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Effect of Different Dietary Threonine Levels on Optimal Growth Performance and Intestinal Morphology in 1-14 Days Old Ross 308 Broilers

■Author(s)

Najafi R[I]
Ahmar R[I]
Tazehkand GN[II]

[I] Department of Animal Science, Agriculture Faculty, Urmia University, Urmia, Iran
[II] Department of Basic Science, Veterinary Faculty, Urmia University, Urmia, Iran

■Mail Address

Corresponding author e-mail address
Ramin Najafi
Urmia University - animal science
Pardis Nazloo Agriculture Faculty Urmia
57153 Iran (the Islamic Republic of)
Tel: (+98) 9121433581
Email: dr.najafiramin@gmail.com

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SUMMARY

This experiment was carried out to evaluate the effects of four levels of dietary threonine (Thr) supplementations on growth performance, gut size and histomorphometric alterations of small intestines in broiler chicks in 1-14 days. Two hundred eighty-eight Ross 308 one-day-old male broiler chicks were randomly assigned to four treatments with six replicates (12 birds per replication) received a common diet based on corn, wheat and soybean meal that met nutrients requirement of Ross 308. Birds were fed dietary treatments consisting of four levels of standardized digestible (SD) Thr: control diets (Basal diets) containing 0.65% SD Thr, 0.89% SD Thr (nutrients requirement of Ross 308), 0.93% and 0.97% SD Thr. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured at 1, 7 and 14 days old. Morphometric analysis of small intestine was carried out to investigate the effect of Thr levels on development of small intestines in starter period of broiler chicks. Results indicated that Thr levels improved FCR (quadratic effect, p=0.044) and BWG (quadratic effect, p=0.0009) for broilers given the diets containing 0.89% SD Thr in the starter period compared to other treatments (p<0.05). Furthermore, villus height and muscular thickness in duodenumand muscular thickness in jejunum were increased by this treatment (p<0.05). Villus width was increased by Thr treatments comparing to control group, as well. In conclusion, broiler performance and intestinal morphometry were improved by Thr supplementation which were efficiently obtained by 0.89% SD Thr in the first two weeks of the broiler´s diet.

INTRODUCTION

Accretion of feed ingredients cost and excretion of nitrogen into the environment are two important issues which world poultry nutritionists try to solve. Broiler's diets contain high levels of crude protein (CP) which is the most expensive diet ingredient after energy; also CP is the basic source of nitrogen excretion to environment. Cereals in comparison with soybean meal, corn or sorghum have lower cost and are extensively used as feed ingredients in broiler's diets, but we know that cereal's protein content is low. Fortunately, most of the amino acids are available in synthetic form and recent researches show that we may decrease crude protein levels of diet with limited amino acids supplementation (Kidd *et al*., 2001; Namroud *et al*., 2008). Currently, the commercially available amino acids for broiler's diets are methionine (Met), lysine (lys), and threonine (Thr) and their inclusion reduce dietary crude protein; keeping in mind that the Trp and Val are essential. Threonine is considered to be the third limiting amino acid in broiler's nutrition after total sulphur amino acids (TSAA) and lysine under practical feeding conditions (Kiddand Kerr, 1996). Threonine, like lysine, is limited in most cereals. Wheat, wheat midds, sorghum (milo), barley, meat and bone meals are low in Thr and their use may cause Thr to be a pressure point in poultry rations (Kiddand Kerr, 1996). The consequence is that if TSAA and lysine requirements are enough, maximum performance depends on the adequate supply of dietary Thr. Threonine serves as a variety of other functions in the organism, which sometimes makes difficult comparison and combination of trial results. For example, Thr plays an important role in feather synthesis not only as a component of the feather protein, but also as a precursor of glycine and serine. This implies an interaction between feather growth potential and dietary glycine, serine and Thr (Lemme, 2001).

There is scientific corroboration of Thr participation both in the composition of immunoglobulins as in the composition of mucin. It is one of the most important amino acids at the intestinal level for maintenance purposes too. The Gastrointestinal tract (GIT) is the main digestive and absorptive organ that permits the uptake of dietary substances into systemic circulation and it also excludes pathogenic compounds simultaneously (Gaskins, 1997). The GIT develops rapidly during the first few days post hatch; it was observed that duodenal villus growth was completed by day 7, whereas development of jejunum and ileum is continued until day 14 (Uni *et al*., 1998). In addition to this, it has been estimated that more than half of the dietary Thr consumed by a piglet or a human is retained at the intestinal level to fulfill these gut maintenance functions and is primarily used in the synthesis of mucin (Corzo *et al*., 2007).

Mucin is a glycoprotein which is produced by goblet cells that are distributed along the villi (Uni *et al*., 2003). Development of intestinal tract in the first few days post hatch can increase feed efficiency and growth in market age broiler chicken. Therefore, few evidence is available regarding the effects of Thr supplementation in gut development during early ages in broiler chickens. Hence, this study was conducted to investigate the effects of Thr on growth performance and development of small intestines during starter period in broiler chicks fed with wheat-corn-soybean meal based diets.

MATERIAL AND METHODS

Birds and management

A total of two hundred eighty-eight Ross 308, one-day-old male broiler chicks were obtained from a commercial hatchery, randomly allotted to four treatments with each treatment replicated six times (12 birds per replicate). Treatments consisted of four levels of SD Thr and six repetitions per treatment.

Diets

Before diet formulation, the content of amino acids of corn, wheat and soy bean meal were analysed (Evonik Degussa Lab[1]Germany). The formulation of the diets was calculated according to the standardized digestible (SD) amino acids content (Table 1). All the birds were fed ad libitum from 1 to 14 days of age with basal diets containing wheat, corn and soybean meal in mash form (Table 2). Based on Thr content of the diet, four groups were identified: 0.65% SD Thr (control diet containing 0.65% SD Thr without Thr supplementation), 0.89% SD Thr (standard recommended level of SD Thr for Ross 308 broilers), 0.93% SD Thr and 0.97% SD Thr containing higher levels of Thr in the diet.

Table 1 – Standardized digestible amino acid analysis of ingredients (%)*

Parameter	Soybean meal	Wheat	Corn
Dry matter	90.39	91.20	90.80
Crude protein**	43.50	9.981	8.011
Methionine	0.538	0.146	0.197
Cystine	0.531	0.211	0.144
Methionine + Cystine***	1.069	0.361	0.343
Lysine	2.355	0.260	0.201
Threonine	1.454	0.252	0.270
Tryptophan	0.525	0.110	0.080
Arginine	2.885	0.410	0.490
Isoleucine	1.743	0.304	0.295
Leucine	2.921	0.590	1.090
Valine	1.824	0.384	0.475
Histidine	1.060	0.211	0.200
Phenylalanine	1.941	0.391	0.421

*Evonik Degussa. Animal Nutrition Services. Amino acids and more. Lab code: NW2011/67882

** Standardized to dry matter content of 88.00%

***Met + Cys estimated with separate calibration equation

Performance parameters determination (body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)) and gut histomorphometrical examinations, were measured from 1 to 14 days. Chicks that died during the experiment were weighted and used to adjust weight gain and feed consumption data. At 15 days of age, two birds from each replicate, close to the average, per pen were randomly selected for

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Table 2 – Ingredients and calculated contents of the SD Thr deficient basal diet fed during 1 to 14 d of age

Ingredients	%
Yellow corn	38.30
Soybean meal	37.50
Wheat	15.00
Soybean oil	4.00
Vitamin-mineral premix[1]	0.5
Calcium carbonates	1.60
Dicalcium phosphate	1.90
NaHCO3	0.20
Sodium chloride	0.20
DL-methionine	0.40
L-lysine. HCl	0.40
L-threonine	0
Total	100
Calculated composition	%
ME, kcal/kg	2.93
Cp	21.06
Lys	1.31
Met	0.47
Thr	0.65
Met + Cys	0.99
Trp	0.21
Val	0.91
Arg	1.46
Ile	0.80
Gly + Ser	1.16
Phe + Tyr	1.24

[1] Provides per kg of diet : Vit A: 12000iu , Vit D3:5000iu , Vit E : 75 iu, Vit K3: 3 mg, Vit B1: 3 mg, VitB2: 8 mg, Vit B6: 5 mg, VitB12: 0.016 mg, Pantothenic acid: 13 mg, Niacin: 55 mg, Folic acid: 2 mg, Biotin: 0.2 mg, Cu: 16 mg, İ: 1.2mg, Se: 0.3 mg, Mn: 120 mg, Fe: 40 mg, Zn: 100 mg

processing (12 birds per treatment). However, healthy looking birds were considered for careful selection. The intestines were removed and the length of different segments of gut were measured and presented as a percentage of the small intestine, duodenum (from the craniodorsal sac of gizzard to the distal point of entry of the bile ducts), jejunum (from entry of the bile ducts to Meckel's diverticulum), ileum (from Meckel's diverticulum to the ileocecal junction) and rectum, then the guts were gently flushed with physiological saline solution to remove the intestinal content. For histomorphological analysis, approximately two cm of the middle portion of the duodenum, jejunum and ileum were excised and fixed in 10% buffered formalin for one week. Tissues were dehydrated by immersing through a series of alcohols of increasing concentration, infiltrated with xylene, and embedded in paraffin. The rotary type microtome was used for cutting the paraffin sections. The blocks were properly trimmed and the sections of seven micrometre thickness were cut. The tissue sections were stained by haematoxylin and eosin and periodic acid shift (PAS) for measurement of villus height, villus width, muscular thickness, goblet cell numbers and crypt depth (Uni *et al.*, 1995). All of the parameters were determined using an Image Analyser coupled with a Microscope (Image focus V2, Build707023 d). Twenty readings of those parameters were performed per intestinal segments. All of the specimens were studied by multiple magnifications (400X and 1000X) (Humuson, 1979). The experimental protocols were reviewed and approved by the Animal Care Committee of the Urmia University.

Statistical analyses

Data were statistically analysed as a completely randomized design by analysis of variance (General linear model) using the procedure of SAS software (SAS, 2003). Differences between means were computed using Duncan's multiple-range test. Only quadratic effects are presented on the respective tables, because significance ($p>0.05$) of higher-order polynomials was not observed.

RESULTS

Results indicated that Thr supplementation improved FCR and BWG in broilers fed with diets containing 0.89% SD Thr in the starter diet compared to other treatments. Increasing SD Thr from 0.65 to 0.89 g/kg diet improved BWG and FCR in the second week and 1 to 14 days period ($p<0.05$, quadratic). Increasing SD Thr to 0.97 g/kg diet had no significant improvement in BWG and FCR. Inclusion of threonine to basal diets didn't have significant ($p>0.05$) effect on feed intake at this period (Table 3).

Results of gut parts measurement did not show significant effects of Thr supplementation in this study. As indicated in Table 4, the relative lengths of duodenum, jejunum, ileum, and cecum were not significantly affected by Thr supplementation ($p>0.05$).

The effect of Thr supplementation on villus height, villus width, muscular thickness, goblet cell number and crypt depth of different sections of the small intestine of birds in the starter period is shown in Table 5. Supplementation of 0.89 g/kg diet of SD Thr increased significantly villus height and muscular thickness in the duodenum of broiler chicks and also muscular thickness in jejunum ($p<0.05$). Villus width in jejunum was affected by 0.93% SD Thr ($p<0.05$). None of the various levels of Thr was able to significantly impact goblet cell numbers and crypt depth in the duodenum, jejunum and ileum ($p>0.05$).

Table 3 – Effects of four levels of Thr on Feed intake, body weight gain and feed conversion rate in 1-7, 7-14 and 1-14 d of age

Treatments	FI (g/b)[a]			BWG(g)[b]			FCR[c]		
	1-7	7-14	1-14	1-7	7-14	1-14	1-7	7-14	1-14
Thr 0.65	101.56	255.31	356.88	94.37	157.29^B	251.66^B	1.07	1.63^A	1.42^A
Thr 0.89	102.75	271.50	374.25	100.34	193.05^A	293.40^A	1.02	1.40^B	1.27^B
Thr 0.93	101.31	258.06	359.41	96.32	167.22^B	263.54^B	1.05	1.55^A	1.37^A
Thr 0.97	104.25	271.75	376.01	100.38	165.90^B	267.91^B	1.02	1.64^A	1.40^A
SEM [d]	0.67	3.11	3.49	1.15	3.80	4.41	0.01	0.03	0.02
Quadratic	NA[e]	NA	NA	NA	0.003	0.009	NA	0.001	0.04
R^2	0.128	0.254	0.261	0.249	0.537	0.468	0.204	0.526	0.457

[a] Feed Intake (g/b)
[b] Body Weight Gain (g)
[c] Feed Conversion Rate
[d] Standard Error of means
[e] Not applicable due to lack of significance (p≤0.05) in the regression model
[A–B] Values with no common superscripts differ significantly (p<0.05) when tested with Duncan's multiple range test following ANOVA

Table 4 – Effect of Thr supplementation on relative gut parts size (per total intestine size) of broiler chicks

Treatments[a]	gut parts size (cm)				
	Duodenum	Jejunum	Ileum	Cecum	Rectum
Thr 0.65[b]	15.54	64.56	9.06	7.55	3.27
Thr 0.89	15.52	66.18	8.24	6.80	3.24
Thr 0.93	15.78	64.30	8.79	7.24	3.88
Thr 0.97	14.87	65.20	8.78	7.30	3.83
SEM [c]	0.41	0.63	0.20	0.19	0.12
Quadratic	NA[d]	NA	NA	NA	0.03
R^2	0.071	0.113	0.209	0.187	0.622

[a] Total standardized digestible threonine
[b] Control group
[c] Standard Error of means
[d] Not applicable due to lack of significance (p≤0.05) in the regression model
Values with no common superscripts differ significantly (p<0.05) when tested with Duncan's multiple range test following ANOVA

DISCUSSION AND CONCLUSIONS

The present study showed that 0.89% SD Thr in the diet appears to be adequate to sustain the performance compared to other treatments at day 14. Moghaddam *et al*. (2011) reported that Thr requirement in starter period for Ross (308) broilers, was 0.87 % SD, which was more than that of NRC recommendation (0.80 %). Zaghari *et al*. (2011) found that the inclusion of Thr in diets (between 0.65 and 0.90% SD Thr) can improve broiler performance, which is consistent with present results. However, these results are in disagreement with the findings of Kidd and Kerr (1997), Dozier *et al*. (2000) and Rama Rao *et al*. (2011) who reported that no improvement in performance was observed in broilers fed with diets supplemented with crystalline Thr. These controversial reports may be affected by several important causes which were mentioned here. A point which should be considered in the results of the researches, is the protein content of experimental diets. The amount of protein and digestibility of amino acids of experimental diets can influence Thr requirement of broilers. In our study, SD Thr increased from 0.65 to 0.89 has improved BWG and FCR in the second week and 1 to 14 days during the growth period (p<0.05, quadratic). Zaghari *et al*. (2011) reported that there were significant interactions between CP and Thr for FI, BWG and FCR during 1 to 21 days of age. The interaction between dietary CP and Thr for growth performance was in agreement with works of Nakajima *et al*. (1985) and Holsheimer *et al*. (1994).

Another factor that may affect Thr requirement is the ratio between Thr and Lys. In the present study the ratio 0.67 is closer to the levels suggested by others (Baker and Han, 1994; Mack *et al*. 1999; Rama Rao *et al*. 2011) (0.63, 0.64 and 0.67, respectively). A ratio SD Thr: Lys at 65% optimizes weight gain and feed efficiency of broilers for the overall period of 0-42 days of age, which is in agreement with recent find-

Table 5 – Effect of Thr supplementation on villus height (µm), villus width (µm), muscular thickness (µm), crypt depth (µm), goblet cell number (in each 0.25 mm²) of different sections of small intestine in 14 d of age

Duodenum	villus height	villus width	crypt depth	muscular thickness	Goblet cell number
Thr 0.65[b]	1350.33[B]	101.00	127.00	177.33[B]	634.67
Thr 0.89	1551.67[A]	96.00	124.66	190.66[A]	634.33
Thr 0.93	1313.00[B]	103.67	125.00	162.66[C]	606.67
Thr 0.97	1358.00[B]	104.33	125.00	161.33[C]	625.67
SEM [c]	33.07	2.14	1.14	3.97	9.83
Regression Linear	NA[d]	NA	NA	0.002	NA
Quadratic	0.09	NA	NA	0.09	NA
R^2	0.719	0.212	0.059	0.825	0.121
Jejunum	villus height	villus width	crypt depth	muscular thickness	Goblet cell number
Thr 0.65	1082.00	91.00[C]	104.00	159.33[B]	861.00
Thr 0.89	1069.00	96.66[B]	108.00	163.66[A]	851.67
Thr 0.93	1069.67	101.66[A]	108.00	158.33[B]	869.67
Thr 0.97	1073.33	98.00[B]	109.00	160.00[AB]	868.33
SEM	16.20	1.79	2.60	1.84	10.90
Quadratic	NA	NA	NA	NA	NA
R^2	0.009	0.416	0.049	0.108	0.039
Ileum	villus height	villus width	crypt depth	muscular thickness	Goblet cell number
Thr 0.65	696.33	111.00	89.66	150.00	1037.33
Thr 0.89	679.67	109.00	86.33	146.33	1030.00
Thr 0.93	685.00	104.66	86.66	146.66	1032.33
Thr 0.97	681.00	109.33	91.00	144.66	1050.33
SEM	6.95	2.27	1.70	1.43	18.82
Quadratic	NA	NA	NA	NA	NA
R^2	0.080	0.096	0.123	0.167	0.015

[a] Total standardized digestible threonine [b] Control group

[c] Standard Error of means

[d] Not applicable due to lack of significance (p≤0.05) in the regression model

[A–C] Values with no common superscripts differ significantly (p<0.05) when tested with Duncan's multiple range test following ANOVA

ings (Rostagno *et al*. 2005). Samadi & Liebert (2008) observed that a positive correlation between age and total Thr:Lys requirement (from 73% to 80% with age varying from 0 to 8 weeks in a total basis) in modelling Thr requirement of Ross 308 broilers. Non- starch polysaccharide (NSP) in wheat is another factor which may have influenced our study results. In this experiment, basal diets contained 0.15% wheat, which have no enzyme supplementation and also wheat contains about 11% total NSP, 80% of which is insoluble NSP (Smits & Annison, 1996). On the other hand, viscosity of digesta is a limitation in using wheat in poultry rations. In the two first weeks of age, the gastrointestinal tract, especially the small intestine´s epithelium is not completely matured (cellularity and enzymology), for this reason the chicks can't face any inconvenient material such as NSP in their diets (Peterson *et al*. 1976; Mcnab & Smithard, 1992). Wheat contains variable amounts of NSP, such as arabinoxylans, which can interfere with nutrient digestibility.

Thr is an important amino acid because of the role it plays in the intestinal tracts. It´s role is heavily associated with mucins; Amino acid backbone. Mucins are glycosylated proteins, that are the main component of mucous layers that cover intestinal epithelium. Mucin is secreted by goblet cells. The main function of mucus layer is to protect the epithelium from bacteria, acidic chyme and digestive enzymes. Thr represents more than 40% of their amino acid residues (Bengmark & Jeppsson, 1995). A significant portion of mucin also contains serine (Bengmark & Jeppsson, 1995). Wang *et al*. (2007) reported that a dietary deficiency or excess in Thr reduces the synthesis of mucosal protein and mucins as well as muscle protein in weaned pigs. Threonine can be transaminated to glycine and serine, whereas the reverse pathway has been shown to be negligible (Baker *et al*. 1972). The effect of an oral deficiency of Thr on gut function (mucosal mass, mucin production, small intestine histomorphological parameters) was evaluated by Law *et al*. (2007). Supplementation of SD Thr up to 0.97% had no significant effect on length of duodenum, jejunum, ileum, cecum and colon. Zaghari *et al*. (2011) reported that significant differences were found between interaction of CP and Thr for relative weight and length of duodenum and jejunum. Law *et al*. (2000) and Ball (2001) observed that piglets receiving diets deficient in Thr, decreased intestinal weight and had less intestinal structure development in comparison with control groups.

Results of this study indicated that the inclusion of Thr to the diets, influenced significantly the villus height and muscular thickness in duodenum and villus width and muscular thickness in jejunum of broilers in starter phase ($p<0.05$). Zaghari *et al*. (2011) found that Thr supplementation significantly affected villus height, muscular thickness, goblet cell number and crypt depth in duodenum, jejunum and ileum of broilers at 1 to 21 days of age. However, in this study, no effects of Thr levels on goblet cells were observed. Our results were also in consistence with the report of Ball (2001), who found that piglets receiving Thr deficient diets had lower villus height than those receiving Thr adequate diet. Approximately, 30-50% of arginine, proline, isoleucine, valine, leucine, Met, Lys, phenylalanine, glycine, serine and Thr may be used through the small intestines and as a result would not be available for extra intestinal tissues (Wu, 1998). Not withstanding, several literature references (Stoll *et al*. 1998; Burrin *et al*. 2001; Bertolo *et al*., 1998) describe that about 40-50% of the threonine intake is used in animal's gut. This implies that a part of threonine requirement is not associated with muscle protein deposition, but is essential for adequate gut functions. In fact, the intestine seems to contribute extensively in threonine metabolism. Furthermore, Stoll *et al*. (1998) showed that approximately 90% of threonine used by the intestine was either secreted as mucosal protein or catabolized. The villi height in duodenum was greater than those in the jejunum and ileum, and this is consistent with the major role of duodenum in nutrient absorption (Zaghari *et al*. 2011). Wang *et al*. (2007) reported that both deficiency and excess of dietary Thr, reduced the synthesis of intestinal mucosal protein and mucins in young pigs. Digestive secretions and especially mucins are rich in Thr (Le Bellego *et al*. 2002). Consequently, an increase in dietary provision of Thr and other amino acids can promote mucin synthesis and reequilibrate the gut microbiota in favour of intestinal protection and mucosal healing.

The data of the present experiment suggested that broilers require 0.89% standardized digestible Thr during starter period (1-14 days of age). Intestinal lengths were not affected by Thr supplementation. Data indicated that Thr has a high impact on gut morphology of chicks. Villus height and muscular thickness in duodenum, muscular thickness and villus width in jejunum were affected by dietary Thr supplementation.

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