

INFLUENCE OF *IN OVO* INJECTION WITH VITAMIN D₃ ON SOME PHYSIOLOGICAL PARAMETERS AND BONE DEVELOPMENT OF NORFA CHICKS

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ABSTRACT: This study aimed to assess the effects of *in ovo* injection with vitamin D₃ on Norfa chick's performance. All fertile eggs were kept at incubation room temperature for 12 hours and then incubated in a forced draft incubator at 37.8°C with 55 - 60% relative humidity. Eggs were turned every two hours from the 2nd to 18th day of incubation. In the 1st group, 40 eggs were not injected and considered as negative control, while in the 2nd group, 40 eggs were injected with distilled water (positive control). In the 3rd and 4th groups, 160 and 180 eggs were injected with 50 and 100µL vitamin D₃ (VD₃), respectively. Eggs were injected on the 18th day of incubation to deposit test material in the air sac through the wide end of the egg then the hole was closed by wax.

During the last 3 days of incubation, all eggs were transferred to a separate hatcher at 36.5°C and 75 - 80% relative humidity. At hatch, the chicks were wing-banded, weighed and kept in the brooding house.

The results showed that the chicks produced from eggs injected with 100 µL VD₃ exhibited significantly greater live body weight and body weight gain compared to other groups at different ages. Moreover, the feed conversion ratio and embryonic mortality rate were significantly affected during experimental period. The embryonic mortality rate was significantly increased in all treated-groups compared to negative control group. Immune response at 7 days and 28 days for Norfa chicks injected with VD₃ significantly improved compared to control groups. The highest value for femur and sternum bone length was recorded in chicks injected with 100µL VD₃, while tibia bone length was significantly decreased. Blood AST and ALT levels for chicks injected with 100µL VD₃ significantly increased compared to negative control group. *In ovo* injected VD₃ of 50 and 100 µL increased total serum calcium compared with the control groups. These results indicated that the *in ovo* injection of different levels of vitamin D₃ had a significant effect on white blood cells, lymph count, neutrophil, red blood cells and platelets. However, there was no significant effect of the *in ovo* injection on the levels of hemoglobin and hematocrit. In conclusion, early *in ovo* injection of different levels of vitamin D₃ might be considered a feasible technique for increasing Norfa chicks performance, hatchability, blood parameters, immunity and decreasing mortality.

Key words: *In ovo* injection, VD₃, immunity, bone development, Norfa chicks.

INTRODUCTION

Vitamin D is required for proper embryonic development in chickens. Vitamin D₃ levels in the maternal diet are linked to vitamin D₃ and 25-hydroxy vitamin D₃ (25-OH D₃) levels in the egg yolk (Mattila *et al.*, 1999). The enzyme 25-hydroxyvitamin D-1-hydroxylase, which converts 25-OH D₃ to 1,25(OH)₂ D₃, can be found as early as day 12 of incubation and rises in specific activity as embryonic development advances (Turner *et al.*, 1987). The chick's early

development of vitamin D₃ metabolism exemplifies the relevance of vitamin D₃ to the developing embryo. Commercial *in ovo* injections of 25(OH) D₃ has been shown to increase the hatchability of fertile broiler hatching eggs without compromising the hatchling quality (Bello *et al.*, 2013).

Chicks at hatch have an underdeveloped immune system, making them more susceptible to infection and sickness than adult birds (Lowenthal *et al.*, 1994; Wells *et al.*, 1998). In

the embryogenesis and growth rates of birds, a range of nutrients perform crucial physiological, nutritional, and immunological functions. Injecting these nutrients into the embryo's eggs may be able to assist overcome any constraints imposed by inadequate egg nutrition (Selim *et al.*, 2012). Furthermore, *in ovo* nutrition, such as vitamins and amino acids, might be a different way to improve hatchability and bird quality at hatch, as well as the innate immune system (Ohta *et al.*, 2001).

The importance and necessity of vitamin D₃ for chicken embryonic development, as well as the availability of vitamin D₃ in eggs, are critical for supporting embryo calcium metabolism and maintaining an acceptable level of blood calcium and phosphorus during incubation. Narbaitz *et al.* (1987), Holick and Garabedian (2006) found that calcium and phosphorus absorption varies, with 10 - 15% of dietary calcium and roughly 60% of phosphorus absorption without vitamin D. As a result, vitamin D is essential for the formation and maintenance of healthy bones. Vitamin D receptors have been found in a variety of cells, demonstrating that it affects more than mineral metabolism (Zhang and Naughton, 2010).

Additionally, vitamin D₃ has been found to play a role in calcium absorption and bone mineralization in broiler chickens, as well as having regulatory effects for the immune response and muscle growth (Bronner, 2003; Morris *et al.*, 2014; Vignale *et al.*, 2015, Nordin, 2010, Saunders-Blades and Korver, 2014). The studies concerning the injection of the important vitamins into chicken eggs during the incubation period are scarce. Therefore, the aim of the present experiment was to evaluate the effect of *in ovo* injections with vitamin D₃ on immunity, bone development, blood characteristics, and productivity of Norfa chicks.

MATERIALS AND METHODS

This study was approved by the Department of Poultry and Fish Production, Faculty of Agriculture, Shibin El-Kom, Menoufia University, Egypt. Norfa strain was used as a synthetic local breed of chickens and details of

the formation history of this breed was described by Abdou (1996).

Mating system, experimental design and treatments:

In order to reproduce the next generation, artificial insemination was utilized as the followed mating system. Each family contained one male and three randomly assigned females.

The fertile eggs were collected twice daily and numbered according to their females. All cracked, dirty, and misshapen eggs were culled. The eggs were then kept in the egg storage chamber for 7 days at 15 - 17 °C and 75 - 80% relative humidity.

At 18th day of incubation, 480 eggs were sorted with optical sorting method to determine fertile eggs, 420 fertile eggs were divided into four groups as follow:

1st group: consisted of 40 eggs were not injected and used as a negative control group, 2nd group: consisted of 40 eggs were *in ovo* injected with distilled water (50µL) and used as a positive control group, 3rd group: consisted of 160 eggs were *in ovo* injected with 50 µL vitamin D₃ and 4th group: consisted of 180 eggs were *in ovo* injected with 100 µL vitamin D₃.

Eggs were injected on the 18th day of incubation to deposit test material in the air sac through the wide end of the egg then the hole was closed by wax.

All fertile eggs were incubated in a forced draft incubator at 37.8°C with 55 - 60% relative humidity. The eggs were turned every two hours from the 2nd to 18th day of incubation, then transferred to the hatcher at 36.5°C and 75 - 80% relative humidity. The hatched chicks were wing-banded, weighed individually and transferred to the brooder. The basal diet in this experiment was used to cover the nutrient demands of growing Norfa chicks during 1 - 28 days of age as recommended by (Zanaty and Ibrahim, 2005). The structure and calculated composition of the basal diet are presented in Table 1.

Table 1. Composition of the experimental diet fed growing Norfa chicks during 1 – 28 days of age.

Ingredients	%	Calculated values ³	
Yellow corn (8.8%)	63.84	Crude protein (%)	18.00
Soybean meal (44%)	25.39	ME, kcal/ kg diet	2808
Wheat bran	7.66	C/ P ratio	156.0
Bone meal steamed	2.08	Lysine (%)	0.90
Limestone, ground	0.50	Methionine (%)	0.33
Vit. & Min. Mix. ¹	0.25	Met + cystine (%)	0.62
DL-methionine ²	0.03	Calcium (%)	0.90
Sodium chloride	0.25	Av. phosphorus (%)	0.40
Total	100		

¹Vit. & Min. Mix, at 0.25% of the diet supplies the following per Kg of the diet: Vit. A, 12000 IU; VD₃, 2500 IU; Vit. E, 10 mg; Vit.K₃, 3 mg; Vit. B₁, 1 mg; Vit.B₂, 4 mg; Pantothenic acid, 10 mg; Nicotinic acid, 20 mg; Folic acid, 1 mg; Biotin 0.05 mg; Niacin 40 mg; Vit.B₆, 3 mg; Vit.B₁₂, 20 mg; Choline Chloride, 400 mg; Mn, 62 mg; Fe, 44 mg; Zn, 56 mg; Cu, 5 mg and Se, 0.01 mg.

²DL – methionine: 98% feed grade (98% methionine).

³Calculated according to NRC (1994).

Studied traits:

1. Live body weight:

Individual live body weight was recorded (every two weeks) from hatch to 28 days of age in grams.

2. Feed intake (FI) and feed conversion ratio (FCR):

Feed intake (FI) was measured as the difference between the residual and offered feed. The amount of feed intake per bird per experimental periods were calculated by dividing the total feed intake during the experimental periods (hatch-14, 14-28 and hatch-28 days of age) on the receiving bird number.

The feed conversion ratio was calculated as follows:

$$FCR \left(\frac{\text{feed}}{\text{g}} \text{ weight gain} \right) = \frac{\text{The feed intake (g)/bird/d}}{\text{Body weight gain (g)/bird/d}}$$

Body weight gain was measured as subtraction between the body weights (in gram) at experimental periods (hatch-14, 14-28 and hatch-28 days of age).

3. Hatchability percentage:

Hatchability percentage was calculated for each treatment as follows:

$$\text{Hatchability (\%)} = \frac{\text{no of hatched chicks}}{\text{no of fertile eggs}} \times 100$$

4. Embryonic mortality rates:

The numbers of fertile eggs were determined by candling of all eggs on the day 18th of incubation and cracking the remaining eggs after hatching. The percentage of the embryonic mortality was calculated for as follows:

$$\text{Embryonic mortality (\%)} = \frac{\text{no of dead embryos}}{\text{no of fertile eggs}} \times 100$$

5. Mortality percentage:

The mortality percentage of hatched chicks was calculated during the brooding period for 28 days by using the following formula:

$$\text{Mortality (\%)} = \frac{\text{no of dead chicks}}{\text{no of hatched chicks}} \times 100$$

6. Titration of antibodies:

Antigen immunization:

The SRBC antigen was immunized using a modified version of the method of Siegel and Gross (1980). At 4 weeks of age, the primary antibody response was assessed for each individual at 7 days post-immunization. To stimulate the initial antibody response, each chick was given an intravenous vaccination via

the branchial vein with 0.1 ml of 2.5 % SRBC solution.

Calculation of antibody titers:

The secondary antibody response was measured at 7 and 28 days of chick age. The antibody in the blood serum of chicks was evaluated using microtiter hemagglutinin test assay described by Siegel and Gross (1980). Individual titrations of serum samples were performed in 96 well (8 rows by 12 columns, round (U) bottom) assay plates. Only, 50 µl of physiological saline (0.9% NaCl) was added to all 96 - well plates followed with 50 µl of serum sample to first well (row 1). From the first through the eleventh wells, serial dilutions of each serum sample were prepared. This result in dilution ranging from 1:1 to 1:1024, and 50 µl of 2.5% SRBCs suspension was added to all wells. After that, plates were incubated in 37° C for 90 minutes. The last positive dilution exhibiting each behavior expressed a Log₂ values for the reciprocal of highest titer were complete.

7. Blood samples and serum parameters:

Seven birds of average weight from each treatment were randomly chosen at the end of the 7th day and blood was collected from the wing. Whole blood samples obtained were used immediately to determine haematological parameters in the whole blood. The haematological analysis was performed by using Hemavet 950 (Drew Scientific Inc., Waterbury, CT) immediately after blood collection. The hematological analysis included white blood cell (WBC), lymph count (LYM), heterophil count (Het), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), (RDW-CV) and liver function tests, ALT and AST according to Kim *et al.* (2013).

Serum calcium:

For serum calcium measurement, blood samples corresponding to one millilitre of blood were taken from the axillary vein of randomly selected birds at 7 days of age. To extract the serum, blood was deposited in 1.5 ml Eppendorf

tubes and centrifuged at 3000 rpm for 15 minutes. A commercial kit (Labtest ®) was used to assess serum calcium levels.

8. Bone length:

The average length of femur, sternum and tibia (cm) were measured at 28th days after slaughter for 5 chicks from each group.

Statistical analysis:

Data were examined for normality using the Shapiro–Wilk test prior to the analysis and all percentages were subjected to arcsine transformation. According to the following statistical model, data were analyzed using one-way analysis of variance:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: Y_{ij} – observation, μ – overall mean, T_i – treatment fixed effect T_1 to T_4 and e_{ij} – residual of the model. Duncan’s Multiple Range Test (Duncan, 1955) checked the significance ($P < 0.01$) of the differences between treatment means. Statistical analyses were performed by using SPSS software (SPSS, 1999).

RESULTS AND DISCUSSIONS

Productive Performance:

The productive performance of the Norfa chicks injected with different levels of VD₃ is presented in Tables 2 and 3. The live body weight and body weight gain of chicks was significantly increased by *in ovo* injection of 100 µL /egg VD₃ levels at different ages.

Chicks hatched from eggs injected with 100 µL VD₃ exhibited a significantly greater LBW than chicks in the other groups at all different ages. Nevertheless, the lowest LBW and BWG at different ages were obtained in chicks produced from eggs injected with distilled water. These findings agreed with those of Yarger *et al.* (1995) and Fritts *et al.* (2003), they found significant increase in body weight and breast muscle yield for broiler chickens treated with vitamin D₃.

Atencio *et al.* (2005) found that dietary vitamin D₃ levels increased from 125 IU/kg to

2,000 IU/kg for broiler hens resulting in improved body weight gain for their chicks from hatch to 16 days of age. Moreover, Papešová *et al.* (2008) found that broilers supplied with 25OH D₃ in the food gained much more weight than those in the control groups. Contrarily, Bello *et al.* (2014) found that 25 (OH) D₃ dissolved in vaccine diluent buffer had no effects on growth after hatch and that the hatchability

was significantly higher than those in the control group. Supplementing broiler chicks with 25 (OH) D₃ also boosted pectoral muscle growth (Hutton *et al.*, 2014). Other studies have shown that vitamin D₃ affects embryonic and future development, with this action most likely linked to the vitamin's structure (Chen and Bosmann 1965; Imrie *et al.*, 1967).

Table 2. Live body weight and body weight gain of Norfa chicks as affected by in ovo injection with VD₃ at different ages (Means \pm S.E.).

Groups	Live body weight (g)			Body weight gain (g)		
	one day	14d	28d	1-14d	14-28d	1-28d
Negative control (not injected)	31.5 \pm 0.7 ^b	151.7 \pm 1.7 ^c	430.8 \pm 5.7 ^c	120.1 \pm 10.7 ^{bc}	279.2 \pm 2.9 ^{bc}	399.3 \pm 3.8 ^b
Positive control (distilled water)	31.5 \pm 0.6 ^b	149.7 \pm 1.7 ^c	425.3 \pm 9.0 ^d	118.3 \pm 10.3 ^c	275.51 \pm 3.0 ^c	393.8 \pm 3.9 ^c
VD ₃ (50 μ L /egg)	32.8 \pm 0.3 ^b	157.9 \pm 0.5 ^b	444.2 \pm 2.8 ^b	125.1 \pm 10.2 ^{ab}	286.23 \pm 3.3 ^b	411.3 \pm 4.4 ^b
VD ₃ (100 μ L /egg)	35.2 \pm 0.2 ^a	166.8 \pm 0.8 ^a	488.5 \pm 1.6 ^a	131.6 \pm 10.54 ^a	301.6 \pm 2.9 ^a	433.2 \pm 4.5 ^a
P-Value	0.000	0.000	0.022	0.000	0.031	0.001
Significance	**	**	*	**	*	*

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

* Significant differences at $P \leq 0.05$. ** Highly Significant differences at $P \leq 0.01$.

Table 3. Feed intake and feed conversion ratio of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means \pm S.E.).

Groups	Feed intake (g)			Feed conversion ratio (g feed/g gain)		
	1-14d	14-28d	1-28d	1-14d	14-28d	1-28d
Negative control (not injected)	288.3 \pm 2.3 ^b	795.6 \pm 3.9 ^b	1046.2 \pm 5.3 ^b	2.4 \pm 0.1 ^a	2.85 \pm 0.2 ^a	2.62 \pm 0.2 ^a
Positive control (distilled water)	281.5 \pm 2.9 ^c	768.7 \pm 4.2 ^d	1015.9 \pm 5.2 ^d	2.4 \pm 0.2 ^{ab}	2.79 \pm 0.3 ^b	2.58 \pm 0.3 ^b
VD ₃ (50 μ L /egg)	287.7 \pm 2.0 ^b	778.5 \pm 4.1 ^c	1028.3 \pm 5.4 ^c	2.30 \pm 0.2 ^c	2.72 \pm 0.2 ^c	2.50 \pm 0.2 ^c
VD ₃ (100 μ L /egg)	296.1 \pm 2.3 ^a	802.3 \pm 4.6 ^a	1044.1 \pm 5.6 ^a	2.25 \pm 0.1 ^d	2.66 \pm 0.2 ^d	2.41 \pm 0.2 ^d
P-Value	0.000	0.034	0.012	0.000	0.000	0.000
Significance	**	*	*	**	**	**

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

* Significant differences at $P \leq 0.05$. ** Highly Significant differences at $P \leq 0.01$.

Feed intake and feed conversion ratio:

Feed intake and feed conversion ratio for Norfa chicks injected with 100 µL VD₃ were significantly increased at different age compared with those in the other groups (Table 3).

From different period of age, chicks produced from eggs injected with tested vitamin D₃ exhibited a better feed conversion ratio than those in the control groups. In contrast, feed conversion ratio for chicks produced from eggs injected with 100 µL VD₃ was significantly best throughout different experimental periods.

From 1 to 14 and 14 to 28 days of age, the feed intake produced from eggs injected with 100µL of VD₃ was increased by about 2.7% and 0.8 %, while feed intake from 1 to 28 days of age for those injected with 100µL of VD₃ was decreased by 0.2 %, also 50µL VD₃ and distilled water was decreased by 1.7 and 2.9 %, respectively with those of the control groups, which could explain why the current study's results are different.

These findings are consistent with those of Saunders-Blades and Korver (2014), who discovered that broiler breeder chicks supplemented with 34.5g 25-OH D₃/liter water outperformed control chicks. They also found that supplementing the diet with 3,000 IU/kg vitamin D₃ had no influence on body weight or feed efficiency at 7 days of age.

Yarger *et al.* (1995); Mitchell *et al.* (1997); and Aburto *et al.* (1998) observed similar results, reporting that feed efficiency and breast muscle yields for broilers given a diet supplemented with 25-OH D₃ were considerably enhanced when compared to control groups.

Hatching traits:

The hatchability and embryonic mortality rates are presented in Table 4. The results showed a significant increase in the hatchability percentage in the control groups compared to *in ovo* injected groups. The early embryonic mortality rate was significantly increased for

embryos produced from eggs injected groups compared to control groups. Generally, the negative control and distilled water-injected groups exhibited the highest hatchability percentage and the lowest early embryonic mortality rate.

Contrarily, the late embryonic mortality was increased via *in ovo* injection of 100µL VD₃ compared to control groups. Moreover, 50µL VD₃ injected group showed a significant increase in late embryonic mortality and piped dead compared to positive control group (Table 4). This may be due to the infection resulted from the manual injection of the eggs.

Vitamin D₃ is widely recognized for its importance and requirement in chicken embryo development, and its presence in eggs is important for regulating embryo calcium metabolism during incubation (Narbaitz, 1987). As a result of the lack of vitamin D in the hatching eggs, there is an increase in embryonic mortality and chick deformities (Sunde *et al.*, 1978; Elaroussi *et al.*, 1993). Stevens *et al.* (1984) linked vitamin D₃ insufficiency to decreased hatchability and higher late embryo mortality, which might contribute to poor post-hatch chick performance. The widely variable hatchability percentages seen in the diluent-injected control groups might be another factor contributing to the differences in the research' outcomes. In this study, the diluent-injected treatment group had a lower percent hatchability than the 25OH D₃-injected treatment group, which had a hatchability of 90%. Similar trend were also observed by Bello *et al.* (2013).

The studies did not indicate at what stage of embryo development the losses occurred or why the hatchability was already decreased in similar study, Saunders-Blades and Korver, (2014) found that the adding 25-OH D₃ decreased early embryonic mortality in broiler breeders fed a diet containing 3,000 IU/kg D₃ from 6.22 % to 4.37 % when compared with non-treated group. As a result, 25-OH D₃ appears to have some embryonic development protective effects.

Table 4. Percentages of hatchability, embryonic mortality, piped dead and dead in shell of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means ± S.E.).

Groups	Hatchability (%)	Embryonic mortality (%)			Piped dead (%)	Dead in shell (%)
		Total	Early 0-7d	Late 7-14d		
Negative control (not injected)	90.0±0.79 ^a	10.0 ±0.23 ^c	-	-	7.5±0.17 ^{bc}	2.5±0.06 ^c
Positive control (distilled water)	82.5±0.98 ^b	17.5 ±1.2 ^b	-	5.0±0.5 ^b	10.0±0.6 ^a	2.5±0.06 ^c
VD ₃ (50 µL /egg)	81.24±0.98 ^b	18.76±0.16 ^b	3.13±0.02 ^a	5.63±0.02 ^b	6.88±0.05 ^c	3.13±0.08 ^b
VD ₃ (100 µL /egg)	77.78±1.22 ^c	22.22±0.00 ^a	2.22±0.00 ^b	8.33±0.0 ^a	7.22 ±0.00 ^{bc}	4.44 ±0.00 ^a
P-Value	0.000	0.000	0.000	0.000	0.000	0.000
Significance	**	**	**	**	**	**

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

* Significant differences at $P \leq 0.05$. ** Highly Significant differences at $P \leq 0.01$.

Mortality rate (%):

The weekly mortality rate for embryos was altered by *in ovo* injection with different levels of vitamin D₃, this was due to the reduced cumulative mortality percentage of the 100µL of VD₃ injected group (Table 5). The chicks that had 50µL of VD₃ injection had a higher percent mortality rate than the negative and positive control groups on a weekly basis (Table 5). In contrast to the current findings, embryonic mortality and chick abnormalities increased when hatching eggs were low in vitamin D. (Sunde *et al.*, 1978; Stevens *et al.*, 1984; Elaroussi *et al.*, 1993). Dietary 25-OH-D₃ had a linear influence on mortality in 1 to 21-day-old broiler chicks (Han *et al.*, 2016). Wang *et al.* (2016) found that level of D₃ or 1-OH- D₃ reduced mortality ($P \leq 0.05$). As a consequence, when diets were supplied with varying VD₃ levels, there was no substantial improvement in mortality overall (0 – 31 d), and there was no correlation between mortality rates and dietary VD₃ levels in both stages, Cho *et al.* (2020).

Immunological Assessment:

The antibody titers of SRBCs estimated 7 and 28 days of age were significantly ($P \leq 0.05$) improved in the chicks hatched from eggs injected with different levels of vitamin D₃ compared to control groups. Higher levels of primary and secondary antibody titers were

recorded in chicks hatched from eggs injected with 50µL and 100µL of V D₃ in comparison with both control groups (Table 6).

Different vitamin D₃ sources have an impact on humoral immunity. According to prior studies, the ability of macrophages to phagocytize sheep red blood cells *in vitro* was decreased in chicks deficient in vitamin D. (Aslam *et al.*, 1998). Chou *et al.* (2009) reported no significant change in percent phagocytosis when fed vitamin D and 25-OH D₃ levels similar to those reported in the present study while reporting a 10% difference between the two treatments. Vitamin D and its metabolites have a role in phagocytosis in chicks, although the reasons behind this remain unclear. Furthermore, maternal age appears to impact the effect of vitamin D on chicks' early phagocytic capabilities. These findings imply that vitamin D₃ levels and metabolites have an impact on chicken immunity. Additionally, changes in small intestine morphology and humoral immunity are connected to higher breast meat yield and improved broiler growth performance (Wang *et al.*, 2019). As a consequence, better small intestine shape and immunology in birds might explain an improvement in breast meat yield and growth performance in response to an *in ovo* injection of 25OH D₃. *In ovo* injection of a 0.5 ml solution containing 0.4 mg of 25OH D₃ and 6 mg of vitamin K resulted in an increase in antibody titers against Newcastle Disease in

hatchlings (Abbasi *et al.*, 2017). As a result, increasing Ig levels in early-hatch broilers during their first two weeks of life may be beneficial to their immunity. These findings imply that giving early post-hatch broilers 2.4 mg of 25OH D₃ *in ovo* may be an effective way to boost humoral immunity.

Haematological parameters:

The haematological parameters affected by the *in ovo* injection of different levels of vitamin D₃ are presented in Table 7. No significant differences in HGB and HCT were found in chicks treated with different levels of vitamin D₃

and the control groups. However, significant increments in WBCs and LYM were noted in the chicks hatched from eggs injected with 100µL of VD₃ compared with the negative and positive control groups.

Moreover, *in ovo* injection of 100µL VD₃ administration elevated the WBC count and LYM to a higher level in comparison to both control groups. The highest RBCs, HCT and RDW-CV values were recorded in the chicks hatched from eggs injected with 50µL of VD₃ and positive control groups among other experimental groups (Table 7).

Table 5. Percentages of weekly mortality (%) of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means ± S.E.).

Groups	Mortality rate at				Cumulative mortality rate (%)
	7 days	14 days	21days	28 days	
Negative control (not injected)	8.33±0.02 ^b	11.14±0.03 ^b	5.59±0.07 ^b	8.33±0.02 ^a	33.39 ±0.13 ^b
Positive control (distilled water)	15.15±0.03 ^a	15.15 ^a ±0.09	6.06±0.03 ^a	6.06±0.03 ^b	42.42 ±0.18 ^a
VD ₃ (50 µL /egg)	2.31±0.01 ^c	6.92±0.01 ^c	3.85±0.03 ^c	3.08±0.05 ^c	16.16 ±0.09 ^c
VD ₃ (100 µL /egg)	4.29±0.01 ^c	5.00±0.01 ^c	2.86±0.01 ^c	3.57±0.01 ^c	15.71 ±0.01 ^c
P-Value	0.000	0.000	0.000	0.000	0.000
Significance	**	**	**	**	**

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

* Significant differences at $P \leq 0.05$. ** Highly Significant differences at $P \leq 0.01$.

Table 6. Antibody titer at 7 and 28 days of age of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means ± S.E.).

Groups	Titter at 7 days of age	Compared to primary antibody titers (%)	Titter at 28 days of age	Compared to primary antibody titers (%)
Negative control (not injected)	3.26 ±1.16 ^b	33.61	8.00±1.60 ^c	82.47
Positive control (distilled water)	2.74 ±0.73 ^c	28.25	8.00±1.60 ^c	82.47
VD ₃ (50 µL /egg)	3.94 ±1.07 ^b	40.62	12.80±3.20 ^b	131.96
VD ₃ (100 µL /egg)	5.49 ±1.14 ^a	56.60	25.6 ±6.43 ^a	264.23
P-Value	0.049		0.032	-
Significance	*		*	-

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.05$.

* Significant differences at $P \leq 0.05$.

Table 7. Haematological parameters of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means ± S.E.).

Groups	(WBC) (10 ³ /ul)	(LYM) (10 ³ /uL)	(Het) (10 ⁹ g/l)	(RBC) (10 ⁶ /ul)	(HGB) (g/dl)	(HCT) (L/L)	(RDW-CV) (%)
Negative control (not injected)	247.5 ^d ±6.3	633.0 ^b ±11.5	12.7 ^b ±1.93	107.5 ^b ±5.84	6.1±0.4	113.5±6.9	15.7 ^b ±1.97
Positive control (distilled water)	305.7 ^b ±18.0	673.2 ^a ±7.8	18.2 ^a ±0.48	126.2 ^a ±5.3	7.0±0.1	124.50±8.9	21.0 ^a ±1.29
VD ₃ (50 µL /egg)	278.7 ^c ±9.7	651.2 ^{ab} ±18.7	18.2 ^a ±0.6	116.2 ^{ab} ±5.7	6.4±0.6	116.25±2.6	18.0 ^{ab} ±0.82
VD ₃ (100 µL /egg)	320.0 ^a ±16.2	682.5 ^a ±8.5	17.7 ^a ±0.25	111.2 ^{ab} ±4.6	6.6±0.23	113.00±3.6	16.5 ^b ±0.87
P-Value	0.01	0.05	0.007	0.05	0.37	0.53	0.05
Significance	*	*	**	*	N.S.	N.S.	*

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

* Significant differences at $P \leq 0.05$. ** Highly Significant differences at $P \leq 0.01$.

N.S. = No Significant differences.

These finding suggest that thrombocyte concentration and heterophil to lymphocyte ratio were not significantly influenced by source or level of vitamin D, with no interaction between source and level of vitamin D, in contrast to the present study. At 42 days, the number of white blood cells (10³/L of blood) in birds fed diets supplemented with 25-OH- D₃ was significantly greater than in birds fed VIT- D₃. However, there was no consistent pattern in the effects of vitamin D on white blood cell concentration (Fritts *et al.*, 2004). The major impacts of source and level could not be determined due to significant source by level interactions.

Total calcium and liver function:

Serum total calcium was lower in both negative and positive control groups in comparison to different levels of vitamin D₃ groups (Table 8). *In ovo* injection of 50µL VD₃ administration elevated the serum calcium to a higher level in comparison to both control groups. Moreover, *in ovo* injection with different levels of VD₃ administration elevated the liver enzymes were highly significant in comparison with the positive control. The highest AST and ALT values were recorded in the chicks hatched from eggs injected with 100µL of VD₃ among all experimental groups (Table 8).

The present findings were consistent with the results of Whitehead *et al.* (2004) discovered that with 5 mg vitamin D₃/ kg, blood serum calcium concentrations adjusted to pH 7.4 were lower

than with higher vitamin D₃ concentrations, and that this depression was higher in the diet with lower Ca/ av P. With increasing dietary vitamin D intake, plasma 25-hydroxyvitamin D₃ concentrations increased.

According to Lohakare *et al.* (2005), blood calcium levels were greater in chicks fed a diet containing supplemental VC and 1,800 IU of VD during the first week than in chicks fed 200 IU of VD. The reverse tendency was noticed during the second week, and it remained unaffected during the third week. During the first and second weeks, there was a significant interaction between VC and VD levels and blood calcium levels. As a result, this could indicate a larger 25-OH D₃ reserve at hatch. According to Jamroz *et al.* (2004), stored 25-OH D₃ (yolk sac or tissue) may be readily available to the fast-growing chick, implying a delay in the development of the mechanisms for liver 25-OH D₃ production by dietary vitamin D₃.

As a result, it is well known that adding 1-OH D₃ increased plasma phosphorus content, Ca and Pi absorption is enhanced by 1-OH D₃. Improved phosphate utilisation by promoting calcium absorption and lowering calcium restriction on endogenous phytase are two possible effects of 1-OH D₃ on phosphorus (Ebrahimi *et al.*, 2016). According to Kalantar *et al.* (2019), *in ovo* injection of Q10 at 0.1 and 0.2 mL per egg resulted in substantial increases in serum enzyme activity in both 1 - 21 and 22 - 42 day old mice .

Table 8. Total calcium and liver enzymes of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means ± S.E.).

Groups	Total calcium (mg/dL)	AST (U/L)	ALT (U/L)
Negative control (not injected)	8.1±0.06 ^d	213.0±0.58 ^c	45.0±0.29 ^d
Positive control (distilled water)	8.7±0.12 ^c	63.0±0.43 ^d	73.0±0.35 ^b
VD ₃ (50 µL /egg)	15.3±0.17 ^a	241.0±0.87 ^b	56.0±0.23 ^c
VD ₃ (100 µL /egg)	10.3±0.14 ^b	283.0±0.29 ^a	77.0±0.40 ^a
P-Value	0.000	0.000	0.000
Significance	**	**	**

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

** Highly Significant differences at $P \leq 0.01$.

Bones length:

Data in Table 9 revealed that the femur and sternum length in the 100µL of VD₃ injected group were significantly longer than that of the control groups, while insignificantly affected tibia bone length.

The obtained findings were consistent with the results of the current study, rapid bone formation and production occurs in broilers up to 28 days of age (Leslie *et al.*, 2006), and BMD, BMC, and cross-sectional bone areas in chicks increase rapidly from 2 to 3 weeks of age (Kim *et al.*, 2011). Han *et al.* (2016) discovered a correlation between 25-OH- D₃ or vitamin D₃ levels and the weight, length, ash weight, and percentage of ash, Ca, and P in the tibia. The weight, length, ash weight, and percentage of ash, Ca, and P of the metatarsus increased linearly when the 25-OH- D₃ or vitamin D₃ level increased. In 1- to 21-day-old broilers, levels of D₃ or 1-OH- D₃ had a significant impact on bone quality (tibia, femur, and metatarsus) (Wang *et al.*, 2016).

As a result, Mustafa *et al.* (2019) found that tibia length was significantly ($P \leq 0.05$) higher in the groups of *in ovo* injected with Cole VD₃ and their mix at 0 and 35 days post-hatch, indicating that breaking strength was increased in the Ca,

Cole VD₃ and their mix groups at both ages when compared to the control group and eggs injected with SDW. However, there were no significant variations in tibia width across any of the groups. Tibia bone was remarkably influenced by *in ovo* injection of Ca, Cole VD₃. According to Salim *et al.* (2019), bone mineral content (BMC) and bone mineral density (BMD) from both the tibia and femur of broiler chicks were significantly higher in HY-D2, HY- D₃, and PC treatments than in NC and HY-D1 treatments; however, bone area from both the femur and tibia of broiler chicks was significantly improved in only HY-D2 at 14- d.

Even though there were no variations in bone length or diameter, vitamin D₃ injection significantly increased bone strength and relative weight (Zamani *et al.*, 2019). At day 14, both tibia and toe ash content did not respond to changes in dietary VD₃ levels above 500 IU/kg, according to Cho *et al.* (2020). Only the group of birds given VD₃ at 200 IU/kg of diet had a significant effect on tibia ash. Only the tibia ash content was affected by dietary VD₃ levels at 31 d. Tibia ash did not respond to an increase in dietary VD₃ concentration above 500 IU/kg. In response to dietary VD₃ levels, a linear trend in tibia ash was found.

Table 9. Bone length (cm) of Norfa chicks as affected by *in ovo* injection with different levels of vitamin D₃ (Means ± S.E.).

Groups	Femur bone length (cm)	Sternum bone length (cm)	Tibia bone length (cm)
Negative control (not injected)	5.50±0.06 ^{ab}	7.57±0.23 ^b	6.10±0.15
Positive control (distilled water)	5.37±0.03 ^b	7.23±0.15 ^b	5.43±0.03
VD ₃ (50 µL /egg)	5.50±0.06 ^{ab}	7.70±0.15 ^{ab}	5.63±0.34
VD ₃ (100 µL /egg)	5.67±0.09 ^a	8.13±0.07 ^a	5.90±0.23
P-Value	0.046	0.026	0.227
Significance	*	*	N.S.

^{a, b, c} Means in the same column bearing different superscripts are significantly different at $P < 0.01$.

* Significant differences at $P \leq 0.05$. ** Highly Significant differences at $P \leq 0.01$.

N.S. = No significant differences.

Conclusion and applications:

The present results imply that early *in ovo* treatment of different amounts of vitamin D₃ may be a viable technique for increasing Norfa chick performance, hatchability, decreased mortality, and improved several blood parameters, as well as immunity. At 7 and 28 days of age, chicks produced from eggs injected with 100 µL of vitamin D₃/ egg had a substantially higher body weight, femur, sternum bone length, WBCs, LYM, heterophil, RBCs, and enhanced immunological response than chicks in the other treatments. During the trial, the feed conversion ratio and cumulative embryonic death rate were also considerably influenced. When compared to the control, *in ovo* injection of 100 µL of VD₃ enhanced liver enzyme and serum calcium levels.

REFERENCES

- Abbasi, T.; Shakeri, M.; Zaghari, M. and Kohram, H. (2017). Growth performance parameters, bone calcification and immune response of *in ovo* injection of 25-hydroxycholecalciferol and vitamin K3 in male Ross 308 broilers. *Theriogenology*, 90: 260 – 265.
- Abdou, F. H. (1996). Improving endogenous chickens breeds: Experience from Egypt, Norway and Tanzania. *Egyptian J. Anim. Prod.*, 33: 567 - 576.
- Aburto, A.; Edwards, Jr. H. M. and Britton, W. M. (1998). The influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25-hydroxycholecalciferol, and 1,25 dihydroxycholecalciferol in young broiler chickens. *Poult. Sci.*, 77: 585 – 593.
- Aslam, S. M.; Garlich, J. D. and Qureshi, M. A. (1998). Vitamin deficiency alters the immune responses of broiler chicks. *Poult. Sci.*, 77: 842 – 849.
- Atencio, A.; Edwards, Jr H. M. and Pesti, G. M. (2005). Effect of the level of cholecalciferol supplementation of broiler breeder hen diets on the performance and bone abnormalities of the progeny fed diets containing various levels of calcium or 25-hydroxycholecalciferol. *Poult. Sci.*, 84: 1593 1603.
- Bello, A.; Hester, P. Y.; Gerard, P. D. and Zhai, W. (2014). Effects of commercial *in ovo* injection of 25- hydroxycholecalciferol on bone development and mineralization in male and female broilers. *Poult. Sci.*, 93: 2734 – 2739.
- Bello, A.; Zhai, W.; Gerard, P. D. and Peebles, E. D. (2013). Effects of the commercial *in ovo* injection of 25-hydroxycholecalciferol on the hatchability and hatching chick quality of broilers. *Poult. Sci.*, 92: 2551 – 2559.

- Bronner, F. (2003). Mechanisms and functional aspects of intestinal calcium absorption. *J. Exp. Zool.*, 300: 47 - 52.
- Chen, PS. Jr and Bosmann, HB. (1965). Comparison of the hypercalcemic action of vitamins D₂ and D₃ in chicks and the effect on tetracycline fixation by bone. *J. Nutr.*, 87 (2): 148 – 154.
- Cho, T. Z. A.; Sadiq, M. B.; Srichana, P. and Anal, A. K. (2020). Vitamin D₃ enhanced intestinal phosphate cotransporter genes in young and growing broilers. *Poult. Sci.*, 99: 2041 – 2047
- 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. *Poult. Sci.*, 88: 2333 – 2341.
- Duncan, D.B. (1955). Multiple range and multiple f. *Test Biometrics*, 11: 1.
- Ebrahimi, H.; Shariatmadari, and Karimi Torshizi, M.A. (2016). Dietary supplementation and *in ovo* injection of 1 α -OH D₃ in a low-calcium and low-phosphorous diets for broilers. *J. Appl. Anim. Res.*, 44 (1) :113 – 117.
- Elaroussi, M. A.; Deluca, H. F.; Forte, L. R. and Biellier, H. V. (1993). Survival of vitamin D-deficient embryos: Time and choice of cholecalciferol or its metabolites for treatment *in ovo*. *Poult. Sci.*, 72: 1118 – 1126.
- Fritts, C. A. and Waldroup, P. W. (2003). Effect of source and level of vitamin D on live performance and bone development in growing broilers. *J. Appl. Poult. Res.*, 12: 45 – 52.
- Fritts, C. A.; Erf, G. F.; Bersi, T. K. and Waldroup, P. W. (2004). Effect of source and level of vitamin d on immune function in growing broilers. *J. Appl. Poult. Res.*, 13: 263 – 273.
- Han, J. C.; Chen, G. H.; Wang, J. G.; Zhang, J. L.; Qu, H. X.; Zhang, C. M.; Yan, Y. F. and Cheng, Y. H. (2016). Evaluation of relative bioavailability of 25-hydroxycholecalciferol to cholecalciferol for broiler chickens. *Asian Australas. J. Anim. Sci.*, 29 (8): 1145 - 1151.
- Holick, M.F. and Garabedian, M. (2006). Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In *Primer on the metabolic bone diseases and disorders of mineral metabolism*. 6th edition. Edited by: Favus MJ. Washington, DC: J. BMR., 6: 129-137.
- Hutton, K. C.; Vaughn, M. A.; Litta, G.; Turner, B. J. and Starkey, J. D. (2014). Effect of vitamin D status improvement with 25-hydroxycholecalciferol on skeletal muscle growth characteristics and satellite cell activity in broiler chickens. *J. Anim. Sci.*, 92: 3291 - 3299.
- Imrie, M.; Neville, P.; Snellgrove, A.T. and DeLuca, H. (1967). Metabolism of vitamin D₂ and vitamin D₃ in the rachitic chick. *Arch Biochem Biophys.*, 120 (3): 525 – 532.
- Jamroz, D.; Wertelecki, T.; Wiliczekiewicz, A.; Orda, J. and Skorupinska, J. (2004). Dynamics of yolk sac resorption and post-hatching development of the gastrointestinal tract in chickens, ducks and geese. *J. Anim. Physiol. Anim. Nutr.*, (Berl.) 88: 239 – 250.
- Kalantar, M.; Hosseini, S. M.; Hosseini, M. R.; Kalantar, M. H.; Farmanullah and Yang, L. G. (2019). Effects of *in ovo* injection of coenzyme Q10 on hatchability, subsequent performance, and immunity of broiler chickens. *Biomed Res. Int.*, Article ID 7167525, :1 - 8.
- Kim, M. J.; Parvin, R.; Mushtaq, M. M. H.; Hwangbo, J.; Kim, J. H.; Na, J. C.; Kim, D. W.; Kang, H. K.; Kim, C. D.; Cho, K. O.; Yang, C. B. and Choi, H. C. (2013). Growth performance and hematological traits of broiler chickens reared under assorted monochromatic light sources. *Poult. Sci.*, 92: 1461 – 1466.
- Kim, W. K.; Bloomfield, S. A. and Ricke, S. C. (2011). Effects of age, vitamin D₃, and fructooligosaccharides on bone growth and skeletal integrity of broiler chicks. *Poult. Sci.*, 90: 2425 – 2432.

- Leslie, M. A.; Coleman, R. A.; Moehn, S.; Ball, R. O. and Korver, D. R. (2006). Relationship between bicarbonate retention and bone characteristics in broiler chickens. *Poult. Sci.*, 85: 1917 – 1922.
- Lohakare, J. D.; Kim, J. K.; Ryu, M. H.; Hahn, T.W. and Chae, B. J. (2005). Effects of vitamin C and vitamin D interaction on the performance, immunity, and bone characteristics of commercial broilers. *J. Appl. Poult. Res.*, 14: 670 – 678.
- Lowenthal, J. W.; Connick, T. E.; McWaters, P. G. and York, J. J. (1994). Development of T cell immune responsiveness in the chicken. *Immunol. Cell. Biol.*, 72: 115 – 122.
- Mattila, P.; Lehtikoinen, K.; Kiiskinen, T. and Piironen, V. (1999). Cholecalciferol and 25 hydroxycholecalciferol content of chicken egg yolk as affected by the cholecalciferol content of feed. *J. Agric. Food Chem.*, 47: 4089 – 4092.
- Mitchell, R. D.; Edwards, Jr. H. M. and McDaniel, G. R. (1997). The effects of ultraviolet light and cholecalciferol and its metabolites on the development of leg abnormalities in chickens genetically selected for a high and low incidence of tibial dyschondroplasia. *Poult. Sci.*, 76: 346 – 354.
- 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. *Poult. Sci.*, 93: 1951 - 1956.
- Mustafa, M. A. G. N.; Mustafa, A.G. and Rasheed, R. S. (2019). Effect of calcium and cole vit D₃ *in ovo* injection on hatchability, bone and blood biochemical development at posthatch. *Iraqi J. Agric. Sci.*, 50 (3): 850 – 856.
- Narbaitz, R.; Tsang, C. P. and Grunder, A. A. (1987). Effects of vitamin D deficiency in the chicken embryo. *Calcif. Tissue. Int.*, 40: 109 - 113.
- Nordin, BE. (2010). Evolution of the calcium paradigm: the relation between vitamin D, serum calcium and calcium absorption. *Nutr. J.*, 2: 997 - 1004.
- NRC, (1994). Nutrient requirements of poultry. 9th rev. ed. Washington (DC): National Academy Press.
- Ohta, Y.; Kidd, M.T. and Ishibashi, T. (2001). Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos and chicks after *in ovo* administration of amino acids. *Poult. Sci.*, 80: 1430 – 1436.
- Papešová, L.; Fučíková, A.; Pípalová, M. and Tupý, P. (2008). The synergic effect of vitamin D₃ and 25-hydroxycholecalciferol/calcidiol in broiler diet. *Sci. Acta. Boh.*, 3: 273 – 277.
- Salim, H. M.; Zaman, M. A.; Beg, M. A. H. and Khaleduzzaman, A. B. M. (2019). Effect of supplemental 25-ydroxycholecalciferol on live performance, bone development, and mineral utilization of broiler chickens Fed Low Dietary Ca and P. *EC Nutrition.*, 14 (3): 227 - 238.
- Saunders-Blades, J. L. and Korver, D. R. (2014). The effect of maternal vitamin D source on broiler hatching egg quality, hatchability, and progeny bone mineral density and performance. *J. Appl. Poult. Res.*, 23: 773 – 783.
- Selim, Sh. A.; Gaafar, K.M. and El – ballal, S. S. (2012). Influence of *in ovo* administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. *Emir. J. Food Agric.*, 24: 264 – 271.
- Siegel, P.B and Gross, W.B. (1980). Production and persistence of antibody response to sheep erythrocytes. 1. Directional selection poultry Science, 59: 1 - 6.
- SPSS Program, 1999. User's guide statistic. Release 10.01, Copyright SPSS Inc., USA.
- Stevens, V. I.; R. Blair and R. E. Salmonm, 1984. Effects of vitamin D₃, calcium, and phosphorus on growth and bone development of market turkeys. *Poult. Sci.*, 63: 1571 – 1585.
- Sunde, M. L.; Turk, C. M. and DeLuca, H. F. (1978). The essentiality of vitamin D

- metabolites for embryonic chick development. *Science*, 200: 1067 – 1069.
- Turner, R. T.; Graves, J. S. and Bell, (1987). Regulation of 25- hydroxyvitamin D₃ metabolism in chick embryo. *Am. J. Physiol.*, 252: E38 – E43.
- Vignale, K.; Greene, E. S.; Caldas, J. V.; England, J.; Boonsinchai, N.; Sodsee, P.; Pollock, E. D.; Dridi, S. and Coon, C. N. (2015). 25-Hydroxycholecalciferol enhances male broiler breast meat yield through the mTOR pathway. *J. Nutr.*, 145: 855 - 863.
- Wang, J.; Han, J.; Chen, G.; Qu, H.; Wang, Z.; Yan, Y. and Cheng, Y. (2016). Comparison of bioavailability of 1 α -hydroxycholecalciferol and cholecalciferol in broiler chicken diets. *Poult. Sci.*, 53: 22 - 28.
- Wang, X.; Peebles, E. D.; Kiess, A. S.; Wamsley, K. G. S. and Zhai, W. (2019). Effects of coccidial vaccination and dietary antimicrobial alternatives on the growth performance, internal organ development, and intestinal morphology of *Eimeria*-challenged male broilers. *Poult Sci.*, 98: 2054 – 2065.
- Wells, L. L.; Lowry, V. K.; DeLoach, J. R. and Kogut, M. H. (1998). Age-dependent phagocytosis and bactericidal activities of the chicken heterophil. *Dev. Comp. Immunol.*, 22: 103 – 109.
- Whitehead, C.C.; McCormack, H.A.; McTeir, L. and Fleming, R.H. (2004). High vitamin D₃ requirements in broilers for bone quality and prevention of tibial dyschondroplasia and interactions with dietary calcium, available phosphorus and vitamin A. *Br. Poult. Sci.*, 45 (3): 425 – 436.
- Yarger, J. G.; C. A. Saunders; J. L. McNaughton; C. L. Quarles, B. W. Hollis and R. W. Gray, 1995. Comparison of dietary 25-hydroxycholecalciferol and cholecalciferol in broiler chickens. *Poult. Sci.*, 74: 1159 – 1167.
- Zamani, A.; Shariatmadari, F.; Rahimi, S. and M.A. Karimi Torshizi, 2019. Effects of *in ovo* injection of carbohydrates, β -hydroxy- β -ethylbutyrate, and vitamins on ostrich organ weight, bone characteristics, and small intestinal morphology. *Can. J. Anim. Sci.*, 99: 116 – 122.
- Zanaty, G. A. and Ibrahim, Sh. A. M. (2005). Optimum dietary protein and energy levels of Norfa chicks during the periods of zero to eight and eight to sixteen weeks of age. *EPSJ.*, 25 (3): 589 - 612.
- Zhang, R. and Naughton, D. P. (2010). Vitamin D in health and disease: Current perspectives. *J. Nutr.*, 9: 65.

تأثير حقن البيض بفيتامين D₃ علي بعض الصفات الفسيولوجية وتطور عظام كتاكيت نورفا

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الملخص العربي

تهدف هذه الدراسة إلي تقييم تأثير حقن البيض المخصب بفيتامين D₃ على أداء كتاكيت نورفا. تم حفظ جميع البيض المخصب في درجة حرارة غرفة التفريخ لمدة ١٢ ساعة ثم تم تفريخ البيض في مفرخات عند ٣٧.٨ درجة مئوية مع رطوبة نسبية ٥٥ - ٦٠٪. تم تغليب البيض كل ساعتين من اليوم الثاني إلى اليوم الثامن عشر من التفريخ. المجموعة الأولى لم يتم حقن ٤٠ بيضة واعتبرت كنترول سالب، بينما المجموعة الثانية، تم حقن ٤٠ بيضة بالماء المقطر (كنترول موجب). أما المجموعتين الثالثة والرابعة تم حقن ١٦٠ و ١٨٠ بيضة بـ ٥٠ و ١٠٠ ميكروليتر من فيتامين D₃ (VD₃) على التوالي. تم حقن البيض في اليوم الثامن عشر من التفريخ عن طريق إدخال المادة المستخدمة في الغرفة الهوائية من خلال الطرف العريض للبيضة ثم تم إغلاق الفتحة بالشمع.

خلال الأيام الثلاثة الأخيرة من التفريخ، تم نقل جميع البيض إلى فقاسة منفصلة عند درجة حرارة ٣٦.٥ درجة مئوية ورطوبة نسبية ٧٥ - ٨٠٪. عند الفقس، تم ترقيم الكتاكيت في الجناح ووزنها ووضعها في عنابر حضانة الكتاكيت.

أظهرت النتائج أن الكتاكيت الفاقسة من البيض المحقون بـ ١٠٠ ميكروليتر VD₃ أظهرت زيادة معنوية في وزن الجسم الحي وزيادة وزن الجسم المكتسب مقارنة بالمجموعات الأخرى عند مختلف الأعمار. علاوة على ذلك، تأثر معدل التحويل الغذائي ومعدل نفوق الأجنة معنويًا خلال فترة التجربة. لوحظ زيادة معدل نفوق الأجنة بشكل معنوي في المجموعات المعاملة مقارنة بمجموعة الكنترول السالب. تحسنت الاستجابة المناعية معنويًا عند ٧ أيام و ٢٨ يوم لكتاكيت نورفا المحقونة بـ VD₃ مقارنة بمجموعات الكنترول. سجلت أعلى قيمة لطول عظم الفخذ والقص في الكتاكيت المحقونة بـ ١٠٠ ميكروليتر VD₃، بينما انخفض طول عظم الساق بشكل معنوي. ارتفعت مستويات AST و ALT في دم لكتاكيت المحقونة بـ ١٠٠ ميكروليتر VD₃ معنويًا مقارنة بمجموعة الكنترول السالب. في مجموعة البيض المحقون بـ VD₃ (١٠٠ ميكروليتر) ارتفع مستوى الكالسيوم الكلي في الدم مقارنة بمجموعة الكنترول. أشارت هذه النتائج إلى أن حقن البيض بمستويات مختلفة من فيتامين D₃ كان له تأثير معنوي على خلايا الدم البيضاء، الخلايا الليمفاوية، الخلايا المتعادلة، خلايا الدم الحمراء والصفائح الدموية. ومع ذلك، لم يكن هناك تأثير معنوي لحقن البيض على مستويات الهيموجلوبين والهيماتوكريت. في النهاية يمكن اعتبار الحقن المبكر للبيض بمستويات مختلفة من فيتامين D₃ تقنية مجدية لزيادة أداء كتاكيت نورفا، نسبة الفقس، تحسين صفات الدم والمناعة وخفض معدل النفوق الجيني.