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Standardized Ileal Digestible Threonine Requirements and Its Effects on Performance and Gut Morphology of Broiler Chicks Fed Two Levels of Protein

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ABSTRACT

The objective of this study was to determine the effects of 8 levels of threonine (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 and 1.1%) and 2 levels of protein (17.5 and 20.5%) on growth performance, gut sizes and morphology as well as to estimate Standardized Ileal Digestible (SID) Thr requirements in Ross 308 males at 0 to 21 days of age. Chicks were randomized into 64 battery cages (5 chicks per replicate). FI was lower for broiler given the high CP diets as compared to those fed on low CP diets. BWG and FCR improved up to 0.7% Thr in both CP levels. Fitted broken lines indicated break points at 0.62 and 0.66% SID Thr for weight gain at 17.5 and 20.5% crude protein, respectively. Significant interaction was found between CP and Thr on relative weight and length of duodenum and jejunum ($P< 0.05$). Thr supplementation significantly affected villus height, epithelial thickness, goblet cell number and crypt depth in duodenum, jejunum and ileum ($P< 0.01$). Low CP diets adequate in Lys, Total Sulfur Amino Acid (TSAA) supplemented with Thr may result in optimal BWG and FCR as well as in growth of intestinal length. Such parameters of gut functionality as microvilli height, crypt depth and epithelial thickness seemed to be improved with even higher levels of dietary SID Thr levels.

Keywords: Broiler Chicks, Gut Functionality, Standardized Ileal Digestibility (SID), Threonine.

INTRODUCTION

Synthetic amino acid supplementation to the poultry diet can reduce the cost of feed and total nitrogen leak into the environment. On the other hand supplemental amino acids other than Met and Lys (e.g. Thr, Arg and Val) may be required to support optimal growth and feed conversion as crude protein is decreased. Thr is typically the third limiting amino acid following TSAA and Lys in commercial broiler diets composed of corn or sorghum, soybean meal, and meat meal (Kidd and Kerr, 1996; Kidd, 2000), having an important role in structure and function of gastrointestinal tract (Law *et al*., 2000; Ball, 2001).

There has been much research on Thr and the possible interaction between CP and Thr (Holsheimer *et al*., 1994; Kidd *et al*., 2001; Ciftci and Ceylan, 2004). Holsheimer *et al*. (1994) stated that dietary CP content did not significantly affect Thr requirements. Ciftic and Ceylan (2004) concluded that Thr and CP had a significant interaction on growth performance of broiler chicks. They also stated that Thr supplementation at the level of optimum supply to Low CP-Thr deficient diets resulted in similar growth performance, feed efficiency, carcass traits and meat composition to those of chicks given practical high CP maize- soybean meal diets.

In order to more precisely formulate feed and for a better control of animal

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performance, both the recommendation and feed formulation should be based on digestible rather than total dietary amino acids. Foremost approach to estimate amino acid availability in feed ingredients is ileal digestibility. Standardized ileal digestibility assay eliminates some of the shortcomings of fecal digestibility, correction being made for basal endogenous losses (Lemme *et al*., 2004). There is data set for establishment of the standardized digestibility system in broiler feeding (Lemme *et al*., 2004). Lemme *et al*. (2004) stated that optimum SID Thr levels for broiler chicks at 1-5, 6-14 and 15-35d of age are 0.82, 0.80 and 0.72%, respectively.

Although there has been much research on Thr requirement and its effects on growth performance, literature on SID Thr requirement with different CP levels and also the effects of Thr on digestive tract measurements and gut morphology are sparse as well as limited. The aim of this study was to estimate SID Thr requirement with either low or high CP levels and to evaluate Thr response on growth performance and gut measurement levels as well as morphology of broiler chicks during 0 to 21 days of age.

MATERIALS AND METHODS

Experimental Materials and Procedures

The experiment followed a 2×8 factorial design with two levels of CP (17.5, and 20.5%), and 8 levels of Thr (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, and 1.1%). Three hundred and twenty Ross (308) day-old feather-sexed male broiler chicks with the same average initial body weight (46±0.5 g) were randomly allocated into 16 dietary treatments. Each treatment was assigned to 4 replicate cages with 5 birds each. Each cage contained feeder as well as one tube waterer. Experimental diets, in mash form, and water were offered *ad libitum.* Broiler chicks were provided incandescent light for 24 hours per day. The experimental facilities were a curtain-sided cage with infrared brooding lamps. The temperature was maintained at 33°C for day 1, decreasing at 2°C per week.

The amino-acid contents of the corn and soybean meal were determined using ion-exchange chromatography following hydrolysis with hydrochloric acid for 24 hours at 110°C. The amino acids were identified and quantified using a standard amino-acid solution. Cystine and methionine were analyzed as cysteic acid and methionine sulfone through oxidation with performic acid. Crude protein and dry matter contents of the samples were determined according to AOAC (1990). SID amino acids were calculated using standardized ileal digestibility coefficient of Lemme *et al.* (2004) and by assuming 100% digestibility of synthetic amino acids. Crude protein, standardized ileal CP and amino acid digestibility values (%) of feed ingredients were used for experimental diets. Essential amino acids were stipulated at recommended SID amino acid levels for 0 to 21 days of age (Lemme *et al*., 2004). All diets contained similar ME and electrolyte balance (Table 1).

Cage weight was determined and FI evaluated at 21 day of age. Mortality was recorded throughout the experimental period with dead birds weighed to calculate mortality corrected feed conversion ratios. FCR was calculated by dividing cage FI by cage weight gain.

At 21 day of age, two birds from each cage (8 per treatment) nearest to the mean cage weight were selected and weighed for digestive tract measurements and gut morphology. The birds were killed by use of CO_2 and the digestive tract carefully excised. The intestinal contents gently flushed by distilled water; the empty weight and length of Duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to ileocaecal junction) were recorded. Approximately 1cm lengths of duodenum (midpoint of the pancreatic loop), 2cm length of jejunum (midpoint of jejunum) and 3 cm length of ileum (after Meckel's diverticulum) were

Table 1. Composition and nutrient content of the experimental diets.

Ingredient	Thr percentage in low CP diets								Thr percentage in high CP diets							
	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1
Soybean meal	13.55	13.58	13.61	13.65	13.68	13.71	13.74	13.77	14.33	14.33	14.32	14.32	14.31	14.31	14.30	14.30
Corn	76.69	76.71	76.72	76.73	76.74	76.75	76.76	76.77	70.01	70.04	70.08	70.12	70.15	70.19	70.22	70.26
Corn oil	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.79	0.78	0.78	0.77	0.76	0.75	0.74	0.74
Dicalcium P	2.38	2.38	2.38	2.38	2.38	2.38	2.38	2.38	2.40	2.40	2.40	2.40	2.40	2.40	2.40	2.40
Limestone	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
$NaHCO_3$	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
L-Lys	0.97	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
L-His	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
L-Leu	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.31	0.31	0.31	0.31	0.31	0.31	0.31	031
L-Ile	0.44	0.44	0.44	0.43	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
L-Arg	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
L-Trp	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-Thr	0.01	0.11	0.21	0.31	0.41	0.51	0.61	0.71	0.01	0.11	0.21	0.31	0.42	0.52	0.62	0.72
L-Phe	0.31	0.31	0.31	0.31	0.30	0.30	0.30	0.30	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
L-Glu	1.23	1.09	0.96	0.82	0.69	0.55	0.42	0.28	6.46	6.34	6.21	6.09	5.96	5.84	5.71	5.59
L-Val	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated analysis																
ME, kcal/kg	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
CP %	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
Ca %	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P % available	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Na %	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
DEB mEq / kg[c]	152	152	153	153	153	153	154	154	152	152	152	152	152	152	152	152
SID[d] Lys %	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
SID Met %	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
SID Met+Cys %	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
SID Thr %	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1
SID Trp %	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
SID Ile %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
SID Lue %	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37
SID Arg %	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32
SID Val %	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
SID Phe + Tyr %	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34
SID His %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

[a] Vitamin premix provided the following per kilogram of diet: Vitamin A: 5,600 IU from all *trans*-retinyl acetate; Cholecalciferol: 2000 IU; Vitamin E: 20 IU from all-*rac*-α-tocopherol acetate; Nboflavin: 3.2 mg; Ca pantothenate: 8 mg; Nicotinic acid: 28 mg; Choline Cl: 720 mg; Vitamin B12: 6.4 μg; Vitamin B6: 1.6 mg; Menadione: 1.6 mg (as menadione sodium bisulfate); Folic acid: 0.8 mg; D-biotin: 0.06 mg; Thiamine: 1.2 mg (as thiamine mononitrate); Ethoxyquin: 125 mg.
[b] Trace mineral premix provided the following in milligrams per kilogram of diet: Mn, 40; Zn, 32; Fe, 32; Cu, 3.2; I, 1.2; Se, 0.06.
[c] Represents dietary electrolyte balance as defined by dietary Na+K-Cl (in mEq kg^{-1} of diet).
[d] Represents standardized ileal digestible amino acids.

removed for gut morphological measurements. Intestinal samples were flushed with deionized water and immediately placed in Bouin's fluid. Histological processing was done according to the method of Iji *et al*. (2001) and Wu *et al*. (2004). Measurement of villus height, crypt depth, goblet cell number and epithelium thickness were made at 100 to 400×magnification using computer software (Wu *et al*., 2004). Relative weight of different sections of small intestine were measured as fraction of carcass weight.

Statistical Analysis

The effects of eight levels of threonine and two levels of protein on broiler chicken performance were analyzed using analysis of variance procedure (SAS, 2001). Significant differences were assessed among means using the least-significant difference procedure. The threonine requirements were fitted into one-slope broken-line (Robbins, 1986), exponential and quadratic models (Schuttle and Pack, 1995). When exponential responses occurred, optimization was achieved by extrapolating 95 % of the asymptote.

RESULTS AND DISCUSSION

Analyzed CP, Thr and other essential amino acids were in close agreement with calculated values from the analyzed ingredient compositions (Table 2).

Growth Performance of Broiler Chicks

All performance data for 21 day of age are shown in Table 3. During 0 to 21 days of age supplemented Threonine significantly ($P< 0.001$) improved FI, BWG and FCR. CP level had significant effect on FI ($P< 0.001$), BWG ($P< 0.01$) and FCR ($P< 0.001$). Birds fed high CP diet had lower FI and better BWG and FCR as compared with those fed on low CP diets. As dietary CP decreased most non essential amino acids tended to decline. It is possible that some such non essential amino acid as Gly and Ser become limiting. In agreement with the current study, Waterhouse and Scott (1961) found that the effect of Gly was more pronounced at the lower level of protein. Deficiency of Gly and Ser could be a reason for higher FI and lower performance of broiler chicks fed low CP diet. Another reason for lower FI in chicks fed high CP diet may be due to high Glu content of diet. Although the low and high CP diets contained the same essential amino acid pattern, however, higher CP diet had an excess of non essential amino acid Glu. Dietary CP was increased by the addition of L-Glu. Fancher and Jenson (1989) stated that dietary inclusion of L-Glu for increasing CP level decreased FI.

During 0 to 21 days of age there were significant interactions between CP and Thr for FI ($P< 0.01$), BWG ($P< 0.01$) and FCR ($P< 0.001$). Broiler chickens fed either low or high CP diet supplemented with 0.7 and 0.8% Thr bore significantly higher FI than other treatments. BWG was augmented with 0.7% Thr on both the high and low CP diets. FCR was improved with increasing Thr level on both CP levels. Interestingly, when chickens received 17.5% CP diet supplemented with 0.7% Thr grew similarly to those receiving 20.5% CP. Moreover broiler chicks fed low CP diets supplemented with L-Thr had similar FCR as compared with those fed 20.5% CP. The interaction between dietary CP and Thr for growth performance was in agreement with Nakajima *et al*. (1985) and Holsheimer *et al*. (1994). They stated that supplementation of L-Thr with low CP diet which is adequate in TSAA and Lys for broiler improved FCR and brought about similar growth to the level obtained with a high CP basal diet. In the present experiment performance was not on a very high level which is attributed to feeding mash diets and the semi synthetic character of the diet. Compared to pellets, mash feeding usually results in a lower feed intake and thus growth which might have been strengthened by the diet composition.

Table 2. Results of amino acid analysis (total contents after hydrolysis of protein) compared with amino acid calculated.

Treatment	C/A[a]	Protein and mino acids (%)											
		Protein	Met	Cys	Met+Cys	Lys	Thr	Arg	Ile	Lue	Val	His	Phe
T1	C	16[b]	0.75	0.23	0.98	1.33	0.47	1.37	0.92	1.47	1.07	0.47	0.93
	A	17.57[c]	0.71	0.26	0.96	1.40	0.50	1.37	0.96	1.56	1.09	0.48	0.95
T2	C	16	0.75	0.23	0.98	1.33	0.57	1.37	0.92	1.47	1.07	0.47	0.93
	A	17.49	0.79	0.25	1.04	1.45	0.58	1.44	0.97	1.54	1.16	0.49	0.98
T3	C	16	0.75	0.23	0.98	1.33	0.67	1.37	0.92	1.46	1.07	0.47	0.93
	A	17.66	0.77	0.26	1.02	1.43	0.69	1.41	0.97	1.57	1.11	0.48	0.97
T4	C	16	0.75	0.23	0.98	1.33	0.77	1.37	0.92	1.46	1.07	0.47	0.93
	A	17.87	0.77	0.25	1.02	1.44	0.81	1.42	0.96	1.56	1.14	0.49	0.99
T5	C	16	0.75	0.23	0.98	1.33	0.87	1.37	0.92	1.46	1.07	0.47	0.93
	A	17.68	0.77	0.26	1.03	1.38	0.87	1.42	0.95	1.55	1.14	0.49	0.95
T6	C	16	0.75	0.23	0.98	1.33	0.97	1.37	0.92	1.46	1.07	0.47	0.93
	A	17.78	0.80	0.25	1.05	1.40	0.97	1.43	0.98	1.55	1.37	0.48	0.98
T7	C	16	0.75	0.23	0.98	1.33	1.07	1.37	0.92	1.46	1.07	0.47	0.93
	A	17.67	0.81	0.25	1.06	1.45	1.12	1.45	0.97	1.55	1.14	0.49	0.97
T8	C	16	0.75	0.23	0.98	1.33	1.17	1.37	0.92	1.47	1.07	0.47	0.93
	A	17.68	0.81	0.25	1.06	1.45	1.20	1.46	0.96	1.56	1.14	0.48	0.96
T9	C	19	0.75	0.23	0.98	1.33	0.47	1.37	0.92	1.46	1.07	0.47	0.94
	A	20.52	0.79	0.24	1.03	1.41	0.49	1.40	0.96	1.55	1.22	0.47	0.97
T10	C	19	0.75	0.23	0.98	1.33	0.57	1.37	0.92	1.46	1.07	0.47	0.94
	A	20.37	0.75	0.25	1.00	1.41	0.58	1.41	0.94	1.53	1.29	0.48	0.97
T11	C	19	0.76	0.22	0.98	1.33	0.67	1.37	0.92	1.46	1.07	0.47	0.94
	A	20.73	0.76	0.26	1.02	1.42	0.66	1.43	0.94	1.56	1.14	0.49	0.95
T12	C	19	0.76	0.23	0.98	1.33	0.77	1.37	0.92	1.46	1.10	0.47	0.94
	A	20.72	0.74	0.25	0.99	1.40	0.77	1.41	0.94	1.54	1.30	0.48	0.97
T13	C	19	0.76	0.23	0.98	1.33	0.87	1.37	0.92	1.46	1.06	0.47	0.94
	A	20.59	0.76	0.25	1.01	1.41	0.86	1.40	0.94	1.54	1.30	0.48	0.97
T14	C	19	0.76	0.22	0.98	1.33	0.97	1.37	0.92	1.46	1.07	0.47	0.94
	A	20.30	0.73	0.25	0.98	1.39	0.93	1.39	0.95	1.55	1.29	0.47	0.97
T15	C	19	0.76	0.22	0.98	1.33	1.07	1.37	0.92	1.46	1.07	0.47	0.94
	A	20.62	0.79	0.24	1.03	1.42	1.08	1.41	0.93	1.53	1.30	0.48	0.97
T16	C	19	0.76	0.22	0.98	1.33	1.17	1.37	0.92	1.46	1.07	0.47	0.94
	A	20.19	0.75	0.25	1.00	1.39	1.13	1.38	0.93	1.55	1.26	0.47	0.97

[a] C Represents calculated and A represents analysed values.
[b] C values for protein % are based on SID protein.
[c] A values for protein % are based on crude protein content.

Table 3. Effect of different levels of dietary CP (Crude Protein) and threonine on FI (feed intake) and growth performance during 0 to 21 d of age.

Treatment	CP (%)	Thr (%)	Feed intake (g bird^{-1})	Body weight gain (g bird^{-1})	FCR[a] (g g^{-1})
T1	17.5	0.4	381.6^{f*}	144.3^{f}	2.740^{c}
T2	17.5	0.5	628.3^{d}	245.5^{e}	2.590^{c}
T3	17.5	0.6	757.2^{ab}	360.6^{d}	2.120^{b}
T4	17.5	0.7	819.4^{a}	529.4^{a}	1.545^{a}
T5	17.5	0.8	799.0^{a}	509.4^{ab}	1.572^{a}
T6	17.5	0.9	790.7^{a}	514.7^{ab}	1.537^{a}
T7	17.5	1.0	787.0^{ab}	499.1^{ab}	1.580^{a}
T8	17.5	1.1	786.4^{ab}	464.8^{cb}	1.705^{a}
T9	20.5	0.4	292.0^{g}	223.0^{e}	1.305^{a}
T10	20.5	0.5	493.8^{e}	330.9^{d}	1.515^{a}
T11	20.5	0.6	673.9^{cd}	436.9^{c}	1.542^{a}
T12	20.5	0.7	762.7^{ab}	541.1^{a}	1.410^{a}
T13	20.5	0.8	809.9^{a}	519.5^{ab}	1.560^{a}
T14	20.5	0.9	797.8^{a}	512.9^{ab}	1.557^{a}
T15	20.5	1.0	779.2^{ab}	485.6^{abc}	1.607^{a}
T16	20.5	1.1	723.2^{cb}	435.6^{c}	1.695^{a}
SEM			20.5	17.0	0.1200
Main effect means					
CP					
T1 to T8	17.5	All	718.7^{a}	408.5^{b}	1.924^{b}
T9 to T16	20.5	All	666.6^{b}	435.7^{a}	1.524^{a}
SEM			7.2	6.0	0.0424
Thr					
T1 and T9	Both	0.4	336.8^{e}	183.6^{f}	2.022^{c}
T2 and T10	Both	0.5	561.1^{d}	288.2^{e}	2.052^{c}
T3 and T11	Both	0.6	715.6^{c}	398.7^{d}	1.831^{bc}
T4 and T12	Both	0.7	791.0^{ab}	535.3^{a}	1.477^{a}
T5 and T13	Both	0.8	804.4^{a}	514.5^{ab}	1.566^{a}
T6 and T14	Both	0.9	794.2^{ab}	513.8^{ab}	1.547^{a}
T7 and T15	Both	1.0	783.1^{ab}	492.4^{b}	1.593^{ab}
T8 and T16	Both	1.1	754.8^{cb}	450.2^{b}	1.700^{ab}
SEM			14.5	12.0	0.084

[*] Means within the same column with no common superscripts are significantly different (P< 0.05).
[a] Feed intake/growth.

Thr Requirement

Thr requirements within 2 CP levels for optimum FI, BWG and FCR during 0 to 21 days of age were estimated by different models (Table 4). Fitted broken lines estimated 0.62 (r^2= 0.99) and 0.66% (r^2= 0.99) SID Thr requirement for BWG at 17.5 and 20.5% dietary CP, respectively. High CP diets increased Thr requirement for weight gain at 0 to 21 days of age. This supports the reports of Robbins (1987) who found that Thr requirement of chickens fed high CP diet (20% CP) was 29% higher than that of chickens fed low protein diet (15% CP). He suggested that Thr requirement increases as dietary CP increases. Ciftci and Ceylan (2004) reported higher Thr requirement for low CP diets than those of high CP diets for BWG. Davis and Austic (1982) stated that excess of some dietary amino acids or mixtures of amino acids in Thr deficient diets increased the Thr

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Table 4. Estimated SID (Standardized Ileal Digestible) threonine requirements for broiler chicks performance at two dietary CP (Crude Protein) levels.

CP levels (%)	Model	Trait	Regression equation	Requirement
17.5	Broken Line[a]	Weight gain	$y=566.2-1480\ (0.62-X_{Lr})$	0.62 (0.99)[*]
17.5	Broken Line	Feed intake	-	-[**]
17.5	Broken Line	FCR	$y=1.59+4.05\ (0.69-X_{Lr})$	0.69 (0.97)
17.5	Exponential[a]	Weight gain	$y=226.9+341.7[1-e^{-9.57(x-0.4)}]$	0.71 (0.92)
17.5	Exponential	Feed intake	$y=377+424.2[1-e^{-10.28(x-0.4)}]$	0.69 (0.94)
17.5	Exponential	FCR	$y=2.351-0.745[1-e^{-13.56(x-0.4)}]$	0.72 (0.72)
17.5	Quadratic[a]	Weight gain	$y=-504.2+2193X-1424.4X^2$	0.87 (0.86)
17.5	Quadratic	Feed intake	$y=-566.9+3249X-1871.8X^2$	0.87 (0.99)
17.5	Quadratic	FCR	$y=7.124-12.66X+7.075X^2$	0.89 (0.74)
20.5	Broken Line	Weight gain	$y=564.2-1484\ (0.66-X_{Lr})$	0.66 (0.99)
20.5	Broken Line	Feed intake	-	-
20.5	Broken Line	FCR	$y=1.56+0.257(0.70-X_{Lr})$	0.70 (0.99)
20.5	Exponential	Weight gain	$y=325+233.9[1-e^{-11.1(x-0.4)}]$	0.76 (0.70)
20.5	Exponential	Feed intake	-	-
20.5	Exponential	FCR	-	-
20.5	Quadratic	Weight gain	$y=-778.4+3100.5X-1755.3X^2$	0.88 (0.90)
20.5	Quadratic	Feed intake	$y=-858.2+3830.9X-2185.9X^2$	0.87 (0.85)
20.5	Quadratic	FCR	-	-

[a] Broken-line, exponential and quadratic models were described by Robbins (1986) and Schutte and Pack (1995).
[*] Values in parentheses show r^2.
[**] Represents data that did not fit the models.

requirement. These results were in contrast with results obtained from the present study.

Estimated SID Thr requirements within 2 CP levels based on exponential and quadratic response curves were much higher than those obtained from broken lines (Table 4). Visual assessment suggests responses rather to be one slope broken line while the respective broken-line regression revealing excellent r^2 values than the exponential and quadratic models. Moreover, breakpoints of fitted broken lines predict minimal requirement values, and this is viewed as desirable for calculating AA ratios. Another advantage of the fitted broken-line approach is that the inflection point of the fitted line is established objectively rather than subjectively. The disadvantage of quadratic fits for AA requirement studies is that quadratic fits do not establish objective breakpoints (Baker *et al*., 2002).

Gut Measurements and Histology

Supplementation of Thr up to 0.7% had significantly (P< 0.05) increased weights of duodenum and jejunum (Table 5). Low CP diets significantly reduced (P< 0.05) relative weight of duodenum, jejunum and ileum. Thr supplementation and interaction between CP and Thr had no significant effect on relative weight of ileum (P> 0.05). However, significant differences were found between interaction of CP and Thr for relative weight and length of duodenum and jejunum (P< 0.05). Law *et al.* (2000) and Ball (2001) showed that piglets receiving diets deficient in Thr had decreased intestinal weight and had developed less intestinal structure. The relatively higher duodenal, jejunal and ileunal weight of birds fed high Thr level may lie in the high utilization of some amino acids in the small intestine. Amino acids maintain intestinal viability and mass, in addition to providing energy for normal intestinal function. As gastrointestinal tissues have relatively high protein turnover rate, high protein diet provides a nutrient (CP) for basal

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Table 5. Effect of threonine and CP (Crude Protein) on relative length (cm per kg carcass weight) and weight (% of carcass weight) of different sections of the intestine of broiler chicks.

Treatment	Relative length			Relative weight		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
T1	19.11^{dc*}	41.50^{c}	36.90^{cd}	1.38^{d}	2.29^{d}	1.81
T2	21.62^{abc}	49.31^{b}	43.31^{bc}	1.75^{bc}	2.71^{abcd}	1.70
T3	23.06^{abc}	50.12^{b}	43.75^{abc}	1.75^{bc}	2.44^{cd}	1.73
T4	22.50^{abc}	51.50^{ab}	44.25^{abc}	1.75^{bc}	2.64^{bcd}	1.72
T5	23.65^{ab}	52.82^{ab}	47.00^{ab}	1.57^{cd}	2.73^{abcd}	1.85
T6	22.10^{abc}	53.37^{ab}	48.50^{ab}	1.65^{c}	2.65^{cd}	1.84
T7	23.23^{abc}	52.15^{ab}	46.56^{ab}	1.57^{cd}	2.52^{cd}	1.72
T8	23.37^{abc}	53.15^{ab}	45.31^{abc}	1.56^{cd}	2.39^{cd}	1.73
T9	17.99^{c}	36.50^{c}	32.90^{d}	2.03^{a}	3.12^{a}	1.98
T10	21.56^{bc}	50.12^{b}	40.06^{c}	2.00^{a}	3.10^{ab}	1.94
T11	21.04^{cd}	49.57^{b}	42.58^{bc}	2.04^{a}	2.88^{abc}	1.98
T12	22.42^{abc}	53.56^{ab}	46.06^{ab}	1.94^{abc}	3.12^{a}	2.00
T13	23.05^{abc}	51.5^{ab}	45.56^{abc}	1.78^{abc}	2.78^{abc}	1.77
T14	23.49^{abc}	56.18^{a}	49.37^{a}	1.73^{c}	2.88^{abc}	1.83
T15	23.98^{a}	49.18^{b}	43.43^{bc}	1.79^{abc}	2.60^{bcd}	1.67
T16	22.67^{abc}	53.65^{ab}	45.77^{ab}	1.85^{abc}	2.98^{ab}	1.98
SEM	0.851	1.990	2.020	0.098	0.161	0.116
Main effect means						
CP						
T1 to T8	22.33	50.49	44.44	1.62^{b}	2.55^{b}	1.76^{b}
T9 to T16	22.03	50.03	43.22	1.89^{a}	2.93^{a}	1.89^{a}
SEM	0.300	0.703	0.714	0.034	0.056	0.412
Thr						
T1 and T9	18.55^{c}	39.00^{c}	34.90^{d}	1.71^{ab}	2.71^{ab}	1.89
T2 and T10	21.59^{b}	49.71^{b}	41.90^{c}	1.87^{ab}	2.91^{a}	1.82
T3 and T11	22.04^{ab}	49.84^{b}	43.17^{bc}	1.89^{a}	2.67^{ab}	1.86
T4 and T12	22.46^{ab}	52.53^{ab}	45.16^{abc}	1.89^{a}	2.88^{a}	1.87
T5 and T13	23.35^{a}	52.16^{ab}	46.28^{ab}	1.68^{b}	2.76^{ab}	1.81
T6 and T14	22.79^{ab}	54.78^{a}	48.93^{a}	1.69^{b}	2.77^{ab}	1.84
T7 and T15	23.61^{a}	50.66^{ab}	45.00^{abc}	1.69^{b}	2.56^{b}	1.70
T8 and T16	23.02^{ab}	53.40^{ab}	45.54^{abc}	1.71^{ab}	2.69^{ab}	1.86
SEM	0.601	1.409	1.429	0.069	0.113	0.082

* Means within the same column with no common superscripts are significantly different (P< 0.05).

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metabolism and causes a developed small intestine. On the other hand Thr is of vital nutritional importance, because it is the single most used essential amino acid through a metabolism of portal-drained visera (Schaart *et al*., 2005).

The effect of CP and Thr supplementation, individually or in combination with villus height, epithelial thickness, goblet cell number and crypt depth of different sections of small intestine of birds fed experimental diets are shown in Table 6. Thr supplementation had significant (P< 0.01) effects on villus height, epithelial thicknesses, goblet cell number and crypt depth in duodenum, jejunum and ileum. CP level had significant effects on villus height in duodenum (P< 0.001), jejunum (P< 0.05) and ileum (P< 0.001), goblet cell number in jejunum (P< 0.01) and ileunal crypt depth (P< 0.05). Interaction of CP and Thr was found to be significant on villus height,

Table 6. Effect of dietary threonine, CP (Crude Protein) and their interaction on villus height (µm), epithelial thickness (µm), goblet cell number (per 100 µm villus height) and crypt depth (µm) of duodenum in 21 day of age.

Treatment	Villus height			Epithelial thickness			Goblet cell number			Crypt depth		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
T1	1741.8^{f}	841.2^{cd*}	788.8^{ef}	45.6^{a}	38.2^{abc}	38.4^{ab}	9.6^{a}	10.2^{a}	10.4^{ab}	146.4^{ef}	109.6^{fg}	96.2^{gh}
T2	1743.2^{f}	848.0^{cd}	813.6^{cdef}	41.8^{ab}	39.2^{ab}	35.4^{ab}	9.0^{ab}	9.6^{ab}	11.0^{a}	149.0cdef	115.8^{cdef}	99.2^{efgh}
T3	1747.4^{f}	839.0^{d}	802.8^{def}	38.8^{bc}	39.2^{ab}	36.6^{ab}	8.6^{abc}	9.0^{abc}	8.6^{bcde}	154.6^{bc}	123.2^{bcd}	134.4^{a}
T4	1780.4^{ef}	842.8^{cd}	831.8^{cde}	35.8^{c}	35.8^{bcde}	32.8^{ab}	7.8^{abcd}	9.0^{abc}	7.8^{cdef}	154.2^{bcd}	122.4^{bcde}	134.6^{a}
T5	1787.4^{ef}	854.8^{bcd}	781.0^{f}	38.4^{bc}	39.0^{ab}	34.8^{ab}	8.8^{ab}	8.8^{abc}	9.0^{bc}	146.8^{def}	111.4^{f}	108.4^{def}
T6	1819.6^{ed}	891.8^{a}	779.2^{f}	39.8^{bc}	33.4^{cde}	33.8^{ab}	8.6^{abc}	8.8^{abc}	7.4^{cdef}	151.2^{cde}	115.4^{cdef}	101.4^{defgh}
T7	1910.8^{bc}	894.8^{a}	850.8^{bc}	39.8^{bc}	31.4^{e}	33.6^{ab}	7.6^{bcd}	7.4^{cd}	7.4^{cdef}	150.6^{cde}	123.6^{abcd}	110.0^{cde}
T8	1958.2^{ab}	895.0^{a}	887.0^{ab}	39.0^{bc}	36.0^{bcde}	33.8^{ab}	6.8^{cde}	7.2^{cd}	7.0^{def}	143.6^{f}	124.6^{abc}	123.2^{b}
T9	1767.0^{ef}	845.8^{cd}	807.0^{cdef}	43.2^{ab}	39.8^{ab}	38.6^{ab}	9.6^{a}	8.0^{bcd}	8.8^{bcd}	147.2^{cdef}	113.6^{ef}	105.2^{defg}
T10	1777.0^{ef}	850.8^{bcd}	797.0^{def}	40.8^{b}	36.8^{bcd}	37.2^{ab}	8.4^{abc}	8.4^{abcd}	9.0^{bc}	145.6^{ef}	115.2^{def}	103.4^{defgh}
T11	1860.2^{cd}	860.4^{bc}	801.2^{def}	41.6^{ab}	41.8^{a}	40.4^{a}	9.0^{ab}	8.8^{abc}	8.8^{bcd}	142.4^{f}	101.8^{g}	93.4^{h}
T12	1900.8^{bc}	889.6^{a}	820.8^{cdef}	38.6^{bc}	36.8^{bcd}	37.4^{ab}	9.2^{ab}	8.4^{abcd}	8.8^{bcd}	144.8^{ef}	111.0^{f}	97.6^{fgh}
T13	1948.0^{ab}	868.0^{b}	837.0^{cd}	38.4^{bc}	36.8^{bcd}	36.4^{ab}	8.0^{abcd}	8.4^{abcd}	8.0^{cdef}	148.0^{cdef}	115.8^{cdef}	107.6^{def}
T14	1973.0^{a}	856.2^{bcd}	851.4^{bc}	39.2^{bc}	38.0^{abc}	35^{ab}	7.6^{bcd}	7.4^{cd}	8.0^{cdef}	149.6^{cdef}	121.4^{bcde}	110.8^{cd}
T15	2001.6^{a}	898.8^{a}	920.6^{a}	39.6^{bc}	32.0^{de}	32.2^{b}	6.2^{de}	6.6^{d}	6.8^{ef}	158.4^{ab}	130.0^{ab}	119.8^{bc}
T16	2000.8^{a}	900.8^{a}	914.8^{a}	39.2^{bc}	35.4^{bcde}	38.4^{ab}	5.4^{e}	6.4^{d}	6.6^{f}	163.4^{a}	132.2^{a}	134.0^{a}
SEM	19.56	6.02	13.86	1.44	1.49	2.39	0.57	0.62	0.57	2.33	2.81	3.37
Main effect means												
CP												
T1 to T8	1811.1^{b}	863.4^{b}	816.9^{b}	39.9	36.5	34.9	8.4	8.8^{a}	8.6	149.5	118.25	113.4^{a}
T9 to T16	1903.55^{a}	871.3^{a}	843.8^{a}	40.1	37.2	36.9	7.9	7.8^{b}	8.1	149.9	117.62	109.0^{b}
SEM	6.91	2.13	4.90	0.51	0.53	0.84	0.20	0.22	0.20	0.82	0.94	1.19
Thr												
T1 and T9	1754.4^{e}	843.5^{dc}	798.2^{b}	44.4^{a}	39.0^{ab}	38.5^{a}	9.6^{a}	9.1^{a}	9.6^{ab}	146.8^{c}	111.6^{c}	100.7^{d}
T2 and T10	1760.1^{e}	849.4^{dc}	805.4^{b}	41.3^{b}	38.0^{ab}	36.3^{ab}	8.7^{ab}	9.0^{a}	9.9^{a}	147.3^{c}	115.5^{bc}	101.3^{d}
T3 and T11	1803.8^{d}	840.7^{dc}	802.0^{b}	40.20^{bc}	40.5^{a}	38.5^{a}	8.8^{ab}	8.9^{a}	8.7^{bc}	148.5^{bc}	112.5^{bc}	113.9^{bc}
T4 and T12	1840.6^{dc}	866.2^{b}	826.3^{b}	37.20^{c}	36.3^{b}	35.1^{ab}	8.5^{ab}	8.7^{a}	8.3^{cd}	149.5^{bc}	116.7^{bc}	116.1^{b}
T5 and T13	1867.7^{bc}	861.4^{bc}	804.0^{b}	38.4^{bc}	37.9^{ab}	35.6^{ab}	8.4^{ab}	8.6^{a}	8.5^{bc}	147.4^{c}	113.6^{bc}	108.0cd
T6 and T14	1896.30^{b}	874.0^{b}	815.3^{b}	39.5^{bc}	35.7^{b}	34.4^{ab}	8.1^{b}	8.1^{ab}	7.7^{cde}	150.4^{abc}	118.4^{b}	106.1^{d}
T7 and T15	1956.2^{a}	896.8^{a}	885.7^{a}	39.7^{bc}	31.7^{c}	32.9^{b}	6.9^{c}	7.0^{b}	7.1^{de}	154.5^{a}	126.8^{a}	114.9^{bc}
T8 and T16	1979.5^{a}	897.9^{a}	900.9^{a}	39.1^{bc}	35.7^{b}	36.1^{ab}	6.1^{c}	6.8^{b}	6.8^{e}	153.5^{ab}	128.4^{a}	128.6^{a}
SEM	13.83	4.26	9.80	1.02	1.05	1.69	0.40	0.44	0.41	1.65	1.99	2.38

* Means within the same column with no common superscripts are significantly different ($P< 0.05$).

epithelial thickness, goblet cell number and crypt depth in three sections of the small intestine (Table 6). Broiler chicks fed 17.5% CP diets supplemented with L-Thr had no effect on their gut criteria as compared with those fed 20.5% CP. With respect to microvilli height and crypt depths into single compartments of the small intestine it can be concluded that increasing levels of SID Thr in the diet increases absorptive surface in the gut (Table 5). These results were in consistence with the report by Law *et al.* (2000) and Ball (2001) who found that villus height of piglets receiving Thr deficient diet decreased as compared to those receiving Thr adequate diet. Wu (1998) pointed out that nearly 30-50% of Arg, Pro, Ileu, Val, Leu, Met, Lys, Phe, Gly, Ser and Thr may be used up through the small intestine not becoming available for extra intestinal tissues. Dietary Thr was utilized for protein synthesis in small intestine mucosa with normal protein diet (Schaart *et al.*, 2005). 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 (Table 6).

Goblet cells are producing and secreting mucine and are thus important for digestion. Increasing levels of SID Thr in the diet decreased the number of goblet cells per 100 µm villi height (Table 6). As at the same time villi height increased and also the length of the small intestine, it is not clear whether the total number of goblet cells was reduced or wheather production and secretion of mucin and therefore protection of the gut wall was affected. Mucin contains relatively high levels of Thr which would suggest that at high Thr supply more mucine is produced. However, it is also not known whether the reduced number of goblet cells were possibly more productive. Furthermore the goblet cell number was reduced by higher CP levels in jejunum. Reduced goblet cell number may be related to lower endogenous protein losses in higher CP and Thr levels. As dietary CP increases a lower proportion of endogenous amino acids exists in digesta and excreta (Wu *et al.,* 2004). Broiler chicks fed low CP diets supplemented with higher levels of L-Thr had no significant differences on villus height, epithelial thickness, goblet cell number and crypt depth as compared with those fed 20.5% CP in most sections of the small intestine.

CONCLUSIONS

This study indicated that low CP-Thr-supplemented diets can improve broiler performance. The optimum levels of Thr for growth performance and gut functionality should be between 0.65 and 0.90%. Parameters of gut functionality such as microvilli height, crypth depth and epithelial thickness seemed to be improved with even higher levels of dietary SID Thr whereas the number of mucin producing goblet cells was shrinking. Data indicated that Thr has a high impact on gut functionality of chickens.

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چکیده

در این آزمایش نیاز ترئونین قابل هضم ایلئومی استاندارد شده جوجه‌های گوشتی در دو سطح پروتئین و تاثیر آن بر عملکرد و شکل شناسی روده مورد بررسی قرار گرفت. آزمایش روی جوجه‌های سویه تجارتی راس (۳۰۸) از سن ۰ تا ۲۱ روزگی انجام شد. سطوح ترئونین قابل هضم استاندارد شده ایلئومی خوراک‌های آزمایشی ۰/۴، ۰/۵، ۰/۶، ۰/۷، ۰/۸، ۰/۹، ۱/۰ و ۱/۱ درصد و سطوح پروتئین خام مورد استفاده ۱۷/۵ و ۲۰/۵ درصد بود. آزمایش در تعداد ۶۴ قفس باطری و با استفاده از ۵ قطعه جوجه در هر تکرار انجام شد. مصرف خوراک در جوجه‌هایی که سطح بالای پروتئین را دریافت نموده بودند در مقایسه با گروهی که سطح پائین پروتئین را دریافت نموده بودند، کمتر بود. در هر دو سطح پروتئین با افزایش سطح ترئونین بیش از ۰/۷ درصد، وزن بدن و ضریب تبدیل غذایی بهبود یافت. نیاز ترئونین قابل هضم ایلئومی استاندارد شده برای افزایش وزن بر اساس رابطه تابعیت خط شکسته به ترتیب در سطح ۱۷/۵ و ۲۰/۵ درصد پروتئین ۰/۶۲ و ۰/۶۶ درصد برآورد گردید. اثر متقابل سطح پروتئین و ترئونین بر وزن نسبی و طول دئودنوم و ژژنوم معنی‌دار بود ($P<0/05$). افزایش ترئونین اثر معنی‌داری روی ارتفاع پرز در دئودنوم و ژژنوم، ضخامت اپیتلیوم، تعداد سلول‌های گابلت و عمق کریپت در دئودنوم، ژژنوم و ایلئوم داشت ($P<0/01$). نتایج حاکی از این است که در جیره‌های حاوی پروتئین کم و حاوی مقدار کافی لیزین و آمینواسیدهای گوگرددار افزایش ترئونین موجب بهبود وزن بدن و ضریب تبدیل خوراک، همچنین افزایش طول روده می‌گردد. سایر فراسنجه‌های روده مانند ارتفاع پرز، عمق کریپت و ضخامت اپیتلیوم با افزایش ترئونین قابل هضم ایلئومی استاندارد شده نیز بهبود یافت.

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