Journal Pre-proof

Nano-encapsulated vitamin D_3 supplementation effects on reproductive performance, egg quality, and blood indexes in breeding pigeons

Binbin Guo , Zhengyu Yu , Yunxiang Zhang , Huanxi Zhu , Zhe Chen , Zichun Dai , Xufeng Sun , Leyan Yan

PII: S0032-5791(26)00573-0
DOI: https://doi.org/10.1016/j.psj.2026.106944
Reference: PSJ 106944

To appear in: *Poultry Science*

Received date: 4 March 2026
Accepted date: 15 April 2026

Please cite this article as: Binbin Guo , Zhengyu Yu , Yunxiang Zhang , Huanxi Zhu , Zhe Chen , Zichun Dai , Xufeng Sun , Leyan Yan , Nano-encapsulated vitamin D_3 supplementation effects on reproductive performance, egg quality, and blood indexes in breeding pigeons, *Poultry Science* (2026), doi: https://doi.org/10.1016/j.psj.2026.106944

VD_3 IMPACT ON LAYING AND EGG QULITY IN PIGEONS

Research Note: Nano-encapsulated vitamin D_3 supplementation effects on reproductive performance, egg quality, and blood indexes in breeding pigeons

Binbin Guo[*,1], Zhengyu Yu[*,1], Yunxiang Zhang[†], Huanxi Zhu[*], Zhe Chen[*], Zichun Dai[*], Xufeng Sun[*], Leyan Yan[*,2]

[*]Key Laboratory for Crop and Animal Integrated Farming, Ministry of Agriculture and Rural Affairs, Jiangsu Province Engineering Research Center of Precision Animal Breeding, Institute of Animal Science, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

[†]Jiangsu Key Laboratory of Sericultural and Animal Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212100, China

[1]These authors contributed equally to this work and share first authorship.

[2]Correspondence: yanleyan198469@126.com

ABSTRACT Calcium homeostasis is indispensable for optimal egg production in poultry, and vitamin D_3 (VD_3) plays a pivotal role in regulating calcium metabolism and maintaining overall reproductive health. This study aimed to evaluate the effects of nano-encapsulated VD_3 supplementation in drinking water on reproductive performance, egg quality, endocrine profiles, and antioxidant capacity of breeding White King pigeons. A total of 216 three-year-old pigeon pairs were randomly allocated into four groups, receiving 0, 1,000, 2,000, or 4,000 IU/L of nano-encapsulated VD_3 for 13 weeks. The results demonstrated that a moderate supplementation level (2,000 IU/L) significantly improved egg mass, fertilization rate, plasma concentrations of luteinizing hormone (LH), estradiol (E_2), and testosterone

(T), as well as the activities of antioxidant enzymes [glutathione peroxidase (GSH-Px) and total superoxide dismutase (T-SOD)], while reducing malondialdehyde (MDA) concentrations ($P < 0.05$). This dosage also optimized the growth performance of squabs. Although the highest dosage (4,000 IU/L) further enhanced eggshell thickness and strength ($P < 0.05$), it did not confer additional benefits for reproductive success and slightly reduced squab weight gain. Serum calcium concentrations remained stable across all groups, indicating sufficient dietary calcium supply. Collectively, these findings suggest that a balanced VD_3 supplementation strategy, particularly at 2,000 IU/L, can effectively improve reproductive outcomes and offspring health in breeding pigeons, while excessive supplementation may lead to diminishing returns. This study provides novel insights into the optimal VD_3 requirement for breeding pigeons and offers practical guidance for pigeon production.

Key words: Vitamin D_3, Pigeon, Reproductive performance, Egg quality, Antioxidant capacity

INTRODUCTION

Calcium (Ca) is the main component of avian bones and a critical raw material for eggshells formation. Maintaining calcium homeostasis is essential for sustaining egg production and skeletal health in laying birds. In the absence of sufficient dietary Ca, poultry must rely on bone resorption to meet the Ca demands of eggshell synthesis, leading to skeletal weakness and associated health issues. Vitamin D3 (VD3), also known as cholecalciferol, plays a pivotal role in the metabolism of Ca and phosphorus (P), regulating normal bone development and skeletal integrity. In addition, VD_3 is

involved in various physiological processes, including reproduction, gut health, and immune function (Adhikari et al., 2020; Thamteerasathian et al., 2026).

In poultry, VD_3 can be obtained through two primary sources: endogenously synthesis in the skin upon exposure to sunlight, or exogenously supplementation via diet. While free-range birds can synthesize adequate VD_3 through sunlight exposure, intensive farming practices often restrict sunlight access, making dietary supplementation the primary source of VD_3 for commercially raised poultry. Existing research has shown that that moderate VD_3 supplementation not only enhances growth performance and bone development (Zhou et al., 2024) but also significantly improves reproductive performance, egg production rates, hatchability, and the strength and thickness of hatching eggshells in laying hens (Wen et al., 2019; Jing et al., 2022; Liermann et al., 2023). Conversely, inadequate utilization of Ca and P, coupled with VD_3 deficiency, is associated with reduced egg production, increased cracked eggshells, elevated mortality, and welfare concerns (Lavelin et al., 2000; Whitehead, 2004).

Despite extensive research on VD_3 supplementation in various poultry species, data specific to breeding pigeons remain scarce. Consequently, most dietary formulations for pigeons are extrapolated from the established nutritional standards for chickens, which may not be suitable for pigeons due to their unique physiological characteristics (e.g., crop milk secretion and parental brooding of squabs). To date, there is a lack of scientific data defining the optimal vitamin D_3 requirement for breeding pigeons. Therefore, this study was designed to evaluate the effects of nano-

encapsulated VD_3 supplementation in drinking water on reproductive performance, egg quality, hormone secretion, and antioxidant capacity in breeding White King pigeons, and to determine the optimal VD_3 addition level for practical pigeon production.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee of the Jiangsu Academy of Agricultural Sciences (IACUC number: NKYVET 2014-63). All experimental procedures and management procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals issued by the State Council of the People's Republic of China.

Experimental Design, Birds Management and Dietary Treatments

All experiment pigeons were healthy three years-old White king pigeon pairs produced by a commercial pigeon farm in Nanjing, China. A total of 432 pigeons with similar body weights (479.6 g ~ 527.3, average 509.4±0.5 g) were randomly assigned to four experimental groups in a completely randomized design, with each group consisting 108 pigeons (nine replicates per group, six pairs per replicate). Each pair of pigeon breeders, together with their squabs, was housed in a three-layer, three-dimensional cage (length ×width ×height, 60 * 60 *50 cm) equipped with a perch and nest.

All groups were fed the same basal diet formulated with corn and pea meal, providing 12.2 MJ/kg ME and 15.0% CP (based on our previous research). Pigeons were fed twice daily (at 7:00 a.m. and 3:00 p.m.) and had ad libitum access to

drinking water throughout the experimental period. Each breeding pair reared two squabs, which were nourished with crop milk secreted by the parent pigeons.

The four treatment groups were as follows: Group A (Control): No VD_3 supplementation; Group B: 1,000 IU VD_3 per liter of drinking water; Group C: 2,000 IU VD_3 per liter of drinking water; Group D: 4,000 IU VD_3 per liter of drinking water.

VD_3 was added to the water in the form of nano encapsulated, which was purchased from Shaanxi Jinguan Animal Husbandry Co., Ltd., (Xi'an, China). A 2-week pre-trial period was conducted to acclimate the pigeons to the experimental conditions, followed by a 13-week formal experiment.

Laying Performance and Egg Quality Measurements

Egg production by each pigeon pair was recorded daily. After laying, eggs were marked and incubated in an incubator; hatched squabs were subsequently returned to their original breeding pair or another pair within the same group for rearing. Fertility rate was calculated as (number of fertile eggs at 4 d of incubation/ number of incubated eggs) × 100. Hatchability was calculated as (number of hatched squabs/number of fertile eggs) × 100.

At the end of 13-week experiment, two eggs per replicate (free of shell defects, cracks, and double yolks) were collected for same-day egg quality analysis. The measured parameters included eggshell weight, eggshell thickness, eggshell strength, albumen weight, yolk weight, albumen height, Haugh unit (HU). The maximum transverse (width) and longitudinal (length) lengths of each egg were measured using a vernier caliper (accuracy: 0.1mm), and the egg shape index was calculated as egg

length / egg width. Eggshell thickness was measured at the blunt end, middle, and sharp end of the egg (after removing the albumen and inner shell membrane), and the average value was used for statistical analysis. Albumen weight was calculated by subtracting the sum of yolk weight and eggshell weight from the total egg weight. Eggshell strength was evaluated using an eggshell force gauge model II (Robotmation, Tokyo, Japan). The eggshell ratio, yolk ratio and albumen ratio were calculated as (eggshell weight / total egg weight) × 100, (yolk weight / total egg weight) × 100, and (albumen weight / total egg weight) 100, respectively.

The Haugh unit was calculated using the following formula: $HU=100\times lg(H-1.7*W^{0.37}+7.57)$, where H represents the albumen height (mm), and W denotes egg weight (g).

Hormonal and Biochemical Analyses

At the conclusion of the 13-week experiment (after the first egg laid in each group), and 12 h after feed withdrawal, one pair of pigeons with comparable physiological stages as close as possible were selected from each of the nine replicates per group, weighed and used for blood sample collection. Thus, nine pigeon pairs were collected per group for subsequent hormone and biochemical analyses. Blood samples were collected from the wing vein using heparinized syringes, centrifuged at 3, 000×g at 4 °C for 10 min to harvest plasma samples, which was subsequently stored at -20°C until analysis.

Plasma concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), and testosterone (T) were quantified using enzyme-linked

immunosorbent assay (ELISA) kits (Shanghai Enzyme-Linked Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's protocols. Optical density was read at 450 nm using an Infinite F50 microplate reader (Tecan Group Ltd., Männedorf, Switzerland) within 15 min of substrate addition. Hormone concentrations were derived from standard curves with the following ranges: FSH, 0-8 mIU/mL; LH, 0-160 ng/mL; E_2, 0-64 pmol/L; T, 0-320 pg/mL.

Plasma levels of total antioxidant capacity (T-AOC), glutathione peroxidase activity (GSH-Px), malondialdehyde (MDA) concentration, total superoxide dismutase (T-SOD) activity and Ca were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical Analysis

All data were presented as the mean and standard error of the mean (Mean ± SEM) for each group. Differences among the four groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. All statistical analyses were conducted with SPSS Statistics (version 19.0; IBM SPSS, Armonk, NY, USA). Differences were deemed statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

RESULTS and DISCUSSION

The impacts of nano-encapsulated VD_3 supplementation in drinking water on the reproductive performance, egg quality, and blood indexes of breeding pigeons are summarized in Table 1. Overall, nano-encapsulated VD_3 supplementation exerted a significant yet dose-dependent influence on several key performance metrics. Egg

mass per replicate peaked in the 2000 IU/L group, which was significantly higher than that in the control and other treatment groups ($P < 0.05$), indicating that 2,000 IU/L is the optimal threshold for egg production augmentation in breeding pigeons. This finding is consistent with previous studies showing that adequate VD_3 or VD_3 combined with 25-hydroxycholecalciferol ($25\text{-}OHD_3$) supplementation facilitates Ca and P metabolism, thereby promoting egg production in laying hens (Wen et al., 2019; Jing et al., 2022; Gao et al., 2024). Hrabia et al. (2023) confirmed that the chicken ovary and oviduct express vitamin D receptors (VDR), providing evidence that VD_3 can regulate follicles development in poultry. However, the specific mechanism of action and metabolic pathway of VD in the ovaries of breeding pigeons remain to be further explored.

Fertilization rates were significantly higher in the 1000 IU/L and 2000 IU/L VD_3 groups compared to the control ($P < 0.05$), while the 4000 IU/L group showed a slight but not significant decline. These results suggest that low to moderate VD supplementation may improve sperm motility or egg quality, thereby enhancing fertilization, while excessive VD levels may potentially offset these benefits. Hatchability rates were comparable across all groups, indicating that VD_3 supplementation influences fertilization but does not markedly affect the subsequent embryonic development under the experimental conditions.

A pronounced dose-dependent effect of nano-encapsulated VD_3 supplementation on eggshell characteristics was observed. As VD_3 concentration increased, eggshell ratio ($P < 0.01$), thickness ($P < 0.05$), and strength ($P < 0.01$) improved progressively,

with the highest values observed in the 4000 IU/L group. This aligns with the classical role of VD3's in facilitating calcium deposition, which likely facilitates greater calcium deposition in the shell matrix. Similarly, Wen et al. (2019) reported that dietary VD_3 inclusion levels of 35,014, or 68,348 IU/kg enhanced eggshell breaking strength in laying hens. In contrast, Adhikari et al. (2020) found that different isoforms of dietary vitamin D diets did not affect egg quality in laying hens. These discrepancies may be attributed to differences in VD dosage, formulation (e.g., nano-encapsulated vs. conventional), poultry species, and age.

In contrast to eggshell characteristics, the egg yolk ratio demonstrated a subtle but significant increase in the 2000 IU/L group compared to the control ($P < 0.05$), suggesting that moderate VD_3 supplementation may also promote yolk development. However, egg weight, egg shape index, albumen ratio, albumen height, and Haugh unit remained largely unaffected by VD_3 supplementation, indicating that VD primarily influences eggshell quality and yolk development rather than albumen quality in breeding pigeons.

Due to the unique physiological characteristic of breeding pigeons (i.e., parental feeding of squabs with crop milk), VD_3 supplementation in the drinking water of parent pigeons also indirectly influenced the growth performance of squabs. The body weight (BW) of 28-day-old squabs was positively correlated with parental VD_3 supplementation, peaking in the 2,000 IU/L group ($P < 0.05$), while the 4,000 IU/L group exhibited reduced squab weight gain, suggesting potential toxicity at high VD levels. The improvements in squab weight may be attributed to the role of VD in Ca

metabolism and bone development, which are critical for early growth and development. However, excessive VD supplementation did not yield additional benefits and may have adverse effects on offspring growth.

Administration of nano-encapsulated VD_3 also exerted a significant influence on the endocrine profile of breeding pigeons. As VD_3 supplementation level increased, plasma FSH concentration increased, with the 4000 IU/L group significantly exceeding the control ($P < 0.05$). Compared to the control group, plasma LH and T levels were significantly elevated in the 2000 IU/L group ($P < 0.05$ and $P < 0.01$, respectively), while E_2 concentrations were significantly higher in both the 1,000 IU/L and 2,000 IU/L groups ($P < 0.01$ and $P < 0.05$, respectively). These results suggest that optimal VD_3 dosing potentiate gonadal steroidogenesis, possibly through calcium-mediated enzymatic pathways involved in hormone synthesis. However, a slight decline in LH and T levels was observed in the 4000 IU/L group, indicating a potential threshold beyond which additional VD_3 may not confer further endocrine benefits.

Environmental stresses often induce the generation of reactive oxygen species (ROS) in cells and tissues, leading to oxidative stress, which can accelerate ovarian aging and cause reproductive disorders. In breeding pigeons, plasma GSH-Px and T-SOD activities peaked in the 2,000 IU/L group, coinciding with a significant reduction in MDA concentrations and an increase in T-AOC ($P < 0.05$). This pattern suggests that moderate VD supplementation enhances antioxidant defenses, potentially mitigating oxidative damage during the energetically demanding breeding

period. A similar trend was observed in squabs, with the highest GSH-Px activity in the 2,000 IU/L group. This is consistent with a previous study demonstrating that dietary supplementation with 50 μg/kg 25-OHD and 2,000 IU/kg VD_3 improves antioxidant capacity in aged laying hens (Gao et al., 2024). Interestingly, the lowest MDA concentration in squabs was observed in the 4,000 IU/L group, indicating that higher VD levels may confer additional oxidative protection in offspring. The enhancement of antioxidant enzyme activities (GSH-Px, T-SOD) and reduction in lipid peroxidation (MDA) corroborate previous findings that VD_3 supplementation can strengthen the antioxidant response in poultry, thereby improving overall health and reproductive outcomes.

Contrary to our expectations, serum Ca concentrations remained stable across all groups in both breeding pigeons and squabs, indicating that dietary Ca was sufficient in the basal diet. This suggests that the observed benefits of VD supplementation were likely mediated through mechanisms beyond simple enhancement of Ca absorption, such as regulation of hormone secretion and antioxidant capacity.

CONCLUSION

In conclusion, the findings of this study demonstrate that nano-encapsulated VD supplementation in drinking water at a moderate level (2,000 IU/L) optimally improves egg production, fertilization rate, endocrine profiles, antioxidant defenses, and offspring growth in breeding White King pigeons. While higher supplementation (4,000 IU/L) enhances eggshell integrity and antioxidant status in squabs, it does not confer additional benefits on reproductive success or squab weight gain. Excessive VD_3 supplementation does not translate to better performance and may lead to inefficient resource utilization. Therefore, a supplementation level of 2,000 IU/L is

recommended for practical breeding pigeon production. Future studies should explore a broader range of VD dosages, longer-term effects, and potential interactions with other dietary components (e.g., Ca, P, and other vitamins) to further optimize the nutritional management of breeding pigeons.

ACKNOWLEDGMENTS

The author would like to thank the Key Research and Development Program of Jiangsu Province-Modern Agriculture (No. BE2022315) and Jiangsu Agricultural Science and Technology Innovation Fund (JASTIF) (CX (21) 2013) for financial support of this study.

REFERENCES

Adhikari, R., D. White, J. D. House, and W. K. Kim. 2020. Effects of additional dosage of vitamin D3, vitamin D2, and 25-hydroxyvitamin D3 on calcium and phosphorus utilization, egg quality and bone mineralization in laying hens. Poult. Sci, 99, 364–373.

Gao, S., K. Qiu, J. Zheng, H. Zhang, J. Wang, X. Qi, and S. Wu. 2024. Dietary 25-hydroxycholecalciferol supplementation as a vitamin D3 substitute improves performance, egg quality, blood indexes, jejunal morphology, and tibia quality in late-phase laying hens. Animals 146, 878.

Hrabia, A., K. Kamińska, M. Socha, and M. Grzesiak. 2023. Vitamin d3 receptors and metabolic enzymes in hen reproductive tissues. Int. J Mol. Sci. 24, 17074.

Jing, X., Y. Wang, F. Song, X. Xu, M. Liu, Y. Wei, H. Zhu, Y. Liu, J. Wei, and X. Xu. 2022. A comparison between vitamin D3 and 25-hydroxyvitamin D3 on laying performance, eggshell quality and ultrastructure, and plasma calcium levels in late period laying hens. Animal. 12. 2824.

Lavelin, I., N. Meiri, and M. Pines. 2000. New insight in eggshell formation. Poult. Sci. 79, 1014–1017.

Liermann, W., I. Halle, Frahm J, L. Hüther, S.Weigend, J. Kühn, G. I. Stangl, and S. Dänicke. 2023. Genotype-dependent impact of dietary vitamin d3 on laying

hens. Arch. Anim. Nutr. 77, 205–227.

Thamteerasathian, L., V. Chaiworakul, T. Poeikhampha, C. Kulsing, N. Tungkijanansin, and A. K. Anal. 2026. Vitamin D enrichment of eggs and its effects on performance and intestinal health in late-stage laying hens. Poul. Sci. 105, 106166.

Wen, J., K. A. Livingston, and M. E. Persia2019. Effect of high concentrations of dietary vitamin D3 on pullet and laying hen performance, skeleton health, eggshell quality, and yolk vitamin D3 content when fed to W36 laying hens from day of hatch until 68 wk of age. Poul. Sci. 98, 6713–6720.

Whitehead, C.C. 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. 83, 193–199.

Zhou, X. Y., X. C. Chen, G. S. Fraley, K. Y. Zhang, G. Tian, S. P. Bai, X. M. Ding, J. P. Wang, L. Lv, Y. Xuan, and Q. F. Zeng. 2024. Effects of different dietary vitamin D combinations during the grower phase and the feed restriction phase on growth performance and sternal morphology, mineralization, and related genes expression of bone metabolism in pekin ducks. Poul. Sci. 103, 103291.

Table 1 Effects of feeding different VD_3 levels on laying performance and egg quality of breeding pigeons

Items	VD_3				SEM	*P*
	0 IU/L	1000 IU/L	2000 IU/L	4000 IU/L		
Egg numbers/replicate	25.78^{a}	27.11^{ab}	28.33^{b}	26.78^{ab}	0.55	0.025
Fertilization rate, %	85.27^{a}	87.88^{b}	87.64^{b}	86.35^{ab}	0.60	0.018
Hatchability, %	85.94	87.15	87.24	86.27	0.46	0.165
Egg weight, g	21.46	21.73	21.72	22.24	0.35	0.462
Egg shape index	1.37	1.36	1.37	1.38	0.04	0.762
Egg yolk ratio,%	22.71^{a}	23.26^{ab}	23.30^{b}	23.10^{ab}	0.47	0.047
Albumen ratio,%	70.07	68.75	68.64	68.77	0.54	0.203
Eggshell ratio, %	7.22^{a}	7.80^{bc}	8.06^{bc}	8.13^{c}	0.16	0.004
Eggshell thickness, mm	0.207^{a}	0.219^{ab}	0.219^{ab}	0.224^{b}	0.003	0.025
Eggshell strength, N	11.0^{a}	11.90^{bc}	12.02^{bc}	12.26^{c}	0.18	<0.0001
Albumen height, mm	3.31	3.48	3.45	3.48	0.11	0.603
Haugh unit	74.57	75.60	75.36	75.43	0.83	0.826
BW of 28d squabs, g	449.7^{a}	455.8^{ab}	473.2^{b}	457.7^{ab}	7.16	0.036

$^{a,b,c,\cdots}$Values with different superscript letters within the same row are significantly different ($P < 0.05$).

Table 2 Effects of feeding different VD_3 levels on hormone levels and antioxidant abilities breeding pigeons

Items		VD_3				SEM	*P*
		0 IU/L	**1000 IU/L**	**2000 IU/L**	**4000 IU/L**		
Breeding pigeons	FSH, mIU/mL	7.85[a]	8.50[ab]	8.95[ab]	9.29[b]	0.35	0.042
	LH, ng/mL	174.7[a]	195.1[ab]	226.8[b]	214.1[ab]	12.04	0.027
	E_2, pmol/L	72.62[a]	99.63[c]	92.12[bc]	73.17[a]	3.42	<0.0001
	T (♂), pg/mL	273.8[a]	323.7[b]	354.2[c]	281.0[ab]	14.34	0.004
	Ca, mmol/L	0.93	1.03	1.07	1.06	0.05	0.329
	GSH-px, U/mL	2846.6	2839.5	2866.0	2877.9	93.68	0.991
	T-SOD,U/mL	45.79	43.41	41.64	44.45	2.20	0.615
	MDA, nmol/mL	18.38[a]	11.95[b]	12.78[ab]	13.90[ab]	1.72	0.047
	T-AOC, mM	0.49[a]	0.34[b]	0.42[ab]	0.46[ab]	0.06	0.044
Squabs	Ca, mmol/L	0.91	0.95	0.93	0.95	0.02	0.653
	GSH-px,U/mL	2060.4[a]	2243.5[a]	2872.5[c]	2458.8[ab]	93.26	<0.0001
	T-SOD,U/mL	12.98	12.26	12.35	12.32	0.69	0.877
	MDA, nmol/mL	8.21[a]	7.58[a]	7.54[a]	2.83[c]	0.34	<0.0001
	T-AOC, mM	0.37[a]	0.64[b]	0.49[ab]	0.63[b]	0.07	0.033

[a,b,c,…]Values with different superscript letters within the same row are significantly different (P <0.05);

Abbreviations: FSH: follicle-stimulating hormone, LH: luteinizing hormone, E_2: estradiol, T: testosterone, GSH-Px: glutathione peroxidase activity, T-SOD: total superoxide dismutase, T-AOC: total antioxidant capacity, MDA: malondialdehyde.

Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.