0.06
T-AOC, U/mL	6.47 ^{d} ± 0.17	7.17 ^{c} ± 0.16	8.13 ^{b} ± 0.10	8.90 ^{a} ± 0.10

Means having different superscripts in the same row significantly differ ($p \leq 0.05$). MDA: malondialdehyde; TNF-α: tumor necrosis factor-α; HSP-70; heat shock protein-70; SOD: superoxide dismutase; and T-AOC: total antioxidant capacity.

3.5. Liver and Kidney Function

The effect of feeding CSE at different levels on laying hen liver- and kidney-function-related parameters are presented in Table 6. Firstly, there was no significant effect of CSE supplementation on total protein, albumin, or globulin levels compared with the control. However, the activity of liver ALT and AST enzymes showed a significant decrease with CSE supplementations at 200 mg and 400 mg. Similarly, blood urea concentration decreased significantly by 8%, 11%, and 19% with CSE supplementation at 100 mg, 200 mg, and 400 mg, respectively. Meanwhile, creatinine concentration significantly decreased with the 200 mg and 400 mg CSE supplementation compared with the control. These results indicate that the CSE possesses a hepatoprotective property as well as a renal function amelioration effect, with no influence on plasma protein levels.

Table 6. Liver- and kidney-function-related parameters of laying hens (n = 6) fed different levels of corn silk extract (CSE: 100 mg/kg, 200 mg/kg, or 400 mg/kg diet).

Parameters	Control	CSE-100	CSE-200	CSE-400
Total protein, g/dL	4.89 ^{a} ± 0.12	4.96 ^{a} ± 0.11	5.10 ^{a} ± 0.09	5.26 ^{a} ± 0.22
Albumin, g/dL	2.62 ^{a} ± 0.13	2.35 ^{a} ± 0.13	2.79 ^{a} ± 0.26	2.65 ^{a} ± 0.18
Globulin, g/dL	2.26 ^{a} ± 0.21	2.61 ^{a} ± 0.14	2.32 ^{a} ± 0.28	2.61 ^{a} ± 0.38
ALT, U/mL	13.39 ^{a} ± 0.42	12.15 ^{b} ± 0.17	11.51 ^{b} ± 0.20	10.12 ^{c} ± 0.21
AST, U/mL	30.78 ^{a} ± 1.28	27.03 ^{ab} ± 0.80	23.63 ^{bc} ± 0.96	21.07 ^{c} ± 0.70
Urea, mg/dL	5.88 ^{a} ± 0.05	5.42 ^{b} ± 0.10	5.21 ^{b} ± 0.04	4.78 ^{c} ± 0.08
Creatinine, mg/dL	0.30 ^{a} ± 0.01	0.28 ^{ab} ± 0.004	0.26 ^{bc} ± 0.006	0.24 ^{c} ± 0.004

Means having different superscripts in the same row significantly differ ($p \leq 0.05$). ALT: alanine aminotransferase; AST: aspartate aminotransferase.

4. Discussion

The production of low cholesterol egg is a growing consumer demand, especially for those who suffer from hyperlipidemia or cardiovascular diseases. Egg cholesterol content is considered a limiting factor for egg consumption for individuals having a high risk of cardiovascular disease [4]. Consequently, the reduction in egg cholesterol content is considered to add value for both egg producers and consumers. Apart from this, the CSE hypo-lipidemic as well as antioxidant and anti-inflammatory bioactivities were recently reported [19,25,26,33,34]. Hence, the present study was one of the first to investigate the effect of dietary CSE on egg yolk cholesterol content, egg production performance, egg quality, and redox status of commercial laying hens. The current results demonstrated significant improvement in egg production performance with CSE supplementation. Increasing laying rate as well as improving egg weight and feed conversion ratio was detected in the CSE-supplemented groups. These positive effects on egg production performance parameters can be justified by the antioxidant bioactive properties of the CSE. Al-Harthi [35] reported that, under heat stress conditions, supplementing laying hen diet with natural or

synthetic antioxidants improves production performance and egg quality. Furthermore, Abdel Magied et al. [36] concluded that natural antioxidant sources can be used to improve laying hen productivity during summer seasons. Obianwuna et al. [37] recently reviewed that natural plant antioxidant supplementation might improve the oxidative stability of chicken egg albumen during storage. Meanwhile, feed intake showed unexplained marginal reduction for groups supplemented with 100 mg and 200 mg CSE compared with the control. In contrast, feed intake significantly increased with the 400 mg CSE supplementation level. These conflicting effects could be due to the presence of bioactive compounds that either promote, such as chlorogenic acid and rutin [38,39], or suppress feed intake. However, such a reduction in feed intake had no negative impact on egg production number or egg weight for the 100 mg and 200 mg CSE-supplemented groups (Table 2). A significant hypo-lipidemic effect of the 200 mg and 400 mg CSE supplementation was observed with a significant reduction in plasma triglycerides and LDL-CH levels as well as a significant increase in plasma HDL-CH levels. Furthermore, plasma cholesterol ratio, a sensitive and specific index of cardiovascular risk [40], significantly decreased, confirming the hypo-lipidemic effect of the CSE supplementation. The hypo-lipidemic effect of CSE was reported to be modulated by reduction in mRNA expression levels of hepatic 3-hyroxy-3-methylglutaryl-coenzyme A reductase, farnesoid X receptor, and acyl-CoA: cholesterol acyltransferase genes, which consequently decrease blood and hepatic cholesterol levels [41]. Yan et al. [24] demonstrated that supplementation of moderate (400 mg/kg) and high doses (800 mg/kg) of total flavonoid obtained from CSE significantly decreased serum lipid levels (i.e., triglycerides, total cholesterol, and LDL-CH) in hyperlipidemic-induced rats following a dose-dependent manner. The liver is the site where most of the egg yolk cholesterol is synthesized; afterwords, it is transported via blood stream in the form of lipoproteins and deposited in the ovarian developing follicles [42]. Furthermore, egg yolk cholesterol had low heritability value [43], which implies that it can be manipulated using other environmental parameters such as dietary supplementations. Thus, the linear decrease observed in the liver cholesterol level in the CSE groups is reflected in the linear decrease in egg yolk cholesterol content. The principal phenolic acids detected in CSE were myricetin, chlorogenic acid, rutin, and ferulic and syringic acid (Figure 1). Therefore, the presence of high proportions of phenolic compounds with hypocholesterolemic and anti-hyperlipidemic activity in CSE, such as myricetin [44], chlorogenic acid [45], and rutin [46], played a key factor in the observed low liver cholesterol level and egg cholesterol content.

Poultry antioxidant defense systems include a complex network of endogenously synthesized antioxidant enzymes and exogenously supplied antioxidant materials. A new direction to improve the poultry antioxidant defenses under stress conditions is associated with the activation of a range of vitagenes, gene-coding proteins including SOD and HSP, to maximize internal antioxidant protection and maintain redox balance [47,48]. Ognik et al. [49] reviewed the positive effects of using plant extracts to stimulate antioxidant mechanisms in poultry, which usually demonstrated a modification in antioxidant enzyme activity (e.g., SOD) and an increase in the total antioxidant potential (e.g., T-AOC) as well as a reduction in lipid oxidation products (e.g., MDA). The effect of the moderate (200 mg) and high (400 mg) levels of CSE supplementation used on plasma stress marker concentrations was generally positive, with a significant reduction in MDA, TNF-α, and HSP-70 levels as well as an increase in SOD and total antioxidant activities. Malondialdehyde (MDA) is a well-known lipid peroxidation marker [50,51], whereas HSP-70, a parameter of cell protective and adaptive response, was reported to act as an oxidative injury biomarker [52]. A significant increase in MDA, TNF-α, and corticosterone levels were all demonstrated as stress markers in paraquat-induced oxidative stress in turkey poults [53]. Dietary phytochemical interventions were suggested as an effective strategy to overcome oxidative stress in poultry [54]. Corn silk is a rich source of phenolic and flavonoid components [55]. The bioactive phenolic compounds found in CSE are reported to have an antioxidant effect as well as other bioactive properties [20,56,57]. The antioxidant

properties of CSE are reflected in the observed reduction in oxidative stress markers and the increasing endogenous antioxidant activity. However, the present study was conducted under controlled-environmental conditions with no stress induction except for the internal stress of egg production. Thus, it can be implied that the CSE antioxidant effect will be more pronounced and advantageous when laying hens are subjected to unfavorable oxidative stress conditions.

The reduction in liver enzyme activities detected in the CSE-supplemented groups reflect a hepatoprotective effect. A hepatoprotective effect was reported for myricetin [44], rutin [46], and syringic acid [58]. Furthermore, blood urea concentration is considered a reliable renal function predictor, whereas the elevations of blood urea and creatinine levels are taken as a nephrotoxicity index [59,60]. The present results indicate a significant reduction in blood urea and creatinine levels with CSE supplementation reflecting amelioration of kidney function. Wans et al. [61] reported a protective effect of CSE on nephrotoxicity induced by acetaminophen in rats in response to its antioxidant, anti-inflammatory, and anti-apoptotic protective mechanisms. Moreover, Chen et al. [62] stated that the viability of human renal epithelial cells damaged by nano-calcium oxalate monohydrate crystals increased while the level of ROS decreased with corn silk polysaccharide administration.

5. Conclusions

The present study introduces dietary CSE supplementation as a potential feed additive to modulate egg cholesterol level. CSE supplementation of 200 mg/kg and 400 mg/kg in laying hen diet improved egg production performance and was able to influence plasma and liver lipid profiles. Lowering of total plasma cholesterol, triglycerides, LDL-CH, and liver cholesterol as well as increasing HDL-CH were detected in the CSE-supplemented groups. External and internal egg quality parameters improved when adding the CSE supplementation. Furthermore, a significant reduction in MDA, TNF-α, and HSP-70 levels was detected in the 200 mg and 400 mg CSE-supplemented groups. Meanwhile, corticosterone showed a significant reduction with the 400 mg CSE supplementation. The effect of CSE on plasma and liver cholesterol profiles reflected a significant reduction in egg yolk cholesterol content. Thus, CSE can be safely incorporated into laying hen diets to produce high-quality, functional eggs that are characterized by low cholesterol content in addition to improving laying hen performance under commercial egg production systems.

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