

Assessment of vitamin A requirement of gosling in 0-28 d based on growth performance and bone indexes

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ABSTRACT A dose-response experiment assessing 6 total dietary vitamin A (VA) levels (0, 3,000, 6,000, 9,000, 12,000, and 15,000 IU/kg) was conducted to study the effects of different levels of VA on growth performance and bone indexes of 0 to 28 d goslings. A total of 360 healthy 1-day-old goslings with similar body weights were randomly divided into 6 groups with 6 replicates in each group and 10 goslings in each replicate. The experiment duration was 28 d. The experimental results showed that the VA content in the serum and liver of gosling increased with increasing VA amount ($P < 0.05$). When the diet was supplemented with 9,000 IU/kg VA, the content of vitamin D in serum and the liver was higher than those in other groups ($P < 0.05$). The 28-day body weight and average daily gain in gosling in the 9,000 IU/kg VA group were higher than those in the 0, 3,000 and 15,000 IU/kg VA groups ($P < 0.05$). Growth hormone, thyroxine, and parathyroid hormone levels in the 9,000 IU/kg VA group

were higher than those in the 0, 3,000, 6,000 and 15,000 IU/kg VA groups ($P < 0.05$). The insulin level in the 9,000 IU/kg VA group was higher than those in the other groups ($P < 0.05$). The osteocalcin (BGP) levels in the VA supplementation groups were higher than that in the no VA groups ($P < 0.05$). The tibia length and phosphorus content in the 9,000 IU/kg VA group were higher than those in the 12,000 IU/kg VA group and 15,000 IU/kg VA group ($P < 0.05$). The tibia shear hardness values in the 6,000, 9,000 and 12,000 IU/kg VA groups were higher than those in the 0, 3,000 and 15,000 IU/kg VA groups ($P < 0.05$). The tibia calcium content in the 9,000 IU/kg VA group was higher than those in the 0 and 15,000 IU/kg VA groups ($P < 0.05$). The tibia crude ash content in the 9,000 IU/kg VA group was higher than that in the 15,000 IU/kg VA group ($P < 0.05$). In conclusion, the optimum dietary VA supplementation for 0- to 8 day gosling was 9,000 IU/kg.

Key words: gosling, vitamin A, growth performance, tibia, calcium and phosphorus

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INTRODUCTION

Vitamin A (VA) is an essential fat-soluble vitamin that affects wide range of physiological functions in poultry. Vitamin A is considered to play a very important role in bone development. Vitamin A mainly exists in the liver of animals, and the amount of VA added to the diet directly determines the content of VA in the liver (Ilhan And Bülbül, 2016). When VA is lacking, poultry calcification and bone dysplasia were appeared (Li et al., 2008). When VA is present in excess, animal

leg diseases develop (Stevens et al., 1983; Patriciade and Ligia, 2004). Aburto and Britton (1998) found that excessive VA had adverse effects on the absorption and utilization of vitamin D₃ (VD₃). Vitamin D is related to bone development. In this experiment, different doses of VA were added to the diet to study the effects of VA levels on growth performance and bone development in gosling, and to determine the optimal VA supplementation level in gosling, laying a theoretical foundation for related research on gosling in the future.

MATERIALS AND METHODS

Experimental Design and Diets

The Yangzhou University (Yangzhou, China) Animal Care and Use Committee approved all of the procedures performed in this study.

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Table 1. Composition and nutrient levels of the basal diets of gosling (dry basis).

Item	Content
Ingredients (%)	
Corn	63.00
Soybean meal	30.20
Rice husk	3.20
Methionine	0.10
Salt	0.30
Stone powder	1.10
Calcium hydrogen phosphate	1.10
Premix ¹	1.00
Total	100.00
Nutritional level ²	
ME (MJ/kg)	11.34
Crude protein (%)	18.98
Crude fiber (%)	4.07
Ca (%)	0.83
Total phosphorus (%)	0.56
Effective phosphorus (%)	0.32
Lysine (%)	0.99
Methionine (%)	0.42
Vitamin A (IU/kg)	1,225.00

¹Contain per kilogram of premix: VD 300,000 IU, VE 1,800 IU, VK 150 mg, VB₁ 90 mg, VB₂ 800 mg, VB₆ 320 mg, VB₁₂ 1.2 mg, niacin 45,000 mg, D-pantothenic acid 1,100 mg, folic acid 65 mg, biotin 5 mg, choline 35 g, Fe 6 g, Cu 1 g, Mn 9.5 g, Zn 9 g, Se 30 mg, I 50 mg.

²Vitamin A is converted from carotene, carotene is the measured value.

The experimental setup consisted of 1-day-old Jian-gnan white gosling randomly distributed into 6 experimental groups. The test period lasted for 28 d. The basal diet was supplemented with 6 concentrations of VA (0, 3,000, 6,000, 9,000, 12,000, and 15,000 IU/kg), respectively. A basal corn-soybean meal diet was formulated to provide adequate concentrations of all the nutrients are based on gosling (NRC, 1994) and our laboratory's achievements over the years except for VA (Table 1). Vitamin A (produced by Diesman Vitamin Co., Ltd., Shanghai, China) was added in the form of acetate at a concentration of 1×10^6 IU/g. Vitamin A was purchased from Diesman Vitamin Co., Ltd. All goslings were reared in plastic wire-floor pens. The enclosure was kept clean and well ventilated; the goslings were maintained under conditions of 24 h of light per day, and 29°C. Water and feed were provided ad libitum. No VA was added to the basic premixed diet of gosling.

Sample Collection and Index Determination

Deposition of Vitamin A and Vitamin D in Liver and Serum On the 28th day of the experiment, the goslings were slaughtered and bled, liver samples were placed

into centrifuge tubes to determine the contents of VA and VD in the liver. The determination method was based on the GB/T 5009.82-2016 «determination of VA and vitamin E in food—reversed-phase high-performance liquid chromatography» method (Liang et al., 2019). Enzyme-linked immunosorbent assay was used to determine the values of VA and VD in gosling serum; the kits were purchased from Jiangsu Kete Biotechnology Co., Ltd. (Yancheng, China).

Growth Performance and Hormones All goslings were weighed at 08:00 a.m. on the 28th day, and the average daily gain was calculated. BW was recorded with an electronic platform scale (ACS-30 Shanghai Yousheng Co., Ltd., Shanghai, China).

$$\text{ADG(g)} = (\text{body weight at 28 d(g)} - \text{body weight at 1 d(g)})/28$$

Triiodothyronine, thyroxine (T₄), insulin (TI), growth hormone (GH), parathyroid hormone (PTH), and osteocalcin (BGP) were measured using kits (purchased from Beijing North Biotechnology Research Institute Co., Ltd., Beijing, China). The GC-911 γ radioimmunoassay was produced by Anhui Zhongke Zhongjia Scientific Instrument Co., Ltd. (Anhui, China).

Shank Index and Related Indexes of Calcium and Phosphorus Refer to NY/T823-2004 «poultry production performance terms and measurement statistics» (in China). On the 28th day, the length and circumference of gosling's shank were measured with tape and calipers.

Shank length: the vertical distance between the third and fourth toes.

Shank circumference: the circumference at the middle of the tibia.

The shear hardness of the tibia was measured by an Instron 3367 double-column electronic testing instrument was purchased from Guangzhou Lingtuo Instrument Technology Co., Ltd. (Guangzhou, China).

On the 28th day of the experiment, the gosling blood was collected. Serum calcium and phosphorus were separated and determined by the electrode method. The instrument used is Beckman LX-20 automatic biochemical analyzer of Beckman Coulter company in the United States (Beijing, China).

The tibia was dried at 100°C for 48 h, extracted and defatted with ether for 12 h, and dried to constant weight at 100°C, the ash content of the tibia was

Table 2. Effects of different vitamin A levels on vitamin A and vitamin D contents of gosling on the 28th day.

Item	Vitamin A supplementation level (IU/kg)						SEM	P-value		
	0	3,000	6,000	9,000	12,000	15,000		Vitamin A	Linear	Quadratic
Serum VA content (ng/ml)	120.58 ^d	131.36 ^c	132.46 ^c	135.73 ^b	142.50 ^a	144.11 ^a	1.35	<0.001	<0.001	0.003
Serum VD content (μg/L)	134.45 ^d	134.37 ^d	137.74 ^c	154.61 ^a	144.07 ^b	125.51 ^e	1.59	<0.001	0.890	<0.001
Liver VA content (mg/kg)	1.07 ^e	122.66 ^d	130.72 ^d	206.11 ^c	366.23 ^b	411.07 ^a	24.32	<0.001	<0.001	0.004
Liver VD content (mg/kg)	1.29 ^c	1.40 ^b	1.38 ^b	1.48 ^a	1.41 ^b	1.39 ^b	0.01	<0.001	<0.001	<0.001

^{a-d}The results are mean values, the same letter or no letter in the same column indicates that the difference is not significant ($P > 0.05$), and different lowercase letters indicate significant difference ($P < 0.05$).

Table 3. Effects of different vitamin A levels on growth performance of gosling on the 28th day.

Item	Vitamin A supplementation level (IU/kg)						SEM	P-value		
	0	3,000	6,000	9,000	12,000	15,000		Vitamin A	Linear	Quadratic
Hatch weight (g)	101.20	100.75	101.60	101.72	101.20	100.83	0.14	0.265	0.896	0.109
28d BW (kg)	1.61 ^b	1.63 ^b	1.71 ^{a,b}	1.74 ^a	1.69 ^{a,b}	1.60 ^b	0.02	0.052	0.527	0.004
ADG (g)	53.90 ^b	54.51 ^b	57.31 ^{a,b}	58.60 ^a	56.69 ^{a,b}	53.71 ^b	0.58	0.530	0.525	0.004
ADFI (g)	117.55	119.20	116.24	117.78	115.35	111.63	1.37	0.712	0.181	0.441
F/G (g/g)	2.18 ^a	2.18 ^a	2.03 ^b	2.01 ^b	2.03 ^b	2.06 ^b	0.02	<0.001	<0.001	0.005

^{a,b}The results are mean values, the same letter or no letter in the same column indicates that the difference is not significant ($P > 0.05$), and different lowercase letters indicate significant difference ($P < 0.05$).

measured by heating at 550°C to 600 °C for 24 h. Ash was determined as per GB/T6438-2002, calcium was determined by the EDTA method, and phosphorus was determined by molybdenum yellow colorimetry.

Statistical Analysis SPSS 17.0 (SPSS, 2009) was used to conduct one-way analysis of variance, linear and quadratic analysis. Duncan's method was used for multiple comparisons, and $P < 0.05$ was considered significant. The data analysis results are expressed as mean values and standard errors.

RESULTS

Deposition of Vitamin A and Vitamin D in the Liver and Serum

The effects of different VA levels on VA and VD contents in the liver and serum in the gosling on the 28th day are shown in Table 2. The content of VA in serum in the 12,000 and 15,000 IU/kg VA supplementation groups (VA groups) were higher than those in the 0, 3,000, 6,000, and 9,000 IU/kg VA groups ($P < 0.05$), in the 9,000 IU/kg VA groups were higher than those in the 0, 3,000 and 6,000 IU/kg VA groups ($P < 0.05$), and in the 3,000 and 6,000 IU/kg VA groups were higher than that in the no VA group ($P < 0.05$). The content of serum VD in the 9,000 IU/kg VA group was higher than those in the 0, 3,000, 6,000, 12,000, and 15,000 IU/kg VA groups ($P < 0.05$), in the 12,000 IU/kg VA group was higher than those in the 0, 3,000, 6,000, and 15,000 IU/kg VA groups ($P < 0.05$), in the 6,000 IU/kg VA group was higher than those in the 0, 3,000, and 15,000 IU/kg VA groups ($P < 0.05$), and in the 0 and 3,000 IU/kg VA groups were higher than that in the 15,000 IU/kg VA group ($P < 0.05$). The liver VA content in the 15,000 IU/kg VA group was higher than

those in the 0, 3,000, 6,000, 9,000, and 12,000 IU/kg VA groups ($P < 0.05$), in the 12,000 VA group was higher than those in the 0, 3,000, 6,000, and 9,000 IU/kg VA groups ($P < 0.05$), and in the 9,000 VA group was higher than those in the 0, 3,000, and 6,000 IU/kg VA groups ($P < 0.05$), the VA levels were higher in the VA groups than the in the no VA group ($P < 0.05$). The liver VD content in the 9,000 IU/kg VA group was higher than those in the 0, 3,000, 6,000, 12,000, and 15,000 IU/kg VA groups ($P < 0.05$), and those in the 3,000, 6,000, 12,000, and 15,000 IU/kg VA groups were higher than that in the no VA addition group ($P < 0.05$). There was a linear relationship between dietary VA and serum VA content, liver VA content and liver VD content ($P < 0.05$). There was a quadratic-linear relationship between dietary VA and serum VA content, liver VD content ($P < 0.05$).

Growth Performance and Hormones

Table 3 shows the effects of different VA levels on growth performance of gosling on the 28th day. The BW of gosling fed 9,000 IU/kg VA was higher than those of gosling fed 0, 3,000, and 15,000 IU/kg VA ($P < 0.05$). The average daily gain in gosling fed 9,000 IU/kg VA was higher than the gains in gosling fed 0, 3,000, and 15,000 IU/kg VA ($P < 0.05$). The F/G ratios in gosling fed 0 and 3,000 IU/kg VA were higher than those in 6,000, 9,000, 12,000, and 15,000 IU/kg VA ($P < 0.05$). There was a quadratic-linear relationship between BW and ADG of goslings fed diets supplemented with different levels of VA ($P < 0.05$).

The effect of different VA levels on hormone content of gosling of the 28th day is presented in Table 4. The GH level in the 9,000 IU/kg VA group was higher than those in the 0, 3,000, 6,000, and 15,000 IU/kg VA groups

Table 4. Effects of different vitamin A levels on hormone content of gosling on the 28th day.

Item	Vitamin A supplementation level (IU/kg)						SEM	P-value		
	0	3,000	6,000	9,000	12,000	15,000		Vitamin A	Linear	Quadratic
Growth hormone (ng/mL)	3.93 ^d	4.56 ^d	5.28 ^c	7.04 ^a	6.85 ^{a,b}	6.24 ^b	0.21	<0.001	<0.001	<0.001
Insulin (mIU/L)	4.54 ^e	6.72 ^d	9.23 ^b	10.48 ^a	8.82 ^{b,c}	7.75 ^{c,d}	0.35	<0.001	<0.001	<0.001
T3 (nmol/L)	4.51	4.62	4.71	4.90	4.46	4.33	0.10	0.636	0.554	0.148
T4 (nmol/L)	135.50 ^c	136.61 ^c	136.93 ^c	186.92 ^a	177.89 ^{a,b}	162.63 ^b	4.39	<0.001	<0.001	0.067
PTH (pg/mL)	984.40 ^c	1,166.07 ^{b,c}	1,109.08 ^{b,c}	1,435.38 ^a	1,273.32 ^{a,b}	1,094.30 ^{b,c}	33.02	<0.001	0.021	<0.001
BGP (ng/mL)	14.73 ^b	18.81 ^a	19.07 ^a	18.27 ^a	17.97 ^a	17.55 ^a	0.32	<0.001	0.026	<0.001

^{a-d}The results are mean values, the same letter or no letter in the same column indicates that the difference is not significant ($P > 0.05$), and different lowercase letters indicate significant difference ($P < 0.05$).

Table 5. Effects of different vitamin A levels on shank index of gosling on the 28th day.

Item	Vitamin A supplementation level (IU/kg)						SEM	P-value		
	0	3,000	6,000	9,000	12,000	15,000		Vitamin A	Linear	Quadratic
Shank length (cm)	8.98 ^{a,b}	9.26 ^{a,b}	9.22 ^{a,b}	9.51 ^a	8.74 ^b	8.53 ^b	0.11	0.068	0.087	0.020
Shank circumference (cm)	4.40	4.42	4.43	4.43	4.42	4.32	0.03	0.839	0.475	0.265
Shear hardness value (N)	198.93 ^b	207 ^b	235.85 ^a	238.85 ^a	237.37 ^a	184.57 ^b	5.21	0.001	0.791	<0.001

^{a,b}The results are mean values, the same letter or no letter in the same column indicates that the difference is not significant ($P > 0.05$), and different lowercase letters indicate significant difference ($P < 0.05$).

($P < 0.05$), in the 15,000 IU/kg VA group was higher than those in the 0, 3,000, and 6,000 IU/kg VA groups ($P < 0.05$), and in the 6,000 IU/kg VA group was higher than those in the 0 and 3,000 IU/kg VA groups ($P < 0.05$). T4 in the 9,000 IU/kg VA group was higher than those in the 0, 3,000, 6,000, and 15,000 IU/kg VA groups ($P < 0.05$), and in the 15,000 IU/kg VA group was higher than those in the 0, 3,000, and 6,000 IU/kg VA groups ($P < 0.05$). The level of PTH in the 9,000 IU/kg VA group was higher than those in the 0, 3,000, 6,000, and 15,000 IU/kg VA groups ($P < 0.05$). BGP values in the 3,000, 6,000, 9,000, 12,000, and 15,000 IU/kg VA groups were higher than that in the no VA group ($P < 0.05$). There was a linear relationship between dietary VA and GH, insulin, T4, PTH, BGP ($P < 0.05$). There was a quadratic-linear relationship between dietary VA and GH, insulin, PTH, BGP ($P < 0.05$).

Shank Index and Related Indexes of Calcium and Phosphorus

The effects of different VA levels on shank index in gosling on the 28th day are summarized in Table 5. The shank length in the 9,000 IU/kg VA group was longer than those in the 12,000 IU/kg VA group and 15,000 IU/kg VA group ($P < 0.05$). The tibia shear hardness values in the 6,000, 9,000, and 12,000 IU/kg VA groups were higher than those in the 0, 3,000, and 15,000 IU/kg VA groups ($P < 0.05$). There was a quadratic linear relationship between dietary VA and shank length, tibia shear hardness of gosling ($P < 0.05$).

The effects of different VA levels on calcium and phosphorus related indexes of 28 d gosling are shown in Table 6. The calcium content in the tibia of gosling in the 9,000 IU/kg VA group was higher than those in the 0 and 15,000 IU/kg VA groups ($P < 0.05$). The phosphorus content in the tibia in the 9,000 IU/kg VA group

was higher than those in the 12,000 and 15,000 IU/kg VA groups ($P < 0.05$). The crude ash content in the tibia in the 9,000 IU/kg VA group was higher than that in the 15,000 IU/kg VA group ($P < 0.05$). There was a quadratic linear relationship between dietary VA and calcium, phosphorus, and ash contents in tibia of goslings ($P < 0.05$).

DISCUSSION

Deposition of Vitamin A and Vitamin D in the Liver and Serum

About 20% of VA in the body cannot be absorbed. After 1-2 d of excretion, the other 20 to 50% will be combined or oxidized. After 7 d, it will be excreted in the form of urine or feces. The rest mainly contributes to normal physiological metabolism in the liver. Our previous results showed that VA can be passed on from mother to offspring (Liang et al., 2019). Johansson and Melhus (2001) studied the interaction between VA and VD in the human body, and the results showed that excessive intake of VA could antagonize VD in vivo. Rohde et al. (1999) used mice as an experimental model and showed that there was an antagonistic effect between VA and VD. The results showed that the content of VA in the liver and serum increased with the increase of VA in diet. When 9,000 IU/kg of VA was added, the content of VD in the liver and serum of gosling peaked, and excessive VA in the diet antagonized VD.

Growth Performance and Hormones

As a fat-soluble vitamin, VA can be absorbed through the body's gastrointestinal tract (West and Mehra, 2010). Vitamin A supplementation is closely related to the balance of the gastrointestinal tract (Tian et al., 2017), and gastrointestinal health is the basis for ensuring the healthy growth and development of the animal body.

Table 6. Effects of different vitamin A levels on calcium and phosphorus related indexes in 28 d gosling.

Item	Vitamin A supplementation level (IU/kg)						SEM	P-value		
	0	3,000	6,000	9,000	12,000	15,000		Vitamin A	Linear	Quadratic
Serum calcium content (mmol/L)	2.16	2.19	2.22	2.27	2.16	2.16	0.02	0.356	0.911	0.077
Serum phosphorus content (mmol/L)	1.60	1.61	1.62	1.62	1.62	1.46	0.02	0.251	0.146	0.082
Calcium content of tibia (g/kg)	91.08 ^b	96.88 ^{a,b}	104.73 ^{a,b}	109.40 ^a	95.35 ^{a,b}	93.72 ^b	2.09	0.076	0.739	0.007
Phosphorus content of tibia (g/kg)	44.32 ^{a,b,c}	44.73 ^{a,b}	47.58 ^{a,b}	50.35 ^a	42.67 ^{b,c}	38.27 ^c	0.99	0.004	0.053	0.001
Ash content of tibia (g/100g)	22.28 ^{a,b}	22.03 ^{a,b}	23.97 ^{a,b}	24.71 ^a	22.31 ^{a,b}	20.60 ^b	0.50	0.197	0.490	0.029

^{a-c}The results are mean values, the same letter or no letter in the same column indicates that the difference is not significant ($P > 0.05$), and different lowercase letters indicate significant difference ($P < 0.05$).

Some studies have shown that intestinal mucosal immunity in mice can be improved, and the growth performance of mice can be improved by supplementing an appropriate amount of VA (Goverse et al., 2014). Vahid et al. (2014) showed that the digestion and absorption of nutrients and feed conversion rate in broilers can be improved, when VA is added to the diet of broilers, so that their growth performance is improved. The results showed that the contents of hormones related to growth, calcium and phosphorus in the serum of gosling that received 9,000 IU/kg of VA supplementation were increased, which was beneficial to the growth and development of the gosling. There was no significant difference in BW between the VA deficiency group and the 15,000 IU/kg VA group. This may be because VA can be transferred to offspring through the maternal. Until the gosling reach 28 d of age, the VA transferred from their maternal can maintain its growth temporarily. However, with the increase of feeding time, the VA deficiency group is bound to have different forms of disease and even death. At the same time, when VA was supplemented for a long time, VA in goslings would deposit, we have reason to believe that the suitable dosage of VA for gosling in the later stage will be lower than 9,000 IU/kg.

Shank Index and Related Indexes of Calcium and Phosphorus

Bone strength can reflect the integrity of bone quality. Calcium and phosphorus are mineral elements essential for the growth and development of animal bones and the maintenance of bone mass. The contents of calcium, phosphorus, and ash in the tibia and serum are important indicators that reflect calcium and phosphorus deposition in bone. Li et al. (2008) showed that adding a large amount of VA to the diet could reduce the growth of broilers and increase the incidence of leg diseases. Excessive VA may lead to diseases such as spontaneous bone fracture, abnormal calcium and phosphorus deposition, and reduced bone strength in poultry (Thomas et al., 2017, 2018; Navarro-Valverde et al., 2018). The results of Manston (1966) showed that dietary supplementation with excessive VA reduced intestinal absorption of calcium and phosphorus, and thus reduced the content of serum calcium and phosphorus. Hough et al. (1988) added excessive VA to the diet of rats; the change in serum calcium was no significant, and the serum phosphorus content was higher than that in the control group. The results showed that the contents of calcium, phosphorus, and crude ash in gosling tibia were the highest when 9,000 IU/kg VA was added to the diet of gosling, and there were no significant effect of VA on serum calcium and phosphorus. This may be because in the metabolic process of the body, it will first meet the blood brain balance. Therefore, even if gosling bone calcium and phosphorus contents change abnormally, the body can maintain a relatively stable blood calcium and phosphorus contents through self-regulation.

In summary, VA supplementation in the diet has an effect on the growth of goslings. There was a quadratic-linear relationship between VA supplement and growth and tibia index of gosling. In terms of growth performance and tibia index, the best dietary VA supplementation of 9,000 IU/kg was found for 0-28 d gosling. Part of VA can be transferred from maternal to offspring by eggs; the short-term lack of VA has little effect on the growth and development of newly hatched gosling.

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DISCLOSURES

The authors declare no conflict of interest for this submitted article entitled "Assessment of vitamin A requirement of gosling in 0 to 28 d based on growth performance and bone indexes".

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