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Effects of *In-Ovo* Injection of D_3 Vitamin on Hatchability and Supply Organ Weights in Quail Hatching Eggs

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Abstract

The purpose of this study was to determine the effects of *in-ovo* injection of vitamin D_3 at the day 11 and 14 of embryonic development on hatchability of fertile eggs, embryonic mortality (EM), chick weight, bone length and weight and supply organ weights of Japanese quails. A total of 480 hatching eggs were used in the study. The eggs were randomly divided into 3 groups as 160 eggs in each group with four replicates of 40 eggs each. Eggs were injected at the 11^{th} and 14^{th} day of incubation to deposit test material into amniotic fluid through the blunt end of the egg. The first group without injection (control), while, the second group at 11^{th} day of incubation and third groups 14^{th} day of incubation were injected with 0.2 ml Vit.D_3 (333 IU), for per egg. The results showed that *in-ovo* injection of vitamin D_3 on day 11 and 14 of incubation resulted in significant decreases in the hatchability and led to an increase in EM ($P< 0.05$). However, there was no significant difference for supply organ rate, chick and bone weight and bone length among treatment groups. In conclusion from the present study shows that injection with Vit.D_3 in quail eggs with 333 IU at 11^{th} and 14^{th} day of incubation decreases the hatchability, leads to an increase in EM without affecting the tibia weight and length, and supply organ weights in quail eggs.

Keywords: Vit D_3, Quail hatching eggs, Incubation, Hatchability of fertile eggs, organ weight

Kuluçkalık Bıldırcın Yumurtalarına *In-Ovo* D_3 Vitamini Enjeksiyonunun Çıkış Gücü ve Organ Ağırlığı Üzerine Etkileri

Öz

Bu çalışmanın amacı, kuluçkanın 11. ve 14. günlerinde *in-ovo* D_3 vitamini enjeksiyonunun çıkış gücü, embriyonik ölümleri, civciv ağırlığı, kemik uzunluğu ve ağırlığı ve organ ağırlıkları üzerine etkilerini araştırmaktır. Çalışmada toplam 480 kuluçkalık bıldırcın yumurtası kullanılmıştır. Yumurtalar her bir grupta 160 adet (40 adet/alt grup) kuluçkalık yumurta olacak şekilde üç gruba rastgele dağıtılmıştır. Kuluçkanın 11 ve 14. günlerinde yumurtanın küt ucundan amniyotik sıvı içerisine test materyali enjekte edilmiştir. Birinci grup enjeksiyon yapılmayan (kontrol grubu) diğeri ise kuluçkanın 11. ve 14. günlerinde 0.2 ml Vit D_3 (333 IU) enjekte edilen grup olarak düzenlenmiştir. Kuluçkanın 11. ve 14. günlerinde D_3 vitaminin *in-ovo* enjeksiyonunun kuluçka çıkış gücünü düşürdüğü ve embriyonik ölümleri artırdığı görülmüştür ($P <0.05$). Bununla beraber, gruplar arasında organ ağırlıkları, kemik ağırlığı ve kemik uzunluğunda önemli bir fark olmadığı tespit edilmiştir. Sonuç olarak bu çalışmada kuluçkanın 11. ve 14. günlerinde *in-ovo* D_3 vitamini enjeksiyonu kuluçka randımanını düşürdüğü embriyonik ölümleri arttırdığı fakat kemik ağırlık, uzunluğu ve organ ağırlığını etkilemediği görülmüştür.

Anahtar kelimeler: Vit D_3, kuluçkalık bıldırcın yumurtası, kuluçka, çıkış gücü, organ ağırlığı

Introduction

In-ovo is a technology that consists to administrate nutrients, vitamin and vaccines in the fertile eggs. This was applied for the first time by Sharma and Burmester (1982) injecting the vaccine against Marek's disease into the eggs of turkey. *In-ovo* injections have been administered at 0 day (prior to incubation), 5 days, 10 days or 15 days of incubation in Japanese quail eggs (Romao et al., 2011) and at 18 days of incubation in chicken eggs (Li et al., 2005). Previous studies investigate the effects of *in-ovo* injection of saline and Newcastle disease (ND) vaccine plus saline or industrial diluent (Romao et al., 2011), ascorbic acid (Elibol et al., 2001; Ipek et al., 2004), carbohydrates (Tako et al., 2004; Zhai et al., 2011), amino acids (Ohta et al., 2001; Bhanja et al., 2014; Coskun et al., 2018), vitamins (Bello et al., 2013b), minerals (Oliveira et al., 2015; Yıldız et al., 2018), hormones (Moore et al., 1994), insulin-Like Growth Factor (Kocamis et al., 1999), royal jelly (Moghaddam et al., 2014), pollen extract (Coşkun et al., 2014; 2017), propolis water extract (Aygun, 2016), probiotic (Abdulqader et al., 2018), prebiotic (Maiorano et al., 2012) on hatchability embryo mortality growth and physiological parameters.

Vitamin D is a group of fat-soluble steroids it plays numerous roles as regulatory in immune cells functions (Reinhardt and Hustmyer, 1987), the bones to mobilize calcium and phosphorus, release of insulin in relation to glucose challenge (DeLuca, 1992); magnesium (Mg) absorption, calcium and phosphorus balance (Miller et al., 1965), mobilization of calcium (Garabedian et al., 1974); embryonic development of the chick, stimulated yolk calcium mobilization (Tuan and Suyama, 1996); transport of calcium from eggshell to the embryo (Elaroussi et al., 1993).

In-ovo injection of 1,25-dihydroxyvitamin D_3 at 0.20 µg or 0.60 µg was found to increase hatchability of embryos (Ameenuddin et al., 1983); Injection of vitamin D_3, 25-hydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 into eggs obtained through hens fed 1,25-dihydroxyvitamin at doses of 1,2 µg, 0,5 µg, 0,5 µg respectively, has led to significant increase in the hatchability of fertile eggs (Sunde et al., 1978). Ibrahim et al. (2012) reported that the injection of vitamin D_3 on 17^{th} day of incubation at 180 IU improved the hatchability of fertile eggs. Narbaitz and Tsang (1989) also reported that injection of 10 ng calcitriol, 1 µg 24,25-(OH)2D3, or 2 µg 25OHD3 on the 14^{th} day of incubation improved hatchability and bone weight. Elaroussi et al. (1993) found that the *in-ovo* injection before incubation of 25 ng cholecalciferol, 600 ng 24, 25-dihydroxycholecalciferol [24, 25-(OH)2D3], or 100 ng 1,25-(OH)2D3 into Japanese quail eggs increase hatchability. However, Gonzales et al. (2013) reported that the *in-ovo* injection of 25-hydroxy cholecalciferol (25(OH)D3) at 3 different doses (0.625, 1.250 or 1.875 µg) on the 17^{th} of incubation in broiler eggs did not have any influence on hatchability. Holbrook and Soares (1985) studied the effects of D_3 vitamin in dietary on tibia strength in quail. They concluded that 1, 25(OH) 2D3 had no effect on bone accretion. Frost et al. (1990) reported that tibia weight and tibia breaking strength increased with addition of l, 25-(OH)2 D_3 to the diet. On the other hand, Narbaitz and Tolnai (1978) found that bones alterations realized in the chicken embryos during the second phase of incubation with the injection of 1.25 dihydroxycholecalciferol (1,25(OH)2D3). Bello et al., (2013b) also suggested that the *in-ovo* injection of 25(OH) D_3 at 18^{th} day of incubation had no effect on the bone development when injecting 0.15 to 1.20 µg doses.

The objective of the present study was to evaluate the effects of *in-ovo* injection of vitamin D_3 on the hatchability, embryonic mortality, chick's weight, supply organs and bone weight and length in Japanese quails (*Coturnix japonica*).

Materials and Methods

The experiment was conducted at the incubation laboratory at the Department of Animal Science, Faculty of Agriculture, Selcuk University, Konya, Turkey. Four hundred eighteen (480) Japanese quail (*Coturnix japonica*) eggs obtained from a commercial farm in Adana, Turkey were used in this study. A total of 480 eggs were distributed randomly in 3 groups with 160 eggs per treatment and each treatment contained 4 replications with 40 eggs each. Eggs were incubated in a laboratory type incubator. The temperature and humidity during the first 14 days of incubation were provided as 37.5 °C and 55%, respectively while between the 15 to 18 days of incubation temperature and humidity were 37.2 °C and 75% respectively. Eggs were automatically turned at an angle of 45° every two hours until the end of the 14^{th} day of incubation. At 11^{th} and 14^{th} of incubation, the eggs were removed from the incubator for *in-ovo* injection. After the completion of the injection procedure, the eggs have been placed to the incubator for continuing remaining incubation. The injection procedure has been applied at room temperature. Before beginning the injection eggs were disinfected at the blunt end of the egg with ethanol 70% then perforated with a micro motor (Strong 210, Korea) to allow the injection of the vitamin D_3. The vitamin D_3 was injected (0.20 ml) into the amniotic fluid with a sterile 1ml syringe through the hole.

After the injection, the holes were sealed with glue and the eggs were placed in the incubator. Treatment groups were consisted of: 1- non injected (control), 2- injection of 0.20 ml at 11 days of incubation and 3- injection of 0.20 ml at 14 days of incubation. On each injection day, the experimental eggs were submitted to two different days of injections: injection at 11 days of incubation and injection at 14 days of incubation. After 18 days of incubation, in each group chicks were removed from the incubator and five chicks randomly selected from each group were weighed and necropsied to determine the yolk sac weight (YSW), heart, liver, bone weights and bone lengths. At day 18 of incubation, unhatched eggs were opened to establish the stage of embryonic mortality (Aygun et al., 2012). The stages of embryonic mortality were classified as follows: d 1 to 10 (black-eye visible and embryo without feathers), d 11 to 16 (embryo with feathers and embryo with yolk out), and d 17 to 18 (full-grown embryo dead and with yolk subtracted). Fertility was calculated as the percentage of set eggs. The hatchability was calculated as both set eggs (HS) and the fertile (HF) eggs.

Statistical analysis

The variance analysis technique was performed to compare the means of the studied traits (hatchability, embryonic mortality, chicks weight yolk-free chick weight (YFCW), yolk sac weight (YSW), heart, liver, bone weights and bone length) among the treatment groups. The differences between means of the groups were determined by the Tukey multiple range test.

Results

The effects of *in-ovo* injection of vitamin D_3 during incubation on hatchability and embryonic mortality stages are shown in Table 1. The HS in C (64.90%) differed significantly ($P < 0.05$) from those of *in-ovo* injection of vitamin D_3 in 2 and 3 groups which were calculated as 36.64% and 31.50%, respectively. Beside, significant differences were observed between C (83.31%) and those from *in-ovo* injection of vitamin D_3 at 11th and 14th day of incubation with 48.03% and 40.06% respectively, in terms of HF. There were found

significant differences between treatments in terms of EM. *In-ovo* injection of vitamin D_3 at group2 and group3 had the higher embryonic mortality rate with 40.08% and 48.96%, respectively.

Table 1. The influence of *in-ovo* injection of vitamin D_3 on hatchability and embryonic mortality (%)

Group	Fertility (%)	HS (%)	HF (%)	EM (% of fertile eggs)		
				1 to 9 d	10 to 16 d	17 to 18 d
Control	77.96±3.42	64.90^{a} ±3.83	83.31^{a} ±5.38	3.98±1.21	10.99^{b} ±5.84	1.707±1.85
11thday	76.88±3.42	36.64^{b} ±3.83	48.03^{b} ±5.38	8.57±1.21	40.08^{a}±5.84	3.312±1.85
14th day	79.82±3.42	31.50^{b}± 3.83	40.06^{b} ±5.38	5.30±1.21	48.96^{a}±5.84	5.675±1.85
P-value	0.832	<0.05	<0.05	0.065	<0.05	0.356

^{a,b} Means within column with different superscripts differ significantly (P < 0.05).

The effects of vitamin D_3 injection on chick body weight, YFCW, YSW, supply organ, bone weights and bone lengths are shown in Table 2. YFCW, YSW, supply organ, chick and bone weights, bone length were not significantly affected (P >0.05) by vitamin D_3 injection in this experiment.

Table 2. Effects of *in-ovo* injection of vitamin D_3 on chick weight, supply organ weights and bone weight and length

Group	Chick weight (g)	Yolk-free chick weight (g)	Yolk sac weight (%)	Heart weight (%)	Liver weight (%)	Bone length (mm)	Bone weight (%)
Control	7.78± 0.40	6.80±0.32	12.34±1.28	0.73±0.057	2.134±0.14	18.77±0.87	0.75±0.04
11thday	8.06± 0.40	7.27±0.32	9.80±1.28	0.77±0.057	2.101±0.14	19.87±0.87	0.73±0.04
14th day	8.25± 0.40	7.39±0.32	10.16±1.28	0.86±0.057	2.289±0.14	17.58±0.87	0.65±0.04
P-value	0.717	0.423	0.352	0.274	0.621	0.216	0.344

Discussion

The results in this study has showed that *in-ovo* injection of 333 IU of vitamin D_3 into the amnion at the day 11 and 14 of incubation in quail hatching eggs has negatively impacted on HS, HF and EM in both treatment groups. This result agrees with Elaroussi et al. (1993) who reported that hatchability decreased significantly when cholecalciferol was injected at d 11 or 12 of incubation and Zamani et al. (2018) who found that lower hatchability and higher embryonic deaths by injecting 180 IU vitamin D_3 in the ostrich eggs. However, our results are in disagreement with Sunde et al. (1978), Ameenuddin et al. (1983), Ibrahim et al. (2012), Narbaitz and Tsang (1989) who observed an increase in hatchability by the injection of vitamin D_3 during incubation period. On the other hand Gonzales et al. (2013) reported that *in-ovo* injection of 25-hydroxy cholecalciferol (25(OH)D3) in broiler eggs did not have any influence on HF. The probable reason of negative impacts of D_3 injection on HOF in the study might be a result of high dosage of the solution applied in the study has probably caused hypercalcemia in the embryo. And also injection volume may be increased embryonic mortality. Because Coskun et al. (2018) reported that injection volume has an important effect on hatchability. In this study 0.2 ml vitamin D_3 solution injected into amniotic fluid and this volume must have affected embryonic mortality. Ebrahimnezhad et al. (2011) reported that 0.5 ml *in-ovo* injection into the amniotic fluid caused an allergic cavity, stoppage of breathing, and

embryonic death due to increase in osmotic pressure in eggs. They reported that 0.5 ml injection volume into 60 gr broiler eggs is heavy for embryo, in our study 0.2 ml injection volume into 12 gr egg were used.

The results of this study showed that lower dose from 0.2 ml injection volume should be tested for increasing hatchability and decreasing embryonic mortality. In this experiment YFCW, YSW, supply organ, chick and bone weights, bone length were not significantly affected by vitamin D_3 injection. These results are in agreement with Holbrook and Soares (1985) who found no effect with vitamin D_3 injection on bone; bone length (Zamani et al. 2018). However, they are a disagreement with Zamani et al. (2018), Frost et al. (1990), Narbaitz and Tsang (1989) who found that vitamin D_3 improved tibia bone weight. Our results are in agreement with Bello et al. (2013a) who found no differences on relative yolk sac weight *in-ovo* injection of various forms of vitamin D_3. However a disagreement with Zamani et al. (2018) in the yolk sac weight who found a significantly greater yolk sac residue *in-ovo* fed with vitamin D_3.

Conclusion

The results of this study indicate that injection with vitamin D_3 in quail eggs with 333IU at 11^{th} and 14^{th} of incubation decreases the hatchability of fertile eggs, increases embryonic mortality rates without any influence on the bone length, bone and supply organ weight in quail hatching eggs.

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