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In Ovo Supplementation of 25(OH)D_3 to Broiler Embryos

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ABSTRACT

A dose of 0.3 mL of water solution containing 0.00 (control), 0.625, 1.250 or 1.875 µg of 25-hydroxy cholecalciferol (25(OH)D_3) was administered to 312 fertile eggs derived from 49-w-old Cobb 500 broiler breeders on the 17th day of incubation (DE17) via allantoic cavity. After treatment, eggs were distributed and maintained until hatching in four incubators set at 37.8 ºC and 55% RH. Each incubator received eggs from all treatments, according to a block design with four treatments of 77-79 replicates each. Hatching was checked every two hours from 484h to 512h of incubation to evaluate productivity and chick qualities. Chicks were housed until 10 days of age in heated battery cages according to a block design with four treatments of 10 replicates of six chicks each for performance and mortality evaluation. Mean hatching time of the chicks treated with 25(OH)D_3 during the embryonic phase occurred 4 to 5 h earlier than control group (502:31h), with no effects on hatching or neonate qualities. An inverse linear effect of 25(OH)D_3 dose on chick body weight at hatching was observed, but 10-d-old broiler performance and mortality were not affected. The fast body weight recovery of the broilers obtained from the embryos supplemented with the highest 25(OH)D_3 level was recorded until 10 days of rearing, equaling final mean body weights ($p>0.05$) among experimental groups. The results of this study indicate the potential use of 25(OH)D_3 as exogenous vitamin supplementation to embryos a few days before hatching without affecting neonate qualities and 10-d-old broiler chicken performance.

INTRODUCTION

The *in-ovo* vaccination is becoming a popular management tool in hatcheries. This vaccination system allows the early immunization of the future chicks without affecting the development of the embryos or hatching rate (Ohta *et al*.,2001). This technology allows supplementing nutrients to broiler embryos (Uni *et al.*, 2004; Willemsen *et al*., 2010),as it is known that the nutritional supply from yolk sac is not sufficient to support the fast grow rate broiler chicks present during their neonatal phase (Gonzales *et al.*, 2008b, 2009; Willemsen, *et al.*, 2010).

During the very first week of post-hatching life, the growth of the chick's bone tissues is a priority, demanding an intense mobilization of calcium. Vitamin D_3, as 1,25$(OH)_2D_3$, plays a relevant role in calcium homeostasis by directly controlling its absorption at either intestinal, bone or renal loci. The other metabolic form of vitamin D_3, 24,25$(OH)_2D_3$, is required for the maximum expression of the activity of the enzymes involved in adequate bone mineralization (PizauroJr., 2002).

Vitamin D_3 premixes added to broiler diet are the usual source of vitamin D. However, to be active, vitamin D_3 has to go through a

metabolic pathway, being hydrolyzed in the liver and kidney to 25(OH)$D_3$and 1,25(OH)D_3, respectively. A commercial 25(OH)D_3 product is currently available, and its use as a sole dietetic source of vitamin D_3 during the early growth phase improves the calcification process and broiler performance (Hargis, 2000).

The present study aimed at evaluating the feasibility of *in-ovo* 25(OH)D_3 supplementation, providing a more available source of vitamin D to the embryo in an intent to anticipate its high nutritional requirement for bone growth of chicks during the early stage of the post-hatching phase.

MATERIAL AND METHODS

A total of 312 broiler breeder eggs obtained from 49-week-old Cobb breeders and with 17 days of incubation were individually inoculated via allantoic cavity with 0.3 mL of a water solution containing 0.00, 0.05, 0.10, or 0.15 mg of Rovimix HY-Dâ 1.25% (DMS do Brasil SA, São Paulo, Brazil). These doses corresponded to 0.00, 0.625, 1.250, and 1.875 µg of 25(OH)D_3 or 0,25, 50, and 75 IU of vitamin D_3, respectively.

Vitamin was supplemented by making a small hole on the largeend of eggs (air cell end) by introducing a 25x5 needle attached to a 3-mL syringe. The allantoic cavity was reached from the opposite side of embryo head and located with the aid of a lantern. After supplementation, hot liquid paraffin was used to close the hole.

Eggs were distributed and maintained until hatching in four incubators regulated to maintain the temperature at 37.8 °C and relative humidity at 55%. Each incubator received eggs of all treatments according to a block design with fourtreatments of 79 to 77 replicates each. Before hatching, each egg was segregated using an air-permeable sack. We checked the hatching of the chicks every two hours from 484h to 512h of incubation, measuring hatchability, neonate weight and physical aspects (score 1 = excellent; 2 = good; 3 = bad), navel qualities (score 1 = closed; 2 = semi-open; 3 = open), and embryonic mortality. Newly-hatched chicks were housed in heated battery cages according to a block design, with four treatments of 10 replicates of six chicks each, which were reared up to 10 days in order to evaluate performance (body weight, weight gain) and mortality.

Data were statistically analyzed using SAEG computational program (SAEG, 1999). Kruskal-Wallis was used to test non-parametric data, and analysis of variance (ANOVA) to test parametric data ($p<0.05$), decomposing the degree of freedom of treatment up to the third grade ($p<0.05$). Mortality percentage was arcsine-transformed before ANOVA analysis.

RESULTS AND DISCUSSION

Mean egg weight one day before *in-ovo* supplementation (day 16) was 63.96±0.23g. Irrespective 25(OH)D_3-doses, chicks from supplemented eggs hatched two to four hours earlier than those in the control group (Figure 1). Mean hatching times were 502:31h, 498:22h, 498:58h and 497:47h for 0.00, 0.625, 1.250, and 1.875 µg of 25(OH)D_3 groups, respectively. Hatching rate was not affected ($P>0.05$) by treatment, and 93.67% (74/79), 92.41% (73/79), 88.31% (68/77) and 87.01% (67/77) were recorded according to proportional increases in 25(OH)D_3.

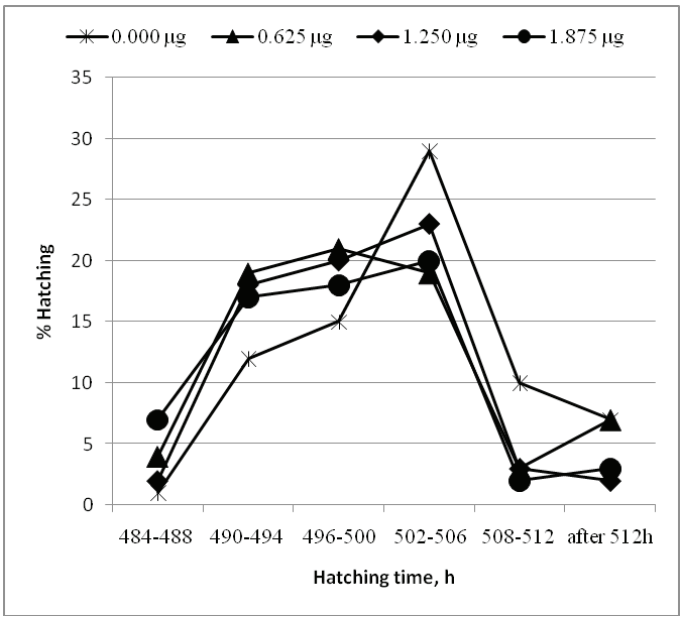

Figure 1 – Distribution of hatching time according to 25(OH)D3 dose (mg/egg) of *in-ovo* supplementation to broiler embryos at 17 days of incubation.

Regardless the treatment, all hatched chicks were in good shape and presented excellent qualities, presenting chick and navel scores close to 1 (Table 1). Vitamin 25(OH)D_3 affected ($p<0.05$) chickweight at the moment of escaping from the egg ($Y = 52.51 – 0.4899x$. $R^2 = 0.73$) and 12h after housing ($Y = 50.65 – 0.0884x$. $R^2 = 0.80$), presenting a linear decrease, proportional to 25(OH)D_3 level increase. However, up to 10 days of rearing, chickens from all treatment groups presented similar body weight and mortality rate ($p>0.05$) (Table 2). Fast body weight recovery of 10-d-old chickens previously treated with the highest level of 25(OH)D_3 was observed.

Table 1 – Body weight and chick and navel quality scores of newly-hatched chicks supplemented *in-ovo*with 25(OH) D_3at 17 days of incubation.

	Body weight		Chick quality	Navelquality
25(OH)D_3	G	%[1]	average score	average score
0.000 µg/egg	51.91 [2]	80.43	1.06	1.21
0.625 µg/egg	51.48	80.64	1.06	1.15
1.250 µg/egg	51.50	80.46	1.12	1.26
1.875 µg/egg	50.26	80.20	1.02	1.09

[1] Related to egg weigh at 16 days of incubation.

[2] Linear effect (P<0.05).Y = 52.51 – 0.4899x. R^2 = 0.73.

Early hatching time and lower weight of neonatal chicks from groups supplied with 25(OH)D_3 suggest increased embryonic metabolic activity, probably stimulated by the supply of exogenous vitamin. In addition to modulating calcium homeostasis and absorbability by inducing calcium carrier protein synthesis, vitamin D also affects at least 30 systems of target cells, stimulating RNAm synthesis and induction of the protein that controls cell functions (Pizauro Jr, 2002). Not surprisingly, adequate vitamin D nutrition, as cholecalciferol (vitamin D_3) or 25-hydroxicolicalciferol 25(OH)D_3, should be essential to achieve the best performance of broiler chicks selected for high grown rate in the very early phase of life.

Some researches support the theory that growth of neonate birds during their first few post-hatch days depends on nutritional elements absorbed from the vitelline residue. However, feed shortage at this time may impair later performance of broilers selected for fast development, suggesting that nutritional input from yolksac is not sufficient to support extreme chick post-hatch growth, as demonstrated in previous studies (Noy & Sklan, 1999, Gonzales *et al.*, 2003, 2008ab). These authors observed poor finisher performance (42-d-old) when broilers were submitted to 24-h of fasting during the neonatal phase, showing that short fasting periods very early in the post-hatch life affect late broiler development. Thus, exogenous supplementation of chicks after 24 hour of hatching is needed for full expression of the genetic potential for growth. However, newly-hatched chicks are rarely housed within this interval of time, which eventually impairs their full development and final performance. According to Sklan (2001), full development imperatively requires metabolic precursors, that is, nutrients, to be available. During the last stage of embryo development, nutrients are derived from the yolk and the albumen, which are rich in protein and lipids. Although calcium is supplied by the shell, in order to be available, it has to be deposited in the yolk and then transferred to the embryo through the blood or Meckel's diverticula. After the abdominal internalization of yolk sac, which occurs around the 20th day of incubation, the only source of nutrients for embryos is the yolk remaining in the gut (Noy & Sklan, 2001). Calcium transport via vitelline or gut cells for embryo bone mineralization or renal reabsorption depend on a calcium-binding-protein expressed by 1.25(OH)D_3 renal metabolite (Hurwitz, 1992, PizauroJr., 2002). This substance – 1.25(OH)D_3 – is obtained in the kidney by the hydrolization of hepatic 25(OH)D_3 deposited in the egg during yolk synthesis under this form or obtained upon cholecalciferol metabolization in the liver (Hurwitz, 1992). In addition to calcium shortage, two other factors maybe implicated in inadequate calcium deposition during final phase of embryo development: 1) insufficient yolk deposition of 25(OH)D_3; and 2) insufficient D_3 and 25(OH) metabolization by the immature liver and kidney of the embryos. As a consequence, post-hatching skeletal growth may be impaired, affecting mainly long bone development. An exogenous source of 25(OH)D_3 supplied to the embryos at this time may prevent post-hatch bone disorders. The high vitamin D requirement of modern broilers is evidenced by the high incidence of skeletal disorders currently observed in broiler flocks (Gonzales *et al.*, 2009; Mendonça Jr, 2009).

Table 2 – Performance and mortality of 1- to 10-d-old broilers according to treatment.

25(OH)D_3	Initial body weight, g[1]	Final body weight, g	Weight gain, g	Feed intake, G	Feed conversion ratio	Mortality rate, % [2]
0.000 µg/egg	50.52 [3]	242.15	192.63	237.49	1.267	6.00
0.625 µg/egg	50.20	242.32	192.13	236.75	1.243	2.00
1.250 µg/egg	50.16	242.60	192.44.	237.40	1.236	0.00
1.875 µg/egg	49.06	250.60	201.54	252.90	1.260	0.00

[1]Immediately before housing.

[2]Arcsine-transformed before ANOVA.

[3] Linear effect (p<0.05).Y = 50.65 – 0.0884x. R^2 = 0.80.

Assuming that *in-ovo* vaccination methods are extensively used in hatcheries and that exogenous 25(OH)D_3 supplementation maybe used without detrimental effects on chick quality and hatchability, this technique could be applied to supply enough vitamin D required for the high calcium deposition of broiler embryos in a more available form. The results of this experiment suggest the possibility of using 25(OH) D_3 *in-ovo* supplementation, with no harmful effects on chick hatchability and quality or on 10-d-old broiler performance. Other trials should be carried out to evaluate the performance of broilers at market age, the development of long bones and the skeleton structure of broilers after *in-ovo* D_3 supplementation.

CONCLUSIONS

The results of this study indicate that vitamin 25(OH) D_3 may be supplemented *in-ovo* to broiler embryos without any influence on hatching rate, neonate quality or 10-d-old broiler performance.

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