

# Effect of 25-Hydroxycholecalciferol (25-OH-D<sub>3</sub>) on Productive Performance and Bone Mineralization in Broiler

Moreno Santiago, Sarzosa David, Naranjo Alexandra, Aragón Eduardo, Quisirumbay-Gaibor Jimmy\*

Veterinary Medicine College, Central University of Ecuador, Quito, Ecuador  
Email: [jquirumbay@uce.edu.ec](mailto:jquirumbay@uce.edu.ec)

Received 1 June 2016; accepted 21 June 2016; published 24 June 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.  
This work is licensed under the Creative Commons Attribution International License (CC BY).  
<http://creativecommons.org/licenses/by/4.0/>



Open Access

---

## Abstract

In order to evaluate the effect of 25-OH-D<sub>3</sub> on productive performance of broilers and bone mineralization, an experiment in which 10,000 newly born Cobb mixed was used. The animals were divided into two groups: experimental received 25-OH-D<sub>3</sub> at a dose of 50 mg/liter and a control group (0 mg/liter). Weight gain and European Production Efficiency Factor (EPEF) were higher ( $P < 0.05$ ) for chickens that received 25-OH-D<sub>3</sub>. Mortality was lower ( $P < 0.05$ ) for the group receiving 25-OH-D<sub>3</sub>. However feed conversion rate (FCR) and feed intake showed no significant difference between treatments. The amount of calcium in tibia was higher in the experimental group ( $P < 0.05$ ). Based on the results obtained, it is concluded that the use of 25-OH-D<sub>3</sub> improves production performance and bone mineral status broiler.

## Keywords

25-Dihydroxycholecalciferol, Broiler, Calcium, Phosphorus

---

## 1. Introduction

The day-to-day poultry industry seeks to satisfy the consumption of animal protein demand growing human populations around the world to meet their nutritional requirements. Scientific breakthroughs have occurred in the poultry area to achieve greater efficiency in production, among them are genetics, health and nutrition have resulted in a bird more weight in less time [1] [2].

The rapid growth of broiler brings disadvantage presentation of metabolic problems that directly affect pro-

\*Corresponding author.

duction costs. These diseases are those affecting the skeletal system and are linked to nutritional factors such as levels of calcium, phosphorus, vitamin D and others [3]. Laying hens they can also be affected after presenting deformities poor nutrition level of the keel bone [4] and increasing the brittleness of the shell [5], problems arise in Turkeys ossification especially during growth [6]. So that nutrition influences the expression of the maximum production potential of the bird by reducing the occurrence of bone diseases, thus preventing injury on performance.

Among all the nutrients that should be included in the feed of broilers to prevent musculoskeletal disorders is vitamin D. Vitamin D is a responsible soluble compound metabolism of calcium and phosphorus in birds, their inclusion in the diet of broilers necessary, because its endogenous production does not meet the nutritional needs generating growth retardation, increased feed conversion and increased mortality [1] [2] [7].

The active form of vitamin D is 1,25-(OH)<sub>2</sub>-D<sub>3</sub> having in its structure two hydroxylations, the first takes place in the liver to result in the formation of 25-OH-D<sub>3</sub> and the second hydroxylation kidney level occurs. Direct administration of 25-OH-D<sub>3</sub> in the diet of broilers is a beneficial way to accelerate the formation of 1,25-(OH)<sub>2</sub>-D<sub>3</sub> avoiding hydroxylation in the liver [1] [2].

There are several advantages of using 25-OH-D<sub>3</sub> when administered orally, has shown greater intestinal absorption compared to vitamin conventional D, improves the morphology of small intestine and humoral immunity as measured by the concentration of IgG and IgA serum level ( $P < 0.1$ ) [8]. In broilers, the use of phytase and 25-OH-D<sub>3</sub> has improved retention of phytic phosphorus in the body [9], decreasing its level of inclusion in the diet, lowering production costs and reduce the amount of P excreted into the environment [10] [11]. Supplementation of 25-OH-D<sub>3</sub> at a dose of 0.21 mg/kg of feed improved by 27% the hydrolysis of phytate phosphorus apparent and increase bone mineralization [12], although other studies these results are inconstant [13].

*In-ovo* administration of 25-OH-D<sub>3</sub> a few days of hatching improves the quality of broilers birth [14]. In addition, other studies have found that increased serum levels of calcium (Ca) and phosphorus (P), improving bone mineralization [15]. In this way, it reduces the levels of Ca and P to include in the diet and the incidence of abnormalities such as tibial discondroplasia in broilers decreases [1] so for example it has been concluded that for every 10 mg/kg of 25-OH-D<sub>3</sub> above 70 mg/kg, the incidence of this disease in 1% and 2% in females and males respectively reduces [16] [17] presenting a better productive performance of the bird [18]. This helps reduce environmental pollution caused by removal of excess phosphorus in excreta thus avoiding huge losses to the national and global poultry industry since phosphorus is one of the most expensive nutrients in animal feed [7] [19].

This study aims to evaluate the effect of oral supplementation of 25-OH-D<sub>3</sub> as a source of vitamin D highly available to promote the proper development of the bones of the bird broiler.

## 2. Materials and Methods

The experiment was conducted at the farm “El despertar” located in Otón, Yaruquí, Pichincha-Ecuador. Necropsy and sampling was conducted at the Laboratory of Pathology, Veterinary Medicine College, Central University of Ecuador. The determination of calcium and phosphorus in bone was conducted at the Laboratory of Analysis Service and Food Research of the National Institute for Agricultural Research (INIAP).

10,000 Cobb broilers were used. The birds were distributed randomly in 2 treatments 5000 chickens each. Each treatment was divided into 5 experimental units of 1000 chickens.

The experimental group received 25-OH-D<sub>3</sub> at a dose of 50 mg/liter through the drinking water, this dose was used in order to overcome the recommendations found in commercial diets seeking higher bone mineral deposition. Besides seeking a new form of administration of this nutrient, since there is no information. 2 treatments received the same balanced food preparation based on corn and soybean. All diets were formulate to meet the Lesson and Summers [1] recommendations (Table 1 and Table 2).

Water and food were administered *ad libitum*. Starter feed from day 0 to 18, growth of 19 to 30 food and end the day 31 to 42 was used.

Production parameters feed intake, body weight, feed conversion ratio (FCR); and European Production Efficiency Factor (EPEF) were obtained through records made during the development work.

$$\text{FCR} = \text{feed intake/body weight}$$

$$\text{EPEF} = [\text{liveability} (\%) \times \text{body weight} (\text{kg}) \times \text{age} (\text{d})] / (\text{FCR} \times 100)$$

20 tibias were analyzed at 35 days of age, corresponding to 10 randomly selected birds per treatment for the determination of calcium and phosphorus. The values of each mineral were determined in atomic absorption spectrophotometer.

**Table 1.** Formula diets used in both treatments.

	Starter (0 - 18 d)	Grower (19 - 30 d)	Finisher (31 - 42 d)
<b>Corn</b>	533	613	693
<b>Soybean meal</b>	342	295	250
<b>Wheat shorts</b>	60	31	0
<b>Fat</b>	28.7	26	23.7
<b>Limestone</b>	15.8	16	16
<b>Dical Phosphate</b>	11.8	10.6	9.9
<b>Salt</b>	4.4	4.2	3.9
<b>DL-Methionine</b>	2.5	2.4	1.7
<b>L-Lysine</b>	0.8	0.8	0.8
<b>Vit-Min Premix**</b>	1	1	1
<b>Total (kg)</b>	1000	1000	1000

\*units/kg feed: Vitamin A (8000UI), Vitamin D<sub>3</sub> (3500 UI), Vitamin E (50 UI), Vitamin K (3 UI), Thiamin (4 mg), Riboflavin (5 mg), Pyridoxine (4 mg), Vitamin B<sub>12</sub> (12 µg), Pantothenic acid (14 mg), Folic acid (1 mg), Biotin (100 µg), Niacin (40 mg), Choline (400 mg); \*\*Iron (20 mg), Zinc (70 mg), Manganese (70 mg), Copper (8 mg), Iodine (0.5 mg), Selenium (0.3 mg).

**Table 2.** Nutrient dense broiler diets.

Nutrient	Starter (0 - 18 d)	Grower (19 - 30 d)	Finisher (31 - 42 d)
<b>Crude Protein (%)</b>	22	20	18
<b>ME (kcal/kg)</b>	3050	3100	3150
<b>Calcium (%)</b>	0.95	0.92	0.89
<b>Av Phosphorus (%)</b>	0.45	0.41	0.38
<b>Sodium (%)</b>	0.22	0.21	0.2
<b>Methionine (%)</b>	0.61	0.58	0.48
<b>Meth + Cystine (%)</b>	0.95	0.88	0.75
<b>Lysine (%)</b>	1.3	1.15	1
<b>Threonine (%)</b>	0.93	0.85	0.78
<b>Tryptophan (%)</b>	0.30	0.27	0.25

### 3. Data Analysis

For variables feed intake, average daily weight gain and feed conversion ratio statistical test “t” of student was used. Variable mortality was analyzed by chi-square test. The analysis was conducted using SPSS version 22.0 (SPSS Inc., Chicago, IL). The level of significance used was 5%.

### 4. Results

Overall mortality showed a significant difference ( $P < 0.05$ ) between treatments being 10.18% for the group receiving 25-OH-D<sub>3</sub> and 13.08% for the control group. Regarding at 42 days of age weight heavier ( $P < 0.05$ ) for the experimental group ( $2105.48 \pm 24.47$  g) was found with a significant difference from the control group ( $2009.96 \pm 30.24$  g).

The control group had an average cumulative retail consumption bird ( $3967.05 \pm 53.79$  g) compared to those who received the 25-OH-D<sub>3</sub> ( $4019.15 \pm 103.36$  g) although this was not found significant differences between treatments ( $P > 0.05$ ). As for FCR was no significant difference ( $P > 0.05$ ) between treatments however the experimental group showed a lower value ( $1.906 \pm 0.063$ ) compared to the control group ( $1.972 \pm 0.022$ ). When analyzing European Production Efficiency Factor (EPEF) there was a significant difference ( $P < 0.05$ ) between the experimental group ( $236.2 \pm 10.89$ ) and control ( $210.8 \pm 4.92$ ).

With respect to calcium content, significant effects between treatments ( $P < 0.05$ ). The content of this element

was higher in the group receiving 25-OH-D<sub>3</sub> ( $19.25 \pm 1.23$ ) versus the control group ( $17.96 \pm 1.30$ ). The phosphorus content also presented significant difference ( $P < 0.05$ ), the control group presented the highest value ( $9.248 \pm 1.64$  vs.  $6.276 \pm 0.52$ ).

## 5. Discussion

The weight difference between treatments at the end of the production cycle agrees with the data obtained by Ledesma *et al.* [15] who obtained greater weight in the group receiving 25-OH-D<sub>3</sub> through food (69 mg/t) even when the chickens were challenged with aflatoxin B1 (AFB1) (350 µg/kg). These data differ from those found by Chou *et al.* [8] who they found no difference between the control group and those receiving 25-OH-D<sub>3</sub> in the diet at a concentration of 69 µg/kg (0 to 21 days of age) and 34.5 µg/kg (22 to 39 days old) but could find that is 25-OH-D<sub>3</sub> decreased feed intake in the growth phase improving feed conversion 5%. In another study by Angel *et al.* [10] also found that the inclusion of 25-OH-D<sub>3</sub> at a dose of 75 µg/kg feed did not improve ( $P > 0.05$ ) live weight of birds when they were sacrificed at 49 days of age, the control group (2916 g) vs. experimental group (2849 g), similar data were found by Roberson *et al.* [9] where there was no difference in body weight between groups when 25-OH-D<sub>3</sub> was administered to several levels 0, 40 and 69 µg/kg of food. No significant difference was found in the study by Fritts y Waldroup [20] in body weight at 21, 42 and 49 days of age among those chickens receiving vitamin D and 25-OH-D<sub>3</sub> to the same dose. Nor existed difference in body weight measured at 17 days of age in broilers reared in battery receiving 25-OH-D<sub>3</sub> at different doses (0, 23, 46, 69, 70, 92 and 250 µg/kg) [21].

The highest content of Ca in tibia ( $P < 0.05$ ) found in chickens receiving 25-OH-D<sub>3</sub> is consistent with data found by Ledesma *et al.* [15] wherein the level of Ca was also higher ( $P < 0.05$ ) in the group receiving 25-OH-D<sub>3</sub> ( $26.61 \pm 21.73$  vs  $22.3 \pm 22.3$ ). However there differences with similar studies in which such Roberson [21] who found that 25-OH-D<sub>3</sub> at different doses (0, 23, 46, 69, 70, 92 and 250 µg/kg) did not change ( $P > 0.05$ ) ash content in tibia in broilers, in this study also found no differences in the levels of Ca and P in plasma ( $P > 0.05$ ). However, in the study by Applegate *et al.* [12] found that the ash level increased by 2.7% ( $P < 0.01$ ) in chicks receiving 25-OH-D<sub>3</sub> at a dose of 0.21 mg/kg through food.

The greater body weight of the birds in the group receiving 25-OH-D<sub>3</sub> could be explained due to higher calcium deposition in bones, which strengthened its locomotor system and allowed them to have better access to feed (higher consumption) and thus reduce mortality in the experimental group.

Regarding the content of phosphorus in warm significant difference ( $P < 0.05$ ) for the group that received 25-OH-D<sub>3</sub> ( $9,248 \pm 1.64$  vs  $6.276 \pm 0.52$ ) opposed to the data found Ledesma *et al.* found [15] that the level of P in tibia was higher ( $P < 0.05$ ) in chickens that received 25-OH-D<sub>3</sub> ( $3.92\% \pm 2.81\%$  vs.  $3.5\% \pm 3.5\%$ ).

## 6. Conclusion

Based on the results obtained in this study, we can conclude that that supplementation of 25-OH-D<sub>3</sub> at a dose of 50 mg/liter through drinking water improves calcium deposition in bones producing a stronger skeletal system that improves production parameters and reduces mortality of the flock that ultimately increases the income for the farm.

## Acknowledgements

The authors thank Dr. Eduardo Aragon intellectual support, Dr. Manuel Acosta and Dr. Alexandra Naranjo for funding to carry out this work.

## References

- [1] Leeson, S. and Summers, J.D. (2009) Commercial Poultry Nutrition. Nottingham University Press, Nottingham.
- [2] Pesti, G.M., Bakalli, R.I., Driver, J.P., Atencio, A. and Foster, E.H. (2005) Poultry Nutrition and Feeding. Trafford Publishing.
- [3] Oviedo-Rondón, E.O., Ferket, P.R. and Havestein, G.B. (2006) Nutritional Factors That Affect Leg Problems in Broilers and Turkeys. *Avian and Poultry Biology Reviews*, **17**, 89-103. <http://dx.doi.org/10.3184/147020606783437921>
- [4] Käppeli, S., Gebhardt-Henrich, S.G., Fröhlich, E., Pfulg, A., Schäublin, H. and Stoffel, M.H. (2011) Effects of Housing, Perches, Genetics, and 25-Hydroxycholecalciferol on Keel Bone Deformities in Laying Hens. *Poultry Science*, **90**,

- 1637-1644. <http://dx.doi.org/10.3382/ps.2011-01379>
- [5] Torres, C.A., Vieira, S.L., Reis, R.N., Ferreira, A.K., Silva, P.X.D. and Furtado, F.V.F. (2009) Productive Performance of Broiler Breeder Hens Fed 25-Hydroxycholecalciferol. *Revista Brasileira de Zootecnia*, **38**, 1286-1290. <http://dx.doi.org/10.1590/S1516-35982009000700018>
- [6] Ferket, P.R., Oviedo-Rondón, E.O., Mente, P.L., Bohórquez, D.V., Santos, A.A., Grimes, J.L. and Felts, V. (2009) Organic Trace Minerals and 25-Hydroxycholecalciferol Affect Performance Characteristics, Leg Abnormalities, and Biomechanical Properties of Leg Bones of Turkeys. *Poultry Science*, **88**, 118-131. <http://dx.doi.org/10.3382/ps.2008-00200>
- [7] Edwards, H.M. (2000) Nutrition and Skeletal Problems in Poultry. *Poultry Science*, **79**, 1018-1023. <http://dx.doi.org/10.1093/ps/79.7.1018>
- [8] Chou, S.H., Chung, T.K. and Yu, B. (2009) Effects of Supplemental 25-Hydroxycholecalciferol on Growth Performance, Small Intestinal Morphology, and Immune Response of Broiler Chickens. *Poultry Science*, **88**, 2333-2341. <http://dx.doi.org/10.3382/ps.2009-00283>
- [9] Roberson, K.D., Ledwaba, M.F. and Charbeneau, R.A. (2005) Studies on the Efficacy of Twenty-Five-Hydroxycholecalciferol to Prevent Tibial Dyschondroplasia in Ross Broilers Fed Marginal Calcium to Market Age. *International Journal of Poultry Science*, **4**, 85-90. <http://dx.doi.org/10.3923/ijps.2005.85.90>
- [10] Angel, R., Saylor, W.W., Mitchell, A.D., Powers, W. and Applegate, T.J. (2006) Effect of Dietary Phosphorus, Phytase, and 25-Hydroxycholecalciferol on Broiler Chicken Bone Mineralization, Litter Phosphorus, and Processing Yields. *Poultry Science*, **85**, 1200-1211. <http://dx.doi.org/10.1093/ps/85.7.1200>
- [11] Angel, R., Saylor, W.W., Dhandu, A.S., Powers, W. and Applegate, T.J. (2005) Effects of Dietary Phosphorus, Phytase, and 25-Hydroxycholecalciferol on Performance of Broiler Chickens Grown in Floor Pens. *Poultry Science*, **84**, 1031-1044. <http://dx.doi.org/10.1093/ps/84.7.1031>
- [12] Applegate, T.J., Angel, R. and Classen, H.L. (2003) Effect of Dietary Calcium, 25-Hydroxycholecalciferol, or Bird Strain on Small Intestinal Phytase Activity in Broiler Chickens. *Poultry Science*, **82**, 1140-1148. <http://dx.doi.org/10.1093/ps/82.7.1140>
- [13] Edwards, H.M. (2002) Studies on the Efficacy of Cholecalciferol and Derivatives for Stimulating Phytate Utilization in Broilers. *Poultry Science*, **81**, 1026-1031. <http://dx.doi.org/10.1093/ps/81.7.1026>
- [14] Gonzales, E., Cruz, C.P., Leandro, N.S.M., Stringhini, J.H. and Brito, A.B. (2013) *In ovo* Supplementation of 25(OH)D3 to Broiler Embryos. *Revista Brasileira de Ciência Avícola*, **15**, 199-202. <http://dx.doi.org/10.1590/S1516-635X2013000300005>
- [15] Ledesma Martínez, N., Casaubon Huguenin, M.T., Moreno Martínez, E., Rosiles Martínez, R., Manuel Petrone, V., Ávila González, E. and Río García, J.C.D. (2006) Efecto del 25-Hidroxicolecalciferol (25-OH-D3) en presencia de aflatoxina B1 en presencia de aflatoxina b1, sobre el comportamiento productivo en el pollo de engorda. *REDVET. Revista Electrónica de Veterinaria*, **VII**, 1-12.
- [16] Zhang, X., Liu, G., McDaniel, G.R. and Roland, D.A. (1997) Responses of Broiler Lines Selected for Tibial Dyschondroplasia Incidence to Supplementary 25-Hydroxycholecalciferol. *The Journal of Applied Poultry Research*, **6**, 410-416. <http://dx.doi.org/10.1093/japr/6.4.410>
- [17] Ledwaba, M.F. and Roberson, K.D. (2003) Effectiveness of Twenty-Five-Hydroxycholecalciferol in the Prevention of Tibial dyschondroplasia in Ross Cockerels Depends on Dietary Calcium Level. *Poultry Science*, **82**, 1769-1777. <http://dx.doi.org/10.1093/ps/82.11.1769>
- [18] Morris, A., Shanmugasundaram, R., Lilburn, M.S. and Selvaraj, R.K. (2014) 25-Hydroxycholecalciferol Supplementation Improves Growth Performance and Decreases Inflammation during an Experimental Lipopolysaccharide Injection. *Poultry Science*, **93**, 1951-1956. <http://dx.doi.org/10.3382/ps.2014-03939>
- [19] Sepúlveda, G., Augusto, C. and Rolando, B.R. (2014) Mecanismos de acción de la vitamina D3, 1- $\alpha$ -hidroxicolecalciferol (1- $\alpha$ -OH-D3,) y 25-hidroxicolecalciferol (25-OH-D3) en gallinas de postura comercial. *CES Medicina Veterinaria y Zootecnia*, **9**, 114-127.
- [20] Bar, A., Razaphkovsky, V., Vax, E. and Plavnik, I. (2003) Performance and Bone Development in Broiler Chickens Given 25-Hydroxycholecalciferol. *British Poultry Science*, **44**, 224-233. <http://dx.doi.org/10.1080/0007166031000087029>
- [21] Roberson, K.D. (1999) 25-Hydroxycholecalciferol Fails to Prevent Tibial Dyschondroplasia in Broiler Chicks Raised in Battery Brooders. *The Journal of Applied Poultry Research*, **8**, 54-61. <http://dx.doi.org/10.1093/japr/8.1.54>