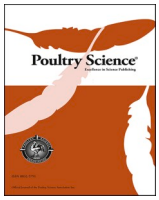
104 (2025) 104780

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj



Full-Length Article

Effects of dietary supplementation levels of vitamin A and vitamin D_3 on growth performance, jejunal function, and tibia development in goslings from 1 to 28 days of age

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ARTICLE INFO

Keywords:
Vitamin A and D_3
Goslings performance
Jejunal function
Tibia mineralization

ABSTRACT

This study explored the interaction effects of dietary Vitamin A ($\mathbf{V_A}$) and Vitamin D_3 ($\mathbf{V_{D3}}$) on growth performance, jejunal function, and tibia development in goslings, aiming to identify any synergistic outcomes that may reshape nutritional strategies for geese production. A total of 540 one-day-old male Jiangnan White goslings with similar body weight (82 ± 5 g) were randomly assigned into 9 treatments with five replicate pens per treatment and 12 birds per pen. The bird trial employed a 3 × 3, two-factorial treatment with three levels of V_A (5000, 7000, and 9000 IU/kg) and three levels of V_{D3} (1000, 1500, and 2000 IU/kg) from one to 28 days of age. Main effects analysis indicated that birds fed 7000 IU/kg V_A exhibited the highest ADG, BW, jejunal maltase activity and IL-10 content ($P < 0.05$), while 9000 IU/kg V_A had the highest SOD activity and content of IL-6 and TNF-α in jejunal mucosa ($P < 0.05$). Both 7000 IU/kg or 9000 IU/kg V_A increased the jejunal IL-1β content, relative expression of tight junction protein 1 (***TJP1***) mRNA, tibia defatted weight and ash weight ($P < 0.05$). Birds fed 2000 IU/kg V_{D3} exhibited the highest ADFI, while both 1500 or 2000 IU/kg V_{D3} increased jejunal maltase activity, and tibia ash content ($P < 0.05$). An interaction between V_A and V_{D3} on ADFI, F/G, jejunal maltase activity, mucosal immune factors (IL-1β, IL-6, IL-10, TNF-α), tibia ash content, and bone morphogenetic protein-2 (***BMP-2***) expression. A simple effects analysis revealed that at a 5000 IU/kg V_A, adding 1000 IU/kg V_{D3} decreased IL-1β, IL-6, TNF-α ($P < 0.05$). At a 7000 IU/kg V_A, adding 1500 or 2000 IU/kg V_{D3} decreased TNF-α, and increased jejunal maltase activity($P < 0.05$). At a 9000 IU/kg V_A, adding 1000 IU/kg V_{D3} decreased ADFI, F/G, jejunal maltase activity, tibia ash, and *BMP-2*, while IL-1β, IL-6, and TNF-α increased ($P < 0.05$). At a 9000 IU/kg V_A, adding 2000 IU/kg V_{D3} increased IL-10 ($P < 0.05$). At a 1000 IU/kg V_{D3}, adding 5000 IU/kg V_A increased F/G, jejunal maltase activity and IL-10, while decreased IL-1β, IL-6, TNF-α ($P < 0.05$), and adding 9000 IU/kg V_A decreased tibia ash and *BMP-2* ($P < 0.05$). At 1500 or 2000 IU/kg V_{D3}, adding 7000 IU/kg V_A increased jejunal maltase activity, IL-10 ($P < 0.05$). At a 2000 IU/kg V_{D3}, adding 9000 IU/kg V_A increased IL-6, and TNF-α ($P < 0.05$). In summary, a dietary level of 7000 IU/kg of V_A and 2000 IU/kg of V_{D3} can be a balanced combination to optimize feed intake and conversion, jejunal function, and tibia mineralization, consequently enhancing growth performance in goslings.

Introduction

Intensive farming impacts geese growth, causing metabolic, intestinal, and bone issues. Vitamin A ($\mathbf{V_A}$) and Vitamin D_3 ($\mathbf{V_{D3}}$) are crucial for bone, intestinal health, and growth, interacting synergistically or antagonistically on nutrient absorption (Blomhoff and Blomhoff, 2006; Norman, 2008).

Vitamin A is crucial for vision, immunity, and poultry development.

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https://doi.org/10.1016/j.psj.2025.104780
Received 30 October 2024; Accepted 3 January 2025
Available online 4 January 2025

Absorbed in the intestine and stored in the liver, it converts to retinoic acid for gene regulation (Tang et al., 1985). V_A deficiency impairs epithelial integrity, nutrient absorption, and growth, while supplementation improves weight, feed efficiency, and immunity, particularly in high-density farming (Vahid et al., 2014; Kucuk et al., 2003). V_A regulates bone remodeling by balancing osteoblast and osteoclast activity; severe deficiency disrupts bone growth, while excess weakens bones (Blomhoff et al., 1991; Thompson and Binkley, 1998), highlighting the need for balance. V_{D3} is essential for calcium (**Ca**) and phosphorus (**P**) metabolism, promoting bone strength and mineralization. It enhances Ca absorption, maintains blood Ca and P levels, and reduces osteoporosis risk (Norman, 2008; Edwards, 2002). V_{D3} supplementation improves tibial strength, mineralization (Jiang et al., 2015; Adhikari et al., 2020), gut health, and immunity by strengthening barriers (Zhu et al., 2015) and reducing oxidative stress (Farhangi et al., 2017). Due to physiological, dietary, and farming methods differences (Chen, 2005), findings in chenkens or ducks may not fully apply to geese. With the national advocacy for green and efficient farming practices, goose farming is transitioning from traditional outdoor systems (such as water surface and free-range grazing) to modern indoor systems, including cage or floor rearing. This transformation has profoundly impacted the demand for vitamins, particularly vitamin D, in geese. Optimizing V_{D3} is crucial for bone and gut health, directly influencing geese growth.

In bone metabolism, V_A and V_{D3} regulate osteoclast and osteoblast activity via the *RANK/RANKL/OPG* pathway and *BMP-2* signaling, critical for bone remodeling and strength (Conaway et al., 2013). V_A increases *OPG* to inhibit bone resorption, while V_{D3} enhances Ca absorption for bone formation, with a balanced ratio optimizing skeletal integrity (Boyle et al., 2003). Excess V_A can weaken bones by reducing V_{D3} effectiveness in Ca absorption (Thompson et al., 1998). Through vitamin D receptor (**VDR**) and the retinoid X receptor (**RXR**), V_A and V_{D3} modulate each other transcriptionally, impacting bone and immune health (Yasmin, 2005). V_A supports epithelial differentiation for nutrient absorption, while V_{D3} regulates Ca transport (Freitas et al., 2021; Uni et al., 2000; Rush et al., 2005). Combined supplementation may improve gut integrity, immune resilience, and growth (Yang et al., 2020). Despite these observations, few studies have focused on $V_A \times V_{D3}$ interactions in geese, and the underlying mechanisms remain unclear.

Studies suggest V_A levels of 5000-9000 IU/kg and V_{D3} levels of 1000-2000 IU/kg meet growth needs for medium and small geese breeds (Zhang et al., 2020). However, most research examined them individually, neglecting their interactions. In broilers, V_A and V_{D3} impact body weight, bone ash, and conditions like hypocalcemia and rickets (Aburto and Britton, 1998a,b; Aburtoetal.,1998). Excessive V_A disrupts V_{D3}-mediated Ca absorption, increasing tibial dyschondroplasia risk (Świątkiewicz et al., 2017). High V_A intake (20,000-35,000 IU/kg) elevates intestinal VDR expression but reduces immune function (Yuan et al., 2014). These findings emphasize the need to explore V_A and V_{D3} interactions and their potential synergistic or antagonistic effects with in geese, considering their unique physiological and nutritional requirements.

This study hypothesizes that dietary V_A and V_{D3} levels interact to influence gosling growth. It investigates the effects of V_A (5000, 7000, 9000 IU/kg) and V_{D3} (1000, 1500, 2000 IU/kg), individually and combined, on growth, jejunal function, and tibial development in Jiangnan White goslings. The findings aim to optimize V_A and V_{D3} supplementation, enhance growth, lower feed costs, and support sustainable geese farming.

Materials and methods

Ethical statement

All procedures of this study were permitted by the Institutional Animal Care and Use Committee (IACUC) of the Yangzhou University Animal Experiments Ethics Committee, with permit number SYXK (Su) IACUC 2020-0910.

Experimental design and animal husbandry

A total of 540 one-day (**D**) -old male Jiangnan White goslings with similar body weights (BW, 82±5 g) were randomly assigned to nine groups, with five replicates per group and 12 goslings per replicate. All goslings were hatched from the same batch provided by Jiangsu Lihua Animal Husbandry Co., Ltd. (Changzhou, China). A 3 × 3, two-factorial treatment was employed, with three levels of V_A supplementation (5000, 7000, and 9000 IU/kg) and three levels of V_{D3} supplementation (1000, 1500, and 2000 IU/kg). V_A and V_{D3} (500,000 IU/g) were sourced from Yangzhou Shuangyang Biotechnology Co., Ltd. (Yangzhou, China). The basal diet was formulated to meet the nutritional requirements of geese, following the NRC (1994) guidelines and based on previous research from our laboratory (Liang et al., 2021). The composition and nutrient levels of the diets are shown in Table 1. The experimental period lasted for 28 D. The birds of each replicate were housed in a plastic wire-floor pen (2 × 0.9 × 1 m) in an environmentally controlled room with feed and water provided *ad libitum*. The lighting schedule was programmed as following: 1 D, 24 h; 2-4 D, 23 h; 5-28 D, 18 h, according to our previous research with modification (Yu et al., 2022). The room temperature was gradually reduced from 31°C to 22°C, with an average decrease of 1-2°C every two days according to birds age.

Growth performance

The body weight and feed intake of each cage were recorded during the 28-D experimental period. BW, (**ADFI**), average daily gain (**ADG**), and the feed/gain ratio (**F/G**) were calculated for the growth performance.

Sample collection

At the end of 28 D of age, all geese were weighed individually after fasting for 6 h. One goose of each pen with a BW closest to the pen's average was selected, and a 4 mL blood sample was collected from the right neck vein for proposed measurements. Then, the bird was exsanguinated by severing the jugular vein and carotid artery. After slaughtering, a segment of the proximal jejunum was collected, the chyme was removed, and the mucosa was carefully scraped into enzyme-free tubes, then immediately stored at −80°C for further analysis of digestive

Table 1
Composition and nutrients of basal diets for 1-28 d geese.

Ingredients (%)		Nutritional level[2]	
Corn	60.5	ME (MJ/kg)	11.4
Soybean meal	29.4	Crude protein (%)	18.8
Wheat bran	6.20	Crude fiber (%)	3.29
Stone powder	1.18	Ca (%)	0.80
Ca hydrogen phosphate	1.32	Total P (%)	0.62
DL-Methionine	0.10	Non-phytate phosphorus (%)	0.32
Salt	0.30	Methionine (%)	0.38
Premix[1]	1.00	Lysine (%)	0.97
Total	100.00	Vitamin A (IU/kg)	1044
		Vitamin D_3 (IU/kg)	not detected

[1] The premix provides per kilogram of diet (without VA and V_{D3}): 18 IU vitamin E (D-a-tocopherol), 1.5 mg vitamin K (coagulation vitamin), 0.6 mg vitamin B_1 (thiamine), 8 mg vitamin B_2 (riboflavin), 3.2 mg vitamin B_6 (pyridoxine), 0.012 mg vitamin B_{12} (cobalamin), 0.045 mg nicotinic acid, 11 mg pantothenic acid, 0.65 mg folic acid, 0.05 mg biotin, 0.45 mg choline, 60 mg Fe (ferrous sulfate), 10 mg Cu (copper sulfate), 95 mg Mn (manganese sulfate), 90 mg Zn (zinc sulfate), 0.5 mg I (potassium iodide), and 0.3 mg Se (sodium selenite).

[2] Measured values except lysine and methionine in the basal ration. V_{D3} less than detection limit (12.5 μg/kg).

enzyme activities, antioxidant capacity, and immune response. After removing the muscle and fascia, the left tibia was used for measuring bone growth parameters before collecting bone marrow (the top side) for gene expression analysis (flash-frozen in liquid nitrogen, ground into powder using a mortar and pestle, and stored at −80°C) . The right tibia was used to determine bone mineralization.

Jejunal and tibia RNA extraction and quantitative RT-PCR

The relative mRNA expression levels of *Occludin* and Tight Junction Protein 1 (***TJP1***) in the jejunal mucosa, as well as OPG, Receptor Activator of Nuclear Factor-κB Ligand (***RANKL***), and Bone Morphogenetic Protein-2 (***BMP-2***) in the bone marrow of the left tibia, were quantified using *β*-actin as the reference gene. The primer sequences were synthesized by Genewiz Biotechnology Co., Ltd. (Suzhou, China) according to NCBI guidelines, and the sequences are listed in Table 2. Total RNA was extracted from the jejunal mucosa and tibial tissue using an RNA extraction kit (cat. No. 19221ES), and the concentration and purity were determined using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). RNA integrity was verified by determining RNA concentration and by 1 % agar gel electrophoresis. Reverse transcription was performed using a commercial kit (cat. No. 11119ES60), and real-time quantitative PCR was conducted on an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific, Waltham, USA) to assess the mRNA expression. The reaction system consisted of a total volume of 20 μL: 10 μL of Hieff qPCR SYBR Green MasterMix (No Rox, cat. No. 11201ES03), 0.4 μL of each forward and reverse primer, 2 μL of cDNA template, and 7.2 μL of nuclease-free water. The reaction conditions were as follows: pre-denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 10 s and annealing at 60°C for 30 s. The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak et al., 2001). All kits were purchased and were used following the protocols provided by a commercial manufacturer (Yeasen Biotechnology Co., Ltd., Shanghai, China).

Jejunal mucosa digestive enzyme activity, antioxidant capacity, and immunity

The jejunal mucosa was weighed and homogenized with a diluent nine times its weight using a fast homogenizer under ice bath conditions. The resulting mixture was centrifuged to remove debris. One part of the supernatant was analyzed, while the rest was stored at −20 °C for further study. The levels of total protein (**TP**, cat. No. A045-3-2), total antioxidant capacity (**T-AOC**, cat. No. A015-2-1), glutathione peroxidase activity (**GSH-Px**, cat. No. A005-1-2), and superoxide dismutase activity (**SOD**, cat. No. A001-1) in all tissue samples were measured using kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The results were normalized against the TP concentration of each sample for intersample comparison. Maltase activity (cat. No. ml063476), sucrase activity (cat. No. ml063476), interleukin (IL)−1β (cat. No. ml061217), IL-6 (cat. No. ml061196), IL-10 (cat. No. ml061196), and tumour necrosis factor-α (**TNF-α**, cat. No. ml036890) were determined using Enzyme-Linked Immunosorbent Assay (***ELISA***) kits from Shanghai Meilian Biotechnology Co., Ltd. (Shanghai, China).

Table 2
Gene-specific primers used for the analysis of mRNA levels using real-time quantitative polymerase chain reaction (RT-qPCR).

Gene name	Gene Bank No	Primer sequence	Product size/bp
TJP1	XM_013177404.1	F: TGAGAGAGTTGTTCTTCGGGAAG R: TCTGTACCAGCATCTCTTGGTTC	278
Occludin	XM_013199669.1	F: TGCTTCCAGCTCCATCCAAG R: CTTGTCGTAGTCGCTCACCA	147
OPG	XM_013185062	F: CATCTCAACACACTGATGGCAAG R: GATGGTGTCTTGGTCTCCATTCT	147
RNAKL	XM_013179680	F: ACCTGACTAAAAGAGGGCTTCAG R: AGTATTTGGTGCTTCCTCCCTTC	102
BMP-2	XM_013182079.1	F: GCACCCAGCACGATGAAAAT R: GACAATGGAGGGTCCGGATT	276
β-actin	XM_013174886.1	F: GCACCCAGCACGATGAAAAT R: GACAATGGAGGGTCCGGATT	150

TJP1: Tight junction protein 1, *OPG*: Osteoprotegerin, *RNAKL*: Receptor Activator of Nuclear Factor-κ B Ligand, *BMP-2*: Bone morphogenetic protein-2.

Tibia growth and mineralization parameters

A DL-3946 high-precision digital vernier caliper (Deli Group, Ningbo, China) was used to measure the whole length and the width of the narrowest part of the left tibial midshaft.

The right tibial midshaft underwent a strength test using an Instron 3367 instrument (Instron Corporation, Norwood, USA). The testing method followed the procedure described by Li et al. (2022). According to Li et al. (2020) and Li et al. (2022), the right tibia was initially dried in an oven at 65°C for 24 h. It was then wrapped in a filter paper bag, tightly secured with degreased cotton thread, and soaked in petroleum ether for 7 D, with the solvent being replaced every 24 h. Following defatting, the tibia was dried again at 65°C for an additional 24 h. The defatted weight of tibia was recorded, and the relative defatted weight of the tibia was calculated as follows:

$$\text{Relative defatted weight (g/kg)} = \frac{\text{Defatted weight (g)}}{\text{Live body weight (kg)}}$$

The dried bones were crushed, charred in an electric furnace, and were burned in a SX2-4-10 muffle furnace at 550–580°C for 12 h (Gengfa Pharmaceutical Equipment Co., Ltd., Shanghai, China). The ash content of the bone samples was weighed and expressed as a percentage of defatted bone weight. Skeletal ash was determined according to GB/T 6438-2007 standard. Add 10 mL of a 1: 3 volume ratio of hydrochloric acid solution and 3-4 drops of concentrated nitric acid to the crucible containing the crude ash. Heat the mixture in the crucible until it boils, then cool it to 60-70°C. Transfer the solution to a 100 mL volumetric flask, rinse the crucible and filter paper with deionized water at 60-70°C, and bring the volume to 100 mL. Mix thoroughly to prepare the Ca and P sample decomposition solution. The Ca was determined by the ethylenediaminetetraacetic acid method based on the GB/T6436–2018 standard. The P was determined via the molybdenum yellow colorimetry method as per the GB/T6437–2018 standard.

Statistical analyses

Statistical analyses were conducted using the Generalized Linear Model (**GLM**) procedure in SPSS 21.0, with multiple comparisons performed using the Tukey's multiple range test. Data are presented as means. Simple effects analysis was conducted for data exhibiting interaction effects and multiple comparisons were performed using the Tukey's multiple range test. The difference was significant when $P < 0.05$.

Results

Growth performance

As shown in Table 3, both dietary level of VA and V_{D3} affected the growth performance of goslings with an interaction effect. Compared with 5000 or 9000 IU/kg V_A, supplementation with 7000 IU/kg of V_A increased the BW at 28 D and the ADG from 1 to 28 D ($P < 0.05$).

Table 3
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on growth performance of goslings from 1 to 28 D of age.

Groups	V_A, IU/kg	V_{D3}, IU/kg	BW, g	ADG, g/d	ADFI, g/d	F/G, g/g
A	5000	1000	1509	51.1	110	2.16^{a}
B		1500	1507	50.7	107	2.10
C		2000	1585	53.6	112	2.10
D	7000	1000	1598	54.1	109	2.03^{b}
E		1500	1579	53.0	112	2.12
F		2000	1615	54.7	115	2.10
G	9000	1000	1512	50.9	103^{B}	2.02^{Bb}
H		1500	1561	52.8	113^{A}	2.15^{A}
I		2000	1564	52.9	113^{A}	2.13^{A}
Main effects						
	5000		1534^{b}	51.8^{b}	110	2.12
V_A	7000		1597^{a}	53.9^{a}	112	2.08
	9000		1546^{b}	52.2^{b}	110	2.10
		1000	1540	52.1	108^{b}	2.07
V_{D3}		1500	1549	52.2	111^{ab}	2.12
		2000	1588	53.7	113^{a}	2.11
SEM			10.5	0.373	0.841	0.012
P-value	V_A		0.026	0.038	0.301	0.315
	V_{D3}		0.111	0.098	0.014	0.172
	$V_A \times V_{D3}$		0.607	0.535	0.044	0.048

Main effects analysis ($n = 15$): different lowercase letters in the same column indicate differences ($P < 0.05$), while the same letters or the absence of letters indicate no difference ($P > 0.05$). The same applies to the tables below.
Rows A-F were analyzed for simple effects ($n = 5$): different uppercase letters indicate differences between different V_{D3} levels at the same VA level ($P < 0.05$), while different lowercase letters indicate differences between different VA levels at the same V_{D3} level ($P < 0.05$). The same applies to the tables below.
BW: Body weight, ADG: Average daily weight gain, ADFI: Average daily feed intake, F/G: Feed intake/ weight gain.

Compared with 1000 IU/kg V_{D3}, adding 2000 IU/kg V_{D3} increased ADFI ($P < 0.05$). There was an interaction effect between V_A and V_{D3} on ADFI and F/G from day 1 to 28 ($P < 0.05$). From the simple effects analysis, at a V_{D3} level of 1000 IU/kg, compared with 7000 or 9000 IU/kg V_A, the addition of 5000 IU/kg V_A increased F/G from 1 to 28 D ($P < 0.05$). However, at 9000 IU/kg V_A, compared with 1500 or 2000 IU/kg V_{D3}, supplementation with 1000 IU/kg V_{D3} decreased ADFI and F/G from day 1 to 28 ($P < 0.05$).

Jejunal mucosa digestive enzyme activity

As shown in Table 4, both dietary level of VA and V_{D3} affected the digestive enzyme activity in the jejunal mucosa of goslings with an interaction effect. Supplementation with 7000 IU/kg of V_A increased maltase activity ($P < 0.05$) compared with 9000 IU/kg. Compared with 1000 IU/kg, supplementation with 1500 or 2000 IU/kg of V_{D3} increased maltase activity ($P < 0.05$). There was an interaction effect between V_A and V_{D3} on maltase activity ($P < 0.05$). From the simple effects analysis, at a V_{D3} level of 1000 IU/kg, compared with 7000 or 9000 IU/kg V_A, the addition of 5000 IU/kg V_A increased maltase activity ($P < 0.05$). At V_{D3} levels of 1500 or 2000 IU/kg, compared with 5000 or 9000 IU/kg V_A, the addition of 7000 IU/kg V_A also increased maltase activity ($P < 0.05$). At V_A levels of 7000 or 9000 IU/kg, the addition of 1000 IU/kg V_{D3} decreased maltase activity ($P < 0.05$).

Table 4
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on maltase and sucrase activity of goslings at 28 D of age.

Groups	V_A, IU/kg	V_{D3}, IU/kg	Maltase, U/mL	Sucrase, U/mL
A	5000	1000	17.6^{a}	81.0
B		1500	18.1^{b}	85.6
C		2000	18.2^{b}	86.3
D	7000	1000	14.3^{Bb}	83.2
E		1500	20.9^{Aa}	86.3
F		2000	20.9^{Aa}	82.2
G	9000	1000	15.6^{Bb}	76.0
H		1500	18.4^{Ab}	84.4
I		2000	17.71^{ABb}	86.5
Main effects				
V_A	5000		18.0^{ab}	84.3
	7000		18.7^{a}	83.9
	9000		17.2^{b}	82.3
V_{D3}		1000	15.8^{b}	80.0
		1500	19.1^{a}	85.4
		2000	18.9^{a}	85.0
SEM			0.358	1.45
P-value	V_A		0.022	0.853
	V_{D3}		< 0.001	0.282
	$V_A \times V_{D3}$		< 0.001	0.796

Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

Jejunal mucosa antioxidant enzymes activity

As shown in Table 5, both dietary level of V_A and V_{D3} affected the antioxidant capacity of the jejunal mucosa in goslings without interaction effect. Compared with 5000 IU/kg V_A, supplementation with 9000 IU/kg of V_A increased mucosal SOD activity ($P < 0.05$).

Immunity in jejunal mucosa

As shown in Table 6, both dietary level of V_A and V_{D3} affected the immune response of the jejunal mucosa in goslings with an interaction effect. Compared with 5000 IU/kg V_A, supplementation with 7000 or 9000 IU/kg of V_A increased IL-1β levels ($P < 0.05$). Compared with 5000 or 7000 IU/kg V_A, supplementation with 9000 IU/kg V_A increased the levels of IL-6 ($P < 0.05$). Compared with 5000 IU/kg V_A, supplementation with 7000 IU/kg V_A increased the levels of IL-10 ($P < 0.05$). Compared with 7000 IU/kg V_A, Supplementation with 9000 IU/kg V_A increased the levels of TNF-α ($P < 0.05$). Notably, there was an interaction effect between V_A and V_{D3} on the levels of IL-1β, IL-6, IL-10, and TNF-α ($P < 0.05$). From the simple effects analysis, at a V_{D3} level of 1000 IU/kg, an increase in V_A supplementation led to higher levels of IL-1β, IL-6, and TNF-α, while IL-10 levels decreased ($P < 0.05$). At a V_{D3} level of 1500 IU/kg, as V_A levels increased, the IL-10 levels first increased and then decreased ($P < 0.05$). ($P < 0.05$). At a V_{D3} level of 2000 IU/kg, the supplementation of 5000 IU/kg V_A increased IL-6, and TNF-α levels, while reducing IL-10 levels ($P < 0.05$). At a V_A level of 5000 IU/kg, increasing V_{D3} supplementation led to higher levels of IL-1β, IL-6, and TNF-α, with a corresponding decrease in IL-10 levels ($P < 0.05$). At a V_A level of 7000 IU/kg, the addition of 1000 IU/kg V_{D3} increased TNF-α levels ($P < 0.05$). At a V_A level of 9000 IU/kg, increasing V_{D3}

Table 5
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on jejunal mucosa antioxidant enzymes activity of goslings at 28 D of age.

Groups	V_A, IU/kg	V_{D3}, IU/kg	SOD, U/mg Prot	T-AOC, U/mg Prot	GSH-Px, U/mg Prot
A	5000	1000	120	4.12	73.7
B		1500	118	4.32	74.6
C		2000	113	4.54	73.3
D	7000	1000	125	4.74	81.8
E		1500	135	4.54	77.0
F		2000	131	4.70	80.6
G	9000	1000	135	4.82	82.3
H		1500	142	4.80	84.4
I		2000	137	4.72	86.7
Main effects					
V_A	5000		117^{b}	4.33	73.9
	7000		130^{ab}	4.66	79.8
	9000		138^{a}	4.78	84.5
V_{D3}		1000	127	4.56	79.3
		1500	132	4.55	78.6
		2000	127	4.65	80.2
SEM			3.37	0.116	2.54
P-value	V_A		0.045	0.306	0.286
	V_{D3}		0.810	0.933	0.971
	$V_A \times V_{D3}$		0.966	0.947	0.989

SOD: Superoxide dismutase, T-AOC: Total antioxidant capacity, GSH-Px: Glutathione peroxidase. Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

Table 6
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on jejunal mucosa immunity of goslings at 28 D of age.

Groups	V_A, IU/kg	V_{D3}, IU/kg	IL-1β, pg/mL	IL-6, pg/mL	IL-10, pg/mL	TNF-α, pg/mL
A	5000	1000	23.0^{Bc}	27.2^{Cc}	74.9^{Aa}	140^{Bb}
B		1500	36.4^{A}	39.8^{B}	53.0^{Bc}	155^{B}
C		2000	39.3^{A}	45.6^{Aa}	52.8^{Bb}	199^{Aa}
D	7000	1000	40.4^{b}	41.4^{b}	63.0^{b}	183^{Aa}
E		1500	38.9	39.4	69.9^{a}	141^{B}
F		2000	32.7	37.5^{b}	68.7^{a}	145^{Bb}
G	9000	1000	48.7^{Aa}	57.4^{Aa}	58.0^{Bb}	202^{Aa}
H		1500	37.3^{B}	41.7^{B}	61.5^{Bb}	180^{AB}
I		2000	32.5^{B}	40.3^{Bab}	71.2^{Aa}	155^{Bb}
Main effects						
V_A	5000		32.9^{b}	37.5^{b}	60.2^{b}	165^{ab}
	7000		37.3^{a}	39.4^{b}	67.2^{a}	156^{b}
	9000		39.5^{a}	46.5^{a}	63.6^{ab}	179^{a}
V_{D3}		1000	37.4	42.0	65.3	175
		1500	37.5	40.3	61.5	159
		2000	34.8	41.11	64.2	166
SEM			1.14	1.25	1.34	4.49
P-value	V_A		< 0.001	< 0.001	0.003	0.019
	V_{D3}		0.148	0.518	0.130	0.118
	$V_A \times V_{D3}$		< 0.001	< 0.001	< 0.001	< 0.001

IL: Interleukin, TNF-α: Tumor necrosis factor α.
Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

supplementation resulted in lower levels of IL-1β, IL-6, and TNF-α, while IL-10 levels increased ($P < 0.05$).

Relative mRNA expression of tight junction protein in jejunal mucosa

As shown in Table 7, both dietary level of V_A and V_{D3} affected the mRNA expression of tight junction proteins in the jejunal mucosa of goslings without interaction effect. Compared with 5000 IU/kg V_A, supplementation with 7000 or 9000 IU/kg V_A increased the relative mRNA expression of *TJP1* in the jejunal mucosa ($P < 0.05$).

Tibia growth parameters

As shown in Table 8, both dietary level of V_A and V_{D3} affected the tibia growth in goslings without interaction effect. Compared with 5000 IU/kg V_A, supplementation with 7000 or 9000 IU/kg V_A increased the defatted weight of the tibia ($P < 0.05$).

Tibia bone mineralization

As shown in Table 9, dietary level V_{D3} affected the bone mineralization of tibia in goslings with an interaction effect of V_A and V_{D3}. Compared with 1000 IU/kg V_{D3}, supplementation with 1500 or 2000 IU/kg V_{D3} resulted in an increase tibia ash content ($P < 0.05$). Compared with 5000 IU/kg V_A, supplementation with 7000 or 9000 IU/kg V_A increased ash weight ($P < 0.05$). An interaction effect was also observed between V_A and V_{D3} on tibia ash content in goslings ($P < 0.05$). From the simple effects analysis, at a 1000 IU/kg V_{D3} level, compared with 5000 IU/kg V_A, supplementation with 9000 IU/kg V_A reduced tibia ash content ($P < 0.05$). At a 9000 IU/kg V_A level, compared with1500 or 2000 IU/kg V_{D3}, supplementation with 1000 IU/kg V_{D3} reduced tibia ash content ($P < 0.05$).

Relative mRNA expression of tibial bone metabolism-related genes in tibia

As shown in Table 10, both dietary level of V_A and V_{D3} affected the

Table 7
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on the relative mRNA expression of tight junction protein in jejunal mucosa of goslings at 28 D of age.

Groups	V_A, IU/kg	V_{D3}, IU/kg	*Occludin*	*TJP1*
A	5000	1000	0.96	0.84
B		1500	0.96	0.89
C		2000	0.90	0.88
D	7000	1000	1.06	1.23
E		1500	0.91	1.26
F		2000	0.94	1.36
G	9000	1000	0.97	1.04
H		1500	0.92	1.89
I		2000	1.03	1.05
Main effects				
V_A	5000		0.94	0.87[b]
	7000		0.97	1.29[a]
	9000		0.97	1.33[a]
V_{D3}		1000	1.00	0.89
		1500	0.93	1.01
		2000	0.96	1.00
SEM			0.060	0.078
P-value	V_A		0.978	0.017
	V_{D3}		0.917	0.159
	$V_A \times V_{D3}$		0.984	0.128

TJP1: Tight junction protein 1.
Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

the expression of tibial bone metabolism-related genes in goslings with an interaction effect. However, an interaction effect between V_A and V_{D3} was observed on the relative expression of *BMP-2* in the tibia of goslings ($P < 0.05$). From the simple effects analysis, at the 1000 IU/kg V_{D3} level, compared with 5000 IU/kg V_A, supplementation with 9000 IU/kg V_A decreased *BMP-2* relative expression ($P < 0.05$). At a 9000 IU/kg V_A level, compared with1500 or 2000 IU/kg V_{D3}, supplementation with 1000 IU/kg V_{D3} reduced *BMP-2* relative expression ($P < 0.05$).

Discussion

Growth performance

Growth performance is a widely used metric for determining V_A and V_{D3} requirements in goslings, particularly during the starter phase (Liang et al., 2021). The three dietary V_A and V_{D3} levels set in this experiment are based on our previous study of the optimal amounts of these vitamins in goslings (Liang et al., 2021; Lin, 2023). Based on examining their interactions, this study also aims to identify the minimum dosage required to maintain unaffected growth performance at relatively lower supplementation levels, with the goal of further promoting energy conservation, emission reduction, and feed cost savings. Supplementing V_A in poultry diets promotes growth by enhancing nutrient digestion, absorption, and feed efficiency (Vahid et al., 2014). Feng et al. (2019) reported that V_A deficiency in starter White Pekin ducks impairs growth, causes dry eye disease, and lowers tissue retinol. Broken-line regression set V_A requirements from hatch to 21 days at 2606 IU/kg for weight gain and 4371 IU/kg for plasma retinol. Excessive V_A can lead to waste, toxicity, and reduced economic benefits in poultry production (Khan et al., 2023). Liang et al. (2021) found that 9000 IU/kg V_A supplementation optimized growth in Jiangnan White geese. For V_{D3}, appropriate inclusion reduces feed intake, but excessive levels may not support growth (Edwards et al., 2002). Sakkas et al. (2019) noted no improvement in broiler growth with V_{D3} levels of 1000–7000 IU/kg, and Rush et al. (2005) reported no significant effects on weight gain or feed-to-meat ratio in Pekin ducks at 826 or 8260 IU/kg. These differences may stem from variations in V_{D3} levels, potentially placing those studies on opposite sides of the quadratic response curve. In our study, goslings showed optimal growth with 7000 IU/kg V_A and 2000 IU/kg V_{D3}, while 9000 IU/kg V_A and 1000 IU/kg V_{D3} resulted in poorer growth, likely due to an excessive V_A:V_{D3} ratio. Veltmann et al. (1986) reported that 45,000 IU/kg V_A significantly antagonized V_{D3}, reducing broiler growth, with the effect intensifying at lower V_{D3} levels (1,000, 200, 100 IU/kg), especially near critical thresholds. Moderate V_A levels (1,500 or 15,000 IU/kg) caused only weak antagonism at low V_{D3} levels. These findings suggest V_A toxicity is influenced by V_{D3} intake, with deficiency worsening its effects. Research indicates a mutual inhibition between V_A and V_{D3}, where each mitigates the other's toxicity under imbalances (Masterjohn, 2007). A high V_A:V_{D3} ratio disrupts their synergy, causing deficiencies and harming growth performance (Khan et al., 2023). This study confirms that an excessive V_A:V_{D3} ratio has adverse effects akin to V_A toxicity.

Jejunal function

Disaccharidase activity determines carbohydrate digestion and transport in poultry. Both V_A excess and deficiency negatively affect disaccharidase activity (Wang et al., 2020b). In our study, 7000 IU/kg V_A supplementation increased maltase activity, enhancing carbohydrate

Table 8
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on tibia growth parameters in goslings at 28 D of age.

Groups	V_A, IU/kg	V_{D3}, IU/kg	Length, mm	Width, mm	Defatted Weight, g	Relative Defatted Weight [1], g/kg
A	5000	1000	110	6.97	4.50	2.97
B		1500	109	7.27	4.48	3.18
C		2000	110	6.88	4.63	2.92
D	7000	1000	111	7.55	5.20	3.25
E		1500	113	7.40	5.19	3.31
F		2000	111	6.88	4.82	2.98
G	9000	1000	112	7.40	4.95	3.28
H		1500	112	7.33	4.99	3.30
I		2000	113	7.46	5.08	3.26
Main effects						
	5000		110	7.04	4.64[b]	3.02
V_A	7000		112	7.28	5.07[a]	3.18
	9000		112	7.40	5.01[a]	3.24
		1000	111	7.30	4.88	3.17
V_{D3}		1500	111	7.33	4.99	3.23
		2000	111	7.08	4.84	3.05
SEM			0.586	0.072	0.065	0.043
P-value	V_A		0.188	0.110	0.015	0.094
	V_{D3}		0.988	0.245	0.583	0.228
	$V_A \times V_{D3}$		0.912	0.279	0.500	0.502

[1] Relative Defatted Weight (g/kg)= Defatted Weight of Tibia (g) ÷ Live Body Weight (kg)
Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

Table 9
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on tibia bone mineralization parameters in goslings at 28 D of age.

Group	V_A, IU/kg	V_{D3}, IU/kg	Ash Weight, g	Ash, %	Ca, %	P, %	Strength, N
A	5000	1000	2.56	57.0^{a}	21.4	9.08	611
B		1500	2.65	55.3	21.1	9.45	621
C		2000	2.55	55.0	20.8	8.64	623
D	7000	1000	2.82	54.2^{ab}	21.0	9.57	563
E		1500	2.95	56.9	20.9	9.05	637
F		2000	2.76	57.2	20.9	9.63	625
G	9000	1000	2.60	52.5^{Bb}	20.4	9.24	559
H		1500	2.83	56.9^{A}	20.7	9.80	639
I		2000	2.95	57.9^{A}	20.8	9.49	657
Main effects							
	5000		2.59^{b}	55.8	21.1	9.06	619
V_A	7000		2.84^{a}	56.1	21.0	9.42	609
	9000		2.79^{a}	55.8	20.6	9.51	618
		1000	2.66	54.6^{b}	20.9	9.30	578
V_{D3}		1500	2.81	56.4^{a}	20.9	9.43	632
		2000	2.75	56.7^{a}	20.9	9.26	635
SEM			0.039	0.216	0.300	0.145	12.6
P-value	V_A		0.012	0.902	0.857	0.443	0.937
	V_{D3}		0.221	0.042	0.994	0.878	0.142
	$V_A \times V_{D3}$		0.321	0.013	0.992	0.544	0.812

Ca: calcium, P: phosphorus.
Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

Table 10
Mean comparation and significance of interaction effect of dietary $V_A \times V_{D3}$ on the relative mRNA expression of bone metabolism genes of tibia in goslings at 28 D of age.

Group	V_A, IU/kg	V_{D3}, IU/kg	*RNAKL*	*OPG*	*BMP-2*
A	5000	1000	1.00	1.04	1.06^{a}
B		1500	1.07	0.96	0.95
C		2000	1.08	1.12	0.85
D	7000	1000	1.10	1.21	0.82^{ab}
E		1500	1.08	1.05	1.02
F		2000	1.08	1.23	1.01
G	9000	1000	1.06	1.10	0.65^{Bb}
H		1500	1.08	1.13	1.05^{A}
I		2000	1.02	1.06	1.05^{A}
Main effects					
	5000		1.05	1.04	0.95
V_A	7000		1.08	1.16	0.95
	9000		1.05	1.10	0.92
		1000	1.05	1.12	0.84
V_{D3}		1500	1.08	1.05	1.01
		2000	1.06	1.14	0.97
SEM			0.035	0.072	0.035
P-value	V_A		0.935	0.810	0.882
	V_{D3}		0.958	0.888	0.104
	$V_A \times V_{D3}$		0.980	0.983	0.028

OPG: Osteoprotegerin, *RNAKL*: Receptor Activator of Nuclear Factor-κB Ligand, *BMP-2*: Bone morphogenetic protein-2.
Main effects analysis: $n = 15$; Rows A-F were analyzed for simple effects: $n = 5$.

digestion and energy provision, thereby supporting gosling growth. The effects of V_{D3} on disaccharidase activity remain unexplored in livestock. V_A supports intestinal epithelial integrity and enzyme-producing cell differentiation (Combs, 2012), while V_{D3} regulates calcium absorption and intestinal structure (Christakos et al., 2016). Both modulate gene expression via retinoic acid and calcitriol, and an imbalanced V_A:V_{D3} ratio disrupts their synergy (Masterjohn, 2007). High V_A or low V_{D3} may alter gut microbiota, damage epithelium, or trigger oxidative stress, further reducing maltase activity (Khan et al., 2023). Their combined influence on intestinal structure, metabolism, and gene regulation likely impacts maltase activity.

Free radical damage is a major factor affecting intestinal mucosa (Yang et al., 2019). Normally, oxygen-free radical production and removal are balanced, but imbalance causes peroxidation damage to cell membranes and organelles (Khan et al., 2013). SOD, GSH-Px, and T-AOC activities reflect the ability to remove free radicals and indicate damage to cell membranes (Riccioni et al., 2015). In this study, dietary V_A significantly increased SOD activity, especially at 9000 IU/kg. V_A and VD inhibit oxidative stress and apoptosis, enhancing antioxidant capacity and reducing immune stress-induced damage (Blaner et al., 2021; Shojadoost et al., 2015; Palace et al., 1999; Wang et al., 2020a). Insufficient V_A or V_D disrupts the oxidative balance, hindering healthy growth (Palace et al., 1999; Kim et al., 2020). The intestinal mucosal immune system serves as the first defense against pathogens, and its compromise increases vulnerability to infections. V_A and V_D play crucial roles in stabilizing mucosal immunity. Yang et al. (2011) found V_A deficiency raised dendritic cells and IL-12 in the mucosa, causing inflammation and damage. V_A supplementation helps combat pathogens by regulating cytokines like IL-4, IL-6, and IFN-γ (Long et al., 2006). V_{D3} reduces pro-inflammatory factors such as IL-1β, IL-2, and IL-6, supporting intestinal health and growth (Shojadoost et al., 2015; Zhao et al., 2014). Our study revealed an interaction between V_A and V_{D3} on mucosal immunity: low V_A with increased V_{D3} raised pro-inflammatory and reduced anti-inflammatory factors, while higher V_A with increased V_{D3} reversed these effects, enhancing immunity. Studies suggest that V_A and V_{D3} jointly regulate intestinal epithelial and mucosal immune systems, influencing the composition of gut microbiota and maintaining intestinal homeostasis (Cantorna et al., 2019). The exact pathways of their interaction, however, require further investigation. Tight junctions prevent pathogenic invasion and abnormal mucosal immune responses (Suzuki et al., 2013). V_A and V_D are vital for maintaining intestinal barrier function, upregulating *TJP1, Occludin, Claudin*, and *ZO-1* expression to mitigate mucosal injury (Lu et al., 2019; Xiao et al., 2018; 2019). In our study, 7000 or 9000 IU/kg V_A improved *TJP1* mRNA expression, strengthened intestinal tight junctions, and protected goslings from mechanical damage.

Tibia development

Bone growth and quality are vital for poultry health, welfare, meat quality, and production potential (Orban et al., 1999). Tibial growth strongly correlates with body weight (Zhang et al., 2019). VA and VD3 are crucial for bone development, with VA balancing bone formation and resorption by regulating osteoblast and osteoclast activity (Ayodeji et al., 2012). In our study, 7000 and 9000 IU/kg V_A increased defatted tibial weight and improved gosling bone growth, similar to Liang et al. (2021), where 9000 IU/kg V_A combined with 3000 IU/kg V_{D3} optimized

tibial shank length and strength. V_{D3} exerts its effects by binding to receptors in the body to maintain normal blood Ca concentrations, regulate Ca balance, and facilitate Ca^{2+} involvement in bone growth (Zhang, 2010). Jiang et al. (2015) reported that increased dietary V_{D3} improved tibial weight and length in broilers at 21 days of age. Lin (2023) demonstrated that V_{D3} supplementation under low Ca conditions significantly increased gosling bone length, width, and weight; however, this effect weakened as Ca levels increased, suggesting that the influence of V_{D3} on bone growth is dependent on dietary Ca levels. Dietary V_{D3} levels did not affect tibial growth in our study. This may be because the dietary Ca level in this study was sufficient to support gosling bone development, thereby diminishing the impact of V_{D3}. However, this study demonstrates that, compared to Liang's findings, reducing V_A and V_{D3} levels from 9000 to 7000 IU/kg and 3000 to 2000 IU/kg, respectively, can still achieve proper tibial growth. This indicates that simultaneously lowering both vitamins is effective, and the tibial index aligns with growth performance.

We found that 7000 and 9000 IU/kg V_A increased tibia ash weight but did not affect tibia ash percentage, which may be because tibia ash percentage sometimes ignores bone size (Li et al., 2015). This may explain that the V_A at 7000 and 9000 IU/kg had a higher defatted weight but ash percentage remained the same. V_D promotes Ca and P absorption in the intestines and kidneys, supporting bone formation, mineralization, and tibial quality by inhibiting osteoclast-mediated resorption (Zhang et al., 2020). Jiang et al. (2015) found that higher V_{D3} intake improved tibial strength in broilers, while Santiago et al. reported 25-OH-D_3 increased tibial Ca and P deposition. In this study, 1500–2000 IU/kg V_{D3} significantly boosted tibial ash content, mineralization, and bone quality, but not affect the Ca and P content, which may be caused by the tibia not being fully calcified because the goslings are too short for its age. Meanwhile, although Ca and P account for a large proportion of the bone ash fraction, there are still other minerals, which we will measure further at a later stage. Studies investigating the effects of V_A on bone mineralization have primarily focused on the impact of excessive intake. High levels of V_A have been shown to disrupt Ca and P deposition, reduce bone Ca, P, and ash content, impair mineralization, decrease bone strength, and elevate fracture risk (Navarro et al., 2018; Thomas et al., 2013, 2017). In this study, 7000 or 9000 IU/kg V_A increased ash weight and promoted mineralization as they were proved to be in the proper dietary range of 6000~12000 IU/kg (Liang et al., 2021). Additionally, our findings revealed that VA and VD3 interact to regulate BMP-2 expression, thereby influencing tibial ash content. Specifically, the combination of 9000 IU/kg VA and 1000 IU/kg VD3 downregulated BMP-2 mRNA expression, potentially inhibiting osteoblast differentiation, reducing the secretion of bone matrix components (such as collagen and minerals), and impairing mineral deposition, ultimately leading to a decrease in ash content. This study demonstrates that reducing VA and VD3 to 7000 and 2000 IU/kg, respectively, supports optimal tibial growth while maintaining overall performance. VD3 was found to enhance tibial ash content and bone quality, with its effects influenced by dietary calcium levels. Moreover, VA and VD3 jointly regulated BMP-2 expression, directly impacting bone matrix secretion. These findings provide valuable insights into optimizing vitamin supplementation to promote bone development and improve resource efficiency.

Conclusion

Addition of 7000-9000 IU/kg V_A of gooslings rations can improve body weight by increasing jejunal digestive enzyme activity and promoting nutrient digestion and absorption. However, the increase in V_A levels also leads to a further increase in pro-inflammatory factors. Adding 2000 IU/kg V_{D3} further promoted feed intake and growth. In addition, V_A and V_{D3} egulate the levels of inflammatory factors and digestive enzyme activities through interactions to maintain organismal growth and development. In particular, it was observed that the combination of 9000 IU/kg V_A and 1000 IU/kg V_{D3} hindered bone mineralization, increased intestinal inflammation, and reduced digestive efficiency, ultimately impairing growth performance. The optimal dietary combination for growth and health was 7000 IU/kg V_A with 2000 IU/kg V_{D3}, which effectively enhanced feed intake, tibia development, and overall jejunal function.

Disclosures

This letter is to certify that the authors of this work do not have a confict of interest or competinginterests in regards to the work contained herein.

Acknowledgments

This work was financially supported by the Project of the China Agriculture Research System (CARS-42-11), the Jiangsu Agricultural Industry Technology System (JATS [2023]453), and the Yangzhou University International Academic Exchange Fund.

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