Effects of dietary supplementation of chitosan on immune function in growing Huoyan geese

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ABSTRACT This present experiment was performed to investigate the effects of dietary supplementation of chitosan (**CS**) on immune function in growing Huoyan geese. A total of 320 28-day-old healthy growing Huoyan geese (sex balance) with similar body weight were randomly allotted into control, CS_{100}, CS_{200}, and CS_{400} groups. Each group includes 4 replicates with 20 geese per replicate, and the feeding trial lasted for 4 wk. The 4 diets contained 0, 100, 200, and 400 mg CS per kg feed, respectively. The results showed that compared with the control group, the relative weight of thymus, serum concentrations of IGF-I, INS, GH, T_3, T_4, IgM, IgG, IgA, complement C3, and IL-2 in CS_{200} group were significantly higher at both 42 and 56 D of age, respectively ($P < 0.05$). In addition, relative weight of bursa of fabricius (**BF**), spleen, serum complement C4, and TNF-a concentrations in CS_{200} group were higher at 56 D of age ($P < 0.05$), no differences were observed at 42 D of age ($P > 0.05$). These results indicated that addition of 200 mg/kg CS enhanced immune organs weight, serum concentrations of immunoglobulins, complements, hormone, as well as cytokines, and improved immune function of growing Huoyan geese.

Key words: chitosan, growing Huoyan goose, immune organ weight, serum immunoglobulins, immune function

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INTRODUCTION

As traditional feed additives, the use of antibiotics can result in residues and bacterial resistance in animal products (Hu et al., 2018). Whereas, chitosan (**CS**) is characterized by non-toxicity, no side effects, no residue and resistance, biodegradability, and good histocompatibility, which derived by the deacetylation of chitin from shells of arthropods (Singla and Chawla, 2001). Meanwhile, CS is widespread and the second most abundant carbohydrate polymer in nature (Knaul et al., 1999). Thus, it is widely used as a dietary additive in livestock due to its beneficial biological properties which alternating to antibiotics. Numerous studies showed that CS plays very important roles, such as promoting animal growth performance (Yang et al., 2012; Hu et al., 2018), regulating fat metabolism (Chiu et al., 2017; Liu et al., 2018), immunity modulation (Chou et al., 2003; Gopalakannan and Arul, 2006; Cha et al., 2008), anti-microbial (Ma et al., 2017; Tsai et al., 2004), antiviral and so on.

Previous research indicated that non-specific immunostimulants are very necessary and useful for improving immunity and disease resistance of animals (Guo et al., 2003). Because CS amino groups are recognized by the immune system, it can stimulate the serum production of circulating antibodies and improve immune response in animals (Li et al., 2015; Tokura et al., 1999). Growing evidences have demonstrated that CS could be used as an immunostimulant for animals (Yoon et al., 2008; Kong et al., 2014). David et al. (2007) observed that CS enhanced antigen-specific serum IgG titers and antigen-specific splenic $CD4^+$ proliferation of mice, and induced both humoral and cell-mediated immune responses. Chi et al. (2017) found that chitosan oligosaccharides (**COS**) increased relative weights of immune organs, and percentages of G_2/M phase thymocytes in broilers. Huang et al. (2007) reported that dietary supplementation with 100 mg/kg COS increased serum concentrations of IgG, IgA, IgM, and immune organ development of broilers, which suggested COS can improve immune response in birds. Li et al. (2015) observed that addition of 500 mg/kg CS affected humoral and cellular immune responses, and improved the antioxidative function in beef cattle. Additionally, results of the study on pig and chick indicated that CS promoted immune function, and improved serum antibody titer (Wang et al., 2003; Tang et al., 2005). Similar results were also reported on dry dairy cows, broilers and weaned piglets (Liu et al., 2007; Li, 2009; Li et al., 2013).

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Table 1. Ingredients and nutrient levels of the basal diets (air-dry basis).

Ingredients, %		Nutrition levels[b], %	
Corn	66.75	ME (MJ/kg)	11.55
Wheat bran	15.00	Crude protein	15.00
Soybean	8.99	Calcium	0.80
Fish meal	3.0	Available phosphorus	0.40
Limestone	0.18	Lysine	0.95
$CaHPO_4$	0.67	Methionine+Cystine	0.67
DL-Methionine	0.08	Threonine	0.16
L-lysine-HCL	0.33		
Premix[a]	5.00		
Total	100		

[a]Premix supplied per kg: 30,000 IU of vitamin A; 5,000 IU of vitamin D_3; 20 IU of vitamin E; 38 mg of vitamin K_3; 5 mg of vitamin B_1; 10 mg of vitamin B_2; 60 mg of nicotinamide; 5 mg of vitamin B_6; 10 mg of D-calcium pantothenate; 3 mg of pyridoxol; 0.1 mg of biotin; 1,000 mg of choline; 1 mg of folic acid; 20 μg of vitamin B_{12}; 5 mg of Cu; 100 mg of Fe; 80 mg of Mn; 100 mg of Zn; 0.1 mg of Se (Na_2SeO_3); 0.15 mg of Co (LCO_4); 0.4 mg of I (KIO_3).
[b]Calculated values.

The Huoyan goose is one of the most famous local goose spices which is characterized by excellent egg laying performance, small body size, high early growth rate, fresh meat quality, and good resistance with crude feed. Numerous evidences demonstrated that CS can improve the immune function in pigs, broiler chickens, beef cattle, cow, as well as mice. Whereas, little is known about the effect of CS on serum immune parameters, hormone concentrations, and relative weight of immune organs in growing Huoyan geese. Hence, the present experiment was aimed to investigate the effects of dietary supplementation of CS on immune function in growing Huoyan geese, and provide a scientific basis for the rational dietary addition of CS in growing Huoyan geese.

MATERIALS AND METHODS

Experimental Design and Diets

All bird handing protocols in this study was approved by the Animal Care and Use Committee of Henan Institute Science and Technology (Xinxiang, PR China). A total of 320 (28 D of age, sex balance) healthy growing Huoyan geese with similar BW were randomly divided into 4 groups (control, CS_{100}, CS_{200}, and CS_{400} groups, respectively). Each group includes 4 replicates with 20 geese per replicate. The control group was fed a basal diet without CS, and CS_{100}, CS_{200}, and CS_{400} groups were fed the same basal diets contained 100, 200, and 400 mg/kg CS, respectively. The basal diet was formulated to meet or exceed the National Research Council (NRC, 1994) nutrient requirements for growing geese. The feeding trail lasted for 4 wk, and the composition and nutrient levels of the basal diet is shown in Table 1. The CS in present study was purchased from Shanghai Lanji technology development Co., Ltd (Shanghai, PR China) with more than deacetylation degree of 90.00%, and less than viscosity of 100 cps. All birds were reared in the same condition, and had ad libitum access to an experimental diet and water via nipple drinkers.

Blood Samples Collection

At 42 and 56 D, 8 experimental geese with similar BW in each group (2 geese per pen, sex balance) were randomly selected to collect blood, respectively. Blood samples were collected and stored according to the methods of Li et al. (2017). Briefly, approximately 2 mL blood was taken immediately via the wing vein from each experimental goose, and allowed to clot for overnight at 4°C. Serum was harvested following centrifugation (3,000 g for 10 min, at 4°C) and then stored at −80°C until analysis.

Relative Weights of Immune Organs

On the last day of each phase, 8 Huoyan geese with average BW in each group were selected to weight, slaughter, and necropsy, respectively. Thymus, spleen, and bursa of fabricius (**BF**) from each goose were weighted, and calculated relative immune organs weight according to the methods of Chi et al. (2017). The formula is as follows:

$$\text{Relative organs weight} = \text{Fresh organ weight (g) /Body weight (kg)}$$

Serum Hormones and Growth Factors

At 42 and 56 D, serum of experimental geese was used to measure insulin-like growth factor I (**IGF-I**), triiodothyronine ($\mathbf{T_3}$), thyroxine ($\mathbf{T_4}$), growth hormone (**GH**), and insulin (**INS**) using the RIA kits (Beijing North Institute of Biotechnology, Beijing, China) in a Gamma-counter (Packard 8500, Packard Instrument Co., Downers Grove, IL, USA).

Serum Immunoglobulins

At 42 and 56 D, serum IgM, IgA, and IgG concentrations of experimental geese were done by goose immunoglobulin ELISA kits which were purchased from Beijing Gersion Bio-Technology Co., Ltd (Beijing, PR China).

Serum Complement C3, C4, and Cytokines

Serum cytokines interleukin (**IL**)-2, IL-4, IL-6, complement C3, C4, and tumor necrosis factor α (**TNF-α**) concentrations were determined using a commercial goose ELISA kit (Shanghai, Antix Biotech Ltd., Co., Shanghai, China) according to the manufacturer's instructions.

Table 2. The effects of chitosan on the relative weight of immune organs in growing Huoyan geese (g/kg).

Item	Groups				SEM	*P*-value
	Control	CS_{100}	CS_{200}	CS_{400}		
42 D						
Thymus	1.99^{b}	$2.67^{a,b}$	3.34^{a}	$2.96^{a,b}$	0.042	0.021
BF	1.54	1.74	1.81	1.61	0.069	0.148
Spleen	1.59	1.75	1.85	1.81	0.077	0.227
56 D						
Thymus	1.86^{b}	2.31^{b}	3.21^{a}	$3.13^{a,b}$	0.053	0.019
BF	1.38^{b}	$1.51^{a,b}$	1.92^{a}	$1.48^{a,b}$	0.025	0.036
Spleen	1.53^{b}	$1.72^{a,b}$	2.12^{a}	$1.83^{a,b}$	0.051	0.044

In the same column, values with different small letter superscripts mean significant difference ($P < 0.05$). BF, bursa of fabricius.

Statistical Analysis

Statistical analysis of variance (ANOVA) were performed using the one-way ANOVA procedure of SPSS 17.0 (SPSS, 2004). Significant differences among all treatment means were measured at $P < 0.05$ by Duncan's multiple range tests. All data were presented as mean±SEM (standard error of the means).

RESULTS

Relative Weights of Immune Organs

The effects of dietary supplementation of CS on the relative weight of immune organs in growing Huoyan geese are shown in Table 2. Compared with the control group, the CS_{200} group had higher relative weight of thymus at the age of 42 D ($P < 0.05$) and 56 D ($P < 0.05$), respectively. No significant differences were determined among experimental groups (*CS_{100}, CS_{200}, and CS_{400} groups*) at 42 D of age ($P > 0.05$), and between CS_{200} and CS_{400} groups at 56 D of age ($P > 0.05$), respectively. In addition, the relative weight of BF and spleen in the CS_{200} group was significantly higher than that in the control group at 56 D of age ($P < 0.05$), While, no significant differences were found among experimental groups ($P > 0.05$). Meanwhile, there were no significant differences among all groups (*control, CS_{100}, CS_{200}, and CS_{400} groups*) at 42 D of age ($P > 0.05$).

Hormone and Growth Factors

The effects of dietary CS on serum hormonal levels in growing Huoyan geese are shown in Table 3. The geese in CS_{200} group had higher serum concentrations of IGF-I, INS, and T_4 compared with those in the control group at both 42 and 56 D of age ($P < 0.05$). No differences were observed among experimental groups ($P > 0.05$). In addition, serum GH and T_3 levels in CS_{200} group were significantly higher than those in the control, CS_{100}, and CS_{400} groups at both 42 and 56 D of age ($P < 0.05$). No differences were found among the control, CS_{100} and CS_{400} groups ($P > 0.05$).

Table 3. Effects of chitosan on serum hormonal concentrations in growing Huoyan geese.

Item	Groups				SEM	*P*-value
	Control	CS_{100}	CS_{200}	CS_{400}		
42 D						
IGF-I (ng/mL)	58.17^{b}	$63.94^{a,b}$	67.47^{a}	$63.18^{a,b}$	2.10	0.037
GH (ng/mL)	0.75^{b}	0.97^{b}	1.25^{a}	0.72^{b}	0.026	0.043
INS (μmol/mL)	5.79^{b}	$6.03^{a,b}$	7.32^{a}	$6.83^{a,b}$	0.154	0.039
T_3 (ng/mL)	0.69^{b}	0.77^{b}	1.09^{a}	0.76^{b}	0.031	0.049
T_4 (ng/mL)	1.37^{b}	$1.77^{a,b}$	1.95^{a}	$1.64^{a,b}$	0.037	0.041
56 D						
IGF-I (ng/mL)	59.01^{b}	$64.44^{a,b}$	68.12^{a}	$65.21^{a,b}$	3.21	0.042
GH (ng/mL)	0.78^{b}	1.04^{b}	1.41^{a}	1.11^{b}	0.083	0.047
INS (μmol/mL)	6.09^{b}	$7.11^{a,b}$	7.82^{a}	$7.23^{a,b}$	0.144	0.022
T_3 (ng/mL)	0.61^{b}	0.72^{b}	1.14^{a}	0.81^{b}	0.015	0.031
T_4 (ng/mL)	1.59^{b}	$1.92^{a,b}$	2.15^{a}	$1.88^{a,b}$	0.064	0.028

In the same column, values with different small letter superscripts mean significant difference ($P < 0.05$).

Table 4. Effects of chitosan on serum IgG, IgM, and IgA concentrations (mg/mL) in growing Huoyan geese.

Item	Groups				SEM	*P*-value
	Control	CS_{100}	CS_{200}	CS_{400}		
42 D						
IgG	1.31^{b}	$1.82^{a,b}$	2.36^{a}	$1.74^{a,b}$	0.264	0.023
IgM	3.12^{b}	$3.67^{a,b}$	3.85^{a}	$3.41^{a,b}$	0.354	0.019
IgA	2.16^{b}	$2.81^{a,b}$	3.16^{a}	$2.43^{a,b}$	0.211	0.039
C3	1.12^{b}	$1.27^{a,b}$	1.46^{a}	$1.33^{a,b}$	0.332	0.047
C4	1.02	1.06	1.07	1.04	0.171	0.106
56 D						
IgG	1.72^{b}	$2.41^{a,b}$	2.77^{a}	$2.26^{a,b}$	0.189	0.031
IgM	3.81^{b}	$4.74^{a,b}$	4.93^{a}	$3.53^{a,b}$	0.366	0.041
IgA	2.51^{b}	$3.12^{a,b}$	3.54^{a}	$2.99^{a,b}$	0.225	0.046
C3	1.23^{b}	$1.35^{a,b}$	1.74^{a}	$1.42^{a,b}$	0.142	0.038
C4	1.13^{b}	$1.34^{a,b}$	1.35^{a}	$1.29^{a,b}$	0.201	0.044

In the same column, values with different small letter superscripts mean significant difference ($P < 0.05$).

Serum Immunoglobulins and Complements

The effects of dietary CS on serum immunoglobulin and complements concentrations in growing Huoyan geese are shown in Table 4. Serum concentrations of IgG, IgM, IgA, and complement C3 in CS_{200} group were significantly higher than that in the control group at both 42 and 56 D of age ($P < 0.05$). While, no differences were observed among experimental groups or among the control, CS_{100}, and CS_{400} groups, respectively ($P > 0.05$). In addition, the geese in CS_{200} group had higher serum complement C4 concentrations than that in the control group at 56 D of age ($P < 0.05$). No differences were determined among all groups at 42 D of age ($P > 0.05$). Meanwhile, no differences were found among experimental groups at 56 D of age ($P > 0.05$).

Serum Cytokines

As shown in Table 5, compared with the control group, serum IL-2 concentration of CS_{200} group was significantly higher at 42 and 56 D of age ($P < 0.05$). No differences were observed among experimental groups. In addition, serum concentration of TNF-a in CS_{200}

Table 5. Effects of chitosan on serum IL-1, IL-2, and TNF-a levels (ng/mL) in growing Huoyan geese.

Item	Groups				SEM	*P*-value
	Control	CS_{100}	CS_{200}	CS_{400}		
42 D						
IL-2	1.34^{b}	$1.66^{a,b}$	1.93^{a}	$1.59^{a,b}$	0.062	0.028
IL-4	5.52	5.89	6.25	5.21	0.081	0.123
IL-6	9.33	9.56	10.48	9.85	0.182	0.524
TNF-a	1.82	2.03	2.10	1.91	0.089	0.795
56 D						
IL-2	1.46^{b}	$1.57^{a,b}$	1.88^{a}	$1.52^{a,b}$	0.054	0.031
IL-4	5.31	5.46	6.18	5.51	0.074	0.094
IL-6	11.02	11.58	12.98	12.16	0.233	0.441
TNF-a	1.59^{b}	$1.84^{a,b}$	2.04^{a}	$1.88^{a,b}$	0.121	0.042

In the same column, values with different small letter superscripts mean significant difference ($P < 0.05$).

group was significantly higher than that in the control group at 56 D of age ($P < 0.05$), while no differences were determined among all groups at 42 D of age ($P > 0.05$). Meanwhile, no differences were found in serum concentrations of IL-4, IL-6 among all groups at 42 and 56 D of age, respectively ($P > 0.05$).

DISCUSSION

Relative Weight of Immune Organs

Previous study demonstrated that thymus, BF and spleen are very vital immune organs for poultry, and they are very important to generate immune response (Chen et al., 2014). Changes of immune organs index will influence immune functions and resistance to disease inpoultry (Huang et al., 2007). In this present study, relative weights of immune organs in growing Huoyan geese were significantly increased by CS (200 mg/kg CS), which suggested that CS could improve immune function of growing Huoyan geese by enhancing immune organs index. Similar results were determined by Huang et al. (2007), who found that 100 or 150 mg/kg COS supplementation enhanced weight of BF and thymus in 21 D of age broilers. Chi et al. (2017) also reported that compared with control group, 350 mg/kg COS supplementation significantly increased relative weights of thymus, BF, and spleen in broiler at 42 D of age. Deng et al. (2008) found that 100 mg/kg COS supplementation improved indices of spleen, thymus, and BF, increased immune function of broiler chickens. Yuan and Chen (2012) determined effects of CS on immune organs in ducks, and found that 2.4 g/kg dietary CS significantly increased immune organs index of ducks. Whereas, other study reported that addition of 50 mg/kg COS significantly decreased relative weight of spleen in broiler chickens (Wang et al., 2003). Inconsistent research results in immune organs index might be due to species, ages, differential molecular weights of CS, dosage, as well as duration of feeding CS (Chi et al., 2017).

Hormone and Growth Factors

GH and IGF-I are very important to control growth and metabolism of animals. Thyroid hormones (T_3 and T_4) could increase basal energy expenditure by influencing protein, carbohydrate and lipid metabolism pathway (Miao et al., 2008). INS mainly inhibits gluconeogenesis, and enhances glycogen synthesis (Chung et al., 1983). In the present study, it was observed that dietary supplementation of 200 mg/kg CS enhanced the serum concentrations of IGF-I and GH compared with the control group, which suggested an important role of CS in affecting serum hormones and growth factors of growing Huoyan geese. These results indicated that dietary supplementation of CS improved growth performance and protein anabolism in growing Huoyan geese (our previous data) may be due to enhance serum IGF-I and GH concentrations. These results are in accordance with previous reports (Tang et al., 2005), who observed that dietary supplementation of COS increased serum GH and IGF-I concentrations of piglets, which suggested COS may improve growth and feed conversion efficiency of piglets by increasing serum GH and IGF-I levels. Jin (2008) also reported that 200 mg/kg CS supplementation improved growth performance of broiler chickens by increasing serum GH and IGF-I levels. Nowadays, no information has yet been published in the effects of CS on the serum concentrations of INS, T_3, and T_4 in geese. In this experiment, serum INS, T_3, and T_4 levels of growing Huoyan geese were increased by dietary CS. The results suggested that suitable CS supplementation might also improve growth performance, nutrients metabolism in poultry by increasing serum INS, T_3, and T_4 concentrations. As indicated above, however, its mechanism needs to be still identified in further studies of growing Huoyan geese.

Serum Immunoglobulins and Complements

Increasing evidences indicated that CS could stimulate the production of serum antibodies, and served as an immunostimulant for animals (Kong et al., 2014; Li et al., 2015; Tokura et al., 1999). In this study, serum IgG, IgM, IgA, complement C3, and C4 levels of growing Huoyan geese were increased by 200 mg/kg CS supplementation. The results are in accordance with previous reports. Li et al. (2013) reported that dietary CS enhanced serum IgG concentrations. Zhang et al. (2018) observed that COS (0.6 g/kg BW) increased serum concentrations of IgA and IgG of mice. Deng et al. (2008) found that CS improved immune function of chickens by enhancing serum IgM and cytokines concentration. Huang et al. (2007) also demonstrated that CS increased serum IgG, IgM, and IgA levels of broilers, which indicated that CS is helpful for increasing release of serum immunoglobulin of animals. In addition, our previous study showed that CS significantly increased growth performance, digestive enzyme activities and nutrients utilization of growing Huoyan geese (previous data not listed). Combined the above data in this present experiment, these combined results indicated that CS improved growth performance of growing Huoyan geese may be due to increase serum immunoglobulin and complements concentrations. Our results were in accordance with previous reports (David et al., 2007; Huang et al., 2007; Ma et al., 2014; Li et al., 2015), which showed that CS could improve growth performance by increasing remarkably serum IgM, IgA, and IgG concentrations in mice, beef cattle, piglets and broiler, respectively.

Serum Cytokines

ILs, a group of cytokines, play a very important role in immune system of animals (Xu et al., 2018). IL-2, a key cytokine with broad-spectrum immune-regulatory activity, is essential for the development of T cell immune memory (Jain et al., 1995; Yin et al., 2008). TNF-α is an inflammatory cytokine involved in systemic inflammation, which can lead to apoptotic cell death, cell proliferation, differentiation, and inflammation (Basaran et al., 1993; Baek et al., 2007). Numinous evidences showed that CS could improve serum cytokines concentrations. Xu et al. (2018) demonstrated that CS increased the production of serum IL-2 in piglets. Xu et al. (2013) indicated that CS enhanced growth performance of piglets which may be due to improve immune function. Li et al. (2013) reported that serum IL-2, IL-1, and TNF-α levels of piglets were increased by dietary CS. Li (2009) also demonstrated that CS increased serum concentrations of IL-1, IL-2, and TNF-α, and improved growth performance of broilers. Baek et al. (2007) obtained similar results, who reported that CS enhanced serum concentrations of IL-2 and TNF-α in elderly adult. In the present study, 200 mg/kg CS increased the serum concentration of IL-2 and TNF-α, improved growth performance (data not listed) of growing Huoyan geese. Our results are in accordance with these pervious findings, and indicated that CS enhanced the growth performance of growing Huoyan geese probably due to improve the immunostimulatory effects, and increase humoral and cellular immune function.

In our study, the concentration of immune parameters, serum IgM, IgA, IgG, C3, C4, IL-2, and TNF-α, were decreased when the concentration of CS was increased to 400 mg/kg. Similar results were reported in previous study in other farm animals (Li et al., 2013; Li et al., 2015), level of serum IgA, IL-1, IL-2, and TNF-α in piglets tended to be lower in 2,000 mg/kg CS groups than that of 500 and 1,000 mg/kg CS group. Serum IgA and IL-1 concentration of cattle in 500 mg/kg CS group was higher than that in control group, and tended to decrease in 1,000 mg/kg CS group. The reason may be supposed that the absorption of nutrients in the gastrointestinal tract were reduced for its emulsification by high dose of CS, and finally decreased the immune function. Certainly, the mechanism underlying still needs to be proved by further investigation.

SUMMARY

In the present study, dietary supplementation of 200 mg/kg CS increased relative weight of immune organs, and serum concentrations of immunoglobulins, complements and cytokines, as well as enhanced serum hormones levels. However, the positive effect of CS tended to decrease when the concentration of CS increased to 400 mg/kg. These data were suggested that 200 mg/kg CS improved immune function of growing Huoyan geese.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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