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The effect of threonine and vitamin A on intestinal histology of broiler chickens

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Abstract

The aim of this was to find the intestinal histological alterations in chickens under the influence of threonine (Thr) and vitamin A treatment. A total 60 one day old birds were allotted to three treatments. Each treatment was further divided into four replicates of ten chickens each. They were fed 0.80% Thr and 1500 IU/kg vitamin A (control; NRC 1994 recommendation; T1), 0.87% Thr and 6250 IU/kg vitamin A (T2) and 0.94% thr and 11000 IU/kg vitamin A (T3) from the age of 1 to 14 days. Management and husbandry practices were in accordance with current standards. The villus height was significantly high ($P<0.05$) in T2 group with no significant effect on crypt depth. From the present study, it is clear that the higher level of Thr and vitamin A (T2) than the recommended level of NRC (1994) improved the villus height in growing broiler chicks.

Keywords: threonine; vitamin A; intestinal histology; broiler chickens

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Introduction

The intestines of the birds have variety of functions including storage, secretion, digestion and absorption of nutrients. According to some authors, mucosa development is associated with increase in height and density of the villi in broiler birds (Uni et al., 1998; Applegate et al., 1999; Murakami et al., 2007).

Threonine (Thr) was discovered over 60 years ago and is considered to be the third limiting amino acid for broilers fed corn-soybean meal (Montagne et al., 2000). The nutrient Thr must be considered in dietary formulations for commercial broilers because its excess is costly and its deficiency will decrease the sufficiency of total sulfur amino acid (TSAA) and lysine (Lys) use (Kidd, 2000). Proteins and specific amino acids have been shown to alter mucin secretion and may interact directly with goblet cells (Montagne et al., 2000). Studies in piglets showed that up to 90% of dietary Thr is used by the intestine (Schaart et al., 2005). Also, Thr is an integral constituent of intestinal mucin proteins (Van Klinken et al., 1995).

Abnormal intake of dietary vitamin A causes keratinization and drying of the epithelial in the gastrointestinal tract, respiratory tract and ocular surface (Uni et al., 2000). Vitamin A and its derivatives are required for maintenance of various epithelial tissues at the appropriate differentiated stages and small intestine is one of the tissues exhibiting rapid cell proliferation and differentiation (Uni et al., 20000). NRC (1994) requirement for Thr and vitamin A is 0.8% and 1500 IU respectively.

Our aim was to determine the effect of several levels of Thr and vitamin A on intestinal histology in growing broiler chicks. One level is according to the NRC, 1994) recommendations and the other two are the higher levels.

Materials and Methods

A total of 60, one-day-old chicks were placed in pens (0.9×1.2 m), fitted with a tube feeder and nipple water line. Birds were reared at 29 and 32°C from day 1 to 14 respectively. Diets were formulated to provide a minimum of 100% of NRC (1994) amino acid recommendations. The dietary treatments consisted of isonitrogenous corn-soybean meal-based diets as following: 0.80% Thr and 1500 IU/kg Vit. A (NRC,

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1994 requirement; control), 0.87% Thr and 6250 IU/kg Vit. A, and 0.94% Thr and 11000 IU/kg Vit. A. Treatments were achieved by the addition of crystalline L-Thr (98.5% Thr, Degussa Co., Essen, Germany) at the expense of filler in the test diet containing 0.80% Thr (Table 1). Supplementation of L-Glutamic acid served to make the diets isonitrogenous. Corn and soybean meal were analyzed for crude protein (AOAC, 1990).

At day 14, one chicken from each replicate of treatments was killed. Samples of approximately 2 cm were taken from the midpoint between entry of the bile duct and Meckel's diverticulum (jejunum). After flushing with 0.9% (wt/vol) NaCl, samples were fixed in fresh 4% formaldehyde buffer. Jejunum was of particular interest because it is a major site of nutrient absorption in poultry (Horn et al., 2009). After fixation, soaked samples were rinsed several times in absolute alcohol, and then embedded in paraffin. Serial 6-µm longitudinal sections were cut on a rotary microtome (LEICA RM 2145) and placed on glass slides. Then, slides were routinely stained with Gill's haematoxylin and eosin (H&E). The parameters measured were villus height (measured from the tip of the villus to the villus-crypt junction) and crypt depth (measured from the crypt-villus junction to the base of the crypt). Data were analyzed using GLM procedures of SAS software (SAS, 2006) in a completely randomized design. Differences between means were tested using Duncan ′s test (1955). Differences were considered significant at $P<0.05$.

Results

Data on intestinal villus height and crypt depth are presented in Table 2. Dietary treatments affected villus height and crypt depth in jejunum of broiler chicks fed levels of treatments for 14 days. Villus height increased significantly ($P<0.05$) in 6250 IU/kg Vit. A and 0.87% Thr (T2) containing diets in comparison to the other treatments and there was no significant difference between villus height in birds fed 1500 IU/kg Vit. A and 0.80% Thr (T1) and 11000 IU/kg Vit. A and 0.94% Thr (T3). It is important to note that Ross (2007) has estimated Thr requirements 0.14% and vit. A 9500 IU/kg.

Discussion

In the present study, it is clear from the results that linear increase of vitamin A and Thr had no significant effect on villus height. Further, no significant effect of these two essential nutrients produced on crypt depth. Numerically, too the crypt depth decreased in T3. These results were in consistent with the report of Law et al. (2007) and Sepehri et al. (2011) who found that villus height in piglet and broilers receiving Thr deficient diet decreased compare to those receiving the Thr adequate diet. Horn et al. (2009) suggested established a link between dietary Thr and intestinal crude mucin in chicken and Pekin duckling. Azzam et al. (2012) found no effect on gut morphology including villus height, goblet cells and mucosal thickness in Babecok Brown layer supplemented with 0.47, 0.66 and 0.74% Thr. From these results, it can be inferred that the better findings in T2 group may be due to the supplementation of the higher level of Thr than the recommended level.

Up to 90% of dietary Thr is extracted by the portal-drained viscera (versus only about a third for other

Table 1: Basal diet fed of broilers from 1 to 14 day of age

Ingredients	Amount (%)
Corn	60.31
Soybean meal	28.3
Gluten meal	5.79
Poultry oil	1.22
Calcium carbonate	1.7
Dicalcium phosphate	1.19
Sodium chloride	0.23
Sodiun bicarbonate	0.26
Vitamins and minerals[1]	0.4
DL-Met	0.25
L-Lys HCl	0.25
L-Glutamic acid	0.1
Calculated composition	
CP, %	21.22
ME, kcal/kg	2952.95
Ca, %	1
Available P, %	0.45
Lys, %	1.1
TSAA, %	0.83
Thr, %	0.76
Leu,%	2
Val, %	0.9
Trp, %	0.23
Arg,%	1
IIe,%	0.8
His,%	0.5
Phe,%	1

[1]Permix provided the following per kilogram of diet: cholecalciferol 2,200 IU; vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B12, 11µg; choline, 780 mg; riboflavin, 5 mg; niacin, 33 mg; D-biotin, 1 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 7 mg; iodine, 1 mg; selenium, 0.2 mg.

Table 2: Effect of vitamin A and threonine on villus height and crypt depth of broilers

Treatments	Villus Height (µm)	Crypt Depth (µm)
T1	610.34[b]	143.00
T2	635.13[a]	150.07
T3	609.17[b]	144.01
SEM	18.1	5.2
P value	0.05	0.42

T_1: 1500 IU/kg vitamin A & 0.80% threonine, T_2: 6250 IU/kg vitamin A & 0.87% threonine, T_3: 11000 IU/kg vitamin A & 0.94% threonine

essential amino acids) (Stoll et al., 1998). Uni et al. (1998) reported that chicken have a very quick response to deficient vitamin A intake compared to rats and mice. Reports dealing with the effect of vitamin A on the intestinal histology in poultry are scarce. These authors concluded that deficiency of vitamin A probably caused loss of mucosal protein and reduced the height of villus and crypt depth.

Numerous reports in other animal models have documented, both *in vivo* and *in vitro* studies, that vitamin A deficiency or excess affect on the morphology and mucin production in the small intestine. Vitamin A deficiency caused a decreased in the number of goblet cells and villus height in chickens (Sepehri et al., 2010). Vitamin A and its derivates are required for maintenance of various epithelial tissues at the appropriate differentiated stages and small intestine is one of the tissues exhibiting rapid cell proliferation and differentiation (Tei et al., 2000).

From the present study, it is clear that the higher level of Thr and vitamin A than the recommended level of NRC (1994) improved the villus height in growing broiler chicks.

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