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The Threonine Requirements and its Effects on Growth Performance and Gut Morphology of Broiler Chicken Fed Different Levels of Protein

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Abstract: An experiment was conducted to determine the effects of dietary threonine (Thr) and crude protein (CP) on growth performance, gut measurements and morphology and to estimate Standardized Ileal Digestible (SID) Thr requirements of Ross 308 males at 0-21 days of age. The SID Thr levels were 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 and 1.1% with two levels of protein 16 and 19%. Chicks were randomized into 64 battery pens (5 chicks per replicate). FI was lower for broiler given the high CP diets compare to those fed on the low CP diets. BWG and FCR improved up to 0.7% Thr in both CP levels. Weight gain improved non-linearly suggesting optimum dietary SID Thr levels between 0.89 and 0.84% (quadratic) at 16 and 19% ideal protein, respectively. Significant interaction was found between CP and Thr on relative weight and length of duodenum and jejunum ($p<0.05$). Thr supplementation had significant effect on villus height, epithelial thickness, goblet cell number and crypt depth in duodenum, jejunum and ileum ($p<0.01$). Low CP diets adequate in Lys, TSAA supplemented with Thr may result in optimal BWG and FCR as well as growth of intestinal length non-linearly. Parameters of gut functionality such as microvilli height, crypt depth and epithelia thickness seemed to be improved with even higher levels of dietary SID Thr level.

Key words: Broiler chicks, threonine, standardized ileal digestibility, gut functionality

INTRODUCTION

Crystalline amino acids supplementation can reduce the cost of feed and total nitrogen output to 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 behind TSAA and Lys in commercial broiler diets composed of corn or sorghum, soybean meal and meat meal (Kidd and Kerr, 1996; Kidd, 2000) and has an important role in structure and function of gastrointestinal tract (Law *et al*., 2000; Ball, 2001).

There has been much research on Thr and 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 significant interaction on growth performance of broiler chicks. They also stated that Thr supplementation at the level of optimum requirement 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 diet.

In order to more precisely formulate feed and better control of animal 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 and correction is 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 15-35 day 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 effects of Thr on digestive tract measurements and gut morphology is sparse and limited. The aim of this study was to estimate SID Thr requirement with different CP levels and to evaluate Thr response on growth performance and gut measurements and morphology of broiler chicks during 0-21 day of age.

MATERIALS AND METHODS

Experimental materials and procedures: The experiment was 2×8 factorial arrangement with 2 levels of CP (16 and 19%) 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 allotted into 16 dietary treatments. Each treatment was assigned to 4 replicate cage pens with 5 birds in each. Each cage contained one tube feeder and one tube waterer. Experimental diets in mash form and water were offered *ad libitum*. Broiler chicks were provided incandescent light for 24 h per day. The experimental facilities were a curtain-sided cage with

Table 1: Composition and nutrient content of the experimental diets

	Thr percentage in low CP diets								Thr percentage in high CP diets							
Ingredient	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 [1]	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[2]	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%	16	16	16	16	16	16	16	16	19	19	19	19	19	19	19	19
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[3]	152	152	153	153	153	153	154	154	152	152	152	152	152	152	152	152
SID[4] 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
SIDPhe + 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

[1]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; riboflavin, 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. [2]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. [3]Represents dietary electrolyte balance as defined by dietary Na + K-Cl (in mEq/kg of diet). [4]Represents standardized ileal digestible amino acids

infrared brooding lamps. The temperature was maintained at 33°C for 1 day, decreasing 2°C per week. Corn and soybean meal were analyzed for CP and amino acid content. SID amino acids were calculated using standardized ileal digestibility coefficient of Lemme *et al.* (2004) and by assuming 100% digestibility of crystalline amino acids. 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 0-21 day of age (Lemme *et al.*, 2004). All diets had identical ME and electrolyte balance (Table 1).

Pen weight and FI was measured at 21 day of age. Mortality was recorded throughout the experimental period and dead birds were weighed to correct FI data for mortality. FCR was calculated by dividing pen FI by pen weight gain.

At 21 day of age 2 birds from each pen (8 per treatments) nearest to the mean pen weight were selected and weighed for digestive tract measurements and gut morphology. The birds were killed by CO_2 and the digestive tract was 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 1 cm lengths of duodenum (midpoint of the pancreatic loop), 2 cm length of jejunum (midpoint of jejunum) and 3 cm length of ileum (after Meckel's diverticulum) were removed for gut morphological measurement. Intestinal sample 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 100-400×magnification using computer software (Wu *et al*., 2004). Relative weight of different sections of small intestine measured as fraction of carcass weight.

Statistical analysis: Data were statistically analyzed by analysis of variance appropriate for completely randomized design by General Linear Model (GLM) procedure of SAS (2001) to determine the treatments effects means and significant F ratio ($p<0.05$) were separated by the least significant difference test. For calculation of SID Thr requirements, results of performance were fitted into exponential and quadratic models (Schutte and Pack, 1995; Schutte *et al*. 1997). When exponential responses occurred, optimization was calculated 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 in 21 day of age are shown in Table 3. During 0-21 day of age supplemental Thr improved FI ($p<0.0001$), BWG ($p<0.0001$) and FCR ($p<0.0001$). CP level had significant effect on FI ($p<0.0001$), BWG ($p<0.01$) and FCR ($p<0.0001$). Birds fed high CP diet had lower FI and better BWG and FCR compared with those fed on the low CP diet (Fig. 1). As dietary CP decreased most amino acids tend to decline. It is possible that some essential amino acid such as Gly and Ser become limiting. 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 nonessential amino acid Glu. Dietary CP was increased by the addition of L-Glu. Fancher and Jensen (1989) stated that dietary inclusion of L-Glu for increasing CP level decreased FI.

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

		Protein and mino acids (%)											
Treatment	C/A[1]	Protein	Met	Cys	Met+Cys	Lys	Thr	Arg	Ile	Lue	Val	His	Phe
T_1	C	16[2]	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[3]	0.71	0.26	0.96	1.40	0.50	1.37	0.96	1.56	1.09	0.48	0.95
T_2	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
T_3	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
T_4	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
T_5	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
T_6	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
T_7	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
T_8	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
T_9	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
T_{10}	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
T_{11}	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
T_{12}	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
T_{13}	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
T_{14}	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
T_{15}	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
T_{16}	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

[1]C Represents calculated and A represents analyzed values, [2]C values for protein% are based on SID protein,
[3]A values for protein% are based on total protein content

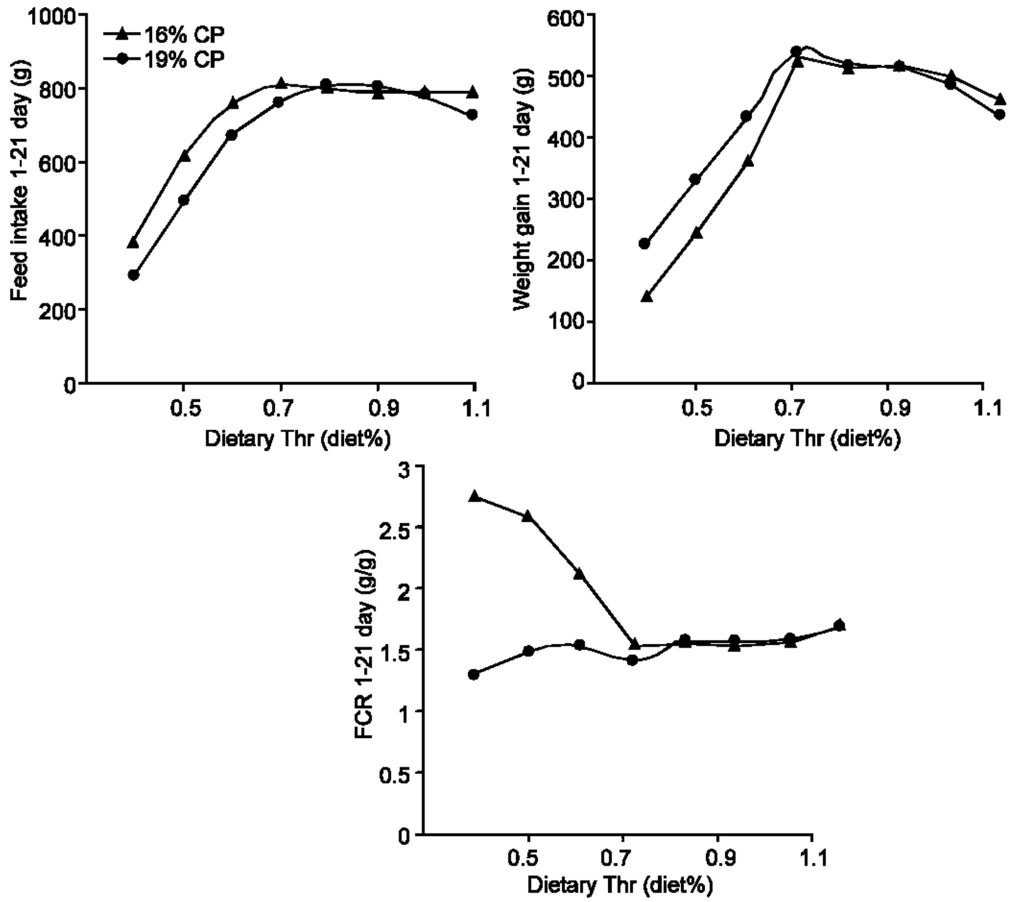

Fig. 1: Feed Intake (FI), Weight Gain (WG) and Feed Conversion Ratio (FCR) responses of male broilers (Ross 308) to graded Standardized Ileal Digestible (SID) threonine (Thr) at 16 and 20% dietary Crude Protein (CP) levels

During 0-21 day of age there were significant interaction between CP and Thr for FI ($p<0.01$), BWG ($p<0.01$) and FCR ($p<0.0001$). Broiler Chickens fed on low or high CP diet supplemented with 0.7 and 0.8% Thr had an increased FI. 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 16% CP diet supplemented with Thr (SID Thr, 0.7%), they grew as well as chickens receiving a 19% CP diet containing 0.7% SID Thr. Moreover broiler chicks fed low CP diets supplemented with L-Thr had similar FCR compared with those fed 19% 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 addition of L-Thr to low CP diet which is adequate in TSAA and Lys for broiler improved FCR and similar growth to level obtained with a high CP basal diet. In our 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.

Thr requirement: Thr requirements of 2 CP levels for FI, BWG and FCR during 0-21 day of age estimated by different models are shown in Table 4. Exponential model for weight gain showed optimum SID Thr level 93% (r^2 = 90) and 77% (r^2 = 86) and quadratic regression revealed 0.89 (r^2 = 96) and 84% (r^2 = 97) at 16 and 19% dietary CP, respectively. Visual assessment suggests responses rather to be quadratic and respective quadratic regression revealed much better r^2 values than exponential model. Low CP diets increased Thr requirement for weight gain at 0-21 day of age. This supports the reports of Ciftci and Ceylan (2004) who estimated higher Thr requirement for low CP diets than that 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 requirement. These results were in contrast with Robbins (1987) who found that Thr requirement of chickens fed high CP diet (20% CP) was 29% higher

Table 3: Effect of different levels of dietary Crude Protein (CP) and Threonine (Thr) on the Feed Intake (FI) and growth performance during 0-21 day of age

Treatment	CP (%)	Thr (%)	Feed intake (g/bird)	Body weight gain(g/bird)	FCR (g/g)
T_1	16	0.4	381.61^f	144.37^f	2.740^c
T_2	16	0.5	628.35^d	245.50^e	2.590^c
T_3	16	0.6	757.26^{ab}	360.64^d	2.120^b
T_4	16	0.7	819.43^a	529.46^a	1.545^a
T_5	16	0.8	799.07^a	509.48^{ab}	1.572^a
T_6	16	0.9	790.70^a	514.70^{ab}	1.537^a
T_7	16	1.0	787.04^{ab}	499.11^{ab}	1.580^a
T_8	16	1.1	786.45^{ab}	464.87^{cb}	1.705^a
T_9	19	0.4	292.08^g	223.00^e	1.305^a
T_{10}	19	0.5	493.86^e	330.96^d	1.515^a
T_{11}	19	0.6	673.98^{cd}	436.94^c	1.542^a
T_{12}	19	0.7	762.75^{ab}	541.14^a	1.410^a
T_{13}	19	0.8	809.91^a	519.52^{ab}	1.560^a
T_{14}	19	0.9	797.89^a	512.94^{ab}	1.557^a
T_{15}	19	1.0	779.25^{ab}	485.69^{abc}	1.607^a
T_{16}	19	1.1	723.23^{cb}	435.63^c	1.695^a
SEM			20.567	17.097	0.1200
Main effect means					
CP					
$T1-T_8$	16	All	718.74^a	408.515^b	1.924^b
T_9-T_{16}	19	All	666.62^b	435.727^a	1.524^a
SEM			7.272	6.044	0.0424
Thr					
T_1 and T_9	Both	0.4	336.84^e	183.69^f	2.022^c
T_2 and T_{10}	Both	0.5	561.10^d	288.23^e	2.052^c
T_3 and T_{11}	Both	0.6	715.62^c	398.79^d	1.831^{bc}
T_4 and T_{12}	Both	0.7	791.09^{ab}	535.30^a	1.477^a
T_5 and T_{13}	Both	0.8	804.49^a	514.50^{ab}	1.566^a
T_6 and T_{14}	Both	0.9	794.29^{ab}	513.82^{ab}	1.547^a
T_7 and T_{15}	Both	1.0	783.14^{ab}	492.40^b	1.593^{ab}
T_8 and T_{16}	Both	1.1	754.84^{cb}	450.25^b	1.700^{ab}
SEM			14.54	12.09	0.084

$^{a-f}$Means within the same column with no common superscript are significantly different ($p<0.05$). FCR = Feed intake/growth

Table 4: Estimated Standardized Ileal Digestible (SID) Threonine (Thr) requirements for broiler chicks performance at 2 dietary Crude Protein (CP) levels

CP levels (%)	Model	Feed intake	Weight gain	FCR
16	Exponential[1]	0.69(0.98)[2]	0.93(0.90)	1.03 (0.89)
16	Quadratic[1]	0.87(0.91)	0.89(0.96)	0.91 (0.94)
19	Exponential	0.82(0.96)	0.77 (0.86)	-[3]
19	Quadratic	0.87(0.99)	0.84 (0.97)	-

[1]Exponential and quadratic models were described by Schutte and Pack (1995). [2]Values in parentheses show r. [3]Represent Data that did not fit to models

than that of chickens fed low protein diet (15% CP). He suggested that Thr requirement increases as dietary CP increases.

Gut measurements and histology: Addition of Thr up to 0.7%, significantly increased relative weight of duodenum and jejunum ($p<0.05$) (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 less well developed intestinal structure. The explanation for relatively higher duodenal, jejunal and ileunal weight of birds fed high Thr level may be lies in the high utilization of amino acids in 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 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 by the metabolism of portal-drained visera (Schaart *et al.*, 2005).

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

	Relative Length			Relative weight		
Treatment	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
T_1	19.11^{dc}	41.50^{c}	36.90^{cd}	1.38^{d}	2.29^{d}	1.81
T_2	21.62^{abc}	49.31^{b}	43.31^{bc}	1.75^{bc}	2.71^{abcd}	1.70
T_3	23.06^{abc}	50.12^{b}	43.75^{abc}	1.75^{bc}	2.44^{cd}	1.73
T_4	22.50^{abc}	51.50^{ab}	44.25^{abc}	1.75^{bc}	2.64^{bcd}	1.72
T_5	23.65^{ab}	52.82^{ab}	47.00^{ab}	1.57^{cd}	2.73^{abcd}	1.85
T_6	22.10^{abc}	53.37^{ab}	48.50^{ab}	1.65^{c}	2.65^{cd}	1.84
T_7	23.23^{abc}	52.15^{ab}	46.56^{ab}	1.57^{cd}	2.52^{cd}	1.72
T_8	23.37^{abc}	53.15^{ab}	45.31^{abc}	1.56^{cd}	2.39^{cd}	1.73
T_9	17.99^{c}	36.50^{c}	32.90^{d}	2.03^{a}	3.12^{a}	1.98
T_{10}	21.56^{bc}	50.12^{b}	40.06^{c}	2.00^{a}	3.10^{ab}	1.94
T_{11}	21.04^{cd}	49.57^{b}	42.58^{bc}	2.04^{a}	2.88^{abc}	1.98
T_{12}	22.42^{abc}	53.56^{ab}	46.06^{ab}	1.94^{abc}	3.12^{a}	2.00
T_{13}	23.05^{abc}	51.5^{ab}	45.56^{abc}	1.78^{abc}	2.78^{abc}	1.77
T_{14}	23.49^{abc}	56.18^{a}	49.37^{a}	1.73^{c}	2.88^{abc}	1.83
T_{15}	23.98^{a}	49.18^{b}	43.43^{bc}	1.79^{abc}	2.60^{bcd}	1.67
T_{16}	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						
T_1-T_8	22.33	50.49	44.44	1.62^{b}	2.55^{b}	1.76^{b}
T_9-T_{16}	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						
T_1 and T_9	18.55^{c}	39.00^{c}	34.90^{d}	1.71^{ab}	2.71^{ab}	1.89
T_2 and T_{10}	21.59^{b}	49.71^{b}	41.90^{c}	1.87^{ab}	2.91^{a}	1.82
T_3 and T_{11}	22.04^{ab}	49.84^{b}	43.17^{bc}	1.89^{a}	2.67^{ab}	1.86
T_4 and T_{12}	22.46^{ab}	52.53^{ab}	45.16^{abc}	1.89^{a}	2.88^{a}	1.87
T_5 and T_{13}	23.35^{a}	52.16^{ab}	46.28^{ab}	1.68^{b}	2.76^{ab}	1.81
T_6 and T_{14}	22.79^{ab}	54.78^{a}	48.93^{a}	1.69^{b}	2.77^{ab}	1.84
T_7 and T_{15}	23.61^{a}	50.66^{ab}	45.00^{abc}	1.69^{b}	2.56^{b}	1.70
T_8 and T_{16}	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

$^{a-d}$Means within the same column with no common superscripts are significantly different ($p<0.05$)

The effect of CP and Thr supplementation, individually or in combination on villus height, epithelial thickness, goblet cell number and crypt depth of different section of small intestine of birds fed experimental diets are shown in Table 6. Thr supplementation had significant effect on villus height, epithelial thickness, goblet cell number and crypt depth in duodenum, jejunum and ileum ($p<0.01$). CP level had significant effect on villus height in duodenum ($p<0.0001$), 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, epithelial thickness, goblet cell number and crypt depth in three sections of the small intestine (Table 6). Broiler chicks fed low CP diets supplemented with L-Thr had no significant gut criteria differences compare with those fed 19% CP in most sections of small intestine. With respect to microvilli height and crypt depths in single compartments of the small intestine in can be concluded that increasing level of SID Thr in the diet increase absorptive surface in the gut (Fig. 2). These results were in consistent with the report by Law *et al.* (2000) and Ball (2001) who found that villus height in piglet receiving Thr deficient diet decreased compare to that receiving the Thr adequate diet. Lower villus height results in decreased intestinal absorptive area. Wu (1998) pointed out that nearly 30-50% of Arg, Pro, Ileu, Val, Leu, Met, Lys, Phe, Gly, Ser and Thr may be used by the small intestine and do not become 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 (Fig. 2).

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 (Fig. 3). 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 and wheather production and secretion of mucin and therefore protection of the gut wall was affected. Mucin contains relatively high levels of

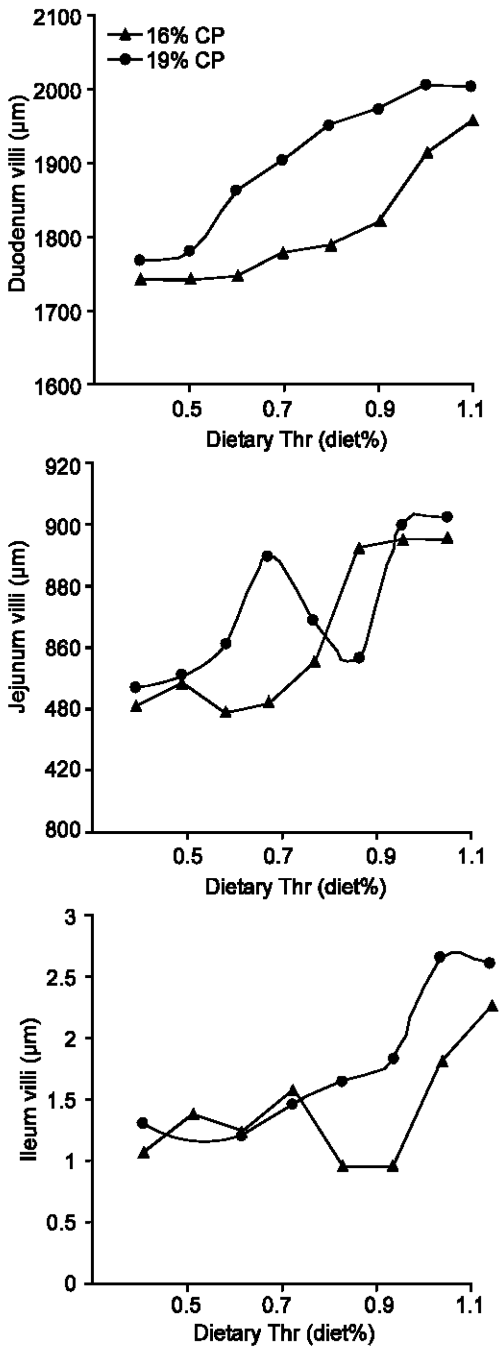

Fig. 2: Microvilli height responses male broilers (Ross 308) in different section of small intestine to graded SID (standardized ileal digestible) threonine (Thr) at 16 and 20% dietary Crude Protein (CP) levels

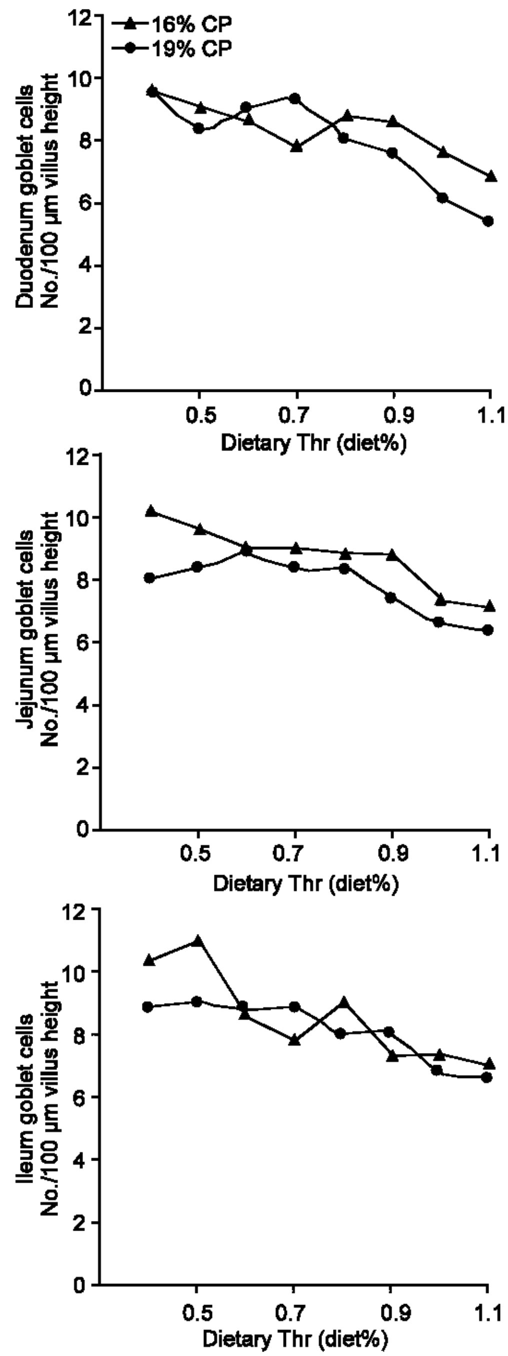

Fig. 3: Responses of number of goblet cell to increasing dietary threonine (Thr) at two protein levels in male Ross 308 broilers

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 reduced by higher CP level in jejunum. Reduced goblet cell number may be related to lower endogenous protein losses in higher CP and Thr level. As dietary CP increases lower proportion of endogenous amino acids exists in digesta and excreta (Wu *et al.*, 2004). Broiler chicks fed low CP diets supplemented with higher level

Table 6: Effect of dietary threonine (Thr), Crude Protein (CP) and their interaction on villus height (µm), epithelial thickness (µm), goblet cell number (per 100 µm villus height) and crypt depth (µm) 0f duodenum in 21 day of age

	Villus height			Epithelia thickness			Goblet cell number			Crypt depth		
Treatment	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
T_1	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}
T_2	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}	149.0^{cdef}	115.8^{cdef}	99.2^{efgh}
T_3	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}
T_4	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}
T_5	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}
T_6	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}
T_7	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}
T_8	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}
T_9	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}
T_{10}	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}
T_{11}	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}
T_{12}	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}
T_{13}	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}
T_{14}	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}
T_{15}	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}
T_{16}	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												
T_1-T_8	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}
T_9-T_{16}	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												
T_1 and T_9	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}
T_2 and T_{10}	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}
T_3 and T_{11}	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}
T_4 and T_{12}	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}
T_5 and T_{13}	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
T_6 and T_{14}	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}
T_7 and T_{15}	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}
T_8 and T_{16}	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

$^{a-h}$Means within the same column with no common superscripts are significantly different ($p<0.05$)

of L-Thr had no significant differences on villus height, epithelial thickness, goblet cell number and crypt depth compare with those fed 19% CP in most sections of small intestine.

Conclusion: The results presented here indicated that low CP-Thr supplemented diets can be successfully used without impairing growth performance when diets are well balanced for amino acids, Thr supplementation at the level of optimum requirement to low CP-Thr deficient diets resulted in similar BWG and FCR relative to those received high CP diets. Optimal SID Thr levels are suggested between 0.70 and 0.93%. 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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