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Effects of Different Levels of Dietary Crude Protein and Threonine on Performance, Humoral Immune Responses and Intestinal Morphology of Broiler Chicks

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ABSTRACT

The present study aimed at investigating the effects of different dietary crude protein (CP) and threonine (Thr) levels on the performance, immune responses and jejunal morphology of broiler chicks. A total of 432 broiler chicks were randomly assigned to a 3×3 factorial arrangement of treatments including three different CP dietary levels (90, 95, and 100% of Ross 308 recommendations) and Thr (100, 110, and 120% of Ross specifications) dietary levels. Performance parameters were recorded for the starter (1-12 days), grower (13-24 days) and finisher (25-42 days) periods. Birds were subjected to different antigen inoculations to evaluate antibody responses. At day 42 of age, two randomly-selected birds per replicate were slaughtered to measure carcass traits. Although Thr dietary supplementation had no marked effect on Newcastle antibody titers, particularly the supplementation of Thr up to 110% of Ross specifications improved ($p<0.05$) antibody titers against sheep red blood cells during both primary and secondary responses. Reduction of dietary CP level resulted in significant decrease in villus height ($p<0.05$) and crypt depth ($p<0.01$) in jejunal epithelial cells, but the supplementation of low-CP diets with Thr up to 110 and 120% of the recommended values allowed overcoming these changes. Except for the starter period, reducing dietary CP level to 90% of Ross recommendations had no harmful effects on performance parameters; however, the best values were obtained with diets containing 110% Thr. The present results indicate that it is possible to reduce dietary CP level up to 10% after the starter period without any detrimental impact on growth performance, and dietary Thr supplementation up to 110% of Ross values may compensate for low CP-induced growth delay in broiler chicks.

INTRODUCTION

Protein and amino acids have various biological functions, including the biosynthesis of tissues, and resulting in diverse economic products in all farm animals (Alleman *et al.*, 2000). The importance of protein in feed formulation is more evident because it is the most costly nutrient in feeds is and dietary crude protein (CP) requirement is high in modern broiler strains as a result of their fast growth rates. In addition to CP, individual amino acids are important to supply nutritional requirements and to support optimal growth performance in modern broiler flocks (Han *et al.*, 1992; Aletor *et al.*, 2000; Kim *et al.*, 2007). Han *et al.* (1992) showed that there are some amino acids that are crucially more important than others, and are known as critical or essential amino acids. During the last few years, attempts have been made to use low-CP diets supplemented with synthetic amino acids for broiler chicks. The reason is that such supplementation can provide significant opportunities for nutritionists to utilize the least-cost formulations in order to decrease feed cost and subsequently to reduce nitrogen excretion to the environment (Kidd *et al.*, 1996; Aletor *et al.*, 2000; Alleman *et al.*, 2000).

Threonine (Thr) is usually known as the third limiting amino acid for broilers, after methionine and lysine, in corn-soybean meal-based diets (Han *et al.*, 1992; Fernandez *et al.*, 1994) and it has an important role in the structure and function of gastrointestinal tract (Law *et al.*, 2000; Ball, 2001). The amount required depends on factors such as dietary CP content, main dietary feed ingredients, and broiler strain, age, and sex (Barkley & Wallis, 2001). The study carried out by Mack *et al.* (1999) showed that weight gain and breast meat percentage increased as a result of Thr supplementation of low-CP diets; consequently, feed conversion ratio (FCR) improved, but there was no noticeable effect on abdominal fat.

Many studies have been conducted to show varying roles of Thr in biological pathways of animals (Defa *et al.*, 1999; Wang *et al.*, 2006, 2007); however, there are few studies on the possible interaction between different dietary CP and Thr levels in broilers (Ciftci & Ceylan, 2004; Jahanian, 2010a,b). Although some first studies did not report any significant effect of dietary CP content on Thr requirements of broilers (Robbins, 1987; Holsheimer *et al.*, 1994), Kidd *et al.* (2001) found a clear interaction between Thr needs and dietary CP levels for feed conversion ratio (FCR), but none for BWG or feed intake. Therefore, the exact relationship between dietary CP and Thr remains unclear. A study performed by Jahanian (2010a) showed a marked increase in weight gain caused by Thr supplementation of deficient diets, especially in high-CP diets. He also showed that, irrespective of dietary CP level, Thr supplementation of deficient diets enhances feed efficiency, particularly when high-CP diets are fed (Jahanian, 2010a). In addition of improving growth performance, Wang *et al.* (2006) and Jahanian (2010a,b) with studies with pigs and broilers, respectively, reported that Thr supplementation of diets enhanced immune functions as measured by antibody responses to different antigens. Furthermore, Jahanian (2010a,b) showed that reducing dietary CP content up to 3 percentage points impaired the immunocompetence of broiler chicks, and concluded dietary CP and individual amino acids have important roles in immune function.

Because of the controversial results obtained relative to the interaction between dietary CP and Thr, the present study was conducted to investigate the effects of different dietary CP and Thr levels on the performance, carcass characteristics, immune responses and intestinal morphology of broilers.

MATERIAL AND METHODS

Birds, diets, and experimental design

The present study was performed at the Poultry Research Station of Isfahan University of Technology (Isfahan, Iran) and all procedures used were approved by Isfahan University of Technology Animal Use and Care Committee. A total of 432 one-day-old Ross 308 broiler chicks were randomly distributed in nine treatments with four replicate pens (12 birds per pen). Treatments consisted of diets containing three different dietary CP levels (90, 95 and 100% of Ross 308 recommendations [Ross Management Manual, 2009] for starter, grower and finisher periods) and three dietary Thr levels (100, 110 and 120% of Ross 308 specifications), and were distributed according to a completely randomized design in a 3×3 factorial arrangement. Of course, the 100% CP and Thr values were calculated after dilution relative to dietary metabolizable energy value, so that dietary CP levels of 23 and 21% for starter and grower, respectively (suggested by Ross manual, 2009) were reduced to 21.90 and 20.34% of diet, and respective 100% Thr values (0.93 and 0.82% of diet) were reduced to 0.89 and 0.79% of diet. Experimental diets were formulated using the analyzed composition of feed ingredients (crude protein, ether extract, crude fiber, total ash and gross energy values by standard protocols of AOAC, 2002) to meet or exceed the nutritional requirements of broilers as suggested by Ross Broiler Management Manual (2009) for the starter (1 to 12 d of age), grower (13 to 24 d of age) and finisher (25 to 42 d of age) phases (Table 1). Dietary CP levels to meet 90, 95 and 100% of Ross 308 specifications were equal to 19.71, 20.80 and 21.90% for starter phase, respectively. The respective values for grower phase were 18.31, 19.32 and 20.34%, and for finisher phase were 17.00, 17.95 and 18.89% of the diet. Three total Thr dietary levels (100, 110 and 120% of Ross 308 specifications) were equal to 0.89, 0.98 and 1.07% of the starter, 0.79, 0.86 and 0.94% of the grower, and 0.71, 0.77 and 0.84% of the finisher diets, respectively. The ratios between individual amino acids (except Thr) and dietary CP were the same for all dietary CP levels. Birds had *ad libitum* access to water and to the experimental diets at all times. Body weight gain (BWG) and feed intake (FI) were regularly measured (1 to 12, 13 to 24 and 25 to 42 d of age). Bird mortality was daily recorded to adjust feed conversion ratio.

Table 1 – Feed ingredients and nutrient composition of basal diets.

Ingredient (%)	Starter (1-12 d of age)	Grower (13-24 d of age)	Finisher (25-42 d of age)
Corn	52.51	56.97	59.03
Soybean meal	39.62	35.22	31.53
Soybean oil	3.04	3.67	5.38
Monocalcium phosphate	1.50	1.30	1.24
Oyster shell	1.81	1.51	1.49
Salt	0.22	0.15	0.18
$NaHCO_3$	0.18	0.30	0.41
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-Methionine	0.34	0.25	0.20
L-Threonine	0.06	0.01	-
L-Lysine.HCl	0.22	0.11	0.05
Nutrient composition (calculated)			
ME_n (kcal/kg)	2880	2980	3100
Crude protein (%)	21.90	20.34	18.89
Methionine	0.68	0.58	0.51
Methionine + Cysteine (%)	1.02	0.90	0.80
Lysine (%)	1.36	1.17	1.03
Arginine (%)	1.51	1.39	1.29
Tryptophan (%)	0.34	0.31	0.29
Threonine (%)	0.89	0.79	0.71
Valine (%)	1.09	1.03	0.96
Calcium (%)	1.00	0.85	0.82
Available phosphorus (%)	0.48	0.43	0.41
Na (%)	0.15	0.16	0.20
Cl (%)	0.21	0.15	0.16
K (%)	0.95	0.88	0.81
DEB (mEq/kg)[3]	249.80	250.30	249.80

[1]Vitamin premix provided the following per kilogram of diet: vitamin A, 9000 IU; cholecalciferol, 2000 IU; vitamin E, 36 IU; vitamin K_3, 2 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; niacin, 30 mg; choline chloride, 250 mg; biotin, 0.1 mg; folic acid, 1 mg; pyridoxine 3.0 mg; vitamin B_{12}, 0.015mg; BHT, 1 mg.
[2]Trace mineral premix provide the following in milligrams per kilogram of diet: iron, 50 mg; zinc, 85 mg; manganese, 100 mg; iodine, 1 mg; copper, 10 mg; selenium, 0.2 mg.
[3]Represents dietary electrolyte balance as defined by dietary Na + K – Cl (in mEq/kg of diet).

Measurements of carcass traits

On the last day of trial (d 42 of age), two birds per pen nearest to the average weight of the same pen were selected to evaluate carcass traits. Feed was removed 3 h before slaughter. Each bird was exsanguinated by cutting the jugular vein and allowed to bled for approximately 2 min. Viscera and abdominal fat were immediately removed and the weights of breast, drumstick, pancreas, and abdominal fat were obtained. Carcass yield and abdominal fat, pancreas, breast and drumstick relative weights were calculated as percentages of live body weight and the average values of two birds per pen was used for analysis of variance (Jahanian *et al.*, 2008).

Antibody responses to different antigens

On day 16, all birds were challenged with Newcastle disease virus (NDV) via cervical injection (Newcastle-Bronchitis vaccines, 2007). Twelve days after antigen inoculation (28 days of age), two birds per replicate were chosen at random and blood samples were collected from the brachial vein. Sera were separated by centrifugation (5000 rpm for 10 min) and antibody titers against NDV were measured by hemagglutination halter test using commercially available V-form ELISA plates according to manufacturer's instructions (Newcastle-Bronchitis vaccines, 2007).

Sheep red blood cells (SRBC) were washed three times in phosphate buffer saline (PBS) and diluted in PBS to a final dilution rate of 7% (vol/vol), as described by van der Zijpp & Leenstra (1980). At 23 and 33 days of age, two birds per replicate were injected with 0.5 mL of 7% SRBC suspension intramuscularly (thigh muscle). Heparinized blood samples were collected at 6 and 12 days after first and second immunizations, respectively. Plasma samples were stored at –20°C until further analysis.

The hemagglutination assay was performed as described by Leshchinsky & Klasing (2001). Briefly, each well of a 96-well plate received 50 µL of diluent buffer containing PBS. The initial well received 50 µL of plasma sample, which was serially doubly diluted by transferring 50 µL to the next wells. Then, 50 µL of 2% SRBC suspension in PBS was added to each well. The plates were shaken, incubated for 1 h at room temperature, and then scored. The agglutination titer was expressed as the $\log_2$ of the highest titer with 50% agglutination.

Measurement of jejunal histological changes

At 42 days of age, two randomly-selected chicks from each replicate were slaughtered to determine the effects of dietary treatments on intestinal morphology as described by Mahdavi *et al.* (2010). Tissue samples were collected and a 2-cm segment of jejunal region anterior to Meckel's diverticulum was excised for light microscopic observations. The histological sections were immediately fixed in 10% formaldehyde solution, and then embedded in paraffin. Transverse and longitudinal 5-µm thick sections were prepared using microtome, stained with hematoxylin-eosin, and examined under a light microscope.

Statistical analyses

All data were analyzed using the General Linear Model procedure of SAS software (SAS Institute, 2001).

The following model was assumed in the analysis of all traits. $Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$, where Y_{ijk}= observed value for a particular parameter, μ= overall mean, A_i= effect of the ith level of dietary CP, B_j= effect of the jth level of dietary Thr, AB_{ij}= the respective interaction between ith and jth levels of dietary CP and Thr, and e_{ijk}= random error associated with the ijkth recording. Treatment means were separated using the Least Significant Difference test at p<0.05 significance level.

RESULTS AND DISCUSSION

Carcass traits and relative organ weights

As shown in Table 2, the lowest CP dietary level caused a significant (p<0.05) decrease in carcass yield; however, the dietary inclusion of different levels of Thr had no marked effect on this parameter. Although the decrease in dietary CP content to 90% of the recommended values had no considerable impact on drumstick and breast relative weights, irrespective of CP level, dietary Thr supplementation up to 110% of Ross recommendation resulted in significant (p<0.05) increase in the relative weights of both drumstick and breast. On the other hand, decrease in dietary CP content caused marked (p<0.05) decline in relative pancreas weight.

These results are in agreement with the findings of Dozier *et al.* (2000b, 2001), who indicated that total carcass yield was not affected by dietary Thr concentration. Alleman *et al.* (2000), however, reported that after reducing dietary CP concentration in broiler chicks, breast meat yield decreased. Similarly, several studies indicated that dietary Thr supplementation markedly increased breast and drumstick yields (Mack *et al.*, 1999; Dozier *et al.*, 2000a,b, 2001; Ciftci & Ceylan, 2004; Jahanian 2010b). Ciftci & Ceylan (2004) showed that increasing dietary Thr concentrations significantly increased breast yield when both high- and low-CP diets were fed, and drumstick yield only with high-CP diets. Some reports suggest that broiler diets containing more Thr than recommended levels improves edible meat percentage. Kidd & Kerr (1997) conducted an experiment to determine Thr dose responses in broilers and proposed that Thr needs for breast meat yield were higher than those reported for weight gain. Also, Chung *et al.* (1995) showed that breast meat yield and drumstick meat increased in

Table 2 – Effects of different levels of dietary CP and Thr on carcass traits, abdominal fat, and pancreas as a percentage of live body weight.

CP level[1] (%)	Thr level[1] (%)	Carcass (%)	Drumstick (%)	Breast (%)	Abdominal fat (%)	Pancreas (%)
100	100	76.20	24.59	26.28	1.78	0.21
	110	77.10	25.83	28.26	1.79	0.21
	120	76.40	26.77	27.56	1.71	0.22
95	100	76.80	25.61	26.43	2.04	0.20
	110	76.50	26.19	27.55	1.95	0.21
	120	76.30	26.10	27.85	1.91	0.18
90	100	74.30	26.07	26.00	2.06	0.18
	110	76.00	26.28	27.06	1.91	0.18
	120	74.50	26.13	27.42	2.00	0.20
CP level						
100		76.40[a]	25.73	27.37	1.76	0.21[a]
95		76.10[a]	25.96	27.27	1.97	0.20[ab]
90		74.60[b]	26.16	26.83	1.99	0.19[b]
Thr level						
100		75.40	25.42[b]	26.24[b]	1.96	0.20
110		76.60	26.10[ab]	27.62[a]	1.88	0.20
120		75.20	26.33[a]	27.61[a]	1.88	0.20
			p-values			
CP		0.03	0.53	0.66	0.36	0.05
Thr		0.27	0.05	0.05	0.86	0.82
CP × Thr		0.87	0.23	0.95	0.99	0.21
SEM		0.009	0.466	0.771	0.212	0.013

[ab]Means with no common superscript within each column are significantly (p<0.05) different.
[1]The CP at the levels of 100, 95 and 90% of Ross 308 recommendations provided 21.9, 20.8 and 19.71% CP for the starter phase; 20.34, 19.32 and 18.31% CP for the grower phase, and 18.89, 17.95 and 17% CP for the finisher phase, respectively. Dietary Thr at the levels of 100, 110 and 120% of Ross 308 specifications provided 0.89, 0.98 and 1.07% Thr for the starter phase; 0.79, 0.86 and 0.94% Thr for the grower phase, and 0.71, 0.77 and 0.84% Thr for finisher phase, respectively.

response to dietary Thr supplementation. Consistently, the present findings suggest that although reducing dietary CP level up to 10% decreased (p<0.05) carcass yield, relative breast and drumstick weights were not influenced by dietary CP content. However, the dietary supplementation with L-Thr at levels at least 10% higher than recommended values markedly increased the relative weights of both carcass sections. It should be noted that Thr needs for breast and drumstick meat yields were higher than recommended values.

As described by earlier researchers (Aletor *et al.*, 2000; Alleman *et al.*, 2000), the relative weight of abdominal fat was numerically increased after feeding low-CP diets. However, Thr supplementation had no marked effect on abdominal fat pad. This finding is consistent with the observations of Mack *et al.* (1999), Dozier *et al.* (2001), Rosa *et al.* (2001) and Jahanian (2010b), who reported that Thr supplementation of diets deficient or sufficient in Thr had no reducing effect on abdominal fat.

As presented in Table 2, reduction of dietary CP content decreased (p<0.05) relative pancreas weight. Danicke *et al.* (2000) suggested that both synthesis and secretion of exocrine pancreas enzymes are up-regulated by feed intake and nutrient presence and density in the intestine. Consistent with the present findings, Swatson *et al.* (2000) indicated that increase in dietary CP level increased relative pancreas weight, in part due to the induction of high amount of enzymes and protein secretion.

Antibody responses to NDV and SRBC

The effect of different levels of dietary CP and Thr on antibody titers against NDV and SRBC are shown in Table 3. Diets with different CP and Thr levels had no marked effect on antibody titers against NDV. The reduction of dietary CP content tended to decrease primary (p=0.16) and secondary (p=0.13) immune responses against SRBC; however, incremental levels of Thr up to 110% of the recommended values enhanced (p<0.05) antibody responses against SRBC inoculation. Kidd *et al.* (1997) evaluated cellular and humoral immunity in broilers fed diets supplemented with Thr and did not observe any improvement in immunocompetence. On the other hand, there are some evidences that Thr modulates immune functions (Bhargava *et al.*, 1971; Wang *et al.*, 2006; Li *et al.*, 2007) and that the immune system is sensitive to dietary Thr intake (Li *et al.*, 1999).

Table 3 – Effects of different levels of dietary CP and Thr on antibody titers against Newcastle and sheep red blood cell (SRBC) of broilers.

CP level[1] (%)	Thr level[1] (%)	Newcastle disease virus (Log_2)	Sheep red blood cell (Log_2) primary responses	Sheep red blood cell (Log_2) secondary responses
100	100	3.50	2.29	1.43
	110	2.38	2.67	1.75
	120	2.75	3.00	2.60
95	100	2.88	1.25	1.43
	110	3.86	1.43	2.71
	120	4.13	3.50	2.60
90	100	3.43	1.40	1.63
	110	3.63	2.00	1.88
	120	3.25	1.80	1.50
CP level				
100		2.88	2.65	1.93
95		3.62	2.06	2.25
90		3.43	1.73	1.67
Thr level				
100		3.27	1.65[b]	1.49[b]
110		3.29	2.03[ab]	2.11[a]
120		3.38	2.77[a]	2.23[a]
p-values				
CP		0.35	0.16	0.13
Thr		0.98	0.05	0.03
CP × Thr		0.44	0.26	0.14
SEM		0.65	0.56	0.35

[ab]Means with no common superscript within each column are significantly (p<0.05) different.

[1]The CP at the levels of 100, 95 and 90% of Ross 308 recommendations provided 21.9, 20.8 and 19.71% CP for the starter phase; 20.34, 19.32 and 18.31% CP for the grower phase, and 18.89, 17.95 and 17% CP for the finisher phase, respectively. Dietary Thr at the levels of 100, 110 and 120% of Ross 308 specifications provided 0.89, 0.98 and 1.07% Thr for the starter phase; 0.79, 0.86 and 0.94% Thr for the grower phase, and 0.71, 0.77 and 0.84% Thr for finisher phase, respectively.

Reduced dietary CP level diminished (p<0.05) anti-SRBC antibody titers, but the dietary supplementation of Thr up to 110% of recommended values resulted in the highest primary and secondary antibody responses. It was reported that Thr is a major component of plasma γ-globulins (Kim *et al.*, 2007) and Thr requirements for antibody production in broilers are higher than those for growth performance (Bhargava *et al.*, 1971; Jahanian, 2010a,b). Wang *et al.* (2006) and Li *et al.* (2007) demonstrated that dietary administration of Thr influences immune system components by increasing serum IgG levels as well as jejunal mucosal concentrations of IgA and IgG. Our findings support previous reports that, irrespective of dietary CP level, Thr supplementation of diets enhances primary immune responses and could preserve memory immune responses for a long time.

Jejunal morphology

The effects of different dietary CP and Thr levels on jejunal villus height (VH), crypt depth (CD), VH to CD ratio, and goblet cell numbers are presented in Table 4. Decrease in dietary CP content caused significant decrease in VH ($p<0.05$) and CD ($p<0.01$); however, VH to CD ratio was not influenced by dietary treatments. Dietary Thr supplementation, especially 110% level, had a significant effect ($p<0.05$) on VH to CD ratio. These changes were mainly due to the marked increase in VH ($p<0.05$) and significant decrease in CD ($p<0.05$). Proteins maintain intestinal viability and mass, in addition to providing energy for normal intestinal functions. The gastrointestinal tissues have relatively high protein turnover rate and high-protein diets provide adequate nutrient (especially CP) for basal metabolism and development of intestinal structure. On the other hand, Thr has special importance as an essential nutrient, because, compared with other amino acids, it has the highest metabolism in the portal-drained viscera (Schaart *et al.*, 2005). Relative to jejunal histological changes, it can be concluded that increasing dietary Thr levels promote an increase intestinal absorptive surface area. These findings confirm the reports by Law *et al.* (2000) and Ball (2001) with piglets and Zaefarian *et al.* (2008) with broilers, who showed that VH in animals fed on Thr-deficient diets decreased in comparison with groups receiving adequate Thr. As described by Wu (1998), 30 to 50% of Thr as well as some other amino acids (Arg, Pro, Ile, Val, Leu, Met, Lys, Phe, Gly, Ser) are directly used by the small intestine and are not available for extra-intestinal tissues. Therefore, it is probable that increases in dietary Thr levels provide adequate concentrations of this critical amino acid for the high turnover of mucosal tissues.

On the other hand, CD was influenced ($p<0.05$) by dietary CP and Thr interaction. Increasing Thr level up to 120% of Ross 308 specifications caused considerable increase in CD when the diets had low CP concentration (90% of recommended values). Our findings showed that the longest villi belonged to the

Table 4 – Effects of different levels of dietary CP and Thr on the histological changes of the jejunal epithelial cells measured at 42 days of age.

CP level[1] (%)	Thr level[1] (%)	Villus height (μm)	Crypt depth (μm)	Villus height / Crypt depth	Goblet cell number
100	100	1582	264[a]	6.03	4.00+[2]
	110	1618	244[ab]	6.63	5.00+
	120	1445	222[bcd]	6.24	4.00+
95	100	1410	240[abc]	5.86	3.00+
	110	1478	215[cd]	6.94	3.00+
	120	1369	206[d]	6.70	4.00+
90	100	1415	218[bcd]	6.65	2.00+
	110	1581	204[d]	7.31	2.00+
	120	1342	240[abc]	5.97	3.00+
CP level					
100		1549[a]	243[a]	6.30	4.33+
95		1419[b]	220[b]	6.50	3.33+
90		1446[ab]	221[b]	6.65	2.33+
Thr level					
100		1469[ab]	241[a]	6.18[b]	3.00+
110		1559[a]	221[b]	6.96[a]	3.33+
120		1385[b]	222[b]	6.30[ab]	3.66+
			p-values		
CP		0.05	0.01	0.57	
Thr		0.02	0.04	0.04	
CP × Thr		0.83	0.02	0.43	
SEM		72.60	10.14	0.40	

[abcd]Means with no common superscript within each column are significantly ($p<0.05$) different.

[1]The CP at the levels of 100, 95 and 90% of Ross 308 recommendations provided 21.9, 20.8 and 19.71% CP for the starter phase; 20.34, 19.32 and 18.31% CP for the grower phase, and 18.89, 17.95 and 17% CP for the finisher phase, respectively. Dietary Thr at the levels of 100, 110 and 120% of Ross 308 specifications provided 0.89, 0.98 and 1.07% Thr for the starter phase; 0.79, 0.86 and 0.94% Thr for the grower phase, and 0.71, 0.77 and 0.84% Thr for finisher phase, respectively.

[2]Number of +'s indicates severity of the histological changes.

birds fed on diets containing high-CP level and 110% Thr. Also, the lowest CD was observed in the group fed on low-CP, highest-Thr diets.

As shown in Table 4, reducing dietary CP decreased the number of goblet cells; whereas increasing dietary Thr levels increased the number of these cells in the jejunal epithelium. Goblet cells are mucin-producing and secreting sites in digestive system. As described in previous studies (Schaart *et al.*, 2005; Kim *et al.*, 2007), one of the primary fates of absorbed Thr is the synthesis of intestinal proteins, which are mainly secreted into the lumen as mucus, whereby protecting the gut from pathogens and anti-nutritional factors. Mucins are glycosylated proteins secreted along the intestinal epithelium and involved in the diffusion and absorption of the nutrients along the digestive tract. Mucins are particularly rich in threonine, proline and serine, with Thr representing as much as 28 to 40% of total amino acid profile of mucins. The jejunal goblet cell numbers was reduced in low-CP diets. Reduced goblet cell numbers may be related to lower endogenous protein losses associated with lower CP levels. Interestingly, increasing dietary Thr level caused a marked increase in goblet cell numbers. Nichols & Bertolo (2008) described that the *de novo* synthesis of mucosal and mucin proteins appeared to be highly sensitive to luminal Thr concentration, which demonstrates the importance of dietary Thr supply for gut metabolism. Our results suggest that although after reduction of dietary CP level the jejunal VH, CD and goblet cell numbers were decreased, but Thr dietary supplementation of diets at at least 110% of recommended values could compensate for these changes.

Performance parameters

Data on performance measurements are presented in Table 5. The present results showed that average daily feed intake (ADFI) was influenced (p<0.01) by dietary

Table 5 – Effects of different levels of dietary CP and Thr on performance parameters of broilers between 1 to 42 d of age.

CP level[1] (%)	Thr level[1] (%)	Average daily weight gain (g/d per bird)				Average daily feed intake (g/d per bird)				Feed conversion ratio (g feed: g gain)			
		1 to 12 d	13 to 24 d	25 to 42 d	1 to 42 d	1 to 12 d	13 to 24 d	25 to 42 d	1 to 42 d	1 to 12 d	13 to 24 d	25 to 42 d	1 to 42 d
100	100	26.31	44.54	86.68	55.56	34.08	85.32	131.69	87.97	1.30	1.92	1.52	1.58
	110	25.10	47.03	95.86	61.28	33.45	84.95	136.81	92.59	1.33	1.81	1.43	1.51
	120	25.69	44.30	91.23	57.81	33.33	83.95	133.23	88.10	1.29	1.82	1.46	1.52
95	100	22.48	43.36	87.07	53.56	32.03	81.07	126.72	83.32	1.43	1.87	1.46	1.55
	110	24.81	44.58	87.37	56.12	33.08	82.79	133.74	87.88	1.33	1.86	1.53	1.57
	120	23.87	47.23	88.02	56.28	33.10	83.57	131.98	87.42	1.39	1.77	1.50	1.55
90	100	23.35	42.96	91.59	56.19	33.01	82.54	134.09	87.87	1.42	1.93	1.47	1.56
	110	23.29	43.30	92.16	56.34	32.88	80.22	133.37	86.65	1.42	1.86	1.45	1.54
	120	22.76	45.40	88.78	56.30	32.24	83.24	130.73	86.57	1.42	1.83	1.48	1.54
CP level													
100		25.7[a]	45.96	91.26	58.22[a]	33.62	84.74[a]	133.91	89.55	1.31[b]	1.85	1.47	1.54
95		23.72[b]	45.06	87.49	55.32[b]	32.74	82.48[b]	130.82	86.21	1.38[a]	1.84	1.50	1.56
90		23.13[b]	43.89	90.84	56.28[b]	32.71	82.00[b]	132.73	87.03	1.42[a]	1.87	1.46	1.55
Thr level													
100		24.05	43.62[b]	88.44	55.10[b]	33.04	82.98	130.83	86.39	1.38	1.91[a]	1.48	1.57
110		24.40	44.97[ab]	91.79	57.91[a]	33.14	82.65	134.64	89.04	1.36	1.85[ab]	1.47	1.54
120		24.11	46.31[a]	89.35	56.80[ab]	32.89	83.59	131.98	87.36	1.37	1.81[b]	1.48	1.54
p-values													
CP		0.01	0.19	0.13	0.01	0.14	0.01	0.60	0.11	0.01	0.63	0.59	0.60
Thr		0.75	0.05	0.22	0.01	0.88	0.60	0.45	0.25	0.72	0.05	0.93	0.20
CP × Thr		0.31	0.73	0.27	0.14	0.43	0.23	0.79	0.37	0.27	0. 83	0.33	0.42
SEM		0.84	1.36	2.39	1.11	0.59	1.14	3.73	19.3	0.03	0.05	0.04	0.02

[ab]Means with no common superscript within each column are significantly (p<0.05) different.

[1]The CP at the levels of 100, 95 and 90% of Ross 308 recommendations provided 21.9, 20.8 and 19.71% CP for the starter phase; 20.34, 19.32 and 18.31% CP for the grower phase, and 18.89, 17.95 and 17% CP for the finisher phase, respectively. Dietary Thr at the levels of 100, 110 and 120% of Ross 308 specifications provided 0.89, 0.98 and 1.07% Thr for the starter phase; 0.79, 0.86 and 0.94% Thr for the grower phase, and 0.71, 0.77 and 0.84% Thr for finisher phase, respectively.

CP level in grower phase; however, different levels of Thr had no pronounced effect on ADFI. Reduction of dietary CP level caused significant (p<0.01) increase in FCR values in starter phase. This change was largely due to the decrease (p<0.01) in BWG during this period. Dietary supplementation of Thr (at least 110% of Ross recommended levels) improved FCR in the grower phase (p<0.05) and also tended to improve it during entire trial period. These improvements were largely due to the marked increase (p<0.05) in BWG. Although the interaction between dietary CP and Thr was not significant for FCR during the starter period, the best FCR values were obtained when the birds fed on high-CP, high-Thr diets. Interestingly, in the grower period, the best FCR and BWG values were obtained in birds fed on diets containing 95% CP level supplemented with 120% Thr.

It seems that the response of broiler performance to different dietary CP and Thr levels was time-dependent, because our findings showed that reducing dietary CP (to 90% of recommended values) decreased BWG only during the starter phase, with no suppressive effect during remaining experimental period. Also, dietary Thr addition at least up to 110% of recommended levels immediately after the starter phase caused a significant improvement (p<0.05) in BWG.

These findings are consistent with those of previous studies that reported improvements in broiler growth performance during the grower (Smith & Waldroup, 1988; Chung *et al.*, 1995; Ciftci & Ceylan, 2004; Jahanian, 2010b) and finisher (Chung *et al.*, 1995) periods in response to incremental levels of dietary Thr. Also, Kidd *et al.* (1996) observed significant improvements in FCR values as a result of dietary Thr supplementation in broilers.

The higher crystalline amino acids content of diet may result in better amino acid availability and better performance because it is assumed that the availability of free crystalline amino acid is higher than that of amino acids in intact proteins. Therefore, our data suggest that after the starter period, reducing dietary CP to 90% of the recommended values had no detrimental impact on broiler performance, with the best result obtained when diets supplemented with Thr at the level of 110% of recommended values. Furthermore, it is probable that Thr supplementation enhances growth performance by improving intestinal health. As previously explained, using at least 110% Thr in experimental diets increased VH to CD ratio. Higher VH to CD ratio may indicate slower tissue turnover, suggesting lower demands to compensate for villus atrophy. Therefore, lower energy would be required to support slower tissue turnover. Taller villi indicate more mature epithelia and enhanced absorptive efficiency due to the increased absorptive surface area. Greater villi heights increase the activity of the enzymes secreted from the tips of the villi (Hampson, 1986), resulting in improved digestibility coefficients. Moreover, it was shown that increasing dietary Thr levels caused a significant increase in goblet cell numbers, improving the protection of intestinal absorptive area due to the production of mucin (Nichols & Bertolo, 2008). The mentioned results suggest that dietary Thr supplementation with at least 110% of recommended values could improve the health status of the gastrointestinal tract and consequent growth performance mainly due to the development of intestinal structures and functions, such as increase in absorptive surface area and goblet cell numbers.

Some of previous findings (Kreukniet *et al.*, 1996; Koenen *et al.*, 2002) suggest that there is a negative relationship between growth performance and stimulation of immune system, but the present data indicate that dietary Thr supplementation not only improved FCR and BWG, but also induced and preserved high antibody levels for a long time. The present findings suggest that Thr broiler needs for antibody production were higher than those for optimum growth performance.

From the present findings, it seems that Ross nutritional specifications for dietary CP content in starter period is precise; after starting period, however, one could reduce dietary CP content up to 10% without any adverse effect on broiler performance. Of course, the best results obtained when diets were supplemented with Thr at the level of 110% of Ross 308 recommendations. It seems that the beneficial impacts of Thr on immune responses, as well as intestinal health indices, may improve broiler performance.

REFERENCES

Aletor VA, Hamid II, Nieb E, Pfeffer E. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient. Journal of the Science of Food and Agriculture 2000;80:547–554.

Alleman F, Michel J, Chagneau AM, Leclercq B. The effects of dietary protein independent of essential amino acids on growth and body composition in genetically lean and fat chickens. British Poultry Science 2000;41:214–218.

AOAC. Official methods of analysis of AOAC International. 17th ed. Washington, DC. 2002.

Ball RO. Threonine requirement and the interaction between threonine intake and gut mucins in pigs. Symposium of the 2001 Degussa; 2001; Banff Pork Seminar. Banff, Alberta. Canada.

Barkley GR, Wallis IR. Threonine requirements of broiler chickens: why do published values differ? British Poultry Science 2001;42:610–615.

Bhargava KK, Hanson RP, Sunde ML. Effects of threonine on growth and antibody production in chicks infected with Newcastle disease virus. Poultry Science 1971; 50:710–713.

Chung TK, Khajarern J, Khajarern S. Effect of dietary threonine on growth performance and carcass characteristics of broiler chickens raised at high temperature. Singapore: Department of Animal Sciences, Faculty of Agriculture, Khan Keen University; 1995. p.1-3.

Ciftci I, Ceylan N. Effects of dietary threonine and crude protein on growth performance, carcase and meat composition of broiler chickens. British Poultry Science 2004;45:280–289.

Danicke S, Bottcher W, Jeroch H, Thielebein J, Simon O. Replacement of soybean oil with tallow in rye-based diets without xylanase increases protein synthesis in small intestine of broilers. The Journal of Nutrition 2000;130:827–834.

Defa L, Xiao C, Qiao S, Zhang J, Johnson EW, Thacker PA. Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. Animal Feed Science and Technology 1999;78:179–188.

Dozier WA, Moran ET, Kidd MT. Threonine requirement of broiler males from 42 to 56 days in a summer environment. Journal of Applied Poultry Research 2000a;9:496–500.

Dozier WA, Moran ET, Kidd MT. Responses of fast- and slow-feathering male broilers to dietary threonine during 42 to 56 days of age. Journal of Applied Poultry Research 2000b;9:460–467.

Dozier WA, Moran ET, Kidd MT. Comparisons of male and female broiler responses to dietary threonine from 42 to 56 days of age. Journal of Applied Poultry Research 2001;10:53–59.

Fernandez RS, Aoyagi S, Han Y, Parsons CM, Baker DH. Limiting order to amino acids in corn and soybean meal for growth of the chick. Poultry Science 1994;73:1887–1889.

Hampson DJ. Alteration in piglet small intestinal structure at weaning. Research in Veterinary Science 1986;40:32–40.

Han Y, Suzuki H, Parsons CM, Baker DH. Amino acid fortification of a low protein corn-soybean meal diet for maximal weight gain and feed efficiency of chicks. Poultry Science 1992;71:1168–1178.

Holsheimer JP, Vereijken PFG, Schutte JB. Response of broiler chicks to threonine supplemented diets to 4 weeks of ages. British Poultry Science 1994; 35:551–562.

Jahanian R. Effects of dietary threonine on performance and immunocompetence of starting broiler chicks. 2nd International Veterinary Poultry Congress; 2010a Feb 20-21; Tehran. Iran. p.200.

Jahanian R. Threonine needs of growing broiler chickens for performance and optimum immunological functions in response to dietary crude protein concentration. 2nd International Veterinary Poultry Congress; 2010b Feb 20-21; Tehran. Iran. p. 200.

Jahanian R, Nassiri-Moghaddam H, Rezaei A. Improved broiler chick performance by dietary supplementation of organic zinc sources. Asian-Australian Journal of Animal Science 2008;21:1348-1354.

Kidd MT, Kerr BJ, Anthony NB. Dietary interactions between lysine and threonine in broilers. Poultry Science 1997;76:608–614.

Kidd MT, Kerr BJ. Threonine responses in commercial broilers at 30 to 42 days. Journal of Applied Poultry Research 1997;6:362–367.

Kidd MT, Kerr BJ, Firman JD, Boling SD. Growth and carcass characteristics of broilers fed low protein, threonine supplemented diets. Journal of Applied Poultry Research 1996;5:180–190.

Kidd MT, Gerard PD, Heger J, Kerr BJ, Rowe DE. Threonine and crude protein responses in broiler chicks. Animal Feed Science and Technology 2001; 94:57–64.

Kim SW, Mateo RD, Yin YL, Wu G. Functional amino acids and fatty acids for enhancing production performance of sows and piglets. Asian-Australian Journal of Animal Science 2007;20:295–306.

Koenen ME, Boonstra-Blom AG, Jeurissen SHM. Immunological differences between layer and broiler-type chickens. Veterinary Immunology and Immunopathology 2002;89:47–56.

Kreukniet MB, Jeurissen SH, Nieuwland MG, Gianotten N, Joling P. The B cell compartment of two chicken lines divergently selected for antibody production: Differences in structure and function. Veterinary Immunology and Immunopathology 1996;51:157–171.

Law G, Adjiri-Awere A, Pencharz PB, Ball RO. Gut mucins in piglets are dependent upon dietary threonine. Advances in Pork Production. Proceedings of the 2000 Banff Pork Seminar 2000;11:10. Abstract.

Lehmann D, Pack M, Jeroch H. Effects of dietary threonine in starting, growing, and finishing turkey toms. Poultry Science 1997;76:696–702.

Leshchinsky TV, Klasing KC. Relationship between the level of dietary vitamin E and the immune response of broiler chickens. Poultry Science 2001;80:1590–1599.

Li DF, Xiao CT, Qiao SY, Zhang JH, Johnson EW, Thacker PA. Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. Animal Feed Science and Technology 1999;78:179–188.

Li P, Yin YL, Li D, Kim SW, Wu G. Amino acids and immune function: a review. British Journal of Nutrition 2007;98:237–252.

Mack S, Bercovici D, De Groote G, Leclercq B, Lippens M, Pack M, Schutte JB, Van Cauwenberghe S. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. British Poultry Science 1999;40:257–265.

Mahdavi AH, Rahmani HR, Nili N, Samie AH, Soleimanian-Zad S, Jahanian R. Effects of dietary egg yolk antibody powder on growth performance, intestinal Escherichia coli colonization, and immunocompetence of challenged broiler chicks. Poultry Science 2010;89:484–494.

Mandal AB, Kaur S, Johri AK, Elangovan AV, Deo C, Shrivastava HP. Response of growing Japanese quails to dietary concentration of L-threonine. Journal of the Science of Food and Agriculture 2006;86:793–798.

Newcastle-Bronchitis vaccines. B1 type, B1 strain, Massachusetts and Connecticut types, live virus. Gainesville: Merial Select; 2007.

Nichols NL, Bertolo RF. Luminal threonine concentration acutely affects intestinal mucosal protein and mucin synthesis in piglets. The Journal of Nutrition 2008; 138:1298–1303.

Plitzner C, Ettle T, Handle S, Schmidt P, Windisch W. Effects of different dietary threonine levels on growth and slaughter performance in finishing pigs. Czech Journal of Animal Science 2007;52:447–455.

Rabbins KR. Threonine requirement of the broiler chick as affected by protein level and source. Poultry Science 1987;66:1531–1534.

Rosa AP, Pesti GM, Edwards HM, Bakalli RI. Threonine requirements of different broiler genotypes. Poultry Science 2001; 80:1710–1717.

Ross. Ross broiler management manual. Alabama: Aviagen; 2009.

Saldana CI, Knabe DA, Owen KQ, Burgoon KG, Gregg EJ. Digestible threonine requirements of starter and finisher pigs. Journal of Animal Science 1994;72:144–150.

SAS Institute. SAS user's guide. Version 8.02 ed. Cary; 2001.

Schaart MW, Schierbeek H, Van der Schoor SRD, Stoll B, Burrin DG, Reeds PG, Van Goudoever JB. Threonine utilization is high in the intestine of piglets. The Journal of Nutrition 2005;135:765-770.

Smith NK, Waldroup P. Investigation of threonine requirements of broiler chicks diets based on grain sorghum and soybean meal. Poultry Science 1988;67:108–112.

Swatson HK, Gous RM, Iji PA. Biological performance and gastrointestinal development of broiler chicks fed diets varying in energy: protein ratios. South African Journal of Animal Science 2000; 30:136–137.

Van der Zijpp AJ, Leenstra FR. Genetic analysis of the humoral immune response of white leghorn chicks. Poultry Science 1980;59:1363-1369.

Waldroup PW, England JA, Kidd MT. An evaluation of threonine requirements of young turkeys. Poultry Science 1998;77:1020–1023.

Wang X, Qiao S, Yin Y, Yue L, Wang Z, Wu G. A deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. The Journal of Nutrition 2007;137:1442–1446.

Wang X, Qiao SY, Liu M, Ma YX. Effects of graded levels of true ileal digestible threonine on performance, serum parameters and immune function of 10–25 kg pigs. Animal Feed Science and Technology 2006; 129:264–278.

Wu G. Intestinal mucosal amino acid catabolism. The Journal of Nutrition 1998; 128:1249–1252.

Zaefarian F, Zaghari M, Shivazad M. The threonine requirements and its effects on growth performance and gut morphology of broiler chicken fed different levels of protein. International Journal of Poultry Science 2008;7:1207–1215.