International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

The Effects of Calcidiol and Betaglucan as Feed Additives on the Performance and Physiological Parameters of Laying Hens

Mitta Agustin[1,2], Meta Eragrosita Ardhityasari[2], Nofitra Dewi Suparno Putri[2], Wira Wisnu Wardani[3], Muhammad Halim Natsir[1], Osfar Sjofjan[1], Eko Widodo[1*]

[1]Department of Animal Nutrition, Faculty of Animal Science, University of Brawijaya, Malang, Indonesia
[2]Nutricell Pacific, Cibis Nine 12th Floor Unit G1, Jakarta, Indonesia
[3]Nutricell Bioscience Molecule Pte Ltd, 10 Anson Road #22-02 International Plaza, Singapore

ABSTRACT: This study aimed to evaluate the effect of adding a combination of calcidiol and betaglucan to feed on the production performance of laying hens aged 0–21 days, immune organ morphometry, and blood hematology of laying hens aged 12 weeks. The study used 500 Day-Old Chicks (DOC) of the Hy-Line Brown strain, divided into two treatments. The data obtained were analyzed using the Independent T-Test and descriptively for variables related to body weight uniformity and mortality. Treatment T0 = basal feed (control), T1 = basal feed supplemented with calcidiol 5.000 IU/kg and betaglucan 40mg/kg in the feed. The results showed that the addition of a combination of calcidiol and betaglucan had a significant effect ($P < 0.01$) on increasing feed efficiency in group T1 during the third week, resulting in a 21.51% improvement in feed conversion and bone marrow density at 12 weeks of age. The treatment did not have a significant effect ($P > 0.05$) on spleen and bursa morphometry, blood hematology, IgG, and IgM levels. Descriptively, the addition of Calcidiol-Betaglucan had a better effect on body weight uniformity, which was 3.84% higher than the control, and total mortality, which was 0.49% lower in T1 than in T0. The conclusion of this study is that the addition of the combination of calcidiol and betaglucan has potential as a functional feed additive to support early growth efficiency and immune system readiness in laying hens.

KEYWORDS: Blood Hematology, Betaglucan, Calcidiol, Immunity, Production Performance.

INTRODUCTION

The starter phase is decisive in pullet rearing. Nutritional management at this stage strongly affects later growth, skeletal strength, immune status, and flock uniformity. During early age, the digestive tract, immune system, and cortical bone develop rapidly and concurrently. This provides the physiological basis for later muscle accretion, medullary bone formation, and reproductive performance. If nutritional support is insufficient, skeletal development may be compromised. This can also reduce uniformity, increase vulnerability to health challenges, and ultimately affect productivity during the laying period.

Selecting feed additives appropriately and including them at the right time is essential. This supports optimal physiological development. Previous studies show that vitamin D_3 supplementation improves egg production and eggshell quality in laying hens. Adequate vitamin D_3 status is linked to improved tibia bone quality during early laying (Li *et al*., 2023). Among vitamin D_3 metabolites, calcidiol (25-hydroxyvitamin D_3; 25-OH-D_3) is widely used in poultry nutrition. It is more bioavailable than cholecalciferol. Calcidiol is produced by hepatic hydroxylation of vitamin D_3, allowing for more efficient metabolic use (Warren and Livingston, 2021).

Calcidiol contributes to calcium homeostasis by regulating parathyroid hormone activity, enhancing calcium mobilization from bone tissue, and reducing calcium excretion through the kidneys, which together increase calcium availability in the circulation (Saunders-Blades and Korver, 2014). During the starter phase, sufficient calcium and phosphorus intake is critical for cortical bone formation, which serves as the structural foundation for medullary bone development in later growth stages. Supplementation with calcidiol has been reported to improve laying performance in laying hens (Yusuf *et al*., 2023) and enhance productivity in broiler breeders (Setyaningsih *et al*., 2022; Setyaningsih *et al*., 2023), indicating its practical relevance across different poultry production systems.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

Alongside skeletal development, rapid maturation of the immune system also occurs during the starter phase. At this stage, pullets are particularly sensitive to environmental and nutritional stress, making immune support an important consideration under commercial conditions. Betaglucan, a complex polysaccharide derived from the cell walls of yeast, fungi, algae, and cereal grains, is widely recognized for its immunomodulatory properties (Vetvicka and Vetvickov, 2014). Dietary inclusion of betaglucan has been shown to enhance immune activity and improve intestinal morphology in poultry, contributing to better health status and survival rates (Bar-Dagan *et al*., 2023).

The combined supplementation of calcidiol and betaglucan reflects a system-based nutritional approach that targets both skeletal development and immune maturation during early growth. By supporting multiple physiological systems at a critical developmental stage, this strategy may induce early life programming effects that positively influence production performance, flock uniformity, and health in later phases. However, information regarding the combined use of calcidiol and betaglucan during the early starter period, as well as its longer term effects on immune related parameters, remains limited.

Therefore, the present study aimed to evaluate the effects of dietary supplementation with a combination of calcidiol and betaglucan on the production performance of laying hens during the starter phase, and to assess its subsequent effects on immune organ morphometry and blood hematology at 12 weeks of age.

MATERIALS AND METHODS

Study Site and Feed Additive

The study was conducted at a commercial layer farm in Kediri, East Java, Indonesia. The animal testing protocol for this study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Brawijaya University (No. 6-KEP-FKHUB-2025). The dietary treatment consisted of a combined feed additive containing calcidiol (25-hydroxycholecalciferol; 25-OH-D_3) and betaglucan. Calcidiol was derived from cholecalciferol (HDM D_3) and formulated using spray-dried beadlet microencapsulation technology to ensure stability during feed processing and pelleting (≤90 °C). The betaglucan component was linear β-1,3-glucan sourced from *Euglena gracilis* (>50% purity), produced through whole-cell fermentation and microencapsulation. Both active ingredients were supplied as a commercial premix Vitagold Prelay Frame A from PT. Nutricell Pacific, as shown in Table 1.

.

Table 1. Content of suplementation

	Amount	Unit
Calcidiol (IU)	2.500.000	IU
Betaglucan (mg)	40.000	mg
Filler		qs

Source: Data Sheet Product of Vitagold Prelay Frame A PT. Nutricell Pacific.

Experimental Design, Birds, and Diets

This experiment was carried out from June 16, 2023, to November 28, 2024, using 500 day-old chicks (DOC) of Hy-Line Brown laying hens with an average initial body weight of 41.0 ± 3.28 g and flock uniformity of 92%. The birds were randomly assigned to two experimental groups: T0 (250 birds) and T1 (250 birds).

The experimental treatments included T0, which received a basal diet without Vitagold Prelay Frame A (control), and T1, which was fed the basal diet supplemented with Vitagold Prelay Frame A at 2 kg/ton from 0 to 6 weeks of age. The basal diet used for T0 consisted of GC101 pre-starter layer feed, while T1 received a modified GC101Y pre-starter feed manufactured by PT. Gold Coin Indonesia (Surabaya, Indonesia). The diets were formulated using corn, soybean meal, rice bran, dried distillers' grains with solubles (DDGS), wheat pollard, meat and bone meal, amino acids, dicalcium monophosphate, vitamins, minerals, enzymes, antioxidants, and antifungal agents. The nutrient composition of the diets is presented in Table 2.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

Table 2. Feed Nutrients Content

Nutrient Compositions	T0	T1
	Basal Feed	Basal Feed + Calcidiol-Betaglucan
Water Content (%)	13.0	13.0
Ash (%)	8.00	8.00
Crude Protein (%)	22.0	22.0
Crude Fat (%)	3.00	3.00
Crude Fiber (%)	6.00	6.00
Calcium (Ca) (%)	0. 80 - 1.20	0. 80 - 1.20
Phospor (P) (%)	0.50	0.50
Calcidiol (IU)	-	5.000
Betaglucan (mg)	-	80.0

Source: Label for GC101 and GC101Y Pre Starter Laying Hen Feed from PT. Gold Coin Indonesia. T0 = control group; T1 = treatment group.

Birds were reared under an open-house system with group cages, feeders, and nipple drinkers, and were provided feed and water ad libitum in accordance with Hy-Line Brown management guidelines. The ambient temperature ranged from 27.3 to 34.5 °C, and the relative humidity ranged from 66.8% to 85.6%, corresponding to Heat Stress Index (HSI) values of 147.9–179.7. with this HSI value falling within the comfortable zone to the dangerous heat stress zone (uncomfortable) (Pakpahan *et al*. 2023; Muhshi *et al*. 2024). Environmental conditions were regulated using curtains, heaters, and ventilation fans.

Data Collection

Production performance was evaluated from 0 to 21 days of age in all birds (n = 250 per treatment). Recorded parameters included total feed intake, body weight gain, feed conversion ratio, body weight uniformity, and cumulative mortality.

At 12 weeks of age, immune organ morphometry was assessed in randomly selected birds (n = 4 per treatment). Birds were euthanized by mechanical decapitation. Tibial bone marrow density was evaluated by measuring medullary bone area across five microscopic fields of view at 400× magnification using Image Raster software. Spleen and bursa of Fabricius weights and dimensions were recorded, and organ indices were calculated relative to live body weight.

Blood samples were collected from the same age group (n = 4 per treatment) via the brachial vein without anesthesia. Hematological and serological analyses were performed using a veterinary hematology analyzer (Mindray BC-2800Vet) and an ELISA reader (LABTRON LMPR-A12). Measured parameters included hemoglobin, total leukocytes, heterophils, lymphocytes, monocytes, IgG, and IgM.

Statistical Analysis

The obtained variable data were statistically analyzed using the Independent T-Test variance analysis with IBM SPSS Statistics v25.0 software. The variable data on body weight uniformity and mortality were analyzed descriptively.

RESULTS

Laying Hen Production Performance

The production performance of the research results with the addition of a combination of Calcidiol 5.000 IU/kg and Betaglucan 80 mg/kg in laying hens aged 0 – 21 days showed a significant difference in feed intake, but no difference in body weight gain, thereby indicating a very significant difference in feed conversion. Descriptively, the percentage of body weight uniformity and the percentage of total mortality in the first three weeks also showed better results, as shown in Table 3.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

Table 3. Effect of Calcidiol-Betaglucan Combination in Feed on Production Performance of 0 – 21 Days Old Laying Hens

Variabels	T0	T1	P-Value
Total Feed Intake (g/head)			
0 – 7 Days	82.7±0.16[b]	78.4±0.15[a]	<0.001
8 – 14 Days	119.1±0.37[a]	113±0.85[b]	<0.001
15 – 21 Days	166±1.07[b]	157±0.67[a]	<0.001
Body Weight Gain (BWG) (g/head)			
0 – 7 Days	33.34±6.62[b]	27.73±4.92[a]	<0.001
8 – 14 Days	52.3±13.28	53.06±8.68	0.450
15 – 21 Days	75.26±29.07	77.84±19.65	0.246
Feed Conversion Ratio (FCR)			
0 – 7 Days	2.59±0.60[a]	2.92±0.55[b]	<0.001
8 – 14 Days	2.45±0.71[b]	2.19±0.37[a]	<0.001
15 – 21 Days	2.79±1.95[b]	2.19±0.79[a]	<0.001
Body Weight Uniformity (BWU) (%)			
0 – 7 Days	91.1	92.84	
8 – 14 Days	90.76	93.94	NA
15 – 21 Days	84.14	90.74	
Total Mortality (%)			
0 – 7 Days	0.56	0.28	
8 – 14 Days	0.24	0.34	NA
15 – 21 Days	0.43	0.12	

T0 = control group; T1 = treatment group; [a-b] Superscript letters in the same row indicate highly significant (P<0.01) and significant (P<0.05). NA = Not analyzed statistically (descriptive).

Total feed intake was significantly influenced by the treatment. In the first week, feed intake differed markedly ($P < 0.01$), with birds in T1 consuming less feed than those in the T0 control group. Similar highly significant differences ($P < 0.01$) were observed in the second and third weeks, where feed intake in T0 remained higher than in T1. Overall, cumulative feed intake during the first three weeks was descriptively lower in T1 than in T0. Body weight gain (BWG) showed a significant difference ($P < 0.01$) in the first week, with higher BWG recorded in T0 compared to T1. However, no significant differences were found between treatments in the second and third weeks. Descriptively, BWG values in T1 were greater than those in T0 during these weeks, suggesting improved growth performance in the treatment group. The feed conversion ratio (FCR) was significantly higher ($P < 0.01$) in T1 than in T0 during the first week. Conversely, in the second and third weeks, FCR differed significantly ($P < 0.01$), with lower values observed in T1 than in T0. Descriptive results indicated that FCR in T1 improved by 10.61% in the second week and by 21.51% in the third week compared with T0. Body weight uniformity and mortality were not statistically analyzed. Nevertheless, descriptively, weekly body weight uniformity was consistently higher in T1 than in T0, with the average uniformity during the first three weeks being 3.84% higher in T1. Mortality in T1 was 50% lower than in T0 during the first week, increased to 41.6% higher in the second week, and declined again in the third week, reaching 72.1% lower than in T0. Overall, cumulative mortality during the first three weeks was 0.49% lower in T1 than in T0.

Immune Organ Morphometry

Immune organ morphometry is a representation of the development of immune status in poultry. The immune organs measured in this study were tibia bone marrow, spleen, and bursa fabricius. The effect of adding a combination of calcidiol and beta-glucan on immune organ morphometry is shown in Table 4.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

Table 4. Effect of Calcidiol-Betaglucan Combination in Feed on Morphometrics of Immune Organs of 12 Weeks Old Laying Hens

Variabels		T0	T1	P-Value
Bone Marrow Density	12 Weeks (%)	26.46±2.34[a]	29.67±0.82[b]	<0.001
Spleen	Weight (g)	5.27±3.88	3.73±0.33	0.459
	Ratio (%)	0.36±0.27	0.25±0.02	0.458
	Diameter (cm)	2.56±0.62	2.25±0.00	0.356
Bursa Fabricius	Weight (g)	0.77±0.34	1.24±1.06	0.429
	Ratio (%)	0.05±0.02	0.08±0.07	0.417
	Diameter (cm)	1.29±0.27	1.61±0.45	0.259

T0 = control group; T1 = treatment group; [a-b] Superscript letters in the same row indicate highly significant (P<0.01).

The density of tibia bone marrow at 12 weeks of age showed significant differences (P<0.01), with T1 having a higher density than T0. There were no significant differences (P>0.05) in weight, diameter, and ratio between T0 and T1 in the spleen morphometric results. Descriptively, T0 had higher values for weight, ratio, and diameter of the spleen compared to T1. Similarly, the bursa fabricius also showed no significant differences (P > 0.05) between T0 and T1; however, descriptively, the values for weight, ratio, and diameter of the bursa fabricius were higher in T1 compared to T0.

Blood Hematology

Immune status is associated with blood hematology parameters, particularly hemoglobin, leukocytes, heterophils, lymphocytes, and monocytes, as well as humoral indicators such as immunoglobulin G (IgG) and immunoglobulin M (IgM). The results of the study on the effect of adding a combination of calcidiol-beta-glucan to the diet of laying hens aged 0–6 weeks on blood hematology are shown in Table 5.

Table 5. Effect of Calcidiol-Betaglucan Combination in Feed on Blood Hematology of 12 Weeks Old Laying Hens

Blood Profile	T0	T1	P-Value
Hemoglobin (g/dl)	20.475±11.65	15.425±4.48	0.449
Leukocytes (x 10^3/mm^3)	19.175±1.04	21.725±3.35	0.197
Heterophils (x 103/mm^3)	33.075±14.69	33.00±10.99	0.994
Heterophils %	26.075±10.70	23.00±4.80	0.619
Lymphocytes (x 10^9/mm^3)	72.08±12.90	81.975±15.33	0.237
Lymphocytes %	230.05±19.26	247.8±18.99	0.361
Monocytes (x 10^9/mm^3)	11.45±5.67	6.03±1.70	0.116
Monocytes %	6.475±3.88	2.425±0.10	0.082
IgG (µg/ml)	6.97±3.62	3.69±3.14	0.275
IgM (µg/ml)	43.32±13.19	51.43±10.47	0.359

T0 = control group; T1 = treatment group.

Table 5 showed no significant differences (P > 0.05) in the overall blood hematology profile. Descriptively, there are differences in some hematological parameters between T0 and T1. Hemoglobin, heterophils, monocytes, and IgG levels were higher

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

in T0 compared to T1, while leukocyte, lymphocyte, and IgM levels were higher in the T1 treatment group compared to the T0 control group.

DISCUSSION

Laying Hen Production Performance

Total Feed Intake

Total feed intake was significantly reduced in T1 during the first week ($P < 0.01$) and remained lower than T0 in the second and third weeks ($P < 0.05$). This response may be associated with the role of calcidiol in enhancing calcium and phosphorus absorption and supporting mineral homeostasis, which can improve skeletal development and nutrient utilization efficiency, thereby reducing feed requirements (Saunders-Blades and Korver, 2014; Wang *et al*., 2020). Improved calcium metabolism has also been reported to reduce physiological stress related to mineral deficiency and optimize growth efficiency (Mutucumarana *et al*., 2014; Roztočilová *et al*., 2024).

The reduced feed intake may further reflect the immunomodulatory and prebiotic effects of betaglucan, which can improve intestinal integrity, modulate gut microbiota, and enhance nutrient utilization efficiency (Murphy *et al*., 2012; Jacob and Pescatore, 2017; Huang *et al*., 2024). Improved gut health may also influence appetite regulation through modulation of satiety-related hormones (Ghazalah *et al*., 2008; Souza *et al*., 2019; Uyanga *et al*., 2023). Although moderate heat stress conditions were indicated by fluctuations in the heat stress index, environmental exposure was similar across treatments; therefore, the observed differences in feed intake were more likely related to supplementation effects rather than environmental variation (Muhshi *et al*., 2024).

Body Weight Gain

Lower body weight gain (BWG) in T1 during the first week may be attributed to reduced feed intake and early physiological adaptation to supplementation. Calcidiol has been reported to influence growth-related hormonal regulation, including growth hormone and insulin-like growth factor-1 activity, which may affect early protein synthesis and growth rate (Kazaev *et al*., 2024). In addition, activation of immune responses induced by calcidiol and betaglucan may increase energy expenditure for immune function, thereby temporarily reducing energy allocation for growth (Bednarczyk *et al*., 2016; Fatemi *et al.*, 2024).

No significant differences in BWG were observed during the second and third weeks ($P > 0.05$), although descriptively higher values were observed in T1. This response may indicate metabolic adaptation to supplementation, reduced inflammatory status, and improved intestinal function, which collectively support nutrient utilization and growth performance (Cox *et al*., 2010; Khan and Mukhtar, 2013; Landy *et al*., 2020).

Feed Conversion Ratio (FCR)

Feed conversion ratio was improved following supplementation, as indicated by significantly lower FCR values in T1 during the second and third weeks. Reduced feed intake without a corresponding reduction in BWG suggests enhanced nutrient utilization efficiency. This effect may be explained by the role of 25-hydroxyvitamin D_3 in increasing intestinal expression of mineral transport proteins and improving calcium and phosphorus absorption, thereby supporting skeletal development and growth efficiency (Zhang *et al*., 2021; Gao *et al*., 2024). Improved calcium metabolism has been associated with reduced physiological stress and enhanced feed efficiency in poultry (El-Gobary *et al*., 2016; Yeh *et al*., 2020).

The improvement in FCR may also reflect the prebiotic effects of betaglucan, which enhance beneficial gut microbiota, improve intestinal morphology, and increase nutrient absorption capacity (Amer *et al*., 2022). These effects can enhance metabolic efficiency and optimize feed utilization, contributing to improved economic returns despite increased feed costs (Tapingkae *et al*., 2017; Da Nóbrega *et al*., 2022).

Body Weight Uniformity

Body weight uniformity was evaluated descriptively and showed higher values in T1, indicating more homogeneous growth. This response may be associated with improved mineral metabolism and skeletal development resulting from adequate vitamin D and calcium status (Akinola and Ebhohon, 2019; Chen *et al*., 2020). In addition, betaglucan may support gut microbiota balance and energy metabolism, thereby promoting consistent nutrient utilization and growth among birds (Wei *et al*., 2022). Improved population uniformity may contribute to more stable production performance in subsequent stages (Wolc *et al*., 2010).

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

Total Mortality Rate

Mortality was low and evaluated descriptively, with lower cumulative mortality observed in T1. This response may reflect improved immune competence associated with calcidiol supplementation, which has been reported to enhance immune function and reduce disease susceptibility in poultry (Wang *et al*., 2012). Adequate vitamin D and calcium status may also improve antioxidant capacity and bone integrity, thereby reducing physiological stress and morbidity risk (Niu *et al*., 2011; Qiu, 2024). Furthermore, betaglucan has been shown to stimulate immune responses and support beneficial gut microbiota, which may contribute to improved health status and reduced mortality (Sokolowicz *et al*., 2018; Liao *et al*., 2023).

Immune Organ Morphometry

Immune organ morphometry reflects the development of immune function in poultry. In the present study, supplementation significantly increased tibial bone marrow density ($P < 0.01$) but did not influence the relative weight or morphometric characteristics of the spleen and bursa of Fabricius. These findings suggest a selective immunomodulatory effect, with a stronger response in the hematopoietic compartment than in secondary lymphoid organs.

Tibial bone marrow is the primary site of hematopoiesis in poultry and produces precursor cells that differentiate into immune cells, including lymphocytes and monocytes/macrophages (Guo *et al*., 2019). In contrast, the spleen functions as a secondary lymphoid organ involved in lymphocyte proliferation and immune activation (Hu *et al*., 2021; Wang *et al*., 2023), whereas the bursa of Fabricius plays a central role in B lymphocyte maturation and antibody production (Gao *et al*., 2022). Therefore, the higher bone marrow density observed in T1 may indicate enhanced hematopoietic activity without substantial changes in peripheral immune organ development.

The increased tibial bone marrow density at 12 weeks may be associated with normal physiological development of the immune and skeletal systems, where early immune maturation is followed by rapid cortical bone development during the growing phase (Regmi *et al*., 2015; Hy-Line International, 2021). Calcidiol has been reported to promote bone mineralization and osteoblastic activity, thereby increasing medullary density (Garcia-Mejia *et al*., 2024; Lyu *et al*., 2024). In addition, betaglucan functions as an immunomodulator by interacting with immune cell receptors such as dectin-1, stimulating cytokine production and phagocytic activity, and supporting osteoprogenitor cell proliferation (Cox and Dalloul, 2010; Lee *et al*., 2005; Purnamasari *et al*., 2022). These mechanisms may contribute to enhanced bone formation and hematopoietic activity without inducing enlargement of secondary lymphoid organs (Soltanian *et al*., 2009; De Marco Castro *et al*., 2020).

No significant differences ($P > 0.05$) were detected in spleen or bursa morphometry between treatments, indicating that lymphoid organ development remained relatively stable. Secondary lymphoid organs generally maintain consistent morphometric characteristics under normal physiological conditions (Moraes *et al*., 2019). Descriptively, the larger spleen observed in T0 may reflect greater immune activation due to environmental or pathogenic challenges, as increased spleen size is commonly associated with active immune responses (Wang *et al*., 2023). Meanwhile, the relatively higher bursa index in T1 may indicate enhanced immunoglobulin production capacity and improved adaptive immune readiness (Gao *et al*., 2022; Zhen *et al.,* 2021).

Blood Hematology

No significant differences were observed in blood hematological parameters between treatments (Table 5). However, several hematological and immunological variables showed descriptive variation in T1 compared with the T0 control group. Hemoglobin concentrations in both groups remained within the normal physiological range for 12-week-old laying hens (10–12 g/dL), consistent with previous findings in chickens receiving calcidiol supplementation (Abun *et al*., 2023).

Total leukocyte counts were descriptively higher in T1, which may reflect increased immune activity. Calcidiol interacts with vitamin D receptors and regulates inflammatory pathways by modulating cytokine expression, including interleukin-6 and interleukin-1β, thereby supporting hematopoiesis and immune cell differentiation (Chou *et al*., 2020). Likewise, betaglucan supplementation has been reported to improve hematological profiles in poultry, particularly by increasing leukocyte numbers (Park *et al.,* 2008).

The proportion of heterophils and the heterophil-to-lymphocyte (H/L) ratio did not differ between treatments and remained within normal limits, suggesting that the birds were not exposed to acute stress and maintained stable immune homeostasis (Politis *et al.,* 2003). This observation aligns with previous reports indicating that immunomodulatory feed additives can enhance immune function without necessarily altering heterophil counts (Herwig *et al.,* 2019). The higher lymphocyte percentage observed in T1

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

may indicate improved immune status associated with calcidiol supplementation and enhanced gut-associated lymphoid tissue integrity, which can contribute to a more balanced immune cell profile and reduced systemic inflammation (Tang *et a*l., 2017; Gao *et al.,* 2024).

Monocyte counts were higher in T0, which may suggest prolonged immune stimulation or chronic inflammatory responses (Osadcha, 2021). In contrast, lower monocyte levels in T1 may indicate that supplementation helped regulate inflammatory responses, likely through the immunoregulatory effects of calcidiol in reducing proinflammatory cytokine expression (Gao *et al.,* 2024).

Immunoglobulin analysis showed higher IgM concentrations in T1, indicating enhanced innate immune responsiveness and early humoral defense (Liu *et al.,* 2018). This effect may be related to betaglucan–induced stimulation of phagocytic activity and cytokine production (Zhen *et al*., 2020). Conversely, IgG levels were descriptively lower in T1 than in T0. Previous studies have shown that betaglucan supplementation may enhance non-specific immune responses without consistently increasing specific antibodies such as IgG (Shanmugasundaram *et al*., 2013). IgM is typically produced during the initial phase of the immune response, whereas IgG predominates during secondary or memory responses (Shlomchik and Weisel, 2019). Therefore, the observed immunoglobulin pattern may indicate that calcidiol–betaglucan supplementation primarily stimulated early immune activation rather than sustained humoral immunity (Soltanian *et al*., 2009; De Marco Castro *et al*., 2020).

Nevertheless, these findings should be interpreted cautiously, considering possible biological and technical variation, particularly the relatively large standard deviation observed in IgG values. Individual differences among birds and sample size limitations may contribute to variability in hematological responses, as previously reported in poultry receiving non-specific immunostimulants, where early immune activation is often more pronounced than antibody class switching (Sharma, 1997; Goodridge *et al*., 2009).

CONCLUSION

The combination supplementation of calcidiol (5,000 IU/kg) and betaglucan (80 mg/kg) in starter feed for laying hens was shown to improve early performance by significantly reducing feed intake and feed conversion ratio (FCR), improving body weight uniformity, and reducing mortality rates during the 0-3 week age period. The treatment group showed increased tibial bone marrow density, lymphocyte levels, and IgM, accompanied by reduced monocyte and IgG levels, indicating activation of the primary immune response and reduced chronic inflammation. This study reveals that the addition of a combination of calcidiol and betaglucan has potential as a functional feed additive to support early growth efficiency and immune system readiness in laying hens.

REFERENCES

1. Abun, A., Supriadi, D., Haetami, K., & Setiatwan, H. (2023). Blood hematology of laying hens given fermented shrimp waste extract. *GSC Biological and Pharmaceutical Sciences, 24*(1), 193–201. https://doi.org/10.30574/gscbps.2023.24.1.0269
2. Akinola, L. A. F., & Ebhohon, S. O. (2019). Influence of different levels of calcium and vitamin D in cassava-based diet on the performances of laying hens. *Nigerian Journal of Animal Production, 46*(1), 157–176. https://doi.org/10.51791/njap.v46i1.2493
3. Amer, S. A., Attia, G. A., Aljahmany, A. A., Mohamed, A. K., Ali, A. A., Gouda, A., Alagmy, G. N., Megahed, H. M., Saber, T., & Farahat, M. (2022). Effect of 1,3-Beta Glucans Dietary Addition on the Growth, Intestinal Histology, Blood Biochemical Parameters, Immune Response, and Immune Expression of CD3 and CD20 in Broiler Chickens. *Animals*, *12*(22), 3197. https://doi.org/10.3390/ani12223197
4. Bar-Dagan, H., Gover, O., Cohen, N. A., Větvička, V., Rozenboim, I., & Schwartz, B. (2023). Betaglucans induce cellular immune training and changes in intestinal morphology in poultry. *Frontiers in Veterinary Science, 9*, 1092812. https://doi.org/10.3389/fvets.2022.1092812
5. Bednarczyk, M., Stadnicka, K., Kozlowska, I., Abiuso, C., Tavaniello, S., Dankowiakowska, A., Slawinska, A., & Maiorano, G. (2016). Influence of different prebiotics and mode of their administration on broiler chicken performance. *Animal, 10*(8), 1271–1279. https://doi.org/10.1017/S1751731116000173

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

6. Chen, C. B., Turner, T. J., Applegate, T. J., Gilberto, L., & Kim, W. K. (2020). Role of long-term supplementation of 25-hydroxyvitamin D_3 on laying hen bone 3-dimensional structural development. *Poultry Science, 99*(11), 5771–5782. https://doi.org/10.1016/j.psj.2020.09.020
7. Chou, P., Chen, Y., Chung, T., Walzem, R. L., Lai, L., & Chen, S. (2020). Supplemental 25-hydroxycholecalciferol alleviates inflammation and cardiac fibrosis in hens. *International Journal of Molecular Sciences, 21*(21), 8379. https://doi.org/10.3390/ijms21218379
8. Cox, C., & Dalloul, R. (2010). Betaglucans as immunomodulators in poultry: Use and potential applications. *Avian Biology Research, 3*(4), 171–178. https://doi.org/10.3184/175815511x12919999481888
9. Da Nóbrega, I. P. T., Reis, M. P., Morillo, F. A. H., de Freitas, L. F. V.-B., Bittencourt, L. C., Fernandes, J. B. K., & Sakomura, N. K. (2022). Dynamics of growth and egg traits in three dietary balanced protein scenarios applied for laying hens. *Animals, 12*, 1371. https://doi.org/10.3390/ani12111371
10. De Marco Castro, E., Calder, P. C., & Roche, H. M. (2020). β-1,3/1,6-glucans and immunity: State of the art and future directions. *Molecular Nutrition & Food Research, 64*(11), 1901071. https://doi.org/10.1002/mnfr.201901071
11. El-Gobary, G. I. A., El-Zoghby, A. F. M., El-Sheikh, N. I., & Hamdy, A. S. (2016). Effect of chito-oligosaccharide as feed additives on egg production and performance of laying hens. *Egyptian Journal of Chemical and Environmental Health, 2*, 183–194. https://doi.org/10.21608/ejceh.2016.247679
12. Fatemi, S. A., Levy, A. W., & Peebles, E. D. (2025). The expressions of the immunity- and muscle development-related genes of 40-day-old broilers are promoted in response to the in ovo and dietary supplemental administration of calcidiol in conjunction with the in ovo administration of Marek's disease vaccine. *Animals, 15*(1), 10. https://doi.org/10.3390/ani15010010
13. Gao, J., Wang, R., Liu, J., Wang, W., Chen, Y., & Cai, W. (2022). Effects of novel microecologics combined with traditional Chinese medicine and probiotics on growth performance and health of broilers. *Poultry Science, 101*(2), 101412. https://doi.org/10.1016/j.psj.2021.101412
14. Gao, S., Qiu, K., Zheng, J., Zhang, H., Wang, J., Qi, X., & Wu, S. (2024). Dietary 25-hydroxycholecalciferol supplementation as a vitamin D_3 substitute improves performance, egg quality, blood indexes, jejunal morphology, and tibia quality in late-phase laying hens. *Animals, 14*(6), 878. https://doi.org/10.3390/ani14060878
15. Garcia-Mejia, R., Sinclair-Black, M., Blair, L., Angel, R., Jaramillo, B., Regmi, P., Neupane, N., Proszkowiec-Weglarz, M., Arbe, Z., Cavero, D., & Ellestad, L. (2024). Physiological changes in the regulation of calcium and phosphorus utilization that occur after the onset of egg production in commercial laying hens. *Frontiers in Physiology, 15*, 1465817. https://doi.org/10.3389/fphys.2024.1465817
16. Ghazalah, A., Elsa, M. A., & Ali, A. M. (2008). Influence of dietary energy and poultry fat on the response of broiler chicks to heat therm. *International Journal of Poultry Science, 7*(4), 355–359. https://doi.org/10.3923/ijps.2008.355.359
17. Goodridge, H. S., Wolf, A. J., & Underhill, D. M. (2009). Beta-glucan recognition by the innate immune system. *Immunological Reviews, 230*(1), 38–50. https://doi.org/10.1111/j.1600-065X.2009.00793.x
18. Guo, L., Jin, H., Luan, Z., Xue, P., Zhou, S., Wang, X., & Qin, N. (2019). Effects of the stems and leaves of *Astragalus membranaceus* on growth performance, immunological parameters, antioxidant status, and intestinal bacteria of quail. *Animal Science Journal, 90*(6), 747–756. https://doi.org/10.1111/asj.13213
19. Herwig, E., Classen, H., Walk, C., Bedford, M., & Schwean-Lardner, K. (2019). Dietary inositol reduces fearfulness and avoidance in laying hens. *Animals, 9*(11), 938. https://doi.org/10.3390/ani9110938
20. Hy-Line Brown. (2021). *Hy-Line Brown conventional system management guide*. Hy-Line International.
21. Jacob, J., & Pescatore, A. (2017). Glucans and the poultry immune system. *American Journal of Immunology, 13*(1), 45–49. https://doi.org/10.3844/ajisp.2017.45.49
22. Kazaev, K. A., Kholodilina, T. N., Sizova, E. A., Lebedel, S. V., & Salnikova, E. V. (2024). Calcium: Physiological role, sources and doses in diets of commercial poultry (Review). *Agricultural Biology, 59*(2), 237–257. https://doi.org/10.15389/agrobiology.2024.2.237eng
23. Khan, S. H., & Mukhtar, N. (2013). Dynamic role of cholecalciferol in commercial chicken performance. *World's Poultry Science Journal, 69*(3), 587–600. https://doi.org/10.1017/S0043933913000597

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

24. Landy, N., Kheiri, F., Faghani, M., & Bahadoran, R. (2020). Investigation of different levels of cholecalciferol and its metabolite in calcium and phosphorus deficient diets on growth performance, tibia bone ash and development of tibial dyschondroplasia in broilers. *Acta Scientiarum. Animal Sciences, 43*(1), e48816. https://doi.org/10.4025/actascianimsci.v43i1.488
25. Lee, D. H., Han, D., Park, B., Baek, H. S., Takatori, K., Aihara, M., & Park, J. (2005). The influences of β-glucan associated with BMP-7 on MC3T3-E1 proliferation and osteogenic differentiation. *Key Engineering Materials, 288–289*, 241–244. https://doi.org/10.4028/www.scientific.net/KEM.288-289.241
26. Li, X., Wang, X., Lv, Y., Ma, W., Wu, X., Zhen, W., & Zhao, F. (2023). Effects of dietary protease supplementation on behaviour, slaughter performance, meat quality and immune organ indices of broilers. *Czech Journal of Animal Science, 68*(6), 255–265. https://doi.org/10.17221/178/2022-CJAS
27. Liao, C., Cui, J., Lei, J., Guo, Y., & Zhang, B. (2023). Effects of *Bacillus subtilis* natto NB205 and its mutant NBMK308 on egg quality in aging laying hens. *Life, 13*(5), 1109. https://doi.org/10.3390/life13051109
28. Liu, X., Canyang, P., Qu, X., Guo, S., Chen, J. F., He, C., & Zhu, S. (2018). Effects of *Bacillus subtilis* C-3102 on production, hatching performance, egg quality, serum antioxidant capacity and immune response of laying breeders. *Journal of Animal Physiology and Animal Nutrition, 103*(1), 182–190. https://doi.org/10.1111/jpn.13022
29. Lyu, Z., Yuan, G., Zhang, Y., Zhang, F., Liu, Y., Li, Y., & Liu, D. (2024). *Anaerostipes caccae* CML199 enhances bone development and counteracts aging-induced bone loss through the butyrate-driven gut–bone axis: The chicken model. *Microbiome, 12*, 215. https://doi.org/10.1186/s40168-024-01920-y
30. Moraes, C., Bueno, J., Nascimento, M., Litz, F., Gotardo, L., & Guimarães, E. (2019). Bone parameters and organ morphometry of two broiler chicken lines exposed to heat for different periods. *Semina: Ciências Agrárias, 40*(5), 2007–2015. https://doi.org/10.5433/1679-0359.2019v40n5p2007
31. Muhshi, M., Rasyaf, M., & Purnama, A. (2024). Assessment of heat stress index and physiological responses in laying hens. *International Journal of Poultry Science, 23*(1), 1–9. https://doi.org/10.3923/ijps.2024.1.9
32. Murphy, P., Bello, F. D., O'Doherty, J. V., Arendt, E. K., Sweeney, T., & Coffey, A. (2012). Effects of cereal beta-glucans and enzyme inclusion on the porcine gastrointestinal tract microbiota. *Anaerobe, 18*(6), 557–565. https://doi.org/10.1016/j.anaerobe.2012.09.005
33. Mutucumarana, R. K., Ravindran, V., Ravindran, G., & Cowieson, A. (2014). Influence of dietary calcium concentration on the digestion of nutrients along the intestinal tract of broiler chickens. *Journal of Poultry Science, 51*(4), 392–401. https://doi.org/10.2141/jpsa.0140022
34. Niu, Z. Y., Min, Y. N., Wang, H. Y., Zhang, J., Li, W. C., Li, L., & Liu, F. Z. (2011). Effects of dietary dihydropyridine on laying performance and lipid metabolism of broiler breeder hens. *South African Journal of Animal Science, 41*(4), 331–336. https://doi.org/10.4314/sajas.v41i4.2
35. Osadcha, Y. (2021). Diagnostic value of integrated immunohematological indices as markers of chronic stress in laying hens. *Animal Biology, 23*(2), 19–25. https://doi.org/10.15407/animbiol23.02.019
36. Pakpahan, A. M., Retnani, Y., Mutia, R., & Wardani, W. W. (2023). Evaluation of the physical quality of supplement wafer and application of supplement on layer performance. *Buletin Peternakan, 47*(3), 190–197. https://doi.org/10.21059/buletinpeternak.v47i3.84653
37. Park, K., Rhee, A., Lee, I., Kim, M., & Paik, I. (2008). Effect of various β-1,3-glucan supplements on the performance, blood parameter, small intestinal microflora and immune response in laying hens. *Korean Journal of Poultry Science, 35*(2), 183–190. https://doi.org/10.5536/kjps.2008.35.2.183
38. Politis, I., Dimopoulou, M., Voudouri, A., Noikokyris, P., & Feggeros, K. (2003). Effects of dietary conjugated linoleic acid isomers on several functional properties of macrophages and heterophils in laying hens. *British Poultry Science, 44*(2), 203–210. https://doi.org/10.1080/0007166031000088316
39. Purnamasari, L., Cruz, J., & Cruz, K. (2022). The potential of beta-glucan as feed additives in broiler chickens: A review. *Jurnal Ilmu Peternakan Terapan, 6*(1), 18–28. https://doi.org/10.25047/jipt.v6i1.3297
40. Regmi, P., DeLand, T. S., Steibel, J. P., Robison, C. I., Haut, R. C., Orth, M., & Karcher, D. (2015). Effect of rearing environment on bone growth of pullets. *Poultry Science, 94*(3), 502–511. https://doi.org/10.3382/ps/peu041

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

41. Roztočilová, A., Šťastník, O., Novotný, J., Pavlata, L., Zeman, L., & Mrkvicová, E. (2023). The influence of calcium intake during the period of sexual maturation of pullets on its retention and eggshell quality of laying hens. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 71*(2), 65–73. https://doi.org/10.11118/actaun.2023.005
42. Saunders-Blades, J. L., & Korver, D. R. (2014). The effect of maternal vitamin D source on broiler hatching egg quality, hatchability, and progeny bone mineral density and performance. *Journal of Applied Poultry Research, 23*(4), 773–783. https://doi.org/10.3382/japr.2014-01006
43. Setyaningsih, N., Sumiati, Jayanegara, A., & Wardani, W. W. (2023). Effects of vitamins D and C supplement on performance, hatchability and blood profiles of broiler breeders. *Journal of World's Poultry Research, 13*(1), 71–80. https://doi.org/10.36380/jwpr.2023.7
44. Setiyaningsih, N., Sumiati, Jayanegara, A., & Wardani, W. W. (2022). Evaluation of Nutricell HYC® (combination of 25OHD$_3$ and vitamin C) supplementation on broiler breeders fertility in laying period. *IOP Conference Series: Earth and Environmental Science, 1020*(1), 012016. https://doi.org/10.1088/1755-1315/1020/1/012016
45. Shanmugasundaram, R., Muthukumar, T., Suryanarayanan, S., & Kamalanathan, V. (2013). Efficacy of yeast-based prebiotic on performance and immune responses of broiler chickens. *Veterinary Immunology and Immunopathology, 156*(3–4), 199–203. https://doi.org/10.1016/j.vetimm.2013.07.008
46. Sharma, J. M. (1997). The structure and function of the avian immune system. *Acta Veterinaria Hungarica, 45*(3), 229–238.
47. Shlomchik, M. J., & Weisel, F. (2019). B cell primary immune responses. *Immunity, 50*(4), 794–811. https://doi.org/10.1016/j.immuni.2019.04.012
48. Sokołowicz, Z., Krawczyk, J., & Dykiel, M. (2018). Productivity of laying hens in different production systems. *Acta Scientiarum Polonorum Zootechnica, 17*(2), 23–30. https://doi.org/10.21005/asp.2018.17.2.03
49. Soltanian, S., Stuyven, E., Cox, E., Sorgeloos, P., & Bossier, P. (2009). Beta-glucans as immunostimulant in vertebrates and invertebrates. *Critical Reviews in Microbiology, 35*(2), 109–138. https://doi.org/10.1080/10408410902753746
50. Souza, R. P., Laurentiz, A. C., Faria, G. A., Filardi, R. S., & Mello, E. S. (2019). Use of soybean gum as an emulsifier in diets for commercial laying hens. *Pesquisa Agropecuária Brasileira, 54*, e00683. https://doi.org/10.1590/S1678-3921
51. Tang, S., Sieo, C., Ramasamy, K., Saad, W., Wong, H., & Ho, Y. (2017). Performance, biochemical and haematological responses, and relative organ weights of laying hens fed diets supplemented with prebiotic, probiotic and synbiotic. *BMC Veterinary Research, 13*, 248. https://doi.org/10.1186/s12917-017-1160-y
52. Tapingkae, W., Panyachai, K., Yachai, M., & Doan, H. V. (2017). Effects of dietary red yeast (*Sporidiobolus pararoseus*) on production performance and egg quality of laying hens. *Journal of Animal Physiology and Animal Nutrition, 102*(1), 337–344. https://doi.org/10.1111/jpn.12751
53. Uyanga, V. A., Sun, L., Liu, Y., Zhang, M., Zhao, J., Wang, X., Jiao, H., Onagbesan, O. M., & Lin, H. (2023). Effects of arginine replacement with L-citrulline on the arginine/nitric oxide metabolism in chickens: An animal model without urea cycle. *Journal of Animal Science and Biotechnology, 14*, 9. https://doi.org/10.1186/s40104-022-00817-w
54. Vetvicka, V., & Vetvickova, J. (2014). Natural immunomodulators and their stimulation of immune reaction: True or false? *Anticancer Research, 34*(5), 2275–2282.
55. Wang, L., Song, Y., Manson, J. E., Pilz, S., März, W., Michaëlsson, K., Lundqvist, A., Jassal, S. K., Barrett-Connor, E., Zhang, C., Eaton, C. B., May, H. T., Anderson, J. L., & Sesso, H. D. (2012). Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: A meta-analysis of prospective studies. *Circulation: Cardiovascular Quality and Outcomes, 5*(6), 819–829. https://doi.org/10.1161/CIRCOUTCOMES.112.967604
56. Wang, J., Qiu, L., Gong, H., Celi, P., Yan, L., Ding, X., Bai, S., Zeng, Q., Mao, X., Xu, S., Wu, C., & Zhang, K. (2020). Effect of dietary 25-hydroxycholecalciferol supplementation and high stocking density on performance, egg quality, and tibia quality in laying hens. *Poultry Science, 99*(5), 2608–2615. https://doi.org/10.1016/j.psj.2019.12.054
57. Wang, S., Li, C., Zhang, C., Liu, G., Zheng, A., Qiu, K., & Chen, Z. (2023). Effects of Sihuang Zhili granules on the diarrhea symptoms, immunity, and antioxidant capacity of poultry challenged with lipopolysaccharide (LPS). *Antioxidants, 12*(7), 1372. https://doi.org/10.3390/antiox12071372

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 09 Issue 02 February 2026
DOI: 10.47191/ijcsrr/V9-i2-43, Impact Factor: 8.048
IJCSRR @ 2026
www.ijcsrr.org

58. Hu, W., Zhang, W., Shah, S. W. A., Ishfaq, M., & Li, J. (2021). *Mycoplasma gallisepticum* infection triggered histopathological changes, oxidative stress and apoptosis in chicken thymus and spleen. *Developmental & Comparative Immunology, 114*, 103832. https://doi.org/10.1016/j.dci.2020.103832
59. Warren, M. F., & Livingston, K. A. (2012). Implications of vitamin D research in chickens can advance human nutrition and perspectives for the future. *Current Developments in Nutrition, 5*(5), nzab018. https://doi.org/10.1093/cdn/nzab018
60. Wei, F., Yang, X., Zhang, M., Xu, C., Hu, Y., & Liu, D. (2022). *Akkermansia muciniphila* enhances egg quality and the lipid profile of egg yolk by improving lipid metabolism. *Frontiers in Microbiology, 13*, 927245. https://doi.org/10.3389/fmicb.2022.927245
61. Wolc, A., White, I. M. S., Avendaño, S., & Hill, W. G. (2010). Genetic and environmental variation in egg quality traits in laying hens. *Poultry Science, 89*(3), 473–479. https://doi.org/10.3382/ps.2009-00274
62. Yeh, Y. L., Chou, P. C., Chen, Y. H., Lai, L. S., Chung, T. K., Walzem, R. L., Huang, S. Y., & Chen, S. E. (2020). Dietary supplementation of 25-hydroxycholecalciferol improves cardiac function and livability in broiler breeder hens—Amelioration of blood pressure and vascular remodeling. *Poultry Science, 99*(7), 3363–3373. https://doi.org/10.1016/j.psj.2020.03.015
63. Yusuf, G. M., Sumiati, Mutia, R., Wardani, W. W., Akbar, I., & Putri, N. D. S. (2023). Effects of vitamin D_3 supplementation on Lohmann laying hens: Performance, egg quality, bone health, and immunity assessments. *Tropical Animal Science Journal, 46*(4), 461–470. https://doi.org/10.5398/tasj.2023.46.4.461
64. Zhang, H., Majdeddin, M., Gaublomme, D., De Meyer, F., Buyse, J., & Everaert, N. (2021). 25-Hydroxycholecalciferol reverses heat-induced alterations in bone quality in finisher broilers associated with effects on intestinal integrity and inflammation. *Journal of Animal Science and Biotechnology, 12*, 104. https://doi.org/10.1186/s40104-021-00627-6
65. Zhen, W., Shao, Y., Wu, Y., Li, L., Pham, V. H., Abbas, W., Wan, Z., Guo, Y., & Wang, Z. (2020). Dietary yeast β-glucan supplementation improves eggshell color and fertile eggs hatchability as well as enhances immune functions in breeder laying hens. *International Journal of Biological Macromolecules, 159*, 607–621. https://doi.org/10.1016/j.ijbiomac.2020.05.134
66. Zhen, W., Liu, Y., Shao, Y., Ma, Y., Wu, Y., Guo, F., Abbas, W., Guo, Y., & Wang, Z. (2021). Yeast β-glucan altered intestinal microbiome and metabolome in older hens. *Frontiers in Microbiology, 12*, 766878. https://doi.org/10.3389/fmicb.2021.766878

Cite this Article: Agustin, M., Ardhityasari, M.E., Suparno Putri, N.D., Wardani, W.W., Natsir, M.H., Sjofjan, O., Widodo, E. (2026). The Effects of Calcidiol and Betaglucan as Feed Additives on the Performance and Physiological Parameters of Laying Hens. International Journal of Current Science Research and Review, 9(2), pp. 991-1002. DOI: https://doi.org/10.47191/ijcsrr/V9-i2-43