

Dietary vitamin A supplementation improves intestinal morphology and immune performance of goslings

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ABSTRACT. A 28-day experiment was conducted to evaluate the effects of dietary vitamin A (VA) levels on intestinal morphology and immune performance of goslings. Healthy, one-day-old goslings (360) of similar body weight were randomly divided into six groups fed diets supplemented with 0 (A), 3 000 (B), 6 000 (C), 9 000 (D), 12 000 (E), and 15 000 (F) IU/kg VA. Compared to group A, villus height in group D, as well as villus width and crypt depth in groups C and D were increased in the duodenum (quadratic effect, $P < 0.05$); villus height and villus width in groups B, C, D and E, and crypt depth in groups C and D were increased in the jejunum (quadratic effect, $P < 0.05$); villus height and villus width in groups C and D, and crypt depth in groups B, C, D, E and F, as well as muscular layer thickness in group D were increased in the ileum (quadratic effect, $P < 0.05$). Serum immunoglobulin A in groups B, C, D and E, and serum immunoglobulin G levels in groups D and E were higher than in group A (quadratic effect, $P < 0.05$). Serum interleukin-1 (quadratic effect, $P < 0.05$) and interleukin-6 (linear and quadratic effect, $P < 0.05$) levels in groups D and E, and serum interleukin-2 level (quadratic effect, $P < 0.05$) in group C were higher compared to group A. In conclusion, dietary supplementation with 6 000–9 000 IU/kg VA improved intestinal morphology and immune performance of goslings.

Introduction

Carotenoids are a group of important natural pigments that are found in the yellow, orange-red and red colouring compounds of animals, higher plants, fungi, and algae. Carotenoids are the main source of vitamin A (VA) in the body and have antioxidant, immune-regulating, anti-cancer, anti-aging and other properties (Hinds et al., 1997). VA is one of the vitamins essential for poultry growth and development (Clagett-Dame and Knutson, 2011), present mainly in animal tissues and in plants in the form of carotene. The effect of VA is mainly

mediated by its active metabolite, retinoic acid (RA), which is a type of hormone molecule that protects the integrity of intestinal mucosa and prevents intestinal epithelial keratinisation (de Medeiros et al., 2018), as well as exerts photoprotective effects on the skin (Cappai et al., 2016). Since normal epithelial development depends on the VA status, it is recognized as an anti-infective vitamin. Moreover, VA is required for innate and adaptive immunity (Stephensen, 2001). VA deficiency can reduce the integrity of mucosal barrier function and increase the susceptibility to animal diseases (Wang et al., 1997; Stephensen, 2001). The lack of

VA was shown to impair phagocyte function and decrease complement activity, leading to increased-sensitivity of rats to *Staphylococcus aureus* infection (Wiedermann et al., 1996). Dietary VA supplementation alleviated immune organ atrophy and growth depression in cockerels infected with velogenic Newcastle disease virus (Okpe et al., 2015).

However, the appropriate dose of VA for optimal growth and development of goslings has not been precisely determined. Furthermore, there are relatively few studies on the nutritional requirements of VA in geese. Our recent work showed that dietary VA at a dose of 9 000 IU/kg had a beneficial effect on the growth of goslings (Liang et al., 2021). This study was conducted to investigate the effects of different dietary VA levels on intestinal morphology and immune performance in goslings to broaden our understanding of the beneficial effects of adequate VA levels and provide a reference for goose production and related research in the future.

Material and methods

Experimental design and diets

All procedures in the present study were approved by the Yangzhou University (Yangzhou, JS, China) Animal Care and Use Committee (permission number SYXK [Su] IACUC 2012-0029). A total of 360 healthy one-day-old Jiangnan white goslings were randomly allocated to 6 experimental groups with six replicates of 10 birds each in a 28-day experiment. The experimental diets were formulated using the corn-soybean meal basal diet supplemented with 0 (control), 3 000, 6 000, 9 000, 12 000, and 15 000 IU/kg VA (groups A, B, C, D, E, and F, respectively). The diets were formulated to meet the nutritional requirements of goslings according to the NRC (1994) recommendations and the results of our laboratory over the years, except for VA (Table 1). VA (Diesman Vitamin Co., Ltd, Shanghai, China) was added in the form of acetate at a concentration of 1×10^6 IU/g. Animals were managed as described in our previous study, which showed that VA supplementation increased body weight and average daily body weight gain, while decreasing the feed to gain ratio over a 28-day period (Liang et al., 2021).

Sample collection and analysis

Intestinal morphology. The goslings were sacrificed after 28 days of feeding. The duodenum, jejunum and ileum were dissected, and the intestinal contents removed. The weight and length of each

Table 1. Composition and nutrient levels of the basal diet of goslings (on dry matter basis)

Items	Contents
Ingredients, %	
corn	63.0
soybean meal	30.2
rice husk	3.20
methionine	0.10
salt	0.30
stone powder	1.10
calcium hydrogen phosphate	1.10
premix ¹	1.00
total	100.0
Nutritional level	
ME, MJ/kg	11.3
crude protein, %	19.0
crude fibre, %	4.07
Ca, %	0.83
total phosphorus, %	0.56
available phosphorus, %	0.32
lysine, %	0.99
methionine, %	0.42
VA, IU/kg	1 225.0

¹ contain per kilogram of premix: IU: vit. D 300 000, vit. E 1 800; mg: vit. K 150, vit. B₁ 90, vit. B₂ 800, vit. B₆ 320, vit B₁₂ 1.2, niacin 45 000, D-pantothenic acid 1 100, folic acid 65, biotin 5, Se 30, I 50; g: choline 35, Fe 6, Cu 1, Mn 9.5, Zn 9; ME – metabolic energy, VA – vitamin A was converted from carotene, which was the measured value

segment were measured. Tissue samples of the duodenum, jejunum and ileum were fixed in a 4% neutral formaldehyde solution (Servicebio, Wuhan, HB, China) and replaced after 24 h. Fixed 5-mm intestinal tissue sections were collected, subjected to alcohol dehydration and cleared with xylene in a JJ-12J dehydrator (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). After wax immersion treatment, a JB-P5 device was used for embedding. The JJ-12J dehydrator and JB-P5 embedding device were purchased from Wuhan Junjie Electronics Co., Ltd. (Wuhan, HB, China). Sections (3 microns) were cut with an RM2016 microtome (Shanghai Leica Instrument Co., Ltd., Shanghai, China). After staining with hematoxylin-eosin (Servicebio, Wuhan, HB, China), the film was sealed with neutral gum. Villus height and width, crypt depth and muscular layer thickness of the duodenum, jejunum and ileum were measured using an LY-WN-HP-SUPER-CCD image analysis system. A Nikon Eclipse Ci Positive Optical Microscope was used (Nikon, Tokio, Japan).

Villus height was defined as the distance from the top of the villus to its root (lamina propria).

Villus width was defined as the distance at its widest part.

Crypt depth was defined as the distance from the root of the villus to the bottom of the intestinal gland.

Muscular layer thickness was defined as the thickness of the circular and longitudinal muscle of the intestine.

Immunity indices. After 28 days of feeding, blood samples were collected from the inferior vein of the gosling wings. Subsequently, the serum was separated using a DL-5M low speed refrigeration centrifuge and stored in a refrigerator until use. The centrifuge was produced by Changsha Xiangyi Centrifuge Instrument Co., Ltd. (Changsha,

Duncan's multiple range test was used for multiple comparisons. A polynomial orthogonal contrast was used to determine the linear and quadratic relationship between dietary VA levels and histological and immunological parameters. The differences were considered statistically significant at $P < 0.05$.

Results

Intestinal length and weight

The effects of different VA levels on intestinal length and weight of 28-day-old goslings are shown in Table 2. The length and weight of the jejunum

Table 2. Effects of different vitamin A (VA) levels on intestinal length and weight of 28-day-old goslings

Items	A	B	C	D	E	F	SEM	P-value	
								linear	quadratic
Duodenum									
length, cm	30.4	31.4	30.9	33.5	31.0	30.9	0.45	0.65	0.18
weight, g	6.42	6.46	6.67	7.65	7.32	7.17	0.15	0.018	0.35
Jejunum									
length, cm	72.6	72.7	73.7	74.8	72.5	71.8	0.60	0.79	0.23
weight, g	17.3	17.4	17.5	18.3	17.6	18.0	0.29	0.47	0.84
Ileum									
length, cm	69.1	69.9	69.5	71.3	70.1	68.9	0.63	0.94	0.38
weight, g	14.6	14.9	14.5	14.7	14.6	14.3	0.17	0.56	0.66

0 (A) – control, 3 000 (B), 6 000 (C), 9 000 (D), 12 000 (E), and 15 000 (F) IU/kg VA; SEM – standard error of the mean; $P > 0.05$

HN, China). Albumin, globulin, and total protein were determined using a fully automatic biochemical analyser (UniCel Dx C 800 Synchron, Beckman Coulter Inc., Brea, CA, USA). Serum levels of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-1 (IL-1), interleukin-2 (IL-2) and interleukin-6 (IL-6) were determined using an enzyme-linked immunosorbent assay (ELISA) for goslings and a microplate reader (Thermo Multiskan Sky, Waltham, MA, USA). The IgA, IgG and IgM ELISA kits (cat. numbers SEKGE-0002, SEKGE-0001, SEKGE-0003) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The IL-1, IL-2 and IL-6 ELISA kits (cat. Numbers H002, H003, H007) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, JS, China). Measurement procedures were carried out according to the kit instructions.

Statistical analysis. Data were analysed using one-way analysis of variance (ANOVA) and the GLM procedure implemented in SPSS 17.0;

and ileum of goslings was not affected by different levels of VA ($P > 0.05$). The effect of increasing dietary VA levels on the weight of the duodenum was linear ($P = 0.018$).

Intestinal morphology

The effect of different VA levels on the intestinal morphology of 28-day-old goslings is shown in Table 3. The height of duodenal villi in group D and the width of villi and depth of crypts in the duodenum in groups C and D were higher than in the control group ($P < 0.05$). The height and width of jejunal villi in groups B, C, D and E, and the depth of crypts in the jejunum in groups C and D were higher than in the control group ($P < 0.05$). The height and width of the ileal villi in groups C and D, the depth of the ileal crypts in groups B, C, D, E and F, and the thickness of the muscle layer in group D were higher than in the control group ($P < 0.05$). The response in the form of changes in villus height, villus width, and crypt depth in the duodenum to increasing VA levels was quadratic ($P < 0.05$). The response in the

Table 3. Effects of different vitamin A (VA) levels on intestinal morphology of 28-day-old goslings, μm

Items	A	B	C	D	E	F	SEM	P-value	
								linear	quadratic
Duodenum									
villus height	950 ^{bc}	1 012 ^{bc}	1 110 ^{ab}	1 211 ^a	1 046 ^{bc}	906 ^c	26.05	0.98	<0.001
villus width	96.0 ^{bc}	106 ^{ab}	114 ^a	115 ^a	99.0 ^{bc}	92.1 ^c	1.97	0.19	<0.001
crypt depth	242 ^c	268 ^{bc}	287 ^{ab}	313 ^a	275 ^{bc}	254 ^{bc}	6.00	0.31	<0.001
muscular layer thickness	273	280	286	262	255	265	5.00	0.18	0.76
Jejunum									
villus height	916 ^b	1 095 ^a	1 134 ^a	1 187 ^a	1 059 ^a	853 ^b	26.22	0.33	<0.001
villus width	92.8 ^c	119 ^b	162 ^a	163 ^a	149 ^a	96.2 ^{bc}	5.87	0.13	<0.001
crypt depth	239 ^c	244 ^c	291 ^{ab}	319 ^a	280 ^{abc}	263 ^{bc}	7.10	0.040	0.002
muscular layer thickness	267	298	286	272	272	274	4.55	0.52	0.35
Ileum									
villus height	721 ^b	750 ^b	897 ^a	938 ^a	853 ^{ab}	755 ^b	21.85	0.18	0.001
villus width	97.9 ^c	111 ^{bc}	125 ^{ab}	128 ^a	106 ^c	111 ^{bc}	2.60	0.20	<0.001
crypt depth	212 ^c	264 ^b	251 ^b	317 ^a	285 ^{ab}	258 ^b	7.09	0.001	<0.001
muscular layer thickness	230 ^b	259 ^{ab}	269 ^{ab}	293 ^a	261 ^{ab}	268 ^{ab}	5.60	0.038	0.021

0 (A) – control, 3 000 (B), 6 000 (C), 9 000 (D), 12 000 (E), and 15 000 (F) IU/kg VA; SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$, means with same superscript lowercase letters indicate not significant difference in the same row ($P > 0.05$)

form of changes in villus height and villus width in the jejunum and ileum to increasing VA levels was also quadratic ($P < 0.05$). The response in the form of changes in crypt depth in the jejunum and ileum, and muscular layer thickness of the ileum to increasing VA levels was linear and quadratic ($P < 0.05$).

Serum immunoglobulin content

Table 4 shows the effects of different levels of dietary VA supplementation on serum IgA, IgG, and IgM concentrations in 28-day-old goslings.

The concentration of IgA in groups B, C, D and E was higher than those in control and group F ($P < 0.05$). IgG levels in groups D and E were higher compared to control and groups B, C, and F ($P < 0.05$). There was a quadratic effect of VA levels on serum IgA and IgG concentrations ($P < 0.05$).

Serum albumin, globulin, and total protein contents

The effects of dietary vitamin A supplementation on albumin, globulin, and total protein contents in 28-day-old goslings are shown in Table 5. There was

Table 4. Effects of different vitamin A (VA) levels on serum immunoglobulin concentration in 28-day-old goslings (ng/ml)

Items	A	B	C	D	E	F	SEM	P-value	
								linear	quadratic
IgA	0.65 ^b	0.84 ^a	0.97 ^a	0.98 ^a	0.89 ^a	0.62 ^b	0.03	0.91	<0.001
IgG	36.3 ^c	35.5 ^c	35.6 ^c	44.1 ^a	39.9 ^{ab}	27.3 ^d	1.16	0.18	<0.001
IgM	15.3	15.8	17.7	16.0	15.8	17.0	0.33	0.33	0.46

0 (A) – control, 3 000 (B), 6 000 (C), 9 000 (D), 12 000 (E), and 15 000 (F) IU/kg VA; IgA – immunoglobulin A, IgG – immunoglobulin G, IgM – immunoglobulin M, SEM – standard error of the mean; ^{a-d} – means within a row with different superscripts are significantly different at $P < 0.05$, means with same superscript lowercase letters indicate not significant difference in the same row ($P > 0.05$)

Table 5. Effects of different vitamin A (VA) levels on serum albumin, globulin, and total protein contents in 28-day-old goslings

Items	A	B	C	D	E	F	SEM	P-value	
								linear	quadratic
Albumin, g/l	8.93	9.37	9.24	9.44	9.26	9.02	0.14	0.90	0.27
Globulin, g/l	18.1	18.4	18.4	18.5	18.4	18.2	0.21	0.90	0.60
Total protein, g/l	27.6	28.3	27.3	27.6	28.7	27.3	0.38	0.98	0.81

0 (A) – control, 3 000 (B), 6 000 (C), 9 000 (D), 12 000 (E), and 15 000 (F) IU/kg VA; SEM – standard error of the mean; $P > 0.05$

no significant effect of dietary VA level on albumin, globulin, and total protein concentrations ($P > 0.05$).

Serum interleukin content

The effects of dietary VA levels on serum interleukin concentration in 28-day-old gosling are summarized in Table 6. IL-1 levels in groups D and E were higher than in control, and groups B, C, and F ($P < 0.05$). IL-2 content in group C was greater in comparison to control and groups B, E, and F ($P < 0.05$). The content of IL-6 in group D group was higher compared to control, and groups B, C, E, and F ($P < 0.05$). IL-6 level in group E was higher compared to control and group B ($P < 0.05$). A quadratic effect was recorded of VA level on serum IL-1 and IL-2 concentrations ($P < 0.05$). There was a linear and quadratic response of serum IL-6 levels to dietary VA dose ($P < 0.05$).

Table 6. Effects of different vitamin A (VA) doses on serum interleukin levels in 28-day-old goslings (pg/ml)

Items	A	B	C	D	E	F	SEM	P-value	
								linear	quadratic
IL-1	0.16 ^b	0.18 ^b	0.17 ^b	0.24 ^a	0.21 ^a	0.16 ^b	0.01	0.11	<0.001
IL-2	0.24 ^b	0.23 ^b	0.29 ^a	0.26 ^{ab}	0.25 ^b	0.24 ^b	0.01	0.78	0.009
IL-6	0.25 ^c	0.24 ^c	0.26 ^{bc}	0.34 ^a	0.29 ^b	0.26 ^{bc}	0.01	0.017	0.002

0 (A) – control, 3 000 (B), 6 000 (C), 9 000 (D), 12 000 (E), and 15 000 (F) IU/kg VA; IL-1 – interleukin-1, IL-2 – interleukin-2, IL-6 – interleukin-6, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Discussion

VA in the diet was mainly absorbed in the proximal part of the intestine (including the duodenum and jejunum adjacent to the duodenum). VA can protect the integrity of the intestinal mucosa and prevent intestinal epithelial keratinisation. VA supports intestinal barrier function by regulating tight junction proteins, thereby exerting beneficial effects on the intestinal epithelium (de Medeiros et al., 2018). Warden et al. (1996) showed that VA deficiency in the diet of mice reduced the height of intestinal villi. Low vitamin A supply in chicken's diet reduced mucosal protein content, villus height, crypt depth, and mucosal enzyme activity, whereas VA repletion increased this activity (Uni et al., 1998). VA deficiency adversely affected enterocyte proliferation and maturation in the chicken intestinal mucosa, thereby affecting the absorption and metabolism of the intestinal tract (Uni et al., 2000). Therefore, the lack of VA inhibited the growth and development

of poultry. Vitamin A is supplied by the egg yolk in the embryonic stage and must be supplemented externally in the diet after hatching. Vitamin A in egg yolk is provided by the maternal diet. Adequate VA levels in poultry diet have been previously studied in chickens and ducks (Sklan et al., 1994; Yuan et al., 2014; Chen et al., 2016; El-Husseiny et al., 2018; Feng et al., 2019). We have investigated the appropriate VA supplementation levels in breeding geese diet, and the effect of maternal VA supplementation on the intestinal morphology of 7-day-old goslings in our previous study. The results showed that 12 000 IU/kg VA in the maternal diet and 9 000 IU/kg VA in the offspring diet had a positive effect on the intestinal morphology of goslings (Yang et al., 2020). Similarly, the present study demonstrated that dietary supplementation of 6 000–9 000 IU/kg VA was adequate for the intestinal development of goslings. Goose is the most capable of utilising crude fibre among poultry, and its fibre digestion and utilisation process differs significantly from other poultry species. The ability of the ileum, cecum and rectum to digest fibre increases with age (Guo et al., 2022). Muscle thickness reflects intestinal motility, and the effect of vitamin A levels on muscle layer thickness in the ileum could be attributed to a well-developed ileum that is prepared to digest crude fibre. The mucosal epithelium is the first barrier of the immune system. Considering the essential function of VA in the mucosal epithelium, inadequate or too low vitamin A levels can lead to impaired immune function and reduced resistance to infections (Wiedermann et al., 1996; Dalloul et al., 2002). Poultry, whose vitamin A intake is higher than the level recommended by the NRC (1994), may exhibit excellent immune performance (Sklan et al., 1994, 1995). VA supplementation was shown to have a beneficial effect on the immune function of laying hens (Lin et al., 2002). Immunoglobulin is the main effector of serum immunity that plays an important role in the body's defence system. IL-1 is one of the most potent mediators of inflammation in the animal body. During the immune response, it can stimulate T cells to secrete IL-2 and express its receptor, as well as cooperate with IL-2 to promote the production of lymphokine-activated killer cells. IL-2 has a broad spectrum of immune-enhancing activity, which is key to ensuring normal immune function of the body. IL-6 can regulate the immune response, acute phase response and hematopoietic function, and plays an important role in the body's anti-infective immune reaction. VA supplementation was demonstrated to improve specific and non-specific immune systems, including cytokine, lymphocyte transformation,

NK cell activity and phagocytosis (Ross, 1992). Carotene (pro-vitamin A) in a natural plant-based diet cannot meet the growth and health needs of animals, whereas VA has been shown to exert adverse effects after chronic excessive intake due to the long half-life of VA in the body (Cappai et al. 2016; Debelo et al., 2017). Kheirouri and Alisadeh (2014) found that VA and zinc deficiency in mice decreased serum and mucosa IgA levels. Guo et al. (2019) showed that dietary VA supplementation dose of 15 000 IU/kg resulted in the highest serum IL-1 concentrations in 21-day-old broilers, while serum IL-1 and IL-6 levels were the highest at 42 days. In contrast, the concentration of serum immune cytokines in broilers supplemented with VA at doses higher than 15 000 IU/kg showed a downward trend, indicating a decrease in the immune function. This was consistent with previous results showing that excessive VA had an adverse effect on the immune performance of chicks and ducks (Freidman et al., 1991; Yuan et al., 2014). Recommended VA doses vary in relation to different physiological conditions and functional requirements (de Medeiros et al., 2018). The results suggest that dietary supplementation with 6 000–9 000 IU/kg VA can improve the immune performance of goslings.

Conclusions

Appropriate dietary VA supplementation is essential for intestinal health and immune function of goslings. According to the results of the present study, the suitable amount of dietary VA supplementation for 0–28-day-old goslings is 6 000–9 000 IU/kg.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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