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Effects of Chito-oligosaccharide Supplementation on Egg Production, Nutrient Digestibility, Egg Quality and Blood Profiles in Laying Hens

Q. W. Meng, L. Yan, X. Ao, H. D. Jang, J. H. Cho and I. H. Kim*

Department of Animal Resource and Science, Dankook University,
No.29 Anseodong, Cheonan, Choongnam 330-714, Korea

ABSTRACT : This study was conducted to evaluate the effects of dietary supplementation with chito-oligosccharide (COS) on egg production, nutrient digestibility, egg quality and blood profiles in laying hens. A total of 240 Hy-line Brown laying hens were randomly allocated into one of the following 5 dietary treatments: i) CON, basal diet; ii) ANT, basal diet+44 mg/kg avilamycin; iii) COS0.2, basal diet+200 mg/kg COS; iv) COS0.4, basal diet+400 mg/kg COS; v) ANTCOS, basal diet+200 mg/kg COS+22 mg/kg avilamycin. The experiment lasted for 6 wk. No change in egg weight ($p>0.05$) was observed during the trial period. Egg production in ANTCOS treatment was improved ($p<0.05$) when compared to CON during weeks 4-6. The birds in the COS0.2, COS0.4 and ANTCOS groups had higher ($p<0.05$) Haugh unit than those fed CON and ANT diets at the end of the 6th wk. The apparent digestibility of nitrogen in CON group was lower ($p<0.05$) than in other treatments. The white blood cell (WBC) concentration of birds in the COS0.4 and ANTCOS group was higher ($p<0.05$) than that of birds in other groups at the end of the 6th wk. In addition, the differences of WBC counts between the beginning and end of the experiment in COS0.4 and ANTCOS groups were higher ($p<0.05$) than in CON and ANT groups. At the end of the experiment, the birds fed ANTCOS diet showed higher ($p<0.05$) total blood protein concentration than those fed CON or ANT diets. In conclusion, dietary supplementation of COS appeared to increase egg production and quality by increasing nutrient digestibility. Additionally, COS improved WBC and total protein concentration. (**Key Words :** Laying Hen, Chito-oligosccharide, Egg Production, Egg Quality)

INTRODUCTION

In the past four decades, antibiotics have been used as feed additives to improve growth and egg production, and to protect animals from pathogenic microorganisms. The general ban on the use of feed antibiotics as microbial performance promoters that is expected to be introduced more widely throughout the world in the coming years, has increased the urgency for research on botanical feed additives (Best, 2000). Therefore, various oligosaccharides are now being added to livestock feed as prebiotics to improve animal health, production, and immune ability and to influence the gut microbiota (White et al., 2002; Lemieux et al., 2003). Certain oligosaccharides are considered to be prebiotic compounds because they are not hydrolyzed in the upper gastrointestinal tract and are able to favorably alter the colonic microflora (Biggs et al., 2007).

Chito-oligosaccharide (COS) is easily obtained by chemical and enzymatic hydrolysis of poly-chitosan which is the second most abundant carbohydrate polymer found in nature (Knaul et al., 1999). However, its application as a nutrient source for animals has been limited due to its insolubility and high viscosity. In contrast, COS has low molecular weight, good solubility and low viscosity (Chae et al., 2005).

COS has been shown to reduce the establishment of pathogens in the intestine (Li et al., 2006) and to enhance immune function in broilers (Wang et al., 2003). In addition, feeding COS to pigs has been found to increase nutrient digestibility (Rozeboom et al., 2005). However, data on the effect of COS in laying hens is still limited; only Spring et al. (2000) suggested that mannan-oligosaccharides (MOS) reduced intestinal *Salmonella* concentrations by 26% in broiler chicks compared with a control diet and subsequently improved growth performance from 0 to 21 d of age (Pelicano et al., 2004). Therefore, this study was conducted to further explore the effects of COS on egg production, nutrient digestibility, egg quality and blood profiles in laying hens.

* Corresponding Author: I. H. Kim. Tel: +82-41-550-3652, Fax: +82-41-565-2949, E-mail: inhokim@dankook.ac.kr
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MATERIALS AND METHODS

Preparation of COS

The COS used in this study, which was prepared and supplied by Easy Bio System, Inc. (Korea), was comprised of: 18.6% crude protein, 14.5% crude fat, 10.7% crude fiber, 22.6% crude ash, 9.0% moisture, 4.3% calcuim, 1.7% phosphorus, 4.0% chitin chitosan, and 3% chitosan oligosaccharide. The product was produced by microbial fermentation by *Aspergillus*, *Aspergillus oryzac*, *Bacillus subtillus*, *Saccharomyces cerevisiae* and *Lactobacillus acidophilus*.

Experiment design, animals and diets

A total of 240 Hy-line Brown laying hens (28-wk-old) were selected for a 6-wk feeding trial. Hens were randomly allocated into 5 dietary treatments with 4 replications per treatment according to a completely randomized block design. Each replication of 12 hens was assigned to six adjacent cages (38.1-cm width×50-cm length×40-cm height) providing two hens per cage. Therefore, each replication represented six cages in which the hens were fed from the same feed trough. The experimental diets were as follow: i) CON, basal diet; ii) ANT, basal diet+44 mg/kg avilamycin; iii) COS0.2, basal diet+200 mg/kg COS; iv) COS0.4, basal diet+400 mg/kg COS; v) ANTCOS, basal diet+200 mg/kg COS+22 mg/kg avilamycin. Hens were housed in a three-tier cage system. An environmentally controlled room was maintained at 21°C by a sensor which monitored the inside temperature and adjusted ventilation fans accordingly to control the temperature. Hens were maintained on a 17 h:7 h light:darkness photoperiod following light stimulation. Feed and water were provided *ad libitum*. All diets were formulated to meet or exceed the NRC (1994) requirements for laying hens. The composition of the experimental diet is shown in Table 1. The Animal Welfare Committee of Dankook University approved the animal care protocol used for this experiment.

Table 1. Diet composition (as-fed basis)

Ingredients	%
Corn	50.40
Soybean meal (CP 46%)	18.70
Wheat grain	10.00
Corn gluten meal	2.00
Wheat bran	5.00
Animal fat	4.40
Limestone	7.50
Tricalcium phosphate (P 18%)	1.40
Salt	0.30
DL-methionine (50%)	0.10
Vitamin premix[1]	0.10
Mineral premix[2]	0.10
Chemical composition[3]	
ME (kcal/kg)	2,904
Crude protein (%)	15.45
Lysine (%)	1.80
Methionine (%)	0.32
Calcium (%)	3.25
Phosphorus (%)	0.61

[1] Provided per kg of complete diet: 125,000,000 IU vitamin A 2,500,000 IU vitamin D_3 10,000 mg vitamin E 2,000 mg vitamin K_3 1,000 mg vitamin B_1 5,000 mg vitamin B_2 1,000 mg vitamin B_6 15 mg vitamin B_{12} 500 mg folic acid 35,000 mg niacin 10,000 mg Ca-Pantothenate and 50 mg biotin.

[2] Provided per kg of complete diet: 8,000 mg Mn 60,000 mg Zn 25,000 mg Cu 40,000 mg Fe; 300 mg Co; 1,500 mg I and 150 mg Se.

[3] Calculated values.

Sampling and measurements

At the initial of the experiment, 8 laying hens were randomly selected from each treatment (two hens in each replication) and blood samples were collected from the wing vein using a sterilized injector. Then, samples were transferred into either vacuum or K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The same laying hens were also sampled at the end of the experiment. For serum analysis, blood samples were centrifuged at 2,000×g at 4°C for 20 min within 1 h of collection to separate the serum. The total protein and albumin in the serum were analyzed using an automatic biochemistry blood analyzer (HITACHI 747, Hitachi, Japan). The concentrations of white blood cells (WBC), red blood cells (RBC) and lymphocytes in the whole blood samples were determined using an automatic blood analyzer (Advia 120, Bayer, Tarrytown, NY, USA).

Daily records of egg production and weekly records of feed consumption were maintained. Egg production was expressed as average hen-day production. A total of 30 salable eggs (no shell defects, cracks or double yolks) were randomly collected at 17:00 h from each treatment (5 per replicate, n = 30) on a weekly basis and used to determine the egg quality at 20:00 h at the same day. Eggshell breaking strength was evaluated using an eggshell force gauge, model II (Robotmation Co. Ltd., Japan). Eggshell thickness was measured on the large end, equatorial region, and small end using a dial pipe gauge (Ozaki MFG. Co. Ltd., Japan). Finally, egg weight, egg yolk color, and Haugh unit (HU) were evaluated using an egg multi-tester (Touhoku rhythm Co. Ltd., Japan).

After the conclusion of the feeding trial, six birds per treatment were randomly chosen for metabolic trials. The selected birds were individually housed in metabolic cages to determine the digestibility of nutrients. Laying hens were fed their respective diets containing chromic oxide (Cr_2O_3 at 0.20% level) for 4 d prior to the collection period. All excreta of the birds were collected daily for 3 d. All the

Table 2. Effects of chito-oligosaccharide on egg production in laying hens[1]

Items	CON	ANT	COS0.2	COS0.4	ANTCOS	SEM[2]
Egg production (%)						
0-3 wk	81.2	83.3	81.6	83.5	84.1	1.68
4-6 wk	81.5[b]	83.8[ab]	82.1[ab]	82.6[ab]	85.4[a]	1.72
Egg weight (g)						
0 wk	57.1	56.5	58.1	56.8	57.4	1.42
3 wk	58.9	58.7	61.5	59.2	60.4	1.72
6 wk	58.7	58.5	61.6	59.8	61.7	1.68

[1] CON = Basal diet; ANT = Basal diet+44 mg/kg avilamycin; COS0.2 = Basal diet+200 mg/kg chito-oligosaccharide; COS0.4 = Basal diet+400 mg/kg chito-oligosaccharide; ANTCOS = Basal diet+200 mg/kg chito-oligosaccharide+22 mg/kg avilamycin.
[2] Standard error of the means.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).

fecal samples along with feed samples, were then analyzed according to AOAC procedures (AOAC, 2000).

Statistical analyses

Statistical analysis was performed by using the GLM procedure in a completely randomized block design with the SAS software program (SAS Institute, 1996). Differences among all treatments were separated by Duncan's multiple range test. Results were expressed as the least squares means and SEM. Probability values less than 0.05 were considered significant.

RESULTS

Egg production

The effects of COS supplementation on egg production in laying hens are presented in Table 2. During weeks 4-6, ANTCOS treatment improved the egg production ($p<0.05$) compared to that of birds fed the control diet. In addition, no effects on egg weight were observed among dietary treatments.

Egg quality

The effects of COS supplementation on egg characteristics in laying hens are shown in Table 3. At the end of the 6th wk, birds in COS0.2, COS0.4 and ANTCOS groups had higher ($p<0.05$) HU as well as larger differences between the beginning and end of the experiment than those in CON and ANT. However, other criteria were unaffected by treatments through the experiment.

Nutrient digestibility

COS0.4 and ANTCOS treatments significantly improved DM digestibility ($p<0.05$) compared to CON treatment (Table 4). The digestibility of N in CON group was lower ($p<0.05$) than other treatments.

Blood profiles

The effects of COS supplementation on blood characteristics in laying hens are shown in Table 5. RBC, lymphocytes and albumin were not influenced by the dietary treatments. However, the WBC concentration of birds in the COS0.4 and ANTCOS group were higher ($p<0.05$) than that of birds in other groups at the end of the 6th wk. In addition, the difference in WBC counts between the beginning and end of the experiment in COS0.4 and ANTCOS groups were significantly higher ($p<0.05$) than that of CON and ANT groups. At the end of the experiment, the birds fed ANTCOS diet showed higher ($p<0.05$) total protein concentration than birds fed CON or ANT diets.

DISCUSSION

Egg production, egg weight and nutrient digestibility

Various oligosaccharides have been classified as prebiotics because of their beneficial effects on gut microflora, which improves health condition and product performance (Stanley et al., 1999; Berry and Lui, 2000). Similarly, previous studies also suggested that COS have antifungal (Hirano and Nagao, 1989) and antimicrobial (Jeon et al., 2000) activities that improved gut health in 196 day-old male broiler chicks (Li et al., 2007), which may promote nutrient digestibility and improve growth performance. In the present study, egg production was increased by the COS supplementation, which may be attributed to the observed improvement of DM and N digestibility in response to COS supplementation. Other study in our laboratory has also reported that DM and N digestibility was improved by COS supplementation in weanling pigs (Chen et al., 2009). Moreover, a considerable improvement in egg production in mannan-oligosaccharide (MOS)-fed broiler breeder hens was observed, which may suggest that COS is beneficial in chickens because of its similar structure to MOS. However, knowledge of the influence of COS supplementation on the laying hen appears limited and we have been unable to find any other study to confirm this result. Further study is needed to demonstrate the effect of COS supplementation in the laying hen.

Table 3. Effects of chito-oligosaccharide on egg quality in laying hens[1]

Items	CON	ANT	COS0.2	COS0.4	ANTCOS	SEM[2]
Egg shell breaking strength (kg/cm^2)						
0 wk	3.24	3.47	3.34	3.56	3.51	0.24
3 wk	3.52	3.29	3.45	3.46	3.53	0.26
6 wk	3.31	3.52	3.80	3.85	3.56	0.24
Difference (0-6 wk)	0.07	0.05	0.46	0.29	0.05	0.41
Egg shell thickness (10^{-2} mm)						
0 wk	35.7	35.1	35.4	35.5	35.2	0.46
3 wk	35.8	35.7	35.3	35.6	36.1	0.44
6 wk	36.1	36.6	36.2	36.3	36.5	0.32
Difference (0-6 wk)	0.40	1.50	0.80	0.80	1.30	0.76
Yolk color unit						
0 wk	8.68	9.00	8.70	8.79	8.90	0.26
3 wk	8.54	8.79	8.87	9.10	9.00	0.22
6 wk	8.75	8.91	9.01	9.11	8.98	0.29
Difference (0-6 wk)	0.07	-0.09	0.31	0.32	0.08	0.07
Albumin height (mm)						
0 wk	8.54	8.68	8.26	8.51	8.36	0.41
3 wk	8.31	8.71	8.56	8.64	8.47	0.36
6 wk	8.64	8.75	8.81	8.77	8.56	0.47
Difference (0-6 wk)	0.10	0.07	0.55	0.26	0.20	0.10
Haugh unit						
0 wk	89.7	90.1	88.6	89.4	90.5	1.89
3 wk	90.1	90.5	91.4	91.6	92.6	1.57
6 wk	90.5^b	90.2^b	92.7^a	92.9^a	93.6^a	1.28
Difference (0-6 wk)	0.80^b	0.10^b	4.10^a	3.50^a	3.10^a	1.09

[1] CON = Basal diet; ANT = Basal diet+44 mg/kg avilamycin; COS0.2 = Basal diet+200 mg/kg chito-oligosaccharide; COS0.4 = Basal diet+400 mg/kg chito-oligosaccharide; ANTCOS = Basal diet+200 mg/kg chito-oligosaccharide+22 mg/kg avilamycin.
[2] Standard error of the means.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).

HU is the measure used most commonly today (Williams, 1992) to measure albumen quality, and, consequently, to judge the freshness of an egg (Eisen et al., 1962). Results from the current study showed greater HU when laying hens were fed diets supplemented with COS, meanwhile, albumin height was also numerically increased in COS groups, which may indicate COS contributes to egg freshness.

Blood profiles

Blood profiles of animals reflect the physiological disposition of their nutrition according to their internal and external environments. Therefore, we measured these characteristics to determine the response by which COS influenced the laying hens. The only change in blood profiles observed in the current study was an increased concentration of WBC when laying hens were fed COS0.4 and ANTCOS diets. However, Chen et al. (2009) reported that 5 g/kg of COS supplementation added in the diet did not affect the concentration of WBC, RBC and lymphocyte as well as total protein in weaning pigs, but obvious effects were observed in the COS group when pigs were challenged with lipopolysaccharide. The variation between the aforementioned studies may also be ascribed to different

Table 4. Effects of chito-oligosaccharide on nutrient digestibility in laying hens[1]

Items	CON	ANT	COS0.2	COS0.4	ANTCOS	SEM[2]
Dry matter	72.1^b	74.9^{ab}	74.6^{ab}	76.8^a	77.4^a	1.25
Nitrogen	61.7^b	64.3^a	63.9^a	65.7^a	67.1^a	2.19

[1] CON = Basal diet; ANT = Basal diet+44 mg/kg avilamycin; COS0.2 = Basal diet+200 mg/kg chito-oligosaccharide; COS0.4 = Basal diet+400 mg/kg chito-oligosaccharide; ANTCOS = Basal diet+200 mg/kg chito-oligosaccharide+22 mg/kg avilamycin.
[2] Standard error of the means.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

Table 5. Effects of chito-oligosaccharide on blood profiles in laying hens[1]

Items	CON	ANT	COS0.2	COS0.4	ANTCOS	SEM[2]
WBC ($\times10^4$/ mm^3)						
0 wk	26.8	28.3	26.9	27.1	28.4	1.49
6 wk	28.7^b	29.1^b	30.2^b	32.7^a	32.9^a	1.89
Difference (0-6 wk)	1.90^b	0.80^b	3.30^{ab}	5.60^a	4.50^a	0.67
RBC ($\times10^6$/ mm^3)						
0 wk	2.38	2.24	2.17	2.23	2.26	0.08
6 wk	2.41	2.19	2.28	2.31	2.30	0.10
Difference (0-6 wk)	0.03	-0.05	0.11	0.08	0.04	0.04
Lymphocyte (%)						
0 wk	66.9	65.8	67.4	67.1	66.9	4.24
6 wk	68.9	69.1	68.7	70.2	71.4	4.98
Difference (0-6 wk)	2.00	3.30	1.30	3.20	4.50	0.89
Total protein (g/dl)						
0 wk	5.36	5.12	5.24	5.31	5.29	0.34
6 wk	5.46^b	5.50^b	5.71^{ab}	5.68^{ab}	5.91^a	0.56
Difference (0-6 wk)	0.10	0.38	0.47	0.37	0.62	0.07
Albumin (g/dl)						
0 wk	2.03	2.18	2.21	2.19	2.09	0.12
6 wk	2.19	2.29	2.26	2.46	2.51	0.11
Difference (0-6 wk)	0.16	0.11	0.04	0.27	0.42	0.04

[1] CON = Basal diet; ANT = Basal diet+44 mg/kg avilamycin; COS0.2 = Basal diet+200 mg/kg chito-oligosaccharide; COS0.4 = Basal diet+400 mg/kg chito-oligosaccharide; ANTCOS = Basal diet+200 mg/kg chito-oligosaccharide+22 mg/kg avilamycin.
[2] Standard error of the means.
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

aspect as well as the environmental conditions employed in each study, as highly healthy conditions can significantly affect the response to supplemented antibiotics. Therefore, the current study may indicate that COS supplementation at 0.4% level could improve the health of laying hens.

Furthermore, significant differences in total protein were observed in the ANTCOS treatment compared with the CON and ANT treatments at the end of the 6th wk, but total protein was not affected by COS supplementation in the current study. This is partially in agreement with Li et al. (2007), who suggested that dietary COS increased the serum total protein in broiler chicks. Moreover, study conducted by Zhou et al. (2009) also reported inclusion of COS increased the RBC concentration in broiler chicks. Therefore, COS supplementation indeed increased the immune related blood cell content in the current study. Meanwhile, no significant difference in total protein was observed in chickens fed the COS diet compared with the CON group. Therefore, the reason for the significant improvement detected in the ANTCOS may be due to the synergestic effect of COS with antibiotics.

CONCLUSION

In conclusion, the results from the current study indicated that dietary supplementation of COS at 200 or 400 mg/kg increased egg production, the immune blood cell counts as well as nutrient digestibility, indicating the COS can be used as a potential alterative to antibiotics in laying hens. However, it is suggested that further research is warranted to elucidate the mechanism by which COS improves the nutrient digestibility, egg quality and production in laying hens.

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