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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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On Top Dose of Vitamin D as the Only Source in the First Week of Broilers

J.A. Giraldo-Carmona[1], W. Narvaez-Solarte[1] and E.A. Diaz-Lopez[2]
[1]Departamento de Salud Animal, Grupo de Investigacion de Nutricion,
Metabolismo y Seguridad Alimentaria, Universidad de Caldas, Manizales, Colombia
[2]Departamento de Produccion Agropecuaria, Universidad de Caldas, Manizales, Colombia

Abstract: The objective of this study was to evaluate the performance of broiler chicks in the first week of age. The study was performed with the inclusion of two vitamin D metabolites as the only source of vitamin D in the broiler's diet. For this purpose, 308 768-day-old male Ross chicks were used, hosted in 96 pens of 0.36 m^2 each. The experiment consisted of six treatments distributed in a completely randomized block design, in a factorial model of 2 x 3. Two vitamin D metabolites 25-hydroxy cholecalciferol and 1,25-dihydroxycholecalciferol and three levels of inclusion of each metabolite (34.5, 69.0 and 93.5 µg/kg) and (0.5, 1.0 and 1.5 µg/kg), respectively. The experiment was performed with 16 replications per treatment and eight animals per experimental unit. The main parameters of study were: final body weight (BW), average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and mortality (M). The birds fed with 25-hydroxy cholecalciferol presented a better ADG ($p = 0.03$) and ADFI ($p = 0.00$) in contrast to the 1,25-dihydroxycholecalciferol, FC ($p = 0.16$) and M ($p = 0.37$) were not affected by the vitamin D metabolite. The use of 34.5 µg/kg of metabolite 25-hydroxy cholecalciferol fed during the first week of life to the broilers show better zootechnical results with respect to 1,25-dihydroxycholecalciferol. This study led to the conclusion that 1,25-dihydroxycholecalciferol is not recommended as the only source of vitamin D when it comes from an herbal source.

Key words: Birds, calcification, cholecalciferol, nutrition, performance

INTRODUCTION

Vitamin D is part of fat-soluble vitamins and should be supplied in the diet when there is an adequate exposure of the body to sunlight. Precursors in the diet are the 7-dehydrocholesterol of animal products and ergosterol of plant products. These precursors must be irradiated by ultraviolet rays in the outer skin layers of the animal to produce Vitamin D_3 or cholecalciferol and vitamin D_2 or ergocalciferol. These molecules are transported to the liver where they encountered the first hydroxylation at the level of carbon 25 forming 25-hydroxy cholecalciferol ($25D_3$). Subsequently, a second hydroxylation take place in the kidney, where different metabolites are generated. One of them, 1,25-dihydroxycholecalciferol ($1,25D_3$) which is the most active form; performing the functions of vitamin D (Norman, 2008; Nelson and Cox, 2008; Collins and Norman, 1991).

Vitamin D mainly acts in three different tissues to regulate calcium and phosphorus homeostasis to perform proper bone mineralization and support other body functions. At the intestinal level, vitamin D increases the absorption of calcium and phosphorus by a gene regulated calcium-binding protein. At the kidney level, vitamin D decreases the excretion of calcium and phosphorus. Vitamin D in bones helps in matrix mineralization and remodeling. In addition, more than 36 different types of cells have the receptor for vitamin D (VDR). Vitamin D is currently consider a hormone because it acts in diverse systems, as cardiac-circulatory, nervous and immune system (Norman, 2008; Nelson and Cox, 2008).

The levels of $25D_3$ in the blood of broiler chickens must be at least 5 ng/ml, if the concentration is below this level vitamin D_3 deficiency occurs producing hypophosphatemia and hypocalcemia (Goff, 1990). The National Research Council (NRC, 1994) recommends that broilers diets must contain at least 200 IU/kg of vitamin D; however, commercial diets are fortified with levels that are between ten and twenty times above the recommended level. Those levels allow to reduce the incidence of rickets and tibialdyschondroplasia; diseases prevalent in the commercial production of broilers (Edwards, 1990; Lofton and Soares, 1986; McNutt and Haussler, 1973).

Cholecalciferol is the form of vitamin D most used in animal diets; however, there are on the market some metabolites that can be used as the $25D_3$ and $1,25D_3$. These metabolites are normally used on top of the diet; which already contains cholecalciferol showing some benefits. The need for this research arises from the few current studies using $25D_3$ and $1,25D_3$ metabolites as a source of vitamin D. This research compares the

Corresponding Author: J.A. Giraldo-Carmona, Departamento de Salud Animal, Grupo de Investigacion de Nutricion, Metabolismo y Seguridad Alimentaria, Universidad de Caldas, Manizales, Colombia

performance of broiler chickens in their first week of age with the inclusion of $25D_3$ and $1,25D_3$ as the only source of vitamin D.

MATERIALS AND METHODS

Aspects of bioethics: The Ethics Committee of the Universidad de Caldas endorsed the implementation of this study, in order to verify the procedures follow the protocol for the use of animals in research.

Location of the study: The research was carried out in the Laboratory of Nutrition and Poultry Health at the Facultad de ciencias agropecuarias de la Universidad de Caldas. Located at a latitude of 5°3'21.63"N, a length of 75°29'33.87"W, at an altitude of 2,130 meters above sea level, with an average temperature of 16.7°C and average annual precipitation around 2000 mm. The experiment was conducted in two environmentally controlled rooms; equipped with 48 vertical pens (0.36 m^2 each), drinking type nipple and a fixed feeder of 0.6 m per pen. The lighting was continuous and the temperature (33±0.5°C) was regulated by a thermostat.

Animals: 308 768-day-old Ross male chicks were used. The chicks were brought from a commercial hatchery, with an initial average weight of 43.97±1.25 g. During the experimental period, the animals consumed water and feed *ad-libitum*.

Diets: The diets were formulated according to recommendations from the NRC (1994) and Rostagno *et al*. (2011); based on corn and soybean meal; varying the amount of supplemental levels of vitamin D metabolites. Two metabolites were used: $25D_3$ with inclusion of 34.5, 69.0 and 93.5 µg/kg of feed and $1,25D_3$ with inclusion of 0.5, 1.0 and 1.5 µg/kg of feed as the only sources of vitamin D. Table 1 presents the experimental diet and the calculated nutritional composition.

Measured variables: On the seventh day of age of the chicks, each experimental unit was weighted. ADFI was determined by adding the amount of daily feed delivered during seven days, minus the uneaten feed collected from the feeders of each experimental unit. ADG was obtained from the difference between the initial average weight and final average weight of the chicks in each experimental unit, divided by the number of days of accommodation. FC was estimated by dividing ADFI by ADG. Mortality, temperature and humidity were recorded daily in each controlled room.

Experimental design and statistical analysis: The birds were distributed in a randomized block designs. The blocking factor was the height of the pen and the controlled environment room in a factorial model 2 x 3.

Table 1: Composition of the basal diet

Ingredient	Pre-starter (1-7 d) %
Corn	57.407
Soybean meal 48%	35.198
Tallow	2.974
Monocalcium phosphate	2.207
Calcium carbonate	0.723
DL-Methionine	0.338
HCL-Lysine	0.300
L-Threonine	0.112
Salt	0.250
Choline chloride	0.040
Vitamin and mineral premix*	0.450
Calculated nutritional composition (%)	
ME Kcal/kg	3000
Crude protein	22.00
Crude fiber	2.468
Fat	5.562
Calcium	0.900
Available phosphorus	0.450
Methionine	0.653
Met+Cis	1.000
Lysine	1.400
Threonine	0.950
Tryptophan	0.270
Linoleic acid	1.459

*Mineral and vitamin composition (mg/kg of feed): Zn 77, Mn 75, Fe 70, Cu 8, I 0.8, Se, 3, thiamine 2, riboflavin 6.5, niacin 40, pantothenic acid 10, pyridoxine 2.5, biotin 0.01, folic acid 1.25, cyanocobalamin 0.08, vitamin E 50, vitamin K 2.5, vitamin A 11,000 UI

Two vitamin D metabolites 25-hydroxy cholecalciferol and 1,25-dihydroxycholecalciferol and three levels of inclusion of feed (34.5, 69.0 and 93.5 µg/kg) and (0.5, 1.0 and 1.5 µg/kg), respectively; for a total of six treatments, each one with 16 replications and 8 birds per experimental unit. The results were subjected to variance analysis, with a significance level of $p<0.05$, in order to compare the means between treatments. The statistical program Stata 12.0 was used (Serial License 30120546473).

RESULTS

Under the evaluated conditions, we found statistically difference between the birds fed with vitamin D metabolites. The birds with $25D_3$ showed ADG ($p = 0.03$) and ADFI ($p = 0.00$) resulting in superior results, the improvements percentage was 3.6 and 6.3%, respectively. On the other hand, FC ($p = 0.16$) and the M ($p = 0.27$) were not affected by the vitamin D metabolite used. When the response of each metabolite was broken down, the metabolite dosage did not show any effect on the evaluated variables ($p>0.05$, refer to Table 2), which denoted that the minimum level of each metabolite was sufficient.

DISCUSSION

Most of the studies with vitamin D metabolites fulfill the animal cholecalciferol requirement, the addition of

Table 2: Final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion (FC) and mortality (M) in broilers fed with two vitamin D metabolites in the pre-starter phase (1 to 7 days of age)

Metabolite	Dose (µg/kg)	FBW (g)	ADG (g)	ADFI (g)	FC	M (%)
25D3	34.5	143.1	14.1	21.5	1.54	2.34
	69.0	143.8	14.1	21.6	1.54	3.12
	93.5	147.7	14.9	22.2	1.5	0.78
	p-value	0.305	0.120	0.202	0.348	0.366
1,25D3	0.5	142.8	14	20.4	1.47	5
	1.0	139.3	13.4	20.5	1.53	2.34
	1.5	142.7	14.1	20.8	1.47	2.68
	p-value	0.386	0.199	0.641	0.163	0.448
25D3		144.9	14.3a	21.8a	1.52	2.08
1,25D3		141.5	13.8b	20.5b	1.49	3.33
	p-value	0.061	0.036	0.000	0.099	0.274
SEM		0.887	0.124	0.134	0.010	0.568

Means followed by different letter within the same column, are statistically different ($p<0.05$)

complementary $25D_3$ and $1,25D_3$ generated extra benefits in performance and bone quality. The genetic lines of broiler chickens have bones with the correct dimensions to support the weight gain. They have a relatively poor bones quality, higher porosity and a lower mineral content; which generates a lower resistance to break (Williams *et al*., 2000). Also, there were some changes in the morphology of the intestines, suggesting an increase in the absorption of nutrients. Likewise, an improvement in the humoral immunity with the addition of $25D_3$; allowing birds to preserve nutrients for other physiological needs in order to maintain a lower serum antibody level (Chou *et al*., 2009). However, even with these associated positive effects, some results did not agree with the performance improvements.

In an experiment carried out by Chou *et al*. (2009) using a dose of 69 µg/kg of $25D_3$ on a basal diet of 3,000 IU/kg of cholecalciferol showed no evidence of improvements in weight gain or feed conversion in the period from 1 to 21 days of age. While Morris *et al*. (2014) used the same dosage of cholecalciferol and $25D_3$ as Chow, they found a better weight gain using the $25D_3$. These results agreed with Rama-Rao *et al*. (2007) who observed that broilers presented better zootechnical performance when consuming $25D_3$ at levels higher than 30 µg/kg. In the case of Whitehead *et al*. (2004), he found that the same effect was achieved with a minimal dose of 125 µg/kg of feed. Broilers fed with $25D_3$ in their diet displayed higher body weight gain, better feed efficiency, higher amount of ashes on bones and better breast meat. Additionally, it was found a lower rate of tibial dyschondroplasia and rickets than the broilers fed with dietary Vitamin D_3. The results from the previous studies support the idea on the importance of the different vitamin D metabolites' bio-potency for poultry nutrition (Fritts and Waldroup, 2003; Yarger *et al*., 1995; McNaughton *et al*., 1977). In this research, the $25D_3$ was used as the only source of vitamin D in broilers' diet. Broilers presented better productive performance than with the $1,25D_3$ metabolite.

The amount of cholecalciferol required in birds changes according to the evaluated variable and the level of calcium and phosphorus in the diet (Whitehead *et al*., 2004; Leeson *et al*., 1995). The $25D_3$ is metabolically stronger than vitamin D_3; the advantages are shown in broiler chicks with best weight gain, higher mineralization of the tibia, lower incidence rate and severity of tibial dyschondroplasia. These results were found when the diet contains low calcium levels (Coto *et al*., 2008; Fritts and Waldroup, 2003).

Mitchell and Edwards (1996) used 5 µg/kg of $1,25D_3$ on a basal diet of 1100 IU of cholecalciferol, three levels of phosphorus (0.45, 0.55 and 0.65%) and the addition or absence of phytase. They found that supplementation with $1,25D_3$ increased the weight of the bird, decreased the incidence of rickets and tibial dyschondroplasia and improved phosphorus usage. Moreover, they generated a synergic effect with the addition of phytase improving even more the weight of the bird, the ashes of the tibia, prevention of rickets and a reduction of phosphorus requirement. While Roberson and Edwards (1996) found no effect on weight and conversion of the birds at 21 days of age using three doses of $1,25D_3$ (3, 6 and 9 µg/kg) in a basal diet of 1100 IU of cholecalciferol. Moreover, Vieites *et al*. (2012) used a dose range of 0 to 5 µg/kg of $1,25D_3$ in a basal diet 1339 IU/kg of cholecalciferol; with diets low in calcium and phosphorus resulting in no influence on performance. This current study used the same commercial vitamin D brand as Vietes *et al*. (2012) experiment. This product comes from an herbal source, suggesting that this product is not an effective precursor of vitamin D; hence, the birds had a lower performance in the seven days period. This became more noticeable after the seventh day, showing symptoms of vitamin D deficiency (data not shown in this report). The deficiency is not noticeable in the first week because until day seven maternal vitamin D starts decreasing and after that point signs of deficiency are shown like: low ionized blood calcium levels and a tendency of the animals of sit quickly after walking some steps (Aslam *et al*., 1998).

Conclusions: In most of the research studied, the metabolites have been analyzed separately: $25D_3$ and $1,25D_3$ showing positive results in the majority of studies. Previously, it was thought that because $1,25D_3$ is the active metabolite, it would have showed a higher efficiency than $25D_3$; however, this study revealed that is was not. Perhaps, in early studies $1,25D_3$ was derived from synthetic sources but in this study it was derived from an herbal source. It is possible that the doses of $1,25D_3$ were low; therefore, future work will aim for higher levels of the metabolite in order to determine the optimal dose for broilers' diet. At the doses used of both metabolites, $25D_3$ showed improvements in animal performance, compare to $1,25D_3$.

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