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Effect of *in ovo* injection of threonine on Mucin2 gene expression and digestive enzyme activity in Japanese quail (*Coturnix japonica*)

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ABSTRACT

A total of 540 Japanese quail eggs were assigned to 9 treatments of 4 replicates to investigate the effect of *in ovo* injection of threonine (THR) on mucin2 (MUC2) mRNA expression and digestive enzyme activity. Treatments were (non-injected) eggs and those *in ovo* injected with saline (0.05 or 0.1 ml) with or without THR (5 mg/ml) in two sites (in or under the air sac). On hatch day, 0.05 ml *in ovo* injected (under the air sac: TUAS) hatchlings were divided into three groups based on NRC recommendations for THR, while all 0.1 ml *in ovo* injected chicks were removed due to low hatchability. The remaining treatments received the NRC recommended diet until day 10 post-hatch. Treatments had no effect on protease and amylase activities, while TUAS increased MUC2 gene expression. In conclusion, the *in ovo* injection of THR increased MUC2 gene expression but had no effect on enzyme activity.

1. Introduction

It has been shown that there is an approximately 48–72 hr gap between hatch time and hatchlings' access to feed and water due to different hatch times, hatchery handling and transport times to the poultry farm (Bhuiyan et al., 2011; Willemsen et al., 2010). This delay results in a lowering of development and function of the gut (Potturi et al., 2005; Yang et al., 2009), thereby reducing final body weight (Noy and Sklan, 1999), decreasing immune response to pathogens (Dibner et al., 1998) retarding growth, and increasing mortality up to 5% (Willemsen et al., 2010). It has thus been suggested that the detrimental effects of this delay could possibly be overcome. Hatchery feeding (Kidd et al., 2007; Willemsen et al., 2010) and *in ovo* feeding (Uni and Ferket, 2004) are two most important clues suggested to overcome the detrimental effects of such delay. Hatchery feeding requires a high level of consistency in nutrients and diet formulation between hatchery management and the rearing farm, something that is practically difficult to achieve (Lilburn, 1998). Sharma and Burmester (1982) first used an *in ovo* method to vaccinate chicks against Marek's disease. In recent decades, many studies have been conducted to evaluate the effects of *in ovo* injection on poultry performance (Ohta and Kidd, 2001; Ohta et al., 2001). Finally, Uni and Ferket (2003) patented "*In ovo* Feeding" and stated that this method could beneficially affect hatchability (Uni et al., 2005), intestinal mucosa, and body weight at hatch and at 35 days of age (Uni and Ferket, 2004). Uni et al. (2003) reported that gastrointestinal functionality of *in ovo* treated chicks was the same as that of 2 day old birds fed immediately after hatching. To investigate the effect of *in ovo* feeding on the performance of chickens, various nutrients such as amino acids (Bhanja and Mandal, 2005; Bhanja et al., 2004, 2010), carbohydrates (Foye, 2005; Tako et al., 2004; Uni and Ferket, 2004), vitamins (Al-Daraji et al., 2012; Bhanja et al., 2007; Nowaczewski et al., 2012) and other nutrients (Moore, 2005; Tako et al., 2005; Zhai et al., 2008) have been administered to different poultry species. Among these nutrients, amino acids and, particularly, threonine (THR) have attracted more attention due to their effect on cellular (Tenenhouse and Deutsch, 1966) and humoral (Takahashi et al., 1994) immune responses, mucin structure (Gum, 1992; Lien et al., 1997), and digestive enzyme activity (Block et al., 1966; Yang et al., 1989). Kadam et al. (2008) reported that *in ovo* injection of THR caused better immunological responses in broilers, while having no effect on digestive enzyme activity. It was also demonstrated that digestive enzymes secreted by the intestinal lumen may degrade the thin mucosal layer of the intestine, enter the epithelial cell walls and cause ischemia (Godl et al., 2002). Similarly, THR can induce mucin secretion which results in an increase in the thickness of the mucosal layer and prevents enzymatic degradation of the intestinal mucous layer (Chang et al., 2012).

These studies were done only with poultry models (Kadam et al., 2008), so consequently there is a dearth of research on *in ovo* administration in quail. Thus, in the current study, we wanted to evaluate the

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Table 1
Description of different treatments used for *in ovo* injection of threonine in Japanese quail.

Treatments	Volume (ml)		Additive		Position		Period	
	0.05	0.1	Threonine	Saline	In the air sac (5 mm)	Under the air sac (8 mm)	Embryonic (E13)	Starter[b] (1–10 d)
Control (non-injected)	–	–	–	–	–	–	✓	✓
5SIAS[a]	✓	–	–	✓	✓	–	✓	✓
5SUAS	✓	–	–	✓	–	✓	✓	✓
1SIAS	–	✓	–	✓	✓	–	✓	–
1SUAS	–	✓	–	✓	–	✓	✓	–
5TIAS	✓	–	✓	✓	✓	–	✓	✓
5TUAS	✓	–	✓	✓	–	✓	✓	✓
1TIAS	–	✓	✓	✓	✓	–	✓	–
1TUAS	–	✓	✓	✓	–	✓	✓	–

[a] 5SIAS: injection of 0.05 ml saline (containing 0.9 g salt/l of distilled water) in the air sac; 5SUAS: injection of 0.05 ml saline under the air sac; 1SIAS: injection of 0.1 ml saline in the air sac; 1SUAS: injection of 0.1 ml saline under the air sac; 5TIAS: injection of 0.05 ml saline containing 5 mg/ml of threonine in the air sac; 5TUAS: injection of 0.05 ml saline containing 5 mg/ml of threonine under the air sac; 1TIAS: injection of 0.1 ml saline containing 5 mg/ml of threonine in the air sac; 1TUAS: injection of 0.1 ml saline containing 5 mg/ml of threonine.

[b] In rearing period (1–10 d), treatment injected 0.1 ml solution was removed due to low hatchability and other groups (except for 5TUAS) were received NRC recommendation (1994) diet. 5TUAS was divided to three groups: first group received all nutrients the same as NRC (1994) recommendations; second group received 10% higher threonine than NRC (1994) recommendations diet; third group received 20% higher threonine than NRC (1994) recommendations diet.

effect of *in ovo* injection of THR on MUC2 gene expression and digestive enzyme activity in quail hatchlings during the rearing period.

2. Materials and methods

2.1. Incubation, injection method and treatments

A total of 540 Japanese quail eggs were set in a single stage incubator. The relative humidity and temperature in the incubator for the hatchery (0–14 days) period were 68% and 37.8 °C, respectively, and for the setter (15–17 days) period were 78% and 36.8 °C, respectively. On day 11 of the embryonic period (E11), eggs were injected with different volumes of solutions containing physiological saline with or without threonine. Gauge 31 needles were used to inject all solutions into the air sac (IAS; depth of injection: 5 mm) or under the air sac (UAS; depth of injection: 8 mm) of quail eggs. The experiment was conducted as a completely randomized design with 9 treatments and 4 replicates of 15 eggs each. Treatments were consisted of (Table 1): (1) non injected (control) group; (2) IAS injection of 0.05 ml saline (containing 0.9 g NaCl/l of distilled water) (5SIAS); (3) UAS injection of 0.05 ml saline (5SUAS); (4) IAS injection of 0.1 ml saline (1SIAS); (5) UAS injection of 0.1 ml saline (1SUAS); (6) IAS injection of 0.05 ml saline containing 5 mg/ml of threonine (5TIAS); (7) UAS injection of 0.05 ml saline containing 5 mg/ml of threonine (5TUAS); (8) IAS injection of 0.1 ml saline containing 5 mg/ml of threonine (1TIAS); (9) UAS injection of 0.1 ml saline containing 5 mg/ml of threonine (1TUAS). Based on the lower hatchability of chicks receiving a 0.1 ml solution of (Kermanshahi et al., under revision) either IAS or UAS, quail hatched from these treatments were removed from the trial. Chicks from all other treatments (except those of 5TUAS group) received a corn-soybean meal basal diet formulated per all the nutrients based on NRC (1994) recommendations (Table 2) up to 10 days of age. In addition, quail from the 5TUAS group were divided into three groups consisting of 4 replicates with 3 quail per replicate and received the following diets: (1) control diet, a corn-soybean meal basal diet containing all nutrients based on NRC (1994) recommendations; (2) control diet except for threonine which was 10% higher threonine than NRC recommendations; (3) control diet except for threonine which was 20% higher threonine than NRC recommendations. Birds had free access to feed and water with a 23L/1D lighting program.

On hatch day and day 10 of the rearing period, 3 quail from each replicate (12 quail from each treatment) were euthanized by CO_2 asphyxiation, the adherent material and contents of the small intestine were carefully removed, and the duodenum, jejunum and ileum were carefully dissected and stored at –70 °C. By mild massaging of the intestine from the end of the duodenum to the ileocecal junction, homogenous digesta were collected and immediately frozen at –70 °C until used. Frozen jujenal samples were then divided into two groups in order to determine enzyme activity for analysis and MUC2 gene expression. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

2.2. Muc2 mRNA expression assay

The assessment of MUC2 gene expression was performed on jejunal samples obtained on the day of hatch and 10 days after hatch. Total RNA was extracted from quail jejunum using the TRIzol

Table 2
Composition of the quail's experimental diet.[c]

Components (%)	1–10 days (starter)
Corn	55.58
Soybean meal (44%)	41.34
Methionine	0.12
Lysine	0.01
Threonine	0.11
Dicalcium phosphate	0.75
CaCO3	1.30
Common salt	0.15
Sodium bicarbonate	0.14
Vitamin premix[a]	0.25
Mineral premix[b]	0.25
Calculated nutrients analysis	
Metabolizable energy (kcal/kg)	2793.92
Crude protein (%)	23.072
Calcium (%)	0.784
Sodium	0.1468
Chlorine	0.1359
Available phosphorous (%)	0.291
Lysine (%)	1.264
Methionine (%)	0.475
Methionine + cysteine (%)	0.848
Arginine (%)	1.509
Threonine (%)	0.98

[a] Each kilogram of vitamin supplement contains: vitamin A, 3,600,000 IU; vitamin D3, 800,000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B2, 2640 mg; vitamin B3, 4000 mg; vitamin B5, 12,000 mg; vitamin B6, 1200 mg; vitamin B9, 400 mg; vitamin B12, 6 mg; biotin, 40 mg; choline chloride, 100,000 mg; antioxidant, 40,000 mg.

[b] Each kilogram of mineral supplement contains: Mn, 40,000 mg; Zn, 33,880 mg; Fe, 20,000 mg; Cu, 4000 mg; I, 400 mg; Se, 80 mg.

[c] T10 and T20 treatments were added 0.001% and 0.002% threonine to diets, respectively, in balance of other nutrients, especially amino acids.