Effects of chitooligosaccharide supplementation on laying performance, egg quality, blood biochemistry, antioxidant capacity and immunity of laying hens during the late laying period

Qianqian Xu\textsuperscript{a}, Mahmoud Mostafa Mohammed Azzam\textsuperscript{b, c}, Xiaoting Zou\textsuperscript{a} and Xinyang Dong\textsuperscript{a}

\textsuperscript{a}Key laboratory for Molecular Animal Nutrition of Ministry of Education, College of Animal Sciences, Zhejiang University, Hangzhou, China; \textsuperscript{b}Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; \textsuperscript{c}Poultry Production Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt

ABSTRACT

The effects of chitooligosaccharide (COS) supplementation in laying hen diets on egg production, egg quality, blood biochemistry, antioxidant capacity and immunity during the late laying period were investigated in this 10-week trial. A total of 3000 Fengda No.1 laying hens, 52 weeks of age, were randomly allocated to three treatment groups, each of which included five replicates of 200 hens. Treatments consisted of the basal diet only or the basal diet supplemented with COS at 75 or 125 mg/kg. Hens fed with both COS supplemented diets had improved hen-day egg production, egg mass and feed conversion ratio than control birds. The higher level of COS supplementation exerted positive effects on eggshell strength and eggshell thickness, but significantly decreased yolk colour. Addition of 125 mg/kg COS was also found to significantly increase serum albumin content and significantly decrease serum cholesterol level. Dietary supplementation with COS significantly decreased serum triglyceride level compared with birds fed the control diet. Moreover, COS significantly increased total antioxidative capacity and significantly decreased malondialdehyde level in serum, but had no significance on the activities of antioxidant enzymes. The only change in blood immune response compounds observed in this study was a significantly increased concentration of C3 when laying hens were fed COS supplemented diets. In conclusion, COS could improve laying performance and egg quality in hens during the late laying period. Inclusion of COS in the diet might be beneficial to hens’ health by lowering serum lipids, enhancing antioxidant activity and slightly enhancing immune ability.

HIGHLIGHTS

- COS improved laying performance and egg quality in hens during the late laying period.
- Inclusion of COS in the diet might be beneficial to hens’ health by lowering serum lipids, enhancing the antioxidant activity and slightly enhancing immune ability.
- The optimum concentration of COS is 75 mg/kg in the basal diet in order to achieve an increased egg production, egg quality and health condition.

Introduction

Prebiotic is defined as a substrate that is selectively utilised by host microorganisms conferring a health benefit (Gibson et al. 2017). Recently, prebiotics are of great interest no longer limited to human food, but also have frequently been used in the economic animal industry as a favourable alternative to enhance animal performance and health (Markowiak and Śliżewska 2018; Pineda-Quiroga et al. 2019). Chitosan which has been studied as a prebiotic (Lee et al. 2002; Liu et al. 2018) has a great potential for application in various industries due to its biocompatibility, biodegradability and low toxicity characteristics (Liaqat and Eltem 2018). However, the insolvibility and high viscosity of chitosan limit its application in animals as a feed additive. Fortunately, chitooligosaccharide (COS), an oligosaccharide that is derived from chitosan (Lodhi et al. 2014), has low molecular weight, good solubility and low viscosity (Chae et al. 2005). Therefore, the application limitation of chitosan was overcome. Moreover, COS contains reactive functional groups (i.e. amino acids and hydroxyl groups), and...
hence possess multiple properties, such as antimicrobial (Holappa et al. 2006), anti-inflammatory (Ma et al. 2011), anti-oxidative (Yen et al. 2008), antitumor (Shen et al. 2009), immunostimulatory (Zaharoff et al. 2007) and hypocholesterolemic (Liu et al. 2008). Due to these properties, COS might be effective as a feed additive for farm animals.

In the field of animal production, supplementation of COS has been shown to have beneficial and biological effects in poultry and pigs. For example, dietary supplementation with 250 mg/kg COS improved growth performance in early weaned pigs by increasing serum growth hormone, upregulating insulin-like growth factor-I mRNA expression and enhancing protein synthesis (Tang et al. 2005). And iron-loaded COS nanoparticles reduce incidence of bacterial chondrocytosis with osteomyelitis in broiler chickens (Yousefi and Saki 2019). Furthermore, previous studies showed that dietary COS can increase nutrient digestibility and weight gain in broilers due to its antifungal and antimicrobial activities (Jeon et al. 2000; Huang et al. 2005). However, studies on the efficacy of dietary COS in the nutrition of laying hens are still limited, and the majority of them were conducted in laying hens during the peak production period (Nogueira et al. 2003; Meng et al. 2010; Yan et al. 2010; Swiatkiewicz et al. 2013). It is known that the performance and egg quality of laying hens in the late period of peak egg production is not as good as the younger ones. Thus researchers seek ways to improve performance of laying hens at this important period (Liu et al. 2013). To the best of our knowledge, so far there has been no other research on the effect of COS dietary supplementation in laying hens during the late laying period. Considering the prebiotic effects of COS (Liu et al. 2018), we hypothesised that COS could enhance performance and health of hens in the late laying period. Therefore, the objectives of conducting the work were to evaluate the effects of COS supplementation in laying hen diets on egg production, egg quality, blood biochemistry, antioxidant capacity and immunity during the late laying period.

Materials and methods

Birds and housing

Fengda No.1 hen is a Chinese indigenous breed, mainly in eastern China. The number of Fengda No.1 hens is more than 10 millions. Age at first egg of Fengda No.1 is 140–150 days. The egg number in 72 weeks of age is 270–280. The average egg weight is 50–51 g. And the feed conversion ratio (FCR) is 2.38–2.42 (Fang 2017). A total of 3000 Fengda No.1 hens, 52 weeks of age, were obtained from a commercial layer farm (Anhui Rongda Poultry Development Co., Ltd., Guangde, China). Hens were randomly allocated to three treatment groups, each of which included five replicates of 200 hens. Four hens were housed per cage under semicontrolled environmental conditions. The size of the cage was 50 × 55 cm (687.5 cm² per each bird). Birds were kept in three-layer complete ladder cages and fed twice daily at 07:00 and 14:00 h. Water was available from a nipple water system at all times. The photoperiod was 16 L:8 D throughout the experiment. The hens were allowed a 14-day adaptation period, and then the study lasted 10 weeks.

Diets and experimental design

The hens were randomly allotted to one of three dietary treatments. The diet formula is shown in Table 1. The experimental diets consisted of a control diet based on corn and soybean meal, and this diet further supplemented with COS at either 75 or 125 mg/kg. The levels of COS were selected according to reported literature (Huang et al. 2005; Yan et al. 2010). All essential nutrients contained in the basal diet met the requirements suggested by the NRC (1994) and the commercial farm (Anhui Rongda Poultry Development Co., Ltd., Guangde, China). The COS was prepared from chitin deacetylated to 90% and was provided by Zhejiang MingZhu Animal Health Products Co. Ltd, China. The average molecular weight of the COS supplement was 1000 Da and the water solubility was greater than 99%.

Table 1. Composition and nutrient levels of the basal diet (air-dry basis).

| Ingredients       | %          | Control diet |
|-------------------|------------|--------------|
| Corn              |            | 63.20        |
| Soybean meal      |            | 23.30        |
| Wheat bran        |            | 1.50         |
| Limestone         |            | 9.30         |
| Soybean oil       |            | 0.30         |
| DL-Methionine     |            | 0.10         |
| CaHPO4            |            | 0.80         |
| Premix*           |            | 1.50         |
| Calculated nutritional level, % |            | 15.57        |
| CP                |            | 11.03        |
| ME, MJ/Kg         |            | 3.42         |
| Calcium           |            | 0.33         |
| Total phosphorus  |            | 0.35         |
| Methionine        |            | 0.78         |
| Lysine            |            |              |

*Premix provided the following per kilogram of diet: retinyl palmitate, 3.96 mg; VD₃, 0.06 mg; VE, 36 mg; VB₁₂, 0.012 mg; NaCl, 3 g; menadione sodium bisulphite, 4 mg; riboflavin, 5.6 mg; thiamine mononitrate, 2 mg; niacin, 30 mg; pantethenic acid, 10 mg; folic acid, 0.5 mg; biotin, 0.22 mg; Mn, 80 mg; Zn, 60 mg; Fe, 80 mg; Cu, 10 mg; I, 0.55 mg; Se, 0.12 mg; choline, 250 mg.
Laying performance parameters and egg quality

Hen-day egg production and egg weight were recorded daily, and feed intake was recorded weekly on a replicate basis. Egg mass was calculated by multiplying egg weight by egg production. Feed conversion ratio was calculated as grams of feed intake per gram of egg mass produced. Mortality and health status were visually checked and recorded daily throughout the whole experimental period. The magnitude of performance parameters such as egg production was adjusted for hen mortalities.

At the end of the experiment, according to previous research on egg quality in laying hens (Li et al. 2018; Zhan et al. 2019), 30 eggs from each treatment (6 eggs per replication, 5 replications per treatment) were randomly collected to assess egg quality parameters. The eggs were weighed and cracked. Albumen height, Haugh units, yolk colour and eggshell strength were measured with the digital egg tester (DET-6000, NABEL, Kyoto, Japan). Eggshell thickness (without the shell membrane) was measured at the middle part of the egg.

Blood sampling and laboratory analyses

At the end of the 10-week experiment, 10 hens per treatment (2 hens per replication) were randomly selected, fasted for 12 h and blood samples were collected from the axillary vein. After centrifugation of blood at 3000 x g for 10 min, serum was separated and stored in 1.5-mL Eppendorf tubes at -80°C. Before analysis, the serum was thawed at 4°C.

Serum concentrations of Ca, P, alkaline phosphatase (ALP), lactic dehydrogenase (LDH), total protein (TP), albumin (ALB), glutamic-oxalacetic transaminase (AST), glutamic-pyruvic transaminase (ALT), cholesterol (CHO), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), glucose, total antioxidative capacity (T-AOC), total superoxide dismutase (T-SOD), CuZn-superoxide dismutase (CuZn-SOD), glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MDA) were measured spectrophotometrically (UV-2000, Unico Instruments Co. Ltd., Shanghai, China) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Calculated globulin (CG) was calculated by subtracting ALB from TP levels. Concentrations of Ca, P, Glucose, CHO, LDL-C, HDL-C and TG were expressed in millimoles per litre of serum. The ALP concentration was expressed in King Units per 100 millilitre of serum. Concentrations of LDH, T-AOC, T-SOD, CuZn-SOD, GSH-Px and CAT were expressed in units per litre or millilitre of serum. Concentrations of TP, ALB, CG and GSH were expressed in grams or milligrams per litre of serum. Concentrations of AST and ALT were expressed in international units per litre of serum. And the MDA concentration was expressed in nanomoles per millilitre of serum.

Serum concentrations of IgA, IgG, IgM, C3 and C4 were determined using standard curves constructed from the standards run on the plate. Concentrations of IgA, IgG, C3 and C4 were expressed in nanograms or micrograms per litre of serum. And the IgM concentration was expressed in micrograms per millilitre of serum.

Statistical analysis

The data were expressed as means ± SEM and analysed statistically by one-way ANOVA, using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL). When significant differences were found (p < .05), Tukey post hoc tests were performed.

Results

The laying performance of hens fed the different diets is presented in Table 2. There was no significance in feed intake or egg weight among three groups. However, dietary supplementation of 75 and 125 mg/kg of COS improved (p < .05) hen-day egg production, egg mass and FCR compared with birds fed the control diet. The hen-day egg production of birds fed 75

| Item                          | COS supplementation, mg/kg | SEM  | p   |
|-------------------------------|----------------------------|------|-----|
| Feed intake, g/hen/day        | 93.47                      | 0.35 | .059|
| Hen-day e.g. g production, %  | 67.83                      | 0.63 | .006|
| Egg weight, g                 | 52.91                      | 0.40 | .124|
| Egg mass, g/hen/day           | 35.89                      | 0.49 | .008|
| FCR                           | 2.60                       | 0.03 | .005|

COS: chitooligosaccharide; FCR: feed conversion ratio.

*Means sharing different letters in the same row are significantly different (p < .05).

Data are means of five replications with 200 hens per replicate.
and 125 mg/kg of COS increased by 3.77 and 4.50%, egg mass was enhanced by 5.02 and 6.38%, and FCR was improved by 3.46 and 5.00%, compared with the control, respectively.

Results of egg quality characteristics are shown in Table 3. Dietary supplementation of COS had no effect on albumen height or Haugh unit. However, the yolk colour was lower (p < .05) in hens fed the 125 mg/kg of COS compared with that of other groups. The eggshell strength of birds fed the control diet and 75 mg/kg of COS did not differ significantly, whereas the eggshell strength of birds in the 125 mg/kg of COS treatment was 10.22% greater (p < .05) than the control. The eggshell thickness of birds in the 75 and 125 mg/kg of COS treatments were higher (p < .05) than those of the control.

The effects of COS on serum biochemical indices are summarised in Table 4. Calcium, P, ALP, LDH, TP, CG, glucose, AST and ALT were not influenced by the dietary treatments. However, the concentration of ALB was enhanced (p < .05) by supplementation with 125 mg/kg of COS compared with the other groups.

The effects of COS supplementation on serum concentrations of lipids are illustrated in Table 5. The serum CHO levels of birds fed the control diet and 75 mg/kg of COS did not differ significantly, whereas the serum CHO levels for birds in the 125 mg/kg of COS treatment decreased (p < .05). Dietary supplementation with 75 or 125 mg/kg of COS decreased (p < .05) the serum TG levels compared with birds fed the control diet. No significant difference was observed in serum LDL-C or HDL-C levels among the three treatments.

Regarding the antioxidant status, increased (p < .05) T-AOC level and decreased (p < .05) MDA content were observed in the serum of birds fed 75 or 125 mg/kg of COS (Table 6). Moreover, The T-AOC in birds fed the 125 mg/kg of COS diet were greater (p < .05) than the values for birds fed the 75 mg/kg of COS diet. Dietary supplementation of COS had no effect on T-SOD, GuZn-SOD, GSH, GSH-Px and CAT.

### Table 3. Effects of chitooligosaccharide on egg quality in laying hensc.

| Item                  | COS supplementation, mg/kg | SEM | p Value |
|-----------------------|----------------------------|-----|---------|
| Albumen height, mm    | 5.25 75 125 0 0 0 0 0     | 0.25| 0.00   |
| Haugh units           | 72.69 74.60 69.66 3.00     | 0.25| 0.00   |
| Yolk colour, points   | 11.21 11.75 9.88 0.25      | 0.25| 0.00   |
| Eggshell strength, Kgf| 4.01 4.19 4.42 0.15        | 0.25| 0.00   |
| Eggshell thickness, mm| 0.35 0.37 0.37 0.01        | 0.25| 0.00   |

COS: chitooligosaccharide; Kgf: kilogram force.

### Table 4. Effects of chitooligosaccharide on serum biochemical indices in laying hens.

| Item                 | COS supplementation, mg/kg | SEM | p Value |
|----------------------|----------------------------|-----|---------|
| Ca, mmol/L           | 4.83 5.03 5.13 0.27        | 0.13| 0.001  |
| P, mmol/L            | 1.52 1.40 1.58 0.13        | 0.13| 0.034  |
| ALP, King Unit/100 mL| 15.80 17.00 17.70 0.73     | 0.73| 0.074  |
| LDH, U/L × 103       | 8.58 9.08 9.33 0.61        | 0.61| 0.486  |
| TP, g/L              | 34.83 35.13 35.88 1.06     | 1.06| 0.607  |
| ALB, g/L             | 18.25 19.92 23.10 1.44     | 1.44| 0.024  |
| CG, g/L              | 16.58 15.21 12.78 1.68     | 1.68| 0.128  |
| AST, IU/L            | 49.47 48.48 49.05 1.84     | 1.84| 0.865  |
| ALT, IU/L            | 15.51 15.62 16.24 0.65     | 0.65| 0.506  |
| Glucose, mmol/L      | 9.55 10.20 10.63 0.87      | 0.87| 0.490  |

ALB: albumin; ALP: alkaline phosphatase; ALT: glutamic-pyruvic transaminase; AST: glutamic-oxaloacetic transaminase; ALB: albumin; COS: chitooligosaccharide; LDH: lactic dehydrogenase; TP: total protein.

### Table 5. Effects of chitooligosaccharide on contents of serum lipids in laying hens.

| Item                  | COS supplementation, mg/kg | SEM | p Value |
|-----------------------|----------------------------|-----|---------|
| CHO, mmol/L           | 2.10 1.80 1.60 0.12        | 0.12| 0.014  |
| LDL-C, mmol/L         | 0.22 0.20 0.22 0.01        | 0.01| 0.228  |
| HDL-C, mmol/L         | 0.20 0.19 0.18 0.01        | 0.01| 0.304  |
| TG, mmol/L            | 18.01 14.22 12.12 0.72     | 0.72| 0.001  |

COS: chitooligosaccharide; CHO: cholesterol; LDL-C: high density lipoprotein cholesterol; HDL-C: low density lipoprotein cholesterol; TG: triglycerides.

### Discussion

Although several researchers have examined laying hen performance response to dietary supplementation of COS (Nogueira et al. 2003; Meng et al. 2010; Yan et al. 2010; Swiatkiewicz et al. 2013), studies on the effect of COS on production traits are continuing, especially regarding to hens at the period of post-peak egg production. In this study, improved production traits of Fengda No.1 layers (54–64 weeks of age), including higher hen-day egg production, increased egg mass, and improved FCR, were obtained in hens fed 75 or 125 mg/kg of COS. However, Meng et al. (2010) found that dietary supplementation of COS at concentrations of 200 and 400 mg/kg had no positive influence on laying intensity in Hy-Line brown layers.
Table 6. Effects of chitooligosaccharide on serum antioxidant capacity in laying hens.

| Item                  | COS supplementation, mg/kg | 0    | 75   | 125  | SEM | p Value |
|-----------------------|----------------------------|------|------|------|-----|---------|
| T-AOC, U/mL           |                            | 7.53 | 8.87 | 10.30| 0.42| .002    |
| T-SOD, U/mL           |                            | 131.4| 133.7| 141.2| 6.43| .312    |
| CuZn-SOD, U/mL        |                            | 114.17| 114.69| 117.93| 3.21| .490    |
| GSH, mg/L             |                            | 11.63| 11.27| 12.02| 0.69| .588    |
| GSH-Px, U/mL          |                            | 1432.21| 1503.26| 1560.67| 53.19| .130    |
| CAT, U/mL             |                            | 13.00| 13.21| 14.30| 0.72| .183    |
| MDA, nmol/mL          |                            | 7.60 | 6.63 | 5.17 | 0.41| .003    |

COS: chitooligosaccharide; CuZn-SOD: CuZn-superoxide dismutase; CAT: catalase; GSH: glutathione; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; T-AOC: total antioxidative capacity; T-SOD: total superoxide dismutase.

Table 7. Effects of chitooligosaccharide on immune ability in laying hens.

| Item        | COS supplementation, mg/kg | 0    | 75   | 125  | SEM | p Value |
|-------------|----------------------------|------|------|------|-----|---------|
| lgA, ng/L   |                            | 41.36| 53.34| 41.27| 5.16| .283    |
| lgG, ng/L   |                            | 70.22| 79.33| 80.94| 7.15| .363    |
| lgM, µg/mL  |                            | 2.67 | 3.04 | 2.97 | 0.39| .625    |
| C3, µg/L    |                            | 173.67| 235.33| 239.00| 19.85| .040    |
| C4, µg/L    |                            | 96.34| 110.50| 104.67| 11.07| .479    |

COS: chitooligosaccharide; C: complement; Ig: Immune globulin.

(27–34 weeks of age). Using the same hybrid, Yan et al. (2010) found that dietary inclusion of COS (100 and 200 mg/kg) linearly improved egg weight in layers (28–34 weeks of age), whereas no significant effect on egg production or FCR was observed. In a recent study with ISA Brown layers (26–55 weeks of age) fed a diet containing a high level of DDGS (20%), the addition of chitosan (0.01%) increased the number of eggs produced and daily egg mass (Swiatkiewicz et al. 2013). These discrepancies among studies could be due to differences in the addition level of COS, the species and physiological status of experimental animals used in different studies. In this study, COS supplementation did not exert a significant effect on feed intake, which indicated that the improvement in egg production could be partially attributed to increased nutrient digestibility. This assumption is supported by Swiatkiewicz et al. (2013), who confirmed that COS supplementation can increase the apparent digestibility of dry matter and nitrogen in laying hens. Considering the prebiotic effects of COS, the enhanced digestibilities of nutrients could be explained by the improved intestinal environment (decreased number of pathogenic bacteria and increased number of beneficial bacteria), reduced intestinal pH and enhanced activities of digestive enzymes in broilers (Huang et al. 2005; Liu et al. 2018; Baghban-Kanani et al. 2019). However, since studies on the efficacy of dietary COS in the nutrition of laying hens is limited, it is difficult to speculate as to the exact mechanisms whereby COS exerts its effects.

Previous studies on the egg quality in layers found that COS had positive influences on the internal egg quality characteristics such as yolk colour and Haugh units in layers during the peak production period, whereas no significant effect on eggshell quality indices was observed (Meng et al. 2010; Yan et al. 2010). Inconsistent with these results, our study showed that COS supplementation exerted negative effect on yolk colour, which was rather astonishing and need further study to give an explanation. Besides, in the current study, dietary supplementation with COS displayed significant beneficial effects eggshell quality reflected by the enhanced eggshell strength and increased eggshell thickness. In general, eggshell quality significantly decreased with increasing age of hens during the late laying period, which usually causes losses in animal production (Rodriguez-Navarro et al. 2002). Thus, the improved eggshell quality could mitigate these losses by reducing egg breakage rate, thereby improving economic returns in animal production. Because the metabolism of Ca is an important factor in the process of formation of the eggshell and the amine groups on chitosan serve as a chelation site for the metal ions (Gotoh et al. 2004). We assume the positive effect of COS on eggshell quality is probably connected with the stimulation of Ca availability. Unexpectedly, in the current study, dietary supplementation of COS had no effect on serum concentration of Ca as well as the activity of ALP, which played an important role in Ca uptake (Nagata et al. 1989). It suggests that COS might regulate eggshell quality via a Ca-independent pathway, but the specific mechanism warrants further research.

Serum biochemistry is considered to be a good indicator of health status. Measurement of such indicators is routinely conducted to evaluate the response of animals to various feed additives (Yan et al. 2010). In this study, treatment with high concentration of COS was found to increase the serum ALB content. Total protein, ALB and CG concentrations usually reflect the hepatic protein metabolic status. Laborde et al. (1995) suggested that the albumin fraction of total protein is an indicator of the long-term protein status. This result implies that dietary supplementation with COS can improve the whole-body protein anabolism in hens during the late laying period. High protein synthesis is a prerequisite for high production of eggs.
in hens. Therefore, more in-depth studies regarding the associations between COS and protein synthesis are needed to confirm if COS could increase egg production by improving whole-body protein anabolism in hens during the late laying period.

Serum concentrations of CHO, TG, LDL-C and HDL-C are parameters used to measure serum lipid levels (Ma et al. 2014). The lipid-lowering effect of COS has been documented in many studies. In the study by Li et al. (2007), 0.01% supplementation with COS in the broiler diet increased serum HDL-C and decreased serum TG and CHO concentrations in comparison with control treatment. And Keser et al. (2012) found that 0.025% supplementation with COS in the broiler diet reduced blood LDL-C concentration. It’s recognised that lower serum TG, CHO, LDL-C and higher serum HDL-C were beneficial for the health of animals (Liu and Kim 2018). Thus, the results obtained from this experiment confirmed the favourable effects of COS on lipid metabolism as reflected by decreased serum TG and CHO concentrations. According to previous studies, the most important mechanism by which COS eliminates CHO would likely be through the reduction of lipid absorption in the intestine by binding bile acids, resulting in increased CHO elimination (Świątkiewicz et al. 2015). Considering that decreased hepatic lipogenesis is normally associated with decreased TG concentrations in blood (Herzberg and Rogerson 1988), we speculate dietary supplementation of COS may decrease the serum TG concentration by inhibiting hepatic lipogenesis.

The antioxidant activity of COS has received much attention in recent years. Some reports showed that COS could enhance the antioxidant activity by reducing serum MDA concentration and elevating the activities of major antioxidant enzymes such as T-SOD, CAT and GSH-Px (Xia et al. 2011; Li et al. 2016). Our results showed that dietary COS significantly increased T-AOC and decreased MDA level in serum, whereas no significance was observed in the determined antioxidant enzymes. it suggests that COS could improve antioxidant status in laying hens by activating non-enzymatic antioxidant system rather than enzymatic antioxidant system. Thus, the antioxidant property of COS holds a great potential for the treatment of oxidative diseases in older laying hens.

Most immunostimulants stimulate the production of immune response compounds, such as globulin and complement proteins that activate the immune system after binding with cell surface receptor proteins of phagocytes or lymphocytes (Zou et al. 2016). Deng et al. (2008) demonstrated that dietary COS had a more pronounced influence on immune function than chlortetracycline and improved the immunity of chickens by increasing the IgM secretion. Huang et al. (2007) evaluated the effect of different dietary levels of COS on immune function in broilers, and found that dietary supplementation with COS increased serum concentrations of IgG, IgA and IgM, as well as enhanced immune organ development, with the greatest response at 0.10% COS addition. However, the only change in blood immune response compounds observed in the current study was an increased concentration of C3 when laying hens were fed 125 mg/kg of COS. On account of the description in a previous study (Chen et al. 2009) that COS supplementation did not influence immunity under non-challenge conditions, we speculate that the discrepancy among the aforementioned studies may possibly be due to the different hygienic conditions employed.

Conclusions

The results from the present experiment showed that COS supplementation in the diet improved laying performance and egg quality in hens during the late laying period. Moreover, supplementation with COS in the diet could lower serum lipids, enhance antioxidant activity and slightly enhance immune ability in hens during the late laying period in order to maintain body health. Therefore, COS might be considered as a new performance enhancer in hens during the late period of peak egg production as reflected by improved egg production and quality, and health condition. Our data suggest that the optimum concentration of COS is 75 mg/kg in the basal diet.

Ethical approval

The experimental protocol utilised in this research were complied with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Animal Science College of Zhejiang University (No. ZJU2013105002), Hangzhou, China.

Disclosure statement

The authors declare that they have no conflicts of interest.

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