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Effect of Vitamin E Supplementation on Growth Performance, Immune Function, Serum Biochemical Parameters, and Antioxidant Capacity of Swan Goose (*Anser cygnoid*)

Jianli Xiong[1,2,*], Hongtao Ren[2], Guanglu Li[2], Xiaochan Gao[2], Yong Huang[2] and Shiyang Gao[2]

[1]*Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang, 621000, Sichuan Province, PR China*
[2]*College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471023, Henan Province, PR China*

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JX designed the experiments. JX, HR, GL, XG, YH and SG performed the experiment. JX and HR analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Key words
Vitamin E, Swan geese, Growth performance, Immunological function, Antioxidant defense

ABSTRACT

The present study aimed to investigate the effects of dietary vitamin E (VE) supplementation on the growth performance, immune function, serum biochemical parameters, and antioxidant capacity of growing swan geese (*Anser cygnoid*). 240 four-week-old healthy swan geese were randomly divided into five groups (n = 48 each). Each group was subdivided into six replicates, with eight geese per replicate. Geese received basal diets, supplemented with either 0 (control), 10, 20, 40, or 80 IU/kg VE. All geese were treated for 12 weeks. The results showed that VE supplementation had no significant effect on body weight, but significantly decreased the average daily feed intake and the feed/gain ratio. Moreover, compared with control, the thymus index, serum SOD and GSH-Px increased significantly, while MDA decreased significantly in the 20, 40, and 80 IU/kg groups. In conclusion, dietary VE supplementation can increase the feed conversion ratio, thymus index, antioxidant defense function, and decrease oxidative damage of growing swan geese.

INTRODUCTION

Vitamin E (VE) is a generic term for a group of lipid-soluble compounds, comprised of four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ). Among these, α-tocopherol is the most abundant form in nature with the highest biological activity (NRC, 1994). VE is a major chain-breaking antioxidant and free radical scavenger in the membranes of cells and sub-cellular organs (Burton *et al*., 1983; Sahin *et al*., 2002). Moreover, VE plays a vital role in cell signaling, by inhibiting or inactivating protein kinase C, modulating gene expression, and inhibiting both cell proliferation and platelet aggregation (Bender, 2003). Dietary VE supplementation is beneficial for the growth, reproduction, and health of poultry. Maternal supplementation with high levels of VE (120-160 mg/kg) enhanced the antioxidant capability and decreased oxidative stress in chicks (Lin *et al*., 2005). VE supplementation increased egg production, as well as both the quality and health of laying hens (Jiang *et al*., 2013). Moreover, VE supplementation increased weight gain and feed intake, and decreased liver lipid peroxidation of White Pekin ducks (Xie *et al*., 2018), and in ovo VE inoculation increased the hatching rate of broiler embryos (Araujo *et al*., 2019). It also increased the egg laying performance, plasma reproductive hormones, and the mRNA expression of reproductive hormone receptor genes in ovaries of Xingguo grey geese (Yin *et al*., 2019). Thus, VE is most commonly added to poultry feed.

Geese are an important poultry meat resource in China, and many breeds are cultured. Although VE is important for poultry, there is no recommendation for VE supplementation for geese in the NRC (1994). Recently, several recommendations for the VE concentration in the feed of different geese breed have been reported. Zhou *et al*. (2012) reported an optimal VE level in the diet of livestock breeders Qingnonghui geese is 42.98 IU/kg (age 0-4 weeks) and 38.92-43.41 IU/kg (aged 5-12 weeks). The lowest level of VE in the diet should be 20 IU/kg. Wang *et al*. (2013) suggested that an optimal dietary VE level for Qingnonghui geese aged 1-12 weeks is 44.31-53.11

* Corresponding author: xjlpanda@126.com
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to achieve the best immune and antioxidant conditions. The minimum dietary VE level in geese aged 1-12 weeks is 6.84 IU/kg according to the serum malondialdehyde content. Yin *et al*. (2019) reported that 80 mg/kg of VE supplementation in the diet was appropriate for Xingguo grey goose. These results showed that the optimal dietary VE levels vary between breeds and ages of geese.

Swan geese, *Anser cygnoid*, were widely domesticated in China. However, until now, the requirement of the specific VE for this species of geese has not been reported. The objectives of this investigation were to study the effects of VE supplementation on the growth performance, slaughter performance, immune function, serum biochemical parameters, and antioxidant capacity of this breed and to identify its VE requirements.

MATERIALS AND METHODS

Experimental animals, design, diets and management

A total of 240 gray geese aged four weeks were randomly assigned to five groups (one control group and four experimental groups). Each group was subdivided into six replicates, with eight birds per replicate. Geese in the control group were fed a basal diet, while geese in experimental groups were fed basal diets supplemented with 10, 20, 40, or 80 IU/kg VE. The basal diet was formulated according to the NRC (1994) to meet the nutritional requirements of geese. DL-α-tocopherol acetate (at an effective substance concentration of 50%) was the source of supplemental VE. Formulation and nutrient composition of the basal diet are shown in Table I. The experimental period lasted 12 weeks. All geese had free access to feed and water throughout the experimental period. All experimental procedures were approved by the animal care and use committee of Henan University of Science and Technology.

Sample collection

Prior to the experiment, geese were weighed (body weight, BW). The average daily gain (ADG), average daily feed intake (ADFI), and the feed / gain ratio (F/G) were measured for each group using the following formulas: ADG = (final weight-initial weight) / days; ADFI = sum of daily feed intake / days; and F / G = ADFI / ADG. After the experiment, 60 geese (two geese per group/replicate) were randomly selected and weighed after fasting for 12 h. Blood samples were obtained from jugular veins, and collected into heparin sodium-anticoagulant tubes, and centrifuged at 3,000 rpm for 10 min to obtain serum. Serum was kept at -20 °C until further analysis (i.e., determination of serum biochemical parameters and antioxidant capacity). After blood collection, geese were euthanized by cervical dislocation. Then, geese were eviscerated, and the slaughter yield, semi-eviscerated carcass yield, and eviscerated carcass yield were measured according to poultry production performance terms and metric statistics methods. The weights of spleen, thymus, and bursa of Fabricius were recorded, and their relative weights were expressed as a percentage of the live BW.

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

Ingredients	Content (%)
Corn	74.62
Soybean meal	19.65
Limestone	1.17
Soybean oil	1
Fish meal	1.00
Calcium hydrogen phosphate	0.89
Salt	0.36
DL-Methionine	0.16
Lysine	0.15
Premix[1)]	1.00
Nutrient levels[2)]	
ME/(MJ/kg)	12.55
Crude protein	15
Calcium	0.74
TP	0.5
Available phosphorus	0.3
Lysine	0.85
Methionine	0.4
Met+Cys	0.725

[1)] The premix provided the following per kg of diets: VA 9000 IU, VD_3 2000 IU, VE 40 mg, VK_3 0.8 mg, VB_1 2.0 mg, VB_2 4.0 mg, nicotinic acid 30 mg, calciuim pantothenate 11 mg, VB_6 4.0 mg, biotin 0.2 mg, folic acid 0.5 mg, VB_{12} 12 μg, Se 0.5 mg, Fe 80 mg, Mn 30 mg, Cu 4 mg, I 0.3 mg. [2)] Nutrient levels were calculated values.

Analysis of serum samples

The serum samples were thawed and analyzed for serum total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholesterol (CHO), low-density lipoprotein cholesterol (LDL), and highly density lipoprotein cholesterol (HDL) by an automatic biochemistry analyzer (Hitachi 7600-020, Hitachi High Technologies, Inc., Ibaraki, Japan). The content of MDA, the total antioxidant capacity (T-AOC), the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were determined using respective diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (version 22.0 for windows, SPSS Inc., Chicago, IL, USA) to estimate the main treatment effect. Differences among treatments were examined using LSD tests. Data are presented as means ± standard errors. The differences were considered to be significant at $P < 0.05$.

RESULTS

Growth performance

VE supplementation had no significant effects ($P > 0.05$) on BW and ADG, but the ADFI and F/G were significantly affected (Table II). ADFI of experimental groups was significantly lower ($P < 0.05$) than that of the control group. Except for the F/G of the 80 IU/kg group, the F/G of all other experimental groups were significantly lower than that of the control group, and the lowest value was found in 40 IU/kg group.

Table II. Effects of vitamin E supplementation on growth performance of swan geese.

Groups	BW (g)	ADG (g/d)	ADFI (g/d)	F/G
0 IU/kg	2348.46±69.95	18.72±0.83	147.80±0.62^{a}	7.93±0.33^{a}
10 IU/kg	2219.42±43.89	17.76±0.52	127.47±0.79^{d}	7.19±0.17^{bc}
20 IU/kg	2356.50±56.18	20.20±0.67	140.14±1.39^{bc}	6.95±0.16^{c}
40 IU/kg	2348.86±71.15	20.29±0.85	138.60±1.73^{c}	6.84±0.21^{c}
80 IU/kg	2248.30±57.72	17.44±0.69	142.62±1.03^{b}	7.86±0.16^{ab}

In the same column, values with no letter or the same letter superscripts mean no significant difference (P>0.05), while with different letter superscripts mean significant difference (P<0.05). The same as below.

Table III. Effects of vitamin E supplementation on slaughter performance (%) of swan geese.

Groups	Slaughter yield	Semi-eviscerated carcass yield	Eviscerated carcass yield
0 IU/kg	88.01±0.99	77.88±1.35	69.43±1.09
10 IU/kg	88.06±0.88	78.79±1.87	68.91±1.06
20 IU/kg	87.99±0.97	79.42±1.19	69.10±1.22
40 IU/kg	88.50±0.93	78.21±1.48	68.57±1.20
80 IU/kg	87.67±0.68	78.69±1.35	68.20±0.44

Slaughter performance

The effects of VE supplementation on growth performance of swan geese are presented in Table III. VE supplementation did not exert significant effects ($P > 0.05$) on slaughter yield, semi-eviscerated carcass yield, and eviscerated carcass yield.

Immune organ indexes

The thymus indexes of experimental groups were higher than those of the control group. Groups that received VE supplementation at 20 IU/kg, 40 IU/kg, and 80 IU/kg had significantly increased thymus indexes ($P < 0.05$), and the maximum was reached in the 40 IU/kg group (Table IV). VE supplementation had no significant effects ($P > 0.05$) on the indices of spleen and bursa of Fabricius.

Table IV. Effects of vitamin E supplementation on immune organ indexes (g/kg) of swan geese.

Groups	Spleen	Bursa of fabricius	Thymus
0 IU/kg	1.11±0.14	0.27±0.03	0.32±0.08^{d}
10 IU/kg	0.92±0.17	0.27±0.03	0.36±0.05^{cd}
20 IU/kg	0.90±0.14	0.28±0.05	0.60±0.09^{bc}
40 IU/kg	1.09±0.10	0.31±0.03	0.89±0.13^{a}
80 IU/kg	1.33±0.14	0.31±0.03	0.63±0.09^{ab}

Serum biochemical parameters

The effects of VE supplementation on serum biochemical parameters of swan geese are presented in Table V. VE supplementation insignificantly influenced ($P > 0.05$) the serum biochemical parameters (TP, ALT, AST, CHO, HDL-C, and LDL-C).

Serum antioxidant capacity

VE supplementation influenced both the SOD and GSH-Px activities, as well as the MDA content in the serum (Table V). The SOD activity was significantly increased ($P < 0.05$) by VE supplementation, and reached a maximum in the 40 IU/kg group. The GSH-Px activity also increased with VE supplementation, but only the groups with higher VE supplementation (20 IU/kg, 40 IU/kg, and 80 IU/kg) showed a significant difference ($P < 0.05$) compared with the control group. The maximum value was reached in the 40 IU/kg group. The MDA content significantly decreased ($P < 0.05$) in all experimental groups, and reached a minimum value in the 40 IU/kg group. VE supplementation did not significantly influence ($P > 0.05$) the activities of CAT and T-AOC (Table V).

DISCUSSION

As an antioxidant, VE can prevent the oxidation of polyunsaturated fatty acids in both the feed and body tissues; thus, VE can improve feed utilization and promote the growth of animals (Li *et al*., 2015). Fan *et al*. (2007) reported that VE supplementation can increase BW, and decrease the F/G of Xupu geese. Zhou *et al*. (2012) reported that the ADG and ADFI of Qingnonghui geese

Table V. Effects of vitamin E supplementation on serum biochemical parameters and antioxidant indices of swan geese.

Items	0 IU/kg	10 IU/kg	20 IU/kg	40 IU/kg	80 IU/kg
Biochemical parameters					
TP/(g/L)	45.90±0.88	44.61±1.15	45.39±1.57	47.30±1.18	45.55±1.49
ALT/(U/L)	22.17±2.23	23.17±1.38	24.00±0.77	22.50±2.67	23.67±2.23
AST/(U/L)	21.67±2.72	22.17±1.27	23.00±3.30	20.67±1.20	22.33±3.05
LDH/(U/L)	249.50±34.40	245.33±6.63	257.50±34.66	187.50±27.37	224.33±28.66
CHO/(mmol/L)	5.15±0.31	5.04±0.52	4.73±0.31	5.43±0.40	4.82±0.17
HDL-C/(mmol/L)	2.90±0.18	2.49±0.19	2.73±0.24	3.24±0.28	2.87±0.19
LDL-C/(mmol/L)	1.78±0.15	1.71±0.12	1.49±0.08	1.83±0.13	1.41±0.08
Antioxidant indices					
SOD/(U/mL)	619.28±54.35[d]	758.80±50.75[c]	853.55±14.74[bc]	994.95±44.59[a]	962.70±37.38[ab]
MDA/(nmol/mL)	7.13±0.07[a]	5.85±0.37[b]	5.69±0.43[b]	5.35±0.37[b]	5.59±0.44[b]
GSH-Px/(U/mL)	251.98±11.06[c]	288.57±13.64[bc]	302.03±13.44[b]	373.39±21.68[a]	305.17±20.87[b]
CAT/(U/mL)	3.11±0.49	2.71±0.41	2.15±0.34	3.26±0.44	3.11±0.32
T-AOC(U/mL)	13.81±0.90	14.05±0.74	15.58±1.18	16.27±0.76	16.96±0.65

(aged 0-4 weeks and 5-8 weeks) were significantly increased by supplementation of VE, while F/G showed no significant change. The results of this study indicated that VE supplementation did not significantly influence BW and ADG, but significantly influenced both ADFI and F/G of swan geese. This may be attributed to the supplementation of VE increasing the feed conversion ratio.

Slaughter performance is a very important indicator to evaluate the production performance of meat poultry, which intuitively reflects the composition of the carcass of the animal body and its meat production performance (Li *et al*., 2017; Yang *et al*., 2015). In this experiment, VE supplementation had no significant effect on the slaughter yield, semi-eviscerated carcass yield, and eviscerated carcass yield. This result agrees with the results of Fan *et al*. (2007).

Birds have central and peripheral lymphoid tissues, which play an important role in the defense of the body against pathogens (Zhou *et al*., 2019). The spleen, bursa, and thymus are the important immune organs of poultry, and their relative weights are positively correlated with the body's cellular and humoral immune functions. Thus, the weight of immune organs can be used as an indicator to evaluate the immune status of poultry (Al-Khalifa *et al*., 2012; Xu *et al*., 2019). The results of the present study indicate that only the thymus index was significantly affected, while the spleen and bursa of Fabricius indices were not significantly influenced by VE supplementation. Wang *et al*. (2013) also found that the thymus index was significantly influenced by VE supplementation in Qingnonghui geese; moreover, they also found that bursa of Fabricius indices were significantly influenced. The thymus is a central immune organ that is mainly involved in humoral immunity. VE supplementation can increase the level of immunoglobulin in the blood (Zduńczyk *et al*., 2013), enhance the body's ability to produce antibodies to various antigens and vaccines, and thus enhance the body's humoral immune response (Wang *et al*., 2009).

The biochemical index in the serum of the animal body can be used as an indicator to reflect the physiological state and health of the body (Lin *et al*., 2012), which can be correlated with the quality of the diet (Ojediran *et al*., 2017). Zhou *et al*. (2012) reported that the TP content of Qingnonghui geese was not significantly affect by VE supplementation, but the serum urea nitrogen, glucose, uric acid content, as well as the serum alkaline phosphatase activity were significantly influenced. The results of the present study show that VE supplementation did not significantly influence the serum biochemical index of swan geese. The reasons need to be explored in the future.

The antioxidant enzyme system plays an important role in maintaining a healthy body. Studies have shown that VE supplementation is closely related to antioxidant function in poultry. Wang *et al*. (2013) reported that VE supplementation significantly increased the serum GSH-Px activity in Qingnonghui geese, and decreased the MDA content. Liu *et al*. (2017) reported that VE supplementation significantly decreased MDA content in serum, significantly increased SOD activity, but insignificantly influenced the T-AOC and GSH-Px activity in Guangxi Sanhuang Chicken. In the present study, VE supplementation significantly increased the serum SOD and GSH-Px activities, significantly decreased the MDA content, but did not significantly influence CAT and T-AOC activities. SOD can remove superoxide anions and protect cells from damage. GSH-Px can prevent the formation of hydroxyl radicals and singlet oxygen, and reduce the accumulation of free radicals. MDA directly reflects the level of oxidized free radicals in the body and the degree of cell damage they caused. The increased SOD and GSH-Px activities, and decreased MDA content indicate that VE supplementation increased the antioxidant function of swan geese.

CONCLUSIONS

The findings of this study indicate that VE supplementation decreases F/G, improves the thymus index, increases SOD and GSH-Px activities, and decreases the MDA content. The best numerical values for the overall growth performance, immune function, and antioxidant capacity were obtained in grey geese that received 40 IU/kg VE supplementation.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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