Journal of Animal and Feed Sciences, 31, 3, 2022, 249–257 https://doi.org/10.22358/jafs/150080/2022
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Nutritional and physiological evaluation of quercetin as a phytogenic feed additive in laying hens

A.S. El-Saadany[1,*], A.M. El-Barbary[1], A. Abd El-Salam[2], M.M. Ahmed[2] and E.Y. Shreif[2]

[1] Agriculture Research Center, Animal Production Research Institute, Poultry Breeding Research Department, Ministry of Agriculture, 12611 Giza, Egypt
[2] Agriculture Research Center, Animal Production Research Institute, Poultry Nutrition Research Department, Ministry of Agriculture, 12611 Giza, Egypt

KEY WORDS: antioxidant, blood parameters, laying hens, quercetin

Received: 5 March 2022
Revised: 11 May 2022
Accepted: 16 May 2022

* Corresponding author:
e-mail: aelsaadany55@gmail.com

ABSTRACT. The objective of the current study was to evaluate the effect of dietary quercetin supplementation on productive and reproductive performance, intestinal bacterial count, and blood biochemical parameters of laying hens. A total of 200 hens at 28 weeks of age were randomly assigned to four treatments (10 hens per treatment, 5 replicates each), group 1 was treated as control (basal diet without any supplementation), group 2, 3 and 4 were fed diets supplemented with 300, 600 and 1200 mg quercetin/kg feed, respectively. Egg production and egg mass increased ($P \leq 0.001$) as a result of dietary supplementation with 300 and 600 mg quercetin/kg feed, while egg weight and feed intake were not affected, but the feed conversion ratio improved. Shell thickness, Haugh unit and yolk colour score were improved by quercetin supplementation. The total count of aerobic, anaerobic and coliform bacteria in the intestine of laying hens was reduced ($P \leq 0.001$) in all quercetin-supplemented groups, and the lowest bacterial count was recorded at 600 mg/kg feed. There was an increase in the total *Lactobacillus* count in the treated groups. Heterophil (H) % was not affected by quercetin addition, while lymphocyte (L) % increased ($P \leq 0.001$) at 300 and 600 mg quercetin/kg feed, hence the H/L ratio was reduced at these doses as compared to the control diet. The inclusion of quercetin in the diet of laying hens increased total plasma antioxidant capacity, superoxide dismutase and high-density lipoprotein cholesterol, while reduced malondialdehyde, total cholesterol and low-density lipoprotein cholesterol levels compared to the control diet. Estradiol-17β and immunoglobulin (IgG and, IgM) levels increased ($P \leq 0.0001$) in response to quercetin treatments, with the highest values recorded in the group supplemented with 600 mg/kg feed. In conclusion, dietary supplementation with quercetin at a dose of up to 600 mg/kg improved productivity and physiological parameters in peak-producing laying hens; Immunity and the count of intestinal bacteria were increased, which in turn translated into overall good health and welfare of chickens.

Introduction

There is a growing interest in the use of phytochemicals in poultry diets as natural additives improving health and performance. The poultry industry would benefit considerably if phytogenic feed additives were used as substitutes for antibiotic or chemotherapeutic substances to satisfy consumer demand for food products without harmful residual substances. Additionally, phytochemicals such as polyphenolic compounds have been shown to exert positive antioxidant effects on animals in terms of

improved performance and production quality (Lee et al., 2013). Therefore, activation of antioxidant mechanisms can provide important strategies to prevent oxidative stress and enhance antioxidant defence in birds.

Quercetin is one of the flavonoids (C15H10O7), which are natural polyphenolic compounds found in plants and plant food sources that have strong antioxidant properties as free radical terminators (Sikder et al., 2014). It is present in various foods such as vegetables, tea, fruits, wine, apples, onions and tomatoes and is known to exert positive effects on poultry production and health (Saeed et al., 2017). Additionally, quercetin is used as a phytogenic additive in chicken feed due to a wide variety of expected beneficial effects on growth performance, oxidation stability, egg and meat quality, immune characteristics and anti-inflammatory properties (Goliomytis et al., 2014). Furthermore, quercetin, one of the six subclasses of flavonoid compounds with a wide range of biological activities, plays a significant role in improving production performance in laying hens (Abdel-Moneim et al., 2020). The addition of quercetin in feed has been reported to increase laying rate and eggshell strength (Liu et al., 2013). Similarly, feeding a diet supplemented with 400 mg/kg quercetin for eight weeks significantly improved laying rate and feed-egg ratio by 6.5 and 22%, respectively, while no significant effects were observed on daily feed intake and egg weight (Yang et al., 2017). Previous studies also demonstrated that the application of quercetin to laying hens lowered their serum cholesterol levels (Iskender et al., 2016) and adjusted the intestinal environment, as it reduced the caecal microflora in terms of absolute aerobes and coliforms, while increasing the number *Bifidobacteria (*Liu et al., 2014).

The positive findings of previous studies led us to hypothesize that quercetin may improve the performance of domestic hens that have a low conversion rate and low egg production, because their egg production curve ends rapidly (Saleh et al., 2008). Moreover, the present study was conducted to evaluate the effect of different quercetin levels on productivity and physiological parameters in laying hens.

Material and methods

Animal ethics and experimental design

The current research was conducted according to the guidelines of the Departmental Committee of Animal and Poultry Production, and the pronouncement of the Ministry of Agriculture in Egypt on animal ethics and welfare (Decree No. 27 (1967) that enforces the humane treatment of animals in general).

A total of 200 hens aged 28 weeks from the Mandarah strain, with an initial body weight of 1650 ± 25 g were used in this study during the spring period (average daily temperature 22–24 °C and relative humidity 65%) at the EL-Sabahia Poultry Research Station (Alexandria), Animal Production Research Institute, Agricultural Research Center. The Mandarah strain has been selected because this breed is recognised in Egypt and is popular among consumers. It is an egg breed, but local breeds have low egg production compared to international breeds, as explained earlier, but they are considered efficient compared to their local counterparts. During the experiment, birds were randomly divided into 4 treatments of 5 replicates (10 hens each) and housed (semi-close system) in floor pens (2.0 m × 1.2 m × 2.0 m). Feed and water were supplied *ad libitum* throughout the experimental period which ended at 42 weeks of age. Artificial lighting was used to provide the birds with 16 h of light per day. The commercial basal corn-soybean diet was formulated to meet the nutrient requirements of chickens according to the Feed Composition Tables for Animals and Poultry Feedstuffs used in Egypt (2001), as shown in Table 1. The birds fed the basal diet were considered a control group (in the form of mash), the other three groups (2, 3 and 4) were fed the basal diet supplemented with 300, 600 and 1200 mg quercetin/kg feed, respectively. Quercetin doses were selected based on our previous studies (Abid et al., 2020; Abdel-Latif et al., 2021).

Table 1. Composition and chemical analysis of the chicken diet

Ingredients, %	Chicken's diet
Yellow corn	63.14
Soybean meal (44%)	27.10
Dicalcium phosphate	1.50
Limestone	7.60
Salt (NaCl)	0.30
DL-methionine	0.06
Vitamin and mineral (premix)[1] free from iodine	0.30
Total	100.00
Chemical analysis[2]	
metabolisable energy, kcal/kg	2719
crude protein, %	17.28
calcium, %	3.22
available phosphate, %	0.44
methionine + cysteine, %	0.57
lysine, %	0.89

[1] supplied per kg diet: IU: vit. A 12 000, vit. D_3 2 200; µg: vit. B_{12} 10; mg: vit. E 10, vit. K_3 2, vit. B_1 1, vit. B_2 5, vit. B_6 1.5, nicotinic acid 30, folic acid 1, pantothenic acid 10, biotin 50, choline 250, copper 10, iron 30, manganese 60, zinc 50, selenium 0.1, cobalt 0.1; [2] according to Feed Composition Tables for Animal and Poultry Feedstuffs Used in Egypt (2001)

Quercetin was added in small amounts to the feed until complete homogeneity was achieved, and then added to the whole diet and mixed well. Quercetin (97%) was purchased from Sigma-Aldrich (Saint Louis, MO, USA) in powder form.

Production and egg quality parameters

Egg weight (EW, g) and egg production (EP, %) were recorded daily. Egg mass (EM) was calculated by multiplying the number of eggs by the average egg weight per hen (g egg/h/d). Daily feed intake (FI) (g/hen/day) was also recorded daily per hen. Feed conversion ratio (FCR) (g feed/g egg) was calculated as the amount of feed consumed divided by egg mass. Twenty-five eggs/group (5 eggs from each replicate) were used to record the weights of yolk, albumen and eggshell (as a percentage of egg weight). Eggshell thickness without inner membranes was measured (mm) with a micrometre and yolk colour intensity was measured based on the standard colour of the yolk using a Roche colour fan with a score range of 1–15 from light yellow to dark yellow. The height of thick albumen (H) and egg weight (W) were used to calculate Haugh units (HU) based on the formula of Haugh (1937):

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37}).$$

Microbial analysis

At the end of the experimental period, 10 hens from each treatment were randomly selected and slaughtered, and the small intestine (from the distal end of the jejunum to the ileocecal junction) was removed from each hen to determine the total number of aerobic bacteria, coliform bacteria and anaerobic bacteria (Clench and Mathias, 1995).

Biochemical parameters

Blood samples were collected at slaughter. Fresh blood samples were used to determine blood morphology, including white blood cell (WBC) count and their fractions (lymphocytes % and heterophils %). Plasma and serum were obtained by centrifugation of blood at 3 000 rpm for 20 min, and subsequently stored at −20 °C for biochemical analysis. Plasma total protein concentration (g/dl) was measured using the Biuret method, as described by Armstrong and Carr (1964). Albumin concentration (g/dl) was estimated calorimetrically and globulin concentration (g/dl) was calculated. Plasma total cholesterol concentration (mg/dl) was determined according to the method of Bogin and Keller (1987). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol concentrations (mg/dl) were determined according to the method described by Lopez-Virella (1977). Serum Ca and P concentrations were measured spectrophotometrically using available commercial kits. Biochemical components were determined using commercial kits manufactured by Diamond Diagnostics (29 Tahreer St. Dokki, Giza, Egypt). Total antioxidant capacity (TAC) was measured according to the method of Koracevic et al. (2001), superoxide dismutase (SOD) was determined as described by Nishikimi et al. (1972), and malondialdehyde (MDA) was measured according to Uohiyama and Mihara (1978). Immunoglobulin G (IgG) and M (IgM) titres were determined using ELISA. Serum samples were used to determine the concentrations of 17-β oestradiol (E_2) (Estradiol ELISA Test Kit with a sensitivity of 6.5 pg/ml) using immunoassay ELISA kits (Fortress Diagnostics Ltd, Antrim, UK).

Statistical analysis

Data were statistically analysed using one-way ANOVA implemented in SAS® software (SAS Institute, 2009). Variables showing significant differences were compared using Tukey's test (SAS Institute, 2009). The following statistical model was applied:

$$Yij = \mu + Ti + eij,$$

where: *Yij* – dependent variable, μ – overall mean, *Ti* – treatment effect and *eij* – random error.

Results

There were no significant ($P \geq 0.05$) differences in the average initial body weight, final body weight, egg weight and feed intake of hens between experimental treatments, but egg production, egg mass and feed conversion ratio improved (Table 2). Additionally, shell thickness, Haugh unit and yolk colour score were affected by quercetin supplementation (Table 3), while this supplementation did not affect yolk, albumen and shell percentage.

As shown in Table 4, the total count of aerobic, anaerobic and coliform bacteria in the intestine of laying hens decreased ($P \leq 0.001$) in all quercetin-supplemented groups compared to the control group. The total *Lactobacillus* count was higher in the treated groups compared to the control group.

Table 5 shows that lymphocyte (L) %, H/L ratio and serum immunoglobulin concentrations (IgG, IgM) were all affected by the addition of quercetin except for the WBC count and heterophil (H) %. Additionally, plasma TAC, SOD and MDA activities improved in all groups supplied with quercetin compared to the control group.

Table 2. Effect of dietary quercetin supplementation on productive performance in laying hens

Trait	Quercetin levels, mg/kg diet				*P*-value
	0	300	600	1200	
Initial body weight, g	1655 ± 30	1669 ± 22	1646 ± 25	1630 ± 24	0.116
Final body weight, g	1717 ± 21	1734 ± 29	1705 ± 31	1687 ± 35	0.641
Egg weight, g	49.35 ± 0.17	50.30 ± 0.24	50.34 ± 0.13	49.22 ± 0.36	0.074
Egg production, %	59.24 ± 0.27^{d}	63.32 ± 0.09^{b}	65.42 ± 0.24^{a}	60.45 ± 0.19^{c}	0.001
Egg mass, g/h/d	29.23 ± 0.14^{c}	31.85 ± 0.19^{b}	32.93 ± 0.06^{a}	29.75 ± 0.25^{c}	0.001
Feed intake, g/h/d	118.93 ± 0.21	118.22 ± 0.10	118.33 ± 0.21	118.85 ± 0.13	0.112
Feed conversion ratio, g/g	4.07 ± 0.02^{a}	3.71 ± 0.02^{b}	3.59 ± 0.13^{c}	3.99 ± 0.05^{a}	0.001

values are expressed as means ± standard error; $^{a–d}$ – means in the same row with different superscripts differ significantly ($P \leq 0.05$)

Table 3. Effect of dietary quercetin supplementation on productive performance in laying hens

Trait	Quercetin levels, mg/kg diet				*P*-value
	0	300	600	1200	
Shell, %	12.03 ± 0.14	12.67 ± 0.18	12.41 ± 0.21	12.23 ± 0.13	0.182
Shell thickness, mm	0.36 ± 0.0034^{c}	0.38 ± 0.0026^{b}	0.41 ± 0.0034^{a}	0.36 ± 0.0026^{c}	0.0001
Haugh unit, %	78.56 ± 1.7^{b}	87.25 ± 2.1^{a}	89.34 ± 2.4^{a}	79.17 ± 3.2^{b}	0.0004
Yolk, %	32.78 ± 0.30	31.36 ± 0.38	30.86 ± 0.34	31.56 ± 0.29	0.091
Albumen, %	55.19 ± 0.31	55.97 ± 0.28	56.73 ± 0.21	56.21 ± 0.21	0.114
Yolk colour score	6.24 ± 0.18^{b}	7.71 ± 0.18^{a}	7.63 ± 0.20^{a}	7.93 ± 0.29^{a}	0.002

values are expressed as means ± standard error; ^{abc} – means in the same row with different superscripts differ significantly ($P \leq 0.05$)

Table 4. Effect of dietary quercetin supplementation on intestine bacterial count [log10 (CFU/g)] in laying hens

Trait	Quercetin levels, mg/kg diet				*P*-value
	0	300	600	1200	
Total aerobic	15.70 ± 0.108^{a}	11.20 ± 0.114^{c}	10.34 ± 0.112^{d}	13.79 ± 0.106^{b}	0.001
Coliform	12.21 ± 0.102^{a}	7.78 ± 0.091^{b}	5.42 ± 0.104^{c}	8.15 ± 0.012^{b}	0.001
Total anaerobic	9.48 ± 0.075^{a}	7.11 ± 0.103^{b}	5.88 ± 0.062^{c}	9.35 ± 0.101^{a}	0.001
Lactobacillus spp.	10.83 ± 0.221^{b}	12.21 ± 0.110^{a}	12.83 ± 0.124^{a}	12.15 ± 0.113^{a}	0.052

CFU – colony-forming unit; values are expressed as means ± standard error; $^{a–d}$ – means in the same row with different superscripts differ significantly ($P \leq 0.05$)

Table 5. Effect of dietary quercetin supplementation on immune and antioxidant responses in laying hens

Trait	Quercetin levels, mg/kg diet				*P*-value
	0	300	600	1200	
WBCs, 10^3/mm^3	23.25 ± 0.20	24.36 ± 0.47	24.60 ± 0.53	24.07 ± 0.45	0.232
Lymphocytes, %	47.70 ± 1.04^{b}	53.86 ± 2.35^{a}	54.80 ± 2.5^{a}	48.06 ± 3.11^{b}	0.001
Heterophils, %	30.20 ± 1.56	28.60 ± 1.29	28.80 ± 1.62	29.40 ± 0.40	0.122
H/L ratio	63.32 ± 3.08^{a}	53.10 ± 3.64^{b}	52.55 ± 3.85^{b}	62.10 ± 3.74^{a}	0.004
IgM, mg/dl	154 ± 0.07^{c}	200 ± 0.07^{b}	282 ± 0.09^{a}	218 ± 0.11^{b}	0.000
IgG, mg/dl	362 ± 0.14^{c}	436 ± 0.22^{b}	536 ± 0.16^{a}	412 ± 0.12^{b}	0.000
TAC, mmol/l	0.512 ± 0.013^{d}	0.760 ± 0.017^{b}	0.830 ± 0.023^{a}	0.658 ± 0.019^{c}	0.000
MDA, µmol/ml	4.17 ± 0.14^{a}	3.50 ± 0.15^{b}	2.62 ± 0.15^{c}	3.30 ± 0.06^{b}	0.000
SOD, U/ml	17.08 ± 0.25^{d}	20.68 ± 0.19^{b}	22.67 ± 0.41^{a}	18.60 ± 0.39^{c}	0.000

WBCs – white blood cells, H/L ratio – heterophils to lymphocytes ratio, IgG – immunoglobulin G, IgM – immunoglobulin M, TAC – total antioxidants capacity, MDA – malondialdehyde, SOD – superoxide dismutase; values are expressed as means ± standard error; $^{a–d}$ – means in the same row with different superscripts differ significantly ($P \leq 0.05$)

Table 6. Effect of dietary quercetin supplementation on selected biochemical blood constituent in laying hens

Trait	Quercetin levels, mg/kg diet				*P*-value
	0	300	600	1200	
Cholesterol, mg/dl	187.96 ± 2.19^{a}	173.54 ± 2.40^{bc}	169.0 ± 1.10^{c}	177.10 ± 1.54^{b}	0.000
HDL, mg/dl	47.22 ± 0.66^{c}	55.98 ± 1.20^{b}	62.28 ± 1.40^{a}	49.88 ± 0.89^{c}	0.000
LDL, mg/dl	102.74 ± 2.99^{a}	92.20 ± 1.06^{b}	89.32 ± 0.57^{b}	100.20 ± 1.71^{a}	0.000
Total protein, g/dl	5.32 ± 0.27^{b}	6.30 ± 0.15^{a}	6.52 ± 0.20^{a}	6.06 ± 0.17^{a}	0.004
Albumin, g/dl	2.86 ± 0.20	3.02 ± 0.15	2.82 ± 0.15	3.02 ± 0.10	0.703
Globulin, g/dl	2.46 ± 0.08^{c}	3.28 ± 0.09^{b}	3.70 ± 0.13^{a}	3.04 ± 0.15^{b}	0.000
Oestradiol-17β, pg/ml	130.40 ± 3.08^{d}	164.0 ± 3.96^{b}	180.80 ± 3.40^{a}	145.20 ± 3.83^{c}	0.000
Ca, mg/dl	15.89 ± 0.30^{c}	16.86 ± 0.17^{b}	18.12 ± 0.33^{a}	17.50 ± 0.23^{ab}	0.000
P, mg/dl	5.42 ± 0.21^{c}	6.56 ± 0.19^{a}	7.08 ± 0.17^{a}	5.98 ± 0.12^{b}	0.000

HDL – high density lipoprotein, LDL – low density lipoprotein; values are expressed as means ± standard error; $^{a–d}$ – means in the same row with different superscripts differ significantly ($P \leq 0.05$)

Quercetin supplementation in laying hens' diet affected plasma total cholesterol, LDL, HDL, total protein, globulin, serum E2, Ca and P levels, but did not affect the concentration of plasma albumin (Table 6).

Discussion

The current study demonstrated that egg production, egg mass and feed conversion were significantly improved by quercetin administration (Table 2). Accordingly, the highest egg production was recorded for 600 mg quercetin/kg feed compared to control. It was evident that the treatment groups supplemented with quercetin had lower feed conversion, which was less apparent at the supplementation level of 600 mg/kg. However, the highest level of inclusion was equally effective as control. Our results indicated that quercetin supplementation at 300 and 600 mg/kg exerted beneficial effects on egg production, egg mass and feed conversion ratio. This observation was consistent with the findings of Liu et al. (2014), who reported that the inclusion of quercetin in the diet of laying hens increased egg production and decreased feed conversion at low levels of quercetin administration (0.2 and 0.4 g/kg). Similarly, feeding a diet supplemented with 400 mg/kg quercetin for 8 weeks significantly improved the laying rate and feed-egg ratio (6.5 and 22%, respectively), while no significant effects were observed for daily FI and egg weight (Yang et al., 2017). Moreover, Abid et al. (2020) found that the addition of low quercetin level (0.4 g/kg) to the ration for laying hens improved hen reproduction, which directly translated into increased production and egg quality; however high quercetin levels (800 or 1200 mg/kg) produced almost identical results to the control treatment. Quercetin is one of the main dietary flavonoids known as phytoestrogens (Lamson and Brignall, 2000) that have the ability to bind to oestrogen receptors and activate oestrogen-responsive genes in chickens (Dusza et al., 2006). Therefore, the improvement in egg production in the present study could be attributed to quercetin, as phytoestrogen may be involved in promoting steroidogenesis resulting in improved egg rate production in Mandarah laying hens. In the current study, the obtained serum oestradiol-17β levels (Table 6) supported this assumption.

The results listed in Table 3 indicated that supplementing quercetin in layer diets had a positive effect on egg quality. The Haugh unit is a well-known indicator of egg freshness and is related to shelf life, therefore it is the most useful index in assessing internal egg quality. The improvement in Haugh unit may indicate that quercetin supplementation improves egg quality by increasing their shelf life. On the other hand, the regulatory effect of quercetin on blood protein may be reflected in the release and precipitation of protein in egg, which in turn affects Haugh units (Abid and Ahmed, 2019a). The data showed that the yolk colour score was affected by dietary quercetin administration, which indicated that the yellowish pigment of quercetin could be responsible for the improved yolk colour. One interesting finding in the present study was that dietary quercetin supplementation significantly improved eggshell thickness, which is important for egg producers and consumer safety (Jones et al., 2002). This increase in eggshell thickness was probably related to higher serum calcium levels associated with phytoestrogens (Gu et al., 2013). On the other hand, phytoestrogens are involved in the regulation of carbonic anhydrase activity via oestrogen receptor-α and oestrogen receptor-β located in the shell gland (Wistedt et al., 2012). Our findings concerning improved egg quality were in line with the results of Ying et al. (2015), who

reported that dietary quercetin supplementation in layers' diets improved eggshell thickness, strength and Haugh unit; similar results were also obtained by Liu et al. (2013, 2014). Moreover, Amevor et al. (2021) observed that the addition of quercetin and a mixture of quercetin and vitamin E to the diet of aging hens resulted in significantly improved egg quality traits, including yolk colour, eggshell thickness and Haugh unit.

The data in Table 4 indicate that quercetin supplementation had a positive effect on intestinal microbiota. The total *Lactobacillus* count was increased ($P \leq 0.05$), while the total counts of aerobic, anaerobic and coliform bacteria were decreased in the treated groups compared to the control group. The lowest bacterial count was recorded at a dose of 600 mg quercetin/kg feed. These results were consistent with the findings of Liu et al. (2014), who observed that dietary quercetin supplementation (200, 400 and 600 mg/kg) improved caecal microflora while the total aerobic and coliform counts decreased with increasing quercetin levels in laying hens. Similarly, the supplementation of quercetin in broiler chicken diet at levels 200, 400 and 800 ppm resulted in a reduction in the total coliform count at 21 and 35 days of age and the total *Clostridium* count at 35 days of age, while *Lactobacillus* count increased in both age groups (Abdel-Latif et al., 2021). One of the mechanisms of quercetin action in improving performance and physiological status involves the reduction of harmful microbiota, which protects laying hens against pathogenic metabolites and free radicals, thereby increasing egg production and efficiency. Quercetin exerts a metabolic prebiotic effect due to its ability to modulate the gut microbiota, which in turn enhances the antioxidant activity of quercetin, which is reflected in birds' health (Lui et al., 2013).

The WBC count and heterophil (H) % were not affected by quercetin supplementation, while lymphocyte (L) % significantly increased at 300 and 600 mg/kg quercetin levels, but not at 1200 mg/kg. Furthermore, the addition of quercetin at 300 and 600 mg/kg feed significantly decreased the H/L ratio compared to the control group. Changes in the number of heterophils, both increase and decrease, were previously observed in various pathological conditions in poultry, and the H/L ratio is an indicator of the immune response (Koncicki and Krasnodebska-Depta, 2005). Thus, the immune response in the current study was enhanced with a decrease in the H/L ratio. Furthermore, the H/L ratio is an important indicator of poultry bird welfare (Table 5).

Dietary quercetin supplementation significantly increased serum immunoglobulin concentrations (IgG, IgM) in laying hens compared to the control group, and the highest value was recorded in the group fed 600 mg quercetin/kg feed (Table 5). This improvement could be due to the structure of quercetin, as flavonoids were shown to affect immune signalling pathways in chickens (Shin et al., 2011). Moreover, a previous study indicated that quercetin showed metabolic prebiotic effects, including enhancing immune system response. The current results were consistent with findings of Liu et al. (2019), who reported that the inclusion of quercetin and vitamin E in chicken diets increased IgA, IgG and IgM levels. The same result was observed by Yang et al. (2020) who found that the addition of quercetin to the diet enhanced the immunoglobulin response in a dose-dependent manner. Moreover, Amevor et al. (2021) demonstrated that supplementation with quercetin alone or in combination with vitamin E improved immunoglobulin (IgA, IgM, and IgG) concentrations in aging hens.

Plasma TAC and SOD values were elevated ($P \leq 0.0001$) at all levels of quercetin supplementation compared to control, but the highest increase was recorded at the 600 mg/kg dose. In contrast, plasma MDA levels decreased in all experimental groups in relation to the control group and the lowest value was recorded for 600 mg/kg. Previous studies have confirmed that quercetin has an antioxidant potential, i.e. it is capable of scavenging reactive oxygen species (Boots et al., 2008), alleviating H_2O_2– induced cell damage and reducing intracellular ROS (reactive oxygen species) levels (Chen et al., 2018). The antioxidant properties of quercetin may be due to the presence of a C-ring along with a high number of hydroxyl groups and conjugated orbitals (Rice-Evans et al., 1997). The present results concerning the increase in plasma TAC and SOD activity, and decreased MDA value were in agreement with the results obtained by Iskender et al. (2016), who reported elevated glutathione peroxidase (GSH-px) and SOD activity, and decreased MDA levels in laying hens fed diets containing 0.5 g quercetin/kg diet. Furthermore, Dong et al. (2020) demonstrated that dietary quercetin at a dose of 400 or 800 ppm limited the increase in serum MDA levels, liver ROS levels and ileal mucosa MDA levels induced by oxidised oil on day 11 in broilers.

The results regarding the effect of dietary quercetin supplementation on plasma total cholesterol, LDL and HDL concentrations in laying hens are presented in Table 6. Total cholesterol levels were

reduced ($P \leq 0.0001$) by the dietary treatment and the lowest value was recorded for the 600 mg/kg dose. Quercetin dietary supplementation in hens at 300 and 600 mg/kg, decreased plasma LDL levels, whereas there was no significant differences between 1200 mg/kg and the control group. Plasma HDL levels were increased in hens fed the basal diet supplemented with 300 and 600 mg quercetin/kg, while no significant differences in HDL values were observed between the highest dose of quercetin (1200 mg) and the control group. These results were consistent with the study of Iskender et al. (2017), who reported that the addition of quercetin to layers' diet (0.5 g/kg) decreased total cholesterol concentration. Qureshi et al. (2011) found that a mixture of quercetin and ɣ-tocotrienol (25 and 50 ppm) added to hens' diet decreased serum total cholesterol, LDL and triglyceride levels. According to Abid and Ahmed (2019b), quercetin dietary supplementation in laying hens (400, 800 and 1200 mg/kg) improved the lipid profile at 50 and 60 weeks of age, whereas total cholesterol, triglyceride, LDL and VLDL levels decreased, except for raising HDL level. The current results on the reduction of plasma cholesterol and LDL levels could be attributed to the regulatory effect of quercetin on cholesterol metabolism and synthesis, whereby quercetin may be associated with the inhibition of HMG-CoA reductase activity – the first step enzyme in cholesterol synthesis (Zhao et al., 2011). Moreover, quercetin may also regulate the expression of LDL receptor genes, leading to an increase in the clearance of circulating LDL cholesterol levels from the blood (Moon et al., 2012).

Plasma total protein and globulin levels were increased by the inclusion of quercetin in the diets of laying hens, while no significant differences in plasma albumin levels were observed between the experimental groups. The highest total protein and globulin levels were recorded for hens fed the basal diet with the addition of 600 mg quercetin/kg. Abid and Ahmed (2019b) supplemented quercetin to the diets of laying hens and also obtained increased serum protein and globulin levels in chickens at 50 and 60 weeks of age. On the other hand, these results contradicted previous findings of Ognik et al. (2016), who showed that quercetin supplementation in turkey hen diets did not exert any effect on plasma total protein and globulin levels. Our result concerning plasma albumin concentrations was consistent with findings of Kim et al. (2015), who demonstrated that the addition of quercetin to the diet of broiler chickens did not influence the level of albumin in blood. Higher plasma total protein and globulin levels recorded in the current study may be attributed to the effect of protein biosynthesis induction by quercetin.

Regarding serum E_2 levels, the result showed that quercetin supplementation of laying hens' diets increased serum E_2 concentration ($P \leq 0.0001$) compared to control, whereas, E_2 level at a dose of 600 mg/kg feed was higher than that at 300 and 1200 mg/kg feed. Our results were in line with the study of Yang et al. (2017), who found that the addition of quercetin at 400 mg/kg feed significantly increased E_2 levels in hens at 45 weeks of age. Similarly, Saleh et al. (2019) reported that dietary supplementation with phytoestrogen (flaxseeds or fenugreek seeds) elevated plasma oestradiol-17β levels in older laying hens. Our results suggested that the estrogenic activity of quercetin increased the secretion of this hormone in laying hens. The positive effects of quercetin in the present study may be attributed to its phytoestrogen properties that exert similar effects to oestrogen produced in the body. By binding to oestrogen receptors, phytoestrogens can stimulate or suppress hormones in a dose-dependent manner (Dusza et al., 2006).

As shown in Table 6, supplementation of quercetin to the layers' diet increased serum Ca and P levels compared to the control group. However, the highest values of Ca and P were recorded in the group supplemented with 600 mg quercetin/kg feed. Our findings were consistent with the results of Yan-li et al. (2013), who found that the addition of quercetin at doses of 200, 400 and 800 mg/kg significantly increased serum calcium content during the peak laying period and the late laying period. Moreover, Ognik et al. (2016) showed that quercetin supplementation to the feed for turkey hens at a dose of 200 g/tonne increased blood phosphorus content, while serum calcium level was not affected. An increase in blood calcium concentration due to quercetin may enhance calcium absorption from intestinal epithelium and stimulate the activity of vitamin D receptor (Inoue et al., 2010). Regarding the beneficial effect of quercetin as a polyphenolic compound on phosphorus availability, Ognik et al. (2016) demonstrated that polyphenolic compounds can prevent the formation of insoluble salts as a result of the reaction of phosphate ions with metals such as iron or copper by forming complex compounds with them, resulting in better phosphorus bioavailability.

Conclusions

It was concluded that the addition of quercetin to laying hens' feed improved egg production rate, feed conversion, egg quality, intestinal bacterial

count, antioxidant status and immune response at doses of 300 or 600 mg/kg, with 600 mg being more effective than the others. The high dose (1200 mg) had a similar effect to the control group. These positive results provided an important reference for quercetin as a promising functional feed additive in the modern poultry industry to mitigate the problems of the sector by improving production, health and welfare of chickens.

Conflict of interests

The Authors declare that there is no conflict of interest.

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