EFFECT OF CALCIUM AND COLE VIT D_3 IN OVO INJECTION ON HATCHABILITY, BONE AND BLOOD BIOCHEMICAL DEVELOPMENT AT POSTHATCH

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ABSTRACT

The aim of this study to investigate the role of in ovo injection in broiler breeder (Ross-308) fertile eggs with calcium, vitamin D_3 and their mixture at 18 d of incubation on hatchability, tibia bone and blood properties post-hatching, included five groups: (non-injected and in ovo injected 100 µL sterilized distill water) controls as well as eggs that were injected with the 100 µL sterilized distill water either 0.8 mg calcium (Ca), 0.8 mg Cole vit D_3 [colecalciferol or 25-OHD_3] and mix of 0.8 mg Ca + 0.8 mg Cole vit D_3. The results showed that an in ovo injection with calcium, Cole vit D_3 and their mix presented significantly higher in hatchability (%), body performances, tibia bone properties [length, breaking strength, Ca and P concentrations, ash %] and blood biochemical analysis [Ca and P minerals concentrations, vit D_3 and parathyroid hormone (PTH)] at 0 and 35 d posthatch. While, blood calcitonin hormone (CT) at 0 and 35 d posthatch and blood Ca: P ratio had recorded lower concentration at 0 posthatch, however had seen non-significant differences among all the groups in tibia width and Ca:P ratio in tibia bone at 0 and 35 d posthatch and also non-significant among all the groups of in ovo injection and control in blood Ca: P ratio at 35 d posthatch. The in ovo injection with Cole vit D_3 and mixture of Ca and Cole vit D3 showed more effectiveness in most characteristics.

Keyword: in ovo, calcium, Vit D_3, hatching, minerals, hormones.

تأثيرحقن البيض بالكالسيوم وكولي فيتامين D_3 في الفقس، وتطور بايوكيميائية العظام والدم بعد الفقس

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المستخلص

تهدف الدراسة الى كشف دور حقن بيض أمهات فروج اللحم (Ross–308) بالكالسيوم وفيتامين D3 ومزيجهما عند عمر 18 يوما من حضن البيض على قابلية الفقس، وزن الأفراخ، طول عظم الساق وخصائص الدم.بعد الفقس وشملت خمسة مجاميع: (مجموعة السيطرة: دون حقن للبيض، حقن البيض100 ميكرولتر من الماء المقطر المعقم، حقن البيض بـ 0.8 ملغم كالسيوم مذاب بـ 100 ميكرولتر من بالماء المقطرالمعقم، 0. حقن البيض بـ 0.8 ملغم كولي فيتامين د3 مذاب بـ 100 ميكرولتر من بالماء المقطرالمعقم، حقن البيض بمزيج 0.8 ملغم كالسيوم + 8 ملغم كولي فيتامين د3 مذاب بـ 100 ميكرولتر من بالماء المقطرالمعقم). أظهرت نتائج حقن البيض بالكالسيوم، كولي فيتامين د3 ومزيجها تفوقا في نسبة الفقس، الأداء الأنتاجي، خصائص عظمة الساق (الطول، قوة امقاومة الكسر، تراكيزالكالسيوم والفوسفور ونسبة الرماد) والتحليل البيوكيميائي للدم [تراكيز (عنصري الكالسيوم والفسفور، فيتامين D3 وهرمون جارات الدرقية [(PTH) عند عمر 0 و 35 يوم بعد الفقس. في حين انخفض تركيزهرمون الكالسيتونين في الدم بعمر 0 و 35 يوم بعد الفقس ونسبة الكالسيوم: الفسفور وسجلت تركيز أقل عند 0 بعد الفقس ، بينما كل مجاميع الدراسة لم تختلف معنويا في عرض الساق ونسبة الكالسيوم: الفسفور في عظمة الساق بعمر 0 و 35 يوما ونسبة الكالسيوم: الفسفور بالدم عند عمر 35 يوما بعد الفقس. أظهر نتائج حقن البيض بـ كولي فيتامين د 3 ومزيج من (الكالسيوم و كولي فيتامين د 3) فعالية أكبر في معظم الصفات المدروسة.

الكلمات المفتاحية: حقن البيض، الكالسيوم، كولي فيتامين د، الفقس، المعادن، الهرمونات.

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INTRODUCTION

Vitamin D plays an essential role in bone mineralization and calcium homeostasis via regulation of parathyroid hormone (PTH) secretion (20). More recently, there has been growing evidence for a role of vitamin D in extra skeletal health (8). Vitamin D plays an important role in maintaining an adequate level of serum calcium and phosphorus. Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed (10). Therefore vitamin D has a great effect in forming and maintaining strong bones. It has also recently been found that vitamin D receptors exist in a variety of cells thus it has a biological effect on more than mineral metabolism (21). The commercial in ovo injection of 25(OH)D3 was reported to improve the hatchability of fertilized broiler hatching eggs without having detrimental effects on hatchling quality (1). Also, in a later related study, it was shown that in ovo injection of up to 1.20 µg of 25-OHD_3 had no detrimental effects on survival or overall posthatch performance, including body weight gain, of broilers (2). The presence of cholecal-ciferol in eggs is very important to support the embryo Ca metabolism during incubation. Therefore, deficiency of this vitamin can lead to reduced hatchability, which can be specially related to late embryo mortality (11). Avian embryos assimilate large amounts of calcium in their bones in a short time. The chicken embryo, for instance, accumulates over 100 mg of calcium from the egg shell across the chorio-allantoic membrane from days 10-12 of embryonic life until hatching day (13). (14) conducted to in ovo vitamin D3 at ages 15, 16, 17 days of incubation increased the rate of calcium mobilization from the egg shell to the embryo and increased calcium concentration in all embryonic compartments. The objective of this study was to investigate the effects of the in ovo injection of 25(OH)D3 on d 18 of incubation on hatchability, posthatch performance, tibia mineralization and hormones concentrations in blood plasma.

MATERIALS AND METHODS

Experimental design

This experiment was conducted at Taqtaq broiler breeder and hatchery farm Erbil/Iraq, 1500 eggs were collected from commercial broiler breeder (Ross 308) hens at age 58 wks. the eggs were randomly allocated to 5 groups each group contain 3 replicates (were equally represented on each of 3 tray levels (blocks) of the incubator). The average weight of egg is 65±2 g. The treatment groups on each tray level were randomly arranged with respect to their arrangement on the other tray levels.

In ovo injection

In ovo injection solution preparation, egg handling, and use of a manual in ovo injection in chorioalantoic membrane **(CAM)** by insulin syringe, eggs of each group application on d 18 of incubation in this study. The treatment groups included: (non-injected and in ovo injected 100 µL sterilized distill water) controls as injected with the 100 µL sterilized distill water of carrying either 0.8 mg calcium (Ca), 0.8 mg Cole vit D_3 (colecalciferol or 25-OHD_3) produced by Sterling UK. and mix of 0.8 mg Ca + 0.8 mg Cole vit D_3 respectively. After the injection, the pinhole site was sealed with sterile paraffin wax and eggs were returned to the incubator. On the 19th day of incubation, eggs were shifted to the hatchery and kept in the respective pedigree hatching boxes Immediately.

Hatchability measurements

On the hatching day, chicks were weighed and hatching of fertile and total eggs, hatch window, total dead embryo and culled chicks percentages were recorded.

Broiler experimental design

sixty chicks of each group replicate were randomly selected and placed in floor pens in a light-controlled research facility. Chicks were placed in pens that corresponded to their respective treatment replicate groups in the hatcher units. Brooding and rearing conditions. The lighting schedule was 22 h light / 2 h darkness at 32-30°C at the first day to 1st wk. Pellet diets and fresh water offered *ad libitum*, all chicks in all groups served the same diets: starter (0-11d) [3100 kcal/kg metabolic energy (ME), 23% crud protein (CP), 4.0% crud fiber (CF)], the grower (12-25d) [2900 kcal/kg ME, 20.5% CP, 4.15% CF] and the finisher (26-35d) [2920 kcal/kg ME, 21.3% CP, 4.45% CF].

Bone measurements

Three chicks/birds from each replicate of treatment groups were randomly selected on d

0 and 35 posthatch they weighed and measured for body length (**BL**) as described by (16) before being euthanized and necropsied. Dried tibias were subjected to breaking strength analysis using the method described by Shim (19). Left tibia bones from birds were subsequently weighed and were dried in a forced-air oven for 24 h at 105°C and weighed. All tibias for chicks at both ages were ether extracted for 12 h extraction before ash obtaining in a muffle furnace at 480°C for 16 h. The mineral contents (Ca and P) of the tibia bone samples were determined by HPLC.

Blood minerals and vitamins determination

blood was sampled from jugular vein of 0 d chicks and the brachial vein of 35 d broiler, for determining blood plasma concentration of total calcium and inorganic phosphorus using kits by spectrophotometer. vitamin D ($1,25(OH)_2D_3$) using kit by HPLC, hormones [parathyroid (PTH) and calcitonin (CT) concentrations were determined using kits by Radio immunoassay- RIA, all kits for chickens produced by Bioscience UK.

Statistical analysis

All data were analyzed for normal distribution using the normal option procedure of SAS software (18). Data were analyzed as a completely randomized design by the GLM procedure of SAS software. Statistical differences were established using a Duncan's Multiple Range Test at the level of $P \leq 0.05$ (4).

RESULTS AND DISCUSSIONS

Table 1. The effect of in ovo injection with calcium, Cole vit D_3 and their combination or mix on hatchability, the results presented significantly ($P \leq 0.05$) higher in hatchability of fertile egg (%), hatchability of total egg (%) and hatch window (h) in the groups of in ovo injection with Ca, Cole vit D_3 and their mix compared with the control (without in ovo injection) and the group of DW (desterilized water in ovo injection). Broiler breeder fertile eggs that were in ovo injected with Ca, Cole vit D_3 and their mix had significantly ($P \leq 0.05$) higher body weight, width of breast and length of shank length of chick at 0 posthatch compared with the control group and eggs that were injected with SDW, also length of broiler was higher in the groups Cole vit D_3 and their mix compared with the other groups of the study. As well as at age 35 d posthatch the groups that were in ovo injected with Ca, Cole vit D_3 and their mix had significantly ($P \leq 0.05$) higher body weight, length of broiler, width of breast and length of shank length compared with the control group and eggs that were injected with SDW (Table 2)

Table 1. Effect of In ovo Injection with Calcium and Cole vit D_3 on some hatching traits

Traits	Treatments					MSE
	C	SDW	Ca	Cole Vit D_3	Mix	
Hatchability of fertile eggs (%)	84.7 [c]	82.9 [c]	86.0 [b]	87.9 [ab]	89.8 [a]	3.11
Hatchability of total eggs (%)	74.3 [c]	73.6 [c]	79.5 [b]	81.8 [ab]	83.6 [a]	2.74
Hatch window (h)	21:00 [c]	21:24 [c]	16:10 [b]	15:47 [ab]	14:05 [a]	0.93
Total dead embryos (1-21) d (%)	18.30 [a]	19.55 [a]	16.27 [ab]	14.65 [b]	13.15 [b]	1.02
Culled chicks (%)	7.40 [a]	6.85 [a]	4.23 [b]	3.55 [b]	3.25 [b]	0.385

C: control (without in ovo injection); SDW: 100 µl sterilized distill water in ovo injection; Ca: 0.8 mg calcium dissolved in 100 µl SDW; Cole Vit D_3: 0.8 mg 25--OHD_3 dissolved in 100 µl SDW; Mix: 0.8 mg Ca + 0.8 mg Cole vit D_3 dissolved in 100µl SDW.

Table 2. Effect of In ovo Injection with Calcium and Cole vit D_3 on body weight and length, width of breast and length of shank at ages 0 and 35 days of posthatch

Age Traits	Treatments					MSE
	C	SDW	Ca	Cole Vit D_3	Mix	
0 day	43.27 [b]	43.10 [b]	44.08 [a]	44.30 [a]	44.85 [a]	1.67
Body weight (g)						
Length of chick (cm)	19.81 [b]	19.75 [b]	19.80 [b]	20.83 [a]	20.97 [a]	1.20
Width of breast (cm)	11.45 [b]	11.56 [b]	12.55 [a]	12.70 [a]	12.67 [a]	0.81
Length of shank (cm)	2.25 [b]	2.10 [b]	2.45 [a]	2.52 [a]	2.58 [a]	0.25
35 day	2529 [c]	2546 [c]	2689 [b]	2710 [ab]	2780 [a]	141
Body weight (g)						
Length of broiler (cm)	43.75 [c]	42.59 [c]	48.90 [b]	50.77 [ab]	52.91 [a]	1.92
Width of breast (cm)	22.13 [b]	21.96 [b]	23.82 [a]	24.10 [a]	24.38 [a]	0.89
Length of shank (cm)	81.33 [c]	80.42 [c]	84.10 [b]	85.93 [ab]	87.35 [a]	2.11

C: control (without in ovo injection); SDW: 100 µl sterilized distill water in ovo injection; Ca: 0.8 mg calcium dissolved in 100 µl SDW; Cole Vit D_3: 0.8 mg 25--OHD_3 dissolved in 100 µl SDW; Mix: 0.8 mg Ca + 0.8 mg Cole vit D_3 dissolved in 100µl SDW.

Table 3. as to tibia length had significantly (P≤ 0.05) higher in the groups of in ovo injected with Cole vit D_3 and their mix at both 0 and 35 d posthatch, so breaking strength had increased in Ca, Cole vit D_3 and their mix groups at both ages compared with the control group and eggs that were injected with SDW. However had seen non-significant differences among all the groups in tibia width.

Table 4. shows that tibia bone affected by in ovo injection with Ca, Cole vit D_3 and their mix, it had significantly (P≤ 0.05) raised in Ca and P concentrations, also in ash percentage at 0 and 35 d posthatch, while non-significant differences among all the groups in Ca:P ratio.

Table 3. Effect of In ovo Injection with Calcium and Cole vit D_3 in different age on tibia weight and length %, and strength at ages 0 and 35 d of posthatch

	Treatments					MSE
Age Traits	C	SDW	Ca	Cole vit D_3	Mix	
0 day	2.65 [b]	2.62 [b]	2.88 [ab]	2.92 [a]	2.97 [a]	0.281
Tibia length (cm)						
Tibia width (cm)	1.78 [a]	1.76 [a]	1.85 [a]	1.89 [a]	1.95 [a]	0.122
Breaking strength (kg/cm^2)	0.890 [b]	0.847 [b]	1.303 [a]	1.308 [a]	1.325 [a]	0.301
35 day	8.89 [b]	8.24 [b]	9.48 [ab]	9.66 [a]	9.73 [a]	0.783
Tibia length (cm)						
Tibia width (cm)	8.13 [a]	8.02 [a]	8.09 [a]	8.13 [a]	8.10 [a]	0.690
Breaking strength (kg/cm^2)	8.205 [b]	8,100 [b]	10.067 [a]	10.843 [a]	10.892 [a]	0.755

C: control (without in ovo injection); SDW: 100 µl sterilized distill water in ovo injection; Ca: 0.8 mg calcium dissolved in 100 µl SDW; Cole Vit D_3: 0.8 mg 25--OHD_3 dissolved in 100 µl SDW; Mix: 0.8 mg Ca + 0.8 mg Cole vit D_3 dissolved in 100µl SDW. The same superscripts within rows means non-significant. [a – c] Means within rows with different superscripts differ significantly at (P≤ 0.05).

Table 4. Effect of In ovo Injection with Calcium and Cole vit D_3 on tibia bone chemical analysis at ages 0 and 35 d of posthatch.

	Treatments					MSE
Age Traits	C	SDW	Ca	Cole Vit D_3	Mix	
0 day	10.33 [b]	10.09 [b]	12.13 [a]	12.42 [a]	12.53 [a]	0.726
Ca (g/g tibia ash)						
P (g/g tibia ash)	6.47 [c]	6.55 [c]	7.69 [b]	8.03 [a]	8.11 [a]	0.703
Ca: p ratio	1.597 [a]	1.540 [a]	1.577 [a]	1.547 [a]	1.545 [a]	0.077
Ash %	37.75 [c]	37.16 [c]	41.29 [b]	40.88 [b]	44.96 [a]	1.402
35 day	11.54 [b]	11.35 [b]	15.00 [a]	14.72 [a]	15.29 [a]	0.810
Ca (g/g tibia ash)						
P (g/g tibia ash)	6.92 [b]	6.89 [b]	8.39 [a]	8.55 [a]	8.83 [a]	0.674
Ca: p ratio	1.668 [a]	1.647 [a]	1.789 [a]	1.722 [a]	1.732 [a]	0.083
Ash %	34.65 [b]	34.02 [b]	39.48 [a]	40.7 [a]	40.10 [a]	1.633

Table 5 clarify posthatch blood minerals, vitamin D_3 and hormones were impacted with the in ovo injection with Ca and Cole vit D_3. The groups of Ca, Cole vit D_3 and their mix were significantly (P≤ 0.05) higher in Ca and P minerals concentrations, vit D_3 [1,25$(HO)_2D_3$] and parathyroid hormone (PTH) at 0 and 35 d posthatch. However, calcitonin hormone was significantly (P≤ 0.05) lower in the groups of Ca, Cole vit D_3 and their mix at 0 and 35 d posthatch, also and Ca: P ratio was decreased in the same groups at 0 posthatch. While, there wasn't any significant differences among the non-injected and injected groups in Ca: P ratio at 35 d posthatch

Table 5. Effect of In ovo Injection with Calcium and Cole Vit D_3 on blood minerals (Ca & P), vit D_3 [1,25$(OH)_2D_3$] and hormones [calcitonin (CT) & parathyroid (PTH)] at 0 and 35 posthatch

Age Traits	Treatments					MSE
	C	SDW	Ca	Cole Vit D_3	Mix	
0 day						
Minerals						
Ca^{+2}(mg/dL)	7.08 ^{b}	7.35 ^{b}	8.07 ^{a}	8.19 ^{a}	8.31 ^{a}	0.633
P (mg/dL)	4.36 ^{b}	4.26 ^{b}	5.61 ^{a}	5.45 ^{a}	5.68 ^{a}	0.425
Ca: P ratio	1.624 ^{b}	1.725 ^{a}	1.439 ^{c}	1.503 ^{c}	1.463 ^{c}	0.106
Vitamine						
D_3 (ng/ml)	125 ^{c}	89.5 ^{d}	162 ^{b}	217 ^{a}	208 ^{a}	17.20
Hormones						
CT (ng/ml)	1.074 ^{a}	1.127 ^{a}	0.895 ^{b}	0.781 ^{c}	0.759 ^{c}	0.093
PTH (ng/ml)	1.18 ^{c}	1.03 ^{c}	2.31 ^{b}	2.34 ^{b}	2.77 ^{a}	0.204
35 day						
Minerals Ca^{+2} (mg/dL)	8.10 ^{c}	7.82 ^{c}	10.18 ^{b}	10.35 ^{ab}	10.93 ^{a}	0.852
P (mg/dL)	5.11 ^{b}	5.02 ^{b}	6.23 ^{a}	6.57 ^{a}	6.89 ^{a}	0.441
Ca: P ratio	1.585 ^{a}	1.558 ^{a}	1.634 ^{a}	1.575 ^{a}	1.586 ^{a}	0.102
Vitamine						
D_3 (pg/ml)	55.4 ^{c}	37.2 ^{d}	74.2 ^{b}	121.0 ^{a}	124.9 ^{a}	3.69
Hormones						
CT (ng/ml)	2.35 ^{a}	2.47 ^{a}	1.16 ^{b}	1.02 ^{b}	0.91 ^{b}	0.082
PTH (ng/ml)	1.95 ^{c}	1.63 ^{c}	2.79 ^{b}	3.18 ^{ab}	3.47 ^{a}	0.205

C: control (without in ovo injection); SDW: 100 µl sterilized distill water in ovo injection; Ca: 0.8 mg calcium dissolved in 100 µl SDW; Cole Vit D_3: 0.8 mg 25--OHD_3 dissolved in 100 µl SDW; Mix: 0.8 mg Ca + 0.8 mg Cole vit D_3 dissolved in 100µl SDW. The same superscripts within rows means non-significant. $^{a-c}$ Means within rows with different superscripts differ significantly at (P≤ 0.05).

The hatchability percentage and body weight of hatched chicks was increased in groups in ovo injected with calcium, phosphorus, and vitamin D complex (6). The functional form of vitamin D in biology is 1,25-$(OH)_2D_3$, the production is very carefully regulated by parathyroid hormone (PTH) in response to serum calcium and phosphate ($PO4^{-3}$) concentra-tions (5). 1,25-$(OH)_2D_3$ is a critical factor in the maintenance of sufficient maternal calcium for transport to the embryo and may play a role in normal skeletal development of the neonate (10). Two hormones, calcitonin and parathyroid function in a delicate relationship with 1,25-$(OH)_2D_3$ to control blood calcium and phosphorus levels (7 & 15). Production rate of 1,25-(OH)2D is under physiological control as well as dietary control. Calcitonin, contrary to the other two, regulates high serum calcium levels by (10) depressing gut absorption, (17) halting bone demineralization, and (10) depressing reabsorption in the kidney. Vitamin D elevates plasma calcium and phosphorus by stimulating specific ion pump mechanisms in the intestine, bone and kidney. These three sources of calcium and phosphorus provide reservoirs that enable vit D to elevate calcium and phosphorus in blood to levels that are necessary for normal bone mineralization and for other functions ascribed to calcium. In the target tissue, the hormone enters the cell and binds to a cytosolic receptor or a nuclear receptor. 1,25-(OH)2D regulates gene expression through its binding to tissue-specific receptors and subsequent interaction between the bound receptor and the DNA (3). The results reported confirms the importance and essentiality of vit D3 and its active metabolites for normal embryonic development by providing the necessary Ca and P needed for normal skeletal development. calcium used for skeleton-genesis comes primarily from the shell, whereas phosphorus is derived mainly from the yolk. Ca mobilization from the shell commences later in incubation and proceeds at a rapid rate resulting in well mineralized embryos. In ovo treatments with vit D3 or metabolites increased the rate of calcium mobilization from the egg shell to the embryo and increased calcium concentration in all embryonic compartments (13). Feeding and in ovo injection of 1α-OHD3 increased breaking

strength, calcium and phosphorous percentage in tibia bone (9). Research has shown that a 1 cm chick length advantage at day of hatch can result in 264 grams more body weight with 45 grams more breast meat yield at 38 days of age, (16). This, and the fact that an optimally developed chick will have a better feed conversion rate.

In a previous study in which Cole Vit D3 and its mix with calcium were used as in ovo injection, it was shown that at a 0.8 mg able to improve the embryonic development, hatchability, body weight at 0 and 35 posthatch of broilers, also increased bone characteristics and breaking strength, thus improved Ca and P in bone and blood plasma also vit D_3, calcitonin and parathyroid hormone concentrations, all these improvement positively reflected in better formation of skeleton and body formation well.

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