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Effects of Calcidiol-Halquinol Combined Supplementation on Growth Performance, Intestinal Morphometry, and Tibia Bone Characteristics in Layer Pullets

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ABSTRACT : The grower phase is a critical period for intestinal and skeletal development in layer pullets, with long-term implications for productivity. This study investigated the impact of dietary supplementation with calcidiol-halquinol on growth performance, intestinal morphology, and tibial bone characteristics in grower pullets raised under antibiotic-free conditions. A total of 900 Hy-Line Brown pullets aged 9–11 weeks were assigned to two dietary treatments: a basal diet (T0) or a basal diet supplemented with calcidiol (5,000 IU/kg) and halquinol (60 mg/kg) (T1). Growth performance data were analysed using a nested randomized complete design, with body weight category included as a random factor nested within dietary treatment. Intestinal morphometric traits and tibia bone parameters were evaluated using independent sample analysis. Pullets fed the supplemented diet exhibited greater body weight gain and improved feed conversion ratio ($P<0.01$), while feed intake was unaffected, and mortality was numerically reduced. Supplementation showed increased duodenal villus density ($P<0.01$) and crypt height ($P<0.05$). Tibia cortical thickness ($P<0.05$) and cortical bone area ($P<0.01$) were also increased, whereas tibia length, weight, and ash content were not affected. It can be concluded that dietary supplementation with calcidiol-halquinol during the grower phase enhanced intestinal development, tibia structural integrity, and improved growth efficiency.

KEYWORDS- Calcidiol-Halquinol, Pullets Performance, Intestinal Morphometry, Tibia Bone Characteristics

I. INTRODUCTION

Eggs are an affordable and nutrient-dense source of animal protein, making them an important food commodity in Indonesia. Egg quality, particularly shell strength and thickness, determines market value by influencing breakage during storage and distribution. Eggshell quality is closely associated with gastrointestinal health and skeletal mineralisation, especially of the tibia, which develops rapidly during the grower phase. Eggshell formation depends on adequate calcium and phosphorus availability and mobilisation, which are regulated by two interconnected physiological systems: the intestinal tract, responsible for mineral absorption, and the skeletal system, particularly the tibia, which serves as the primary mineral reservoir. Both systems undergo intensive development during the grower phase of layer pullets, a critical period for establishing efficient nutrient utilisation and bone integrity that ultimately affects laying performance and eggshell quality.

Following the ban on antibiotic growth promoters (AGPs) in Indonesia (Permentan No. 22/2017), the poultry industry has been required to adopt non-antibiotic nutritional strategies to maintain gut health, skeletal development, and productivity. Calcidiol a biologically active metabolite of vitamin D_3, has been shown to improve intestinal morphology, enhance calcium and phosphorus absorption, and promote tibia mineralisation and structural integrity [1]. In parallel, halquinol, a non-antibiotic antimicrobial feed additive [2], has been reported to improve feed efficiency [3] and growth performance without inducing bacterial resistance [4]. Given their complementary mechanisms, combined calcidiol–halquinol supplementation may exert synergistic effects on intestinal development, tibia quality, and growth performance during the grower phase, thereby supporting improved egg production and eggshell quality during the laying period. Therefore, this study aimed to evaluate the effects of calcidiol–halquinol supplementation on intestinal morphology, bone development, and performance of grower pullets under antibiotic-free production systems.

II. MATERIAL AND METHODS

Calcidiol and Halquinol : Calcidiol used in this study was 25-hydroxycholecalciferol (25-OH-D_3), the primary circulating metabolite of vitamin D_3, produced through enzymatic hydroxylation of cholecalciferol. The product was manufactured using spray-dried beadlet technology to ensure stability and homogenous mixing in feed, with thermal resistance up to 90 °C during pelleting. *Halquinol consisted of a mixture of 5,7-dichloro-8-hydroxyquinoline, 5-chloro-8-hydroxyquinoline, and 7-chloro-8-hydroxyquinoline, synthesized industrially via halogenation of hydroxyquinoline derivatives. Both active ingredients were provided as a commercial premix (Vitagold Prelay Frame; PT Nutricell Pacific, Indonesia) and incorporated into the diet at 2 kg/ton of feed. The composition of the premix is presented in Table 1.*

Table 1. Content of Vitagold Prelay Frame (per/kg).

Composition	Content (kg/product)
Calcidiol (IU)	2,500,000
Halquinol (mg)	30,000
Filler	qs

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

Experimental design and housing : The experiment was conducted in Gedangsewu Village, Pare District, Kediri Regency, East Java, Indonesia, for 21 days. A total of 900 Hy-Line Brown grower pullets aged 9–11 weeks were used and classified into three body weight categories: small (<750 g), medium (750–800 g), and large (>800 g). Initial flock uniformity exceeded 92%. Birds were reared in open-house group pens equipped with feeders and nipple drinkers, and feed and water were provided ad libitum.

Ambient temperature ranged from 27.3 to 34.5 °C with relative humidity of 66.8–85.6%. The heat stress index ranged from 147.9 to 179.7, corresponding to comfortable to moderate heat stress conditions [8][9]. Ventilation fans were used to assist environmental control.

Dietary treatments and experimental design : Two dietary treatments were applied across the three body weight categories. The control diet (T0) consisted of a commercial grower feed (GC102), while the treatment diet (T1) was the same basal feed supplemented with calcidiol–halquinol (GC102Y; PT Gold Coin Indonesia). The treatments were applied from 9 to 11 weeks of age. Experimental groups were arranged as T0K, T0S, T0B, T1K, T1S, and T1B, corresponding to dietary treatment and body weight category. Feed ingredient composition and nutrient content are shown in Table 2.

Table 2. Nutrient Content per kg of Feed

Nutrient		Basal Feed (T0)	Basal Feed + Calcidiol-Halquinol (T1)
Water content (%)	Maximum	13	13
Ash (%)	Maximum	8	8
Crude Protein (%)	Minimum	20	22
Crude Fat (%)	Minimum	3	3
Crude Fiber (%)	Maximum	7	6
Calcium (Ca, %)		0.80 - 1.20	0.80 - 1.20
Phospor (P, %)	-	0.45	0.45
Calcidiol (IU)	-	-	5,000
Halquinol (mg)	-	-	60

Noted: Layer Chicken Grower Feed T0 = control group GC102 and T1 = treatment group GC102Y PT. Gold Coin Indonesia.

Growth Performance Measurements : Total feed intake was calculated weekly as the difference between feed offered and residual feed. Body weight gain (BWG) was determined from weekly body weight measurements between weeks 9 and 11. Feed conversion ratio (FCR) was calculated as feed intake divided by BWG over the experimental period. Body weight uniformity (BWU) was expressed as the percentage of birds within ±10% of the mean body weight. Mortality was recorded daily and expressed as a percentage.

Intestinal Morphometry and Tibia Bone Characteristics : At 12 weeks of age, four pullets per dietary treatment were randomly selected and euthanized by mechanical decapitation. Intestinal samples from the duodenum, jejunum, and ileum were collected for morphometric evaluation following [10]. Parameters measured included villus number, crypt height, and villus surface area, assessed at five fields of view (100× magnification).

Tibia bones were excised to determine length, weight, and tibia weight–to–body weight ratio [11]. Dry matter and ash content were analysed using proximate procedures. Cortical thickness and cortical area were measured at five fields of view (400× magnification) using Image Raster software, based on digital images captured with a Nikon Eclipse Ei light microscope equipped with an Optilab camera, following [12]

Statistical analysis : Data were processed using Microsoft Excel 2016 and analysed with IBM SPSS Statistics v25.0. Growth performance variables were analysed using a nested randomized complete design, with body weight category nested within dietary treatment. Intestinal morphometry and tibia bone parameters measured at 12 weeks were analysed using independent sample t-tests. When significant differences were detected, means were further compared using Duncan's multiple range test at $P < 0.05$ and $P < 0.01$.

III. RESULT

Growth Performance : Data from the study showed a measurable effect of dietary calcidiol 5,000 IU and halquinol 60 mg/kg feed on growth performance. The effect of Calcidiol-Halquinol Combination supplementation on grower growth performance is shown in Table 3.

Table 3. Effect of Calcidiol-Halquinol Combination supplementation on growth performance

Variabels	T0	T1
Total Feed Intake (g/head)	1515.67±56.18	1501.17±57.4
BWG (g/head)	$259.97±30.29^{A}$	$287.41±33.39^{B}$
FCR	$1.46±0.10^{A}$	$1.39±0.11^{B}$
BWU (%)	84.84	84.40
Mortality (%)	0.24	0.17

Note: $^{A-B}$ Superscript letters on the same line indicate significant differences P<0.01; NA = no analysis of variance performed (BWU, Total Mortality); T0 = Control; T1 = Treatment

Total feed intake did not differ between the control (T0) and supplemented (T1) groups (P>0.05). In contrast, birds receiving the supplemented diet showed significantly higher body weight gain (BWG) and improved feed conversion ratio (FCR) compared with the control group (P<0.01). Body weight uniformity (BWU) and mortality were not statistically analysed; however, both parameters were comparable between treatments, with numerically lower mortality observed in the supplemented group. Further analysis based on weight class showed that the response to the addition of the calcidiol–halquinol combination varied between chicken weight classes, as shown in Table 4.

Table 4. Effect of Feed Type Treatment on Weight Groups on the Production Performance of Grower Hens

Treatments	Total Feed Intake (g/head)	BWG (g/head)	FCR	BWU (%)	Total Mortality (%)
Basal Feed					
T0K	1440±49.16	$235.36±16.48^{A}$	$1.50±0.03^{B}$	93.53	0.33
T0S	1462±45.94	$261.36±28.33^{AB}$	$1.42±0.01^{A}$	93.83	0.15
T0B	1645±62.27	$283.18±26.55^{B}$	$1.47±0.02^{AB}$	95.14	0.25
Basal Feed + (Calcidiol-Halquinol)					
T1K	1406±47.71	$262.88±35.70^{A}$	$1.42±0.03^{B}$	94.26	0.23

T1S	1452.5±40.91	289.22±28.01^{AB}	1.36±0.02^{A}	95.05	0.09
T1B	1645±62.27	310.13±20.99^{B}	1.39±0.01^{AB}	96.17	0.18

Note: $^{A-B}$ Superscript letters in the same column indicate significant differences P<0.01; NA = No analysis of variance performed (BWU, Total Mortality)

When performance was further analysed based on body weight category (Table 4), total feed intake differed among weight classes, with large birds consuming more feed than small and medium birds under both dietary treatments. Supplementation did not alter feed intake within the same weight category. Body weight gain increased progressively from small to large birds within each dietary treatment (P<0.01), with the highest BWG recorded in large birds fed the supplemented diet (T1B). Feed conversion ratio was also affected by body weight category (P<0.01), with medium-weight birds exhibiting the most efficient FCR in both dietary treatments. Across all weight categories, supplemented birds tended to show numerically lower FCR values than their respective controls.

Intestinal Morphometry : Intestinal morphometric responses at 12 weeks of age are shown in Table 5.

Table 5. Effect of Calcidiol-Halquinol Combination Supplementation in Grower Hens on Intestinal Morphometry of 12-week-old Pullets.

Variabels		T0	T1
Duodenum	Number of Villi (mm^2)	5.5±0.74^{B}	8.5±1.39^{A}
	Crypt Height (µm)	230.30±51.03^{a}	270.43±26.92^{b}
	Villi Surface Area (µm^2)	446.99±189.80^{B}	289.38±99.65^{A}
Jejunum	Number of Villi (mm^2)	6.85±1.50	8.10±2.34
	Crypt Height (µm)	172.86±49.64	248.11±192.97
	Villi Surface Area (µm^2)	450.81±196.24	361.68±124.12
Ileum	Number of Villi (mm^2)	9.10±3.54	8.35±4.58
	Crypt Height (µm)	196.80±128.23	161.13±52.14
	Villi Surface Area (µm^2)	475.24±145.01	606.69±236.83

Note: T0 = control group; T1 = treatment group; $^{A-B}$ Superscript letters in the same row indicate highly significant (P<0.01). $^{a-b}$ Superscript letters in the same row indicate significant (P<0.05).

Calcidiol–halquinol supplementation significantly increased duodenal villus number (P<0.01) and crypt height (P<0.05) compared with the control group. Conversely, duodenal villus surface area was lower in supplemented birds (P<0.01). No significant differences were detected in jejunal or ileal villus number, crypt height, or villus surface area between treatments. Microscopic observations supported these findings, showing more densely arranged duodenal villi in supplemented birds (Figure 1).

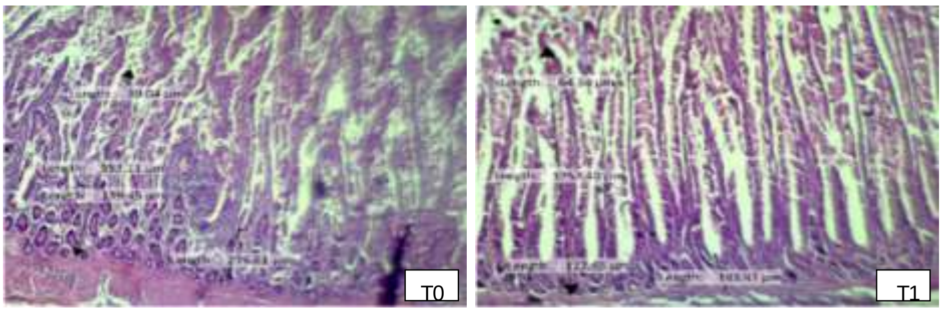

Figure 1: Duodenal morphometry with electron microscope magnification scale 1:100. T0: Morphometry of control duodenum; T1: Morphometry of treated duodenum

Tibia Bone Characteristics

Tibia bone characteristics are presented in Table 6.

Table 6. Effect of Calcidiol-Halquinol Combination Supplementation in Grower Hens on Tibia Bone Characteristics of 12-week-old Pullets.

Variables	T0	T1
Lenght (cm)	11.70±0.08	11.48±0.44
Weight (g)	10.35±0.7	10.70±1.5
Ratio (%)	0.70±0.04	0.72±0.11
Dry matter (%)	66.17±3.57	68.49±2.04
Ash (%)	39.56±3.38	39.31±1.47
Cortical Thickness (µm)	318.95±48.26^{a}	373.83±59.56^{b}
Cortical Area (µm)	942.99±109.34^{A}	1105.42±111.09^{B}

Note: $^{A-B}$ Superscript letters in the same row indicate a highly significant difference P<0.01. $^{a-b}$ Superscript letters in the same row indicate a significant difference P<0.05

Tibia length, weight, tibia-to-body weight ratio, dry matter, and ash content were not affected by dietary treatment (P>0.05). However, cortical bone thickness was significantly greater in the supplemented group (P<0.05), and cortical bone area was markedly increased compared with the control group (P<0.01). These differences were supported by microscopic evaluation of tibial cross-sections, which showed a thicker and denser cortical layer in supplemented birds (Figure 2).

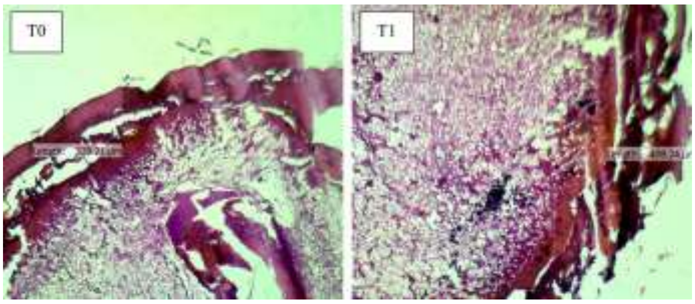

Figure 2. Scanning electron microscopy morphometry of cortical tibia bone at 12 weeks with 1:400x magnification. T0. Control; T1. Treatment.

Cross-sections of tibiae from supplemented chickens showed a cortical layer that appeared thicker and denser than that of the control group, indicating increased cortical bone development at 12 weeks of age.

IV. DISCUSSION

Growth Performance : Calcidiol-halquinol supplementation showed no significant effect on feed consumption, indicating that this additive does not interfere with feed palatability. Higher feed consumption observed in larger birds is consistent with normal physiological requirements, as birds with larger body masses require more nutrients to support maintenance and growth [13][14]. The increased weight gain and feed conversion ratio observed in supplemented birds, despite similar feed intake, suggest better utilization of available nutrients rather than increased feed intake. This response aligns with previous studies showing that calcidiol improves gut function and mineral metabolism, thereby supporting growth efficiency during the growing phase [15][16].Performance differences across weight classes further highlight the role of physiological status in growth efficiency, with medium-weight birds achieving the most favorable feed conversion. The generally lower FCR values observed in supplemented birds across all weight classes suggest that combined supplementation improves digestive efficiency under antibiotic-free production conditions.

The observed performance-enhancing benefits may also be related to the action of halquinol, which has broad antimicrobial activity and helps maintain gut microbiota balance. By limiting subclinical enteric challenges, halquinol can create a more stable gut environment that supports efficient nutrient utilization [17][18]. Importantly, halquinol has been recognized as safe and does not induce resistance, supporting its use in poultry systems where the use of growth-promoting antibiotics is limited [19].

Body weight uniformity and mortality were not statistically analysed; however, both parameters remained comparable between treatments, with numerically lower mortality observed in the supplemented group. These findings indicate that calcidiol–halquinol supplementation improved growth performance and feed efficiency without adverse effects on flock uniformity or survivability.

Intestinal Morphometry : The apparent response in the duodenum suggests that this intestinal segment is particularly sensitive to calcidiol-halquinol supplementation. This may be explained by higher vitamin D receptor expression in the duodenum, which enhances the physiological response to calcidiol compared to more distal intestinal regions [20]. In contrast, the jejunum and ileum are known to exhibit greater functional stability and adaptive capacity, which may explain the limited morphological changes observed in these segments following dietary intervention [21].

Although duodenal villous surface area was reduced in supplemented birds, this change occurred concurrently with an increase in villus density and crypt height. Together, these findings suggest a structural adaptation process rather than impaired absorptive function. A similar pattern has been described as a shift toward a more compact and efficient epithelial structure, supported by increased epithelial turnover and improved mucosal quality [22]. Halquinol may have further contributed to this response by supporting gastrointestinal stability during the growth phase. Its broad antimicrobial activity and ability to modulate the gut microbiota have been linked to improved gut conditions that support efficient nutrient utilization [5]. Furthermore, previous studies have shown that halquinol can influence gastrointestinal motility, increase nutrient-mucosal contact time, and potentially enhance mineral utilization, particularly calcium and phosphorus, which are essential for skeletal development [4][6].

Tibia Bone Characteristics : The results of this study indicate that dietary supplementation with a calcidiol–halquinol combination during the growing period significantly increased tibial cortical thickness and area in 12-week-old hens, while tibial length, bone weight, dry matter ratio, and ash content were unaffected. These findings suggest that supplementation primarily affects cortical bone characteristics rather than overall bone size or composition.Calcidiol, a biologically active metabolite of vitamin D_3, plays a central role in regulating calcium and phosphorus homeostasis, which supports cortical bone mineralization and osteoid matrix development [2][3]. Previous studies have shown that vitamin D_3 and its derivatives can increase cortical bone strength and fracture resistance in the tibia without necessarily increasing bone length or mass [1], which is consistent with the selective cortical response observed in this study.The contribution of halquinol may be related to its antimicrobial properties and its role in maintaining gastrointestinal microbial balance, thereby increasing the efficiency of mineral absorption required for bone formation [5][7]. [4] reported that halquinol can increase nutrient contact time with the intestinal mucosa through modulation of gastrointestinal motility, while [6] showed increased bone density and skeletal characteristics associated with improved mineral metabolism.

V. CONCLUSION

A conclusion section must be included and should indicate clearly the advantages, limitations, and possible applications of the paper. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

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Authors' Contributions : This manuscript was derived from the thesis of Mitta Agustin, supervised by Eko Widodo and Osfar Sjofjan. Mitta Agustin conducted the experiments, analysed the data, and prepared the manuscript. Eko Widodo served as principal investigator and supervised the research and manuscript review. Osfar Sjofjan contributed to research supervision and critical manuscript revision. Meta Eragrosita Ardhityasari assisted with field monitoring and data collection, while Nofitra Dewi Suparno Putri monitored animal health.

Muhammad Halim Natsir contributed to research design analysis. Wira Wisnu Wardani supported supplementation provision and manuscript review. All authors approved the final manuscript.

Competing Interests : The authors declare that there are no competing interests associated with this publication.

Ethical approval : All experimental procedures were conducted in accordance with the institutional animal-care guidelines of Brawijaya University No. 6-KEP-FKHUB-2025. Chickens were handled by trained personnel, and humane endpoints were strictly observed. Housing, feeding, and health-management practices complied with national welfare regulations for poultry research.

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Biographies and Photographs

Agustin, M. was born in Pasuruan, Indonesia, on April 21, 1987. I completed my Bachelor’s degree in Animal Science at Universitas Brawijaya, Malang, in 2008, specializing in Animal Nutrition and Feed Science. After my undergraduate studies, I gained experience in the animal feed and feed additive sector, mainly in quality assurance, technical support, and application of nutritional strategies in poultry production systems. I am currently engaged in managerial roles within the feed additive industry, emphasizing the integration of industry practices with scientific and academic frameworks. In 2023, I continued my academic path by enrolling in the Master’s Program in Animal Science at Universitas Brawijaya. My research interests focus on poultry nutrition, functional feed additives, gastrointestinal health, and mineral metabolism related to skeletal development and production performance in antibiotic-free poultry systems.