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Full Length Research Paper

Effects of dietary supplementation of chitosan on growth performance and immune index in ducks

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The effect of dietary chitosan on growth performance and immune index were investigated in ducks. One-day-old ducks were fed on a control diet or diets containing 0.00, 0.30, 0.60, 1.20, 2.40 and 4.80 g/kg of 85.20% deacetylated chitin (chitosan) or 0.45 g/kg aureomycin for 35 days. The results indicate that the ducks in 1.20 g/kg dietary chitosan group had the superior average daily body gain, average daily feed intake and feed conversion ratios, while the weight of immune organ and body weight adjusted weights for immune organs in 2.40 g/kg dietary chitosan group were higher than those in other groups. Also, lymphocyte proliferation of 1.20 and 2.40 g/kg were higher than those of other groups. It was concluded that 1.20 and 2.40 g/kg dietary chitosan were suitable for ducks to gain a better growth performance and immune function.

Key words: Chitosan, ducks, growth performance, immune function.

INTRODUCTION

Numerous shrimp and crab exoskeletons are discarded by restaurants and the seafood processing industry. Chitosan derived from the chitin is obtained from these discarded exoskeletons by deproteinization, demineralization and deacetylation (Knorr, 1991). It arouses interests because of its low side effects, promoting the growth performance, improving immune functions, inhibiting intestinal tract microbial population, stepping down cholesterol, etc. So, chitosan may have more potential to be utilized as feed additive in the future. A number of studies have been conducted on effective utilization of chitosan as an animal feed supplement. Razdan and Pettersson (1994) showed that 30.00 g/kg dietary chitosan reduced plasma lipid concentrations but resulted in decreased growth performance. However, in broilers given a low concentration of 0.50 to 1.00 g/kg dietary chitosan, growth rate was higher than in the control (Shi et al., 2005). These investigations may suggest that a high dietary chitosan could decrease fat digestibility, but low dietary chitosan results in improving growth performance. The objective of the present study was to evaluate the effect of chitosan on growth performance and immune index in Cherry Volley duck and to determine the appropriate supplemental dosage level of chitosan in ducks.

MATERIALS AND METHODS

A total of 336 one-day-old male Cherry Valley ducks were assigned randomly into 7 groups of 48 ducks each (each group with 6 replications and each replication has 8 ducks). Chitosan was supplemented as the basal diet (Table 1) in quantities of 0.00, 0.30, 0.60, 1.20, 2.40 and 4.80 g/kg, while the last group were fed with the diet supplemented with 0.45 g/kg aureomycin in the basal diet (CG = control group, G1 = group supplemented with 0.30 g/kg chitosan, G2 = group supplemented with 0.60 g/kg chitosan, G3 = group supplemented with 1.20 g/kg chitosan, G4 = group supplemented with 2.40 g/kg chitosan, G5 = group supplemented with 4.80 g/kg chitosan, AG = group supplemented with 0.45 g/kg aureomycin). The ducks were housed in wire pens under light at an average temperature of 30°C. Feed and water were provided *ad libitum.* Average daily body gain (ADG), average daily feed intake (ADFI) and feed conversion ratios (F/G) were measured weekly. At 35 days of the age, all ducks were weight and killed by decapitation under light anaesthesia with diethyl ether. The liver, gizzard, abdominal fat and heart were removed, weighed and the weight was recorded relative to the body weight. Chitosan was provided by Golden-Shell Biochemical Co., Ltd. Zhejiang, China. The degree of deacetylation was 85.20%.

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Table 1. Ingredients and nutrient composition of the diet (g/kg).

Item	Starter (1 to 21 day)	Finisher (21 to 35 day)
Ingredient		
Group maize	605	574
Soybean meal	195	150
Cottonseed meal	20	35
Rapeseed meal	20	35
Wheat bran	100	120
Fish meal	25	0
Rice bran		53
Calcium carbonate	6	8.5
Calcium phosphate tribasic	12	7
Salt	2	2.5
Vitamin / Mineral premix[1]	15	15
Calculated chemical component (g/kg)		
Crude protein	187.20	165.10
Metabolisable energy (MJ/kg)	12.08	11.75
Crude fiber	42.00	43.00
Crude fat	46.00	50.65
Calcium	8.50	7.50
Phosphorus	6.40	5.40

[1]Concentrate mixture including (per kg of diet): retinol 12 mg, pyridoxine 2.5 mg, cholecalciferol 0.02 mg, tocopherol 20 mg, nicotinic acid 50 mg, menadione 2 mg, pantothenic acid 12 mg, cyanocobalamin 12 µg, riboflavin 6 mg, biotin 0.30mg, folic acid 1.10 mg, choline 1500 mg, Fe 80 mg, Zn 100 mg, Cu 10 mg, Mn 40 mg, Se 0.3 mg, I 0.6 mg.

Immune index

Eight ducks (one duck from each replicate) from each group were weighed and killed by decapitation under light anaesthesia with diethyl ether at 7, 21 and 35 days. The thymus, spleen, and bursa of Fabricius (BF) were then collected. Adherent fat was removed, and the tissues were weighed. Body weight adjusted weight for thymus, spleen, and BF (mg/g) were determined.

Lymphocyte proliferation assay

3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay was carried out to determine the lymphocyte proliferation response to stimulation with the T-cell mitogen concanavalin A (10 µg/ml; Sigma Chemical Co., Shanghai, China) at the age of 7, 21 and 35 days. For this, heparinized blood samples (1 ml/ducks) were collected from 8 ducks from each group. Each blood sample was then added into isometric lymphocytes separation medium (Ficoll 1.077; Second Reagent Factory of Shanghai, China). Mononuclear leukocytes were isolated following a 25-min centrifugation at 1 500 × g. The mononuclear leukocytes fraction was then collected from the interphase. The cells were washed twice with RPMI 1640 (GIBCO, Shanghai, China), and the cell suspension was adjusted to 2×10^6 cell/ml. The cell suspensions (0.1 ml/well) were then cultured in 96-well microtiter plates with a predetermined optimal dose of 10 µg/ml of concanavalin A dissolved in RPMI 1640 (concanavalin A-stimulated cultures) or with the same volume of RPMI 1640 instead of concanavalin A (unstimulated cultures). Cells were incubated in a humidified incubator at 5% CO_2 and 39°C for 60 h. The cultures were then pulsed with 15 µL of 5 mg/ml MTT (Sigma Chemical Co.). After 4 h, 10% SDS with 0.01 N HCl (100 µL/well) was added to each well to lyze cells and dissolved the crystals. Two hours later, the cultures were allowed to equilibrate for 5 min at 25°C prior to spectrophotometric analysis at 570 nm (Bio-Rad, Beijing, China) to determine the optical density of each sample. The response to concanavalin stimulation was expressed as the optical density of concanavalin A-stimulated cultures minus the optical density of unstimulated cultures. This assay has previously been reported to induce maximal proliferation (Songlin et al., 2001).

Statistical analysis

All data were shown as means and analyzed by ANOVA using the General Linear model procedure of the SAS Institute (Stokes et al., 2000). The mean values of the 7 groups were only compared with each other at the same point (least significant difference assay). Statements of statistical significance are based on $P<0.05$.

RESULTS

Effects of dietary chitosan on growth performance in ducks

As shown in Table 2, ADG and ADFI increased quadratically with increasing addition of chitosan. ADG and ADFI in ducks fed with diet containing 1.20 g/kg chitosan were higher ($P<0.05$) than those in other groups.

Table 2. The effects of chitosan on growth performance in ducks.

Item	Group							SEM
	CG	G1	G2	G3	G4	G5	AG	
0 to 7 days								
ADG (g)	26.2^{b}	27.1^{b}	27.5^{ab}	28.2^{a}	28.0^{a}	26.9^{b}	27.0^{b}	0.57
ADFI (g)	40.7^{b}	41.5^{ab}	41.7^{ab}	42.2^{a}	42.0^{a}	41.6^{ab}	41.0^{b}	0.86
F/G	1.55^{a}	1.53^{ab}	1.52^{ab}	1.50^{b}	1.50^{b}	1.55^{a}	1.52^{ab}	0.02
8 to 21 days								
ADG (g)	42.3^{b}	43.0^{b}	43.5^{ab}	45.2^{a}	44.2^{a}	43.1^{b}	43.8^{ab}	1.23
ADFI (g)	68.0	69.0	70.0	71.2	69.5	70.0	69.6	3.36
F/G	1.61^{a}	1.60^{a}	1.61^{a}	1.58^{b}	157^{b}	1.62^{a}	1.59^{ab}	0.03
22 to 35 days								
ADG (g)	100.8^{b}	111.2^{b}	112.6^{ab}	123.5^{a}	123.0^{ab}	112.9^{ab}	112.7^{ab}	6.84
ADFI (g)	207.1	230.2	228.5	249.0	255.0	239.2	231.2	11.23
F/G	2.05^{a}	2.07^{a}	2.03^{ab}	2.02^{b}	2.07^{b}	2.12^{ab}	2.05^{ab}	0.06

ADG = Average daily body gain, ADFI = average daily feed intake, F/G = feed conversion ratios. CG = 0.00 g/kg chitosan group, G1= 0.30 g/kg chitosan group, G2 = 0.60 g/kg chitosan group, G3 = 1.20 g/kg chitosan group, G4 = 2.40 g/kg chitosan group, G5 = 4.80 g/kg chitosan group, and AG = 0.45 g/kg aureomycin group. Ducks were weighed on 0, 7, 21 and 35th day. Results are expressed as means. $^{a, b}$ Means at the same row without a common letter are different (P< 0.05); SEM means standard error of the mean.

Compared with the control group, ADG in 1.20 g/kg chitosan group were increased 7.63 (P<0.05), 6.86 (P<0.05) and 22.52% (P<0.05) at 0 to 7, 8 to 21 and 22 to 35 days, respectively; ADFI in 1.20 g/kg chitosan group increased 3.69 (P<0.05), 4.71 (P<0.05) and 20.22% (P<0.05) at 0 to 7, 8 to 21 and 22 to 35 days, respectively while F/G decreased quadratically with increasing addition of chitosan. Ducks supplemented with 1.20 g/kg chitosan had higher F/G than those of the other groups. Compared to the control group, F/G in 1.20 g/kg chitosan group decreased to 4.69, 1.16 and 2.59% at 0 to 7, 8 to 21 and 22 to 35 days, respectively. Compared to the control group, aureomycin supplementation could increase the growth performance; ADG and ADFI were elevated at 4.94 (P< 0.05) and 1.45 (P <0.05), respectively. There were no significant differences among the aureomycin supplemented group and other chitosan supplemented groups.

Effects of dietary chitosan on the weight of immune organ in ducks

Effects of dietary chitosan on the weight of immune organ in ducks are shown in Table 3. The weights of thymus in ducks from G4 were higher (P< 0.05) than those in ducks from CG, G1, G2 and AG for 7, 21 and 35 day. However, there were no differences in thymus weight among G2, G3 and G4 on the 7th day, G3, G4 and G5 on the 21st day and G3 and G4 on the 35th day. Spleen weight in ducks from G4 were higher than those in ducks from other groups, but there were no differences in spleen weight between CG and G4 on the 7th, G3 and G4 on the 21st and among G2, G3 and G4 on the 35th day. The weights of BF in ducks from G4 were higher than CG, G1 and AG. There were no differences in BF weight among G2, G3 and G4 on the 21st and G4 and G5 on the 35th day.

Effect of chitosan on body weight (BW)-adjusted lymphoid organ weights (mg/g of BW) in ducks

Effect of chitosan on body weight adjusted weights for thymus, spleen, and BF are shown in Table 4. Body weight-adjusted thymus weights (mg/g of BW) were higher in G4 ducks than in ducks from other groups on the 7th and 35th day. There were however no differences in BW-adjusted thymus weights among the CG, G2 and G3 on the 7th day. BW-adjusted thymus weights of G5's duck were the lowest among the tested ducks on the 7th and 21st day (Table 3). Body weight-adjusted spleen weights (mg/g of BW) were the highest in G4 ducks on 7th, 21st and 35th day, but there were no differences in BW-adjusted spleen weights among the ducks in G2, G3, G4, G5 and AG.

Effect of chitosan supplementation on ducks blood lymphocyte proliferation

As shown in Table 5, lymphocyte proliferation (optical density measurement) of G3 and G4 were higher than CG and low level chitosan supplemented groups (G1 and G2). Lymphocyte proliferation of G3 and G4 were higher (P <

Table 3. Effects of dietary chitosan on the weight of immune organ in ducks (g).

Item	Group							SEM
	CG	G1	G2	G3	G4	G5	AG	
The weight of thymus								
7 days	0.99^{b}	0.98^{b}	1.04^{ab}	1.06^{ab}	1.12^{a}	0.96^{b}	0.92^{c}	0.11
21days	4.04^{c}	4.10^{c}	4.64^{b}	5.01^{a}	4.96^{a}	5.00^{a}	4.24^{c}	0.32
35 days	6.51^{b}	6.89^{b}	6.96^{b}	7.70^{a}	7.72^{a}	6.59^{b}	5.93^{c}	0.35
The weight of spleen								
7 days	0.25^{ab}	0.21^{c}	0.23^{bc}	0.21^{c}	0.28^{a}	0.19^{c}	0.20^{c}	0.05
21 days	1.12^{c}	1.14^{c}	1.12^{c}	1.48^{ab}	1.61^{a}	1.19^{c}	1.04^{c}	0.09
35 days	2.96^{c}	3.22^{b}	3.47^{ab}	3.69^{a}	3.74^{a}	2.99^{c}	2.87^{c}	0.25
The weight of BF								
7 days	0.45^{c}	0.49^{b}	0.44^{c}	0.48^{b}	0.59^{a}	0.51^{b}	0.51^{b}	0.11
21 days	1.73^{b}	1.63^{c}	1.88^{ab}	2.09^{a}	2.00^{a}	1.58^{c}	1.92^{ab}	0.23
35 days	2.92^{c}	2.99^{c}	3.18^{bc}	3.37^{b}	3.94^{a}	3.61^{ab}	3.17^{bc}	2.29

CG = 0.00, G1= 0.30, G2 = 0.60, G3 = 1.20, G4 = 2.40, G5 = 4.80 g/kg chitosan group, and AG = 0.45 g/kg aureomycin group; BF= bursa of Fabricius. Ducks from each group were weight and killed by decapitation under light anaesthesia with diethyl ether at 7th, 21th and 35th day. The thymus, spleen, and BF were collected and weighed. Results are expressed as means. ^{a,b}Means at the same row without a common letter are different (P <0.05); SEM means standard error of the mean.

Table 4. Body weight adjusted weights for thymus, spleen, and BF (mg/g).

Age (day)	Group							SEM
	CG	G1	G2	G3	G4	G5	AG	
Body weight adjusted weight for thymus								
7	5.40^{b}	5.17^{bc}	5.40^{b}	5.37^{b}	5.71^{a}	4.87^{c}	5.10^{bc}	0.24
21	5.21^{b}	5.18^{b}	5.79^{ab}	6.04^{a}	6.08^{a}	5.29^{b}	6.32^{a}	0.22
35	2.98^{a}	2.93^{a}	2.93^{a}	3.01^{a}	3.04^{a}	2.49^{b}	2.78^{ab}	0.28
Body weight adjusted weight for spleen								
7	1.37^{a}	1.12^{b}	1.21^{ab}	1.09^{b}	1.43^{a}	1.07^{b}	1.03^{b}	0.12
21	1.45^{b}	1.44^{b}	1.39^{b}	1.78^{a}	1.98^{a}	1.30^{c}	1.51^{b}	0.18
35	1.35^{ab}	1.37^{ab}	1.46^{a}	1.44^{a}	1.47^{a}	1.21^{b}	1.26^{b}	0.15
Body weight adjusted weight for BF								
7	2.47^{bc}	2.56^{bc}	2.30^{c}	2.45^{bc}	3.00^{a}	2.69^{b}	2.70^{b}	0.20
21	2.23^{ab}	2.06^{b}	2.34^{ab}	2.51^{a}	2.45^{a}	2.40^{ab}	2.00^{b}	0.17
35	1.34^{b}	1.27^{b}	1.34^{b}	1.32^{b}	1.55^{a}	1.33^{b}	1.52^{a}	0.16

CG = 0.00 g/kg chitosan group, G1= 0.30 g/kg chitosan group, G2 = 0.60 g/kg chitosan group, G3 = 1.20 g/kg chitosan group, G4 = 2.40 g/kg chitosan group, G5 = 4.80 g/kg chitosan group, and AG = 0.45 g/kg aureomycin group; BF= bursa of Fabricius. Ducks from each group were weighed and killed by decapitation under light anaesthesia with diethyl ether at 7, 21 and 35 days. The thymus, spleen, and BF were collected and weighed, body weight adjusted weights for thymus, spleen, and BF (mg/g) were determined. Results are expressed as means. $^{a, b}$ Means at the same row without a common letter are different (P < 0.05); SEM means standard error of the mean.

0.05) than CG, G1 and G2 on the 7th, 21st and 35th day. There were no significant differences among CG, G1 and G2 on the 7th and CG, G1, G2 and CG on 21st and 35th day. As shown in Table 5, Lymphocyte proliferations of the chitosan supplemented groups were not lower than the AG.

DISCUSSION

Many researchers studied the effective utilization of chitosan as an animal feed supplement. Kobayashi et al. (2002, 2006) reported there was no effect on growth performance of broiler chickens fed on a 50 g/kg chitosan

Table 5. Effect of chitosan supplementation on blood lymphocyte proliferation in ducks.

Age (day)	Group							SEM
	CG	G1	G2	G3	G4	G5	AG	
7	0.306	0.322	0.368	0.412^{A}	0.405^{a}	0.395	0.409^{a}	0.000
21	0.416	0.466	0.485	0.525^{A}	0.527^{A}	0.516	0.509	0.006
35	0.448	0.501	0.522	0.535	0.542	0.533	0.526	0.022

CG = 0.00 g/kg chitosan group, G1= 0.30 g/kg chitosan group, G2 = 0.60 g/kg chitosan group, G3 = 1.20 g/kg chitosan group, G4 = 2.40 g/kg chitosan group, G5 = 4.80 g/kg chitosan group, and AG = 0.45 g/kg aureomycin group. Results were expressed as means of the optical density (570 nm). Mean values in a row without the same superscript small letter are different ($P < 0.05$), while those without the same superscript capital letters are significantly different ($P < 0.01$).

diet. Razdan and Pettersson (1994) also showed that 30.00 g/kg dietary chitosan decreased growth performance. However, given a low concentration of 0.50 to 1.00 g/kg, dietary chitosan could gain superior performance than the control groups in broilers (Shi et al., 2005; Khambualai et al., 2008, 2009). In the present study, ADG, ADFI and F/G of the ducks were varying quadratically with the chitosan level in diet. Ducks fed on a 1.20 g/kg chitosan diet gained the superior performance compared with those in other groups. It may be assumed that such a discrepancy is induced by the amount of chitosan in diet (Khambualai et al., 2008). High dietary concentration of chitosan might raise the viscosity and reduce motility in the gastrointestinal tract (Razdan and Pettersson, 1994), thus, resulting in the inhibition of digestive enzyme. However, low concentration of dietary chitosan could elevate nitrogen utilization and amino acid digestibility (Shi et al., 2005) and activate the intestinal villi and the epithelial cells (Khambualai et al., 2008).

Certain polysaccharides have been described to act as potent immunomodulators with specific activity for both T cells and antigen-presenting cells such as monocytes and macrophages. Chitosan is a polysaccharide and so believed could modulate the immune function. The index of immune organs reflected the growing development of thymus, spleen, and BF, which was used to estimate the immune state of birds. In broilers, giving a low concentration of dietary chitosan could increase the weight of immune organs and the BW-adjusted lymphoid organ weights (Zhu et al., 2003; Wang et al., 2003; Shi et al., 2005). In our study, the weight of lymphoid organ and BW-Adjusted lymphoid organ weights were raised in all age stages and all treatment groups when the dietary chitosan level was not exceeding 1.20 mg/kg, which was similar to the predecessors. Lymphocyte proliferation test was another index to estimate the function of chitosan on cellular immunity. Our study investigated the proliferation of peripheral blood lymphocytes of ducks from the chitosan dietary groups, aureomycin group and the control group. The results show a marked increase in proliferative activity in response to dietary chitosan and aureomycin groups compared with the control group. Hence, chitosan could influence the development and function of immune organs, resulting not only in heighten relative weight but also an elevation in cell and antibody production (Gill et al., 1998).

CONCLUSION

The dietary chitosan supplementation therefore provided further insight into the role of growth performance and immune system development in ducks. As expected, most of the effects of dietary chitosan were enhancement of performance and improving the immune function macroscopically. The mechanisms of dietary chitosan on the B-cell and T-cell compartments needs to be further investigated, especially in avian species.

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