Effect of a low crude protein diet supplemented with different levels of threonine on growth performance, carcass traits, blood parameters, and immune responses of growing broilers

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ABSTRACT A study was conducted to evaluate growth performance, carcass traits, blood serum parameters, and immune responses of Ross 308 male broilers fed diets containing 2 different crude protein (CP) levels (97.5 and 100%) and 4 threonine (Thr) levels (100, 110, 120, and 130% of Ross recommendations for starter and grower periods). A completely randomized block design was adopted and main effects (CP and Thr) were arranged in a 2 × 4 factorial approach. Optimum growth performance was achieved when broiler requirements for CP and Thr were 100% satisfied. The 110% Thr inclusion in 97.5% CP diet increased ADG, ADFI, energy intake, and protein intake (Thr, $P < 0.01$; quadratic, P = 0.01). The G:F (linear, $P = 0.05$) and energy efficiency (linear, $P = 0.04$) tended to decreased (Thr, $P = 0.09$) by increasing Thr supplementation level, whereas protein efficiency tended to increase (CP, $P = 0.06$) by reducing CP level. The 110% Thr inclusion in 97.5% CP diet increased eviscerated carcass weight (CP × Thr, $P = 0.03$) and carcass yield (Thr, $P = 0.08$; quadratic, $P = 0.05$). The reduction of CP content promoted fat abdominal deposition (CP, $P = 0.05$). Incremental Thr raised abdominal fat (Thr, $P = 0.01$; linear, $P = 0.01$). The 97.5% CP diets resulted in higher serum concentrations of uric acid (CP, $P = 0.02$), total and high- and low-density lipoprotein-linked cholesterol (CP, $P \leq 0.01$), and alanine aminotransferase (CP, $P = 0.05$) and lower (CP, $P = 0.01$) concentrations of triglycerides and very low density lipoproteins compared with the 100% CP diets. However, the Thr inclusion improved serum lipid profile. Irrespective of CP content, incremental Thr levels up to 120% increased (Thr, $P = 0.01$) broiler immune responses against Newcastle disease virus and sheep red blood cells. In order to reduce dietary CP content, strategies to increase synthetic amino acid availability, such as the use of encapsulated amino acids, should be taken into account.

Key words: Blood serumparameter, carcass traits, growth performance, immune response, protein and threonine

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INTRODUCTION

In poultry nutrition, there is great interest in the use of low crude protein (**CP**) diets for economic and environmental advantages that may result such as reduction of feed cost and total N output to the environment. However, as dietary CP level decreases, essential amino acids also decrease. Therefore, supplemental amino acids other than methionine (**Met**) and lysine (**Lys**), which are the first 2 limiting amino acids in most of plant protein sources, may be required to support optimal growth performance and to ensure maximum economic return (Kidd and Kerr, 1996).

Threonine (**Thr**) is the third limiting amino acid in low-CP poultry diets based on corn-soybean meal (Fernandez et al., 1994). This amino acid plays an important role in the maintenance of intestinal mucosal integrity and barrier function (Mao et al., 2011). A large proportion of dietary Thr is incorporated into intestinal-mucosal proteins, especially mucins, as well as into digestive enzymes in the lumen necessary for nutrient digestion and adsorption (Dozier et al., 2001; Wang et al., 2009). Thus, inadequate provision of dietary Thr may adversely affect the efficiency of nutrient utilization.

Because the Thr requirement of broilers depends on several factors such as age of birds and CP content and main feed ingredients in the diet (Barkley and Wallis, 2001), attention should be paid to interactions between Thr and these factors. To date, few studies have been conducted on the possible interaction between dietary CP content and Thr requirement in broilers and controversial results have been reported (Kidd et al., 2001; Ciftci and Ceylan, 2004; Jahanian, 2010a, 2010b; Abbasi et al., 2014). However, studies have showed that

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dietary CP content can be reduced without any adverse effect on broiler performance when the Thr optimum requirement is satisfied (Ciftci and Ceylan, 2004; Abbasi et al., 2014). Moreover, Jahanian (2010a, 2010b) and have reported that Thr supplementation of low-CP diets enhances immune functions and intestinal health with positive impacts on broiler performance. Further research must be conducted in order to define the optimal levels of Thr for low CP diets capable to satisfy the specific requirements of broilers to guarantee maximum performance and enhance immune system function.

The aim of this study was to investigate the effect of a low-CP diet supplemented with different levels of Thr on growth performance, carcass traits, blood serum parameters, and immune responses of growing broilers.

MATERIALS AND METHODS

Birds and Housing

All procedures were approved by the Animal Care and Welfare Committee of Islamic Azad University.

A total of 240 1-day-old Ross 308 male broilers were obtained from a commercial hatchery (Aviagen, Newbridge, Scotland, UK). The chicks were randomly allocated to 8 dietary treatments. Each treatment was assigned to 3 replicate floor pens with 10 birds per pen. The chicks were weighed and placed in cages which provided a floor area of 10 birds/m^2 at 1 d of age. Initial mean and range of body weight (**BW**) were homogeneous (i.e., 42. 7 ± 0.8 g) for all pens. The cage floors were covered with paper roll litter and the birds remained in the cages until the end of the experiment (42 d). Each pen was equipped with automatic drinkers and one pan feeder. Water and experimental diets were offered ad libitum. House temperature was maintained at 33°C for 3 weeks thereafter was maintained at 23°C by a thermostatically controlled gas heater. Ambient relative humidity was maintained at 55 to 65% by a water spray. The lighting program throughout the study was as follows: 23L:1D in the periods from 1 to 6 d and from 36 to 42 d and 20L:4D in the period from 7 to 35 d.

Experimental Diets

Broiler response to dietary CP and Thr were evaluated from 1 to 21 d (starter period) and from 22 to 42 d (grower period). The treatments consisted of diets containing 2 different dietary CP levels (97.5 and 100% of Ross 308 recommendations for starter and grower periods; Ross, 2007) and 4 dietary Thr levels (100, 110, 120, and 130% of Ross 308 recommendations for starter and grower periods; Ross, 2007). Dietary CP levels to meet 97.5 and 100% of Ross 308 specifications were respectively 21.45 and 22.00% of the diet for starter period, and 19.01 and 19.45% for grower period. The 4 Thr dietary levels (100, 110, 120, and 130% of Ross 308 recommendations) were respectively 0.94, 1.03, 1.13, and 1.22% of the diet for starter period, and 0.74, 0.81, 0.89, and 0.96% for grower period. Experimental diets were formulated to meet or exceed the nutritional requirements of broilers as suggested by Ross (2007) for starter and grower periods. All the experimental diets had same metabolizable energy (**ME**; 3,025 and 3,150 kcal/kg for starter and grower periods, respectively) and essential amino acid content except for Thr. Ingredients, chemical composition and energy of the broiler diets for starter and grower periods are shown in the Table 1.

Performance and Carcass Measurements

Body weight and feed intake were measured on d 21 and 42, respectively at the end of the starter and grower period. On the same days, average daily food intake (**ADFI**), average daily gain (**ADG**), gain to feed ratio (**G:F**), average daily energy intake (**ADEI**), gain to metabolizable energy ratio (**G:ME**), average daily protein intake (**ADPI**), and gain to protein intake ratio (**G:P**) were calculated and corrected for mortality.

On the last day of the experiment (d 42), 1 bird per pen was chosen randomly, weighed, and slaughtered by cervical dislocation to evaluate carcass traits. The weights of the carcass (defeathered) with and without head and drumsticks were recorded. Viscera and abdominal fat were then removed and the carcass yield and the relative weights (as a percentage of eviscerated carcass) of anatomical parts (breast, drumsticks, wings, neck, head, and notarium), abdominal fat and entrails (ventriculus, heart, kidneys, and pancreas), gut tracts (duodenum, jejunum, ileum, colon, right cecum, left cecum, and rectum), and organs related to immune system (thymus, liver, spleen, and bursa of Fabricius) were calculated (Shabani et al., 2015).

Blood Serum Parameters

At the end of the trial (d 42), blood samples (1.5 mL) were collected into EDTA tubes from the broilers by wing vein. Blood serum was separated by centrifugation at 3,000 rpm × 10 min and stored at −18°C until analysis. The serum samples were analyzed for levels of uric acid, total cholesterol, triglycerides, very low density lipoproteins (**VLDL**), cholesterol linked to high density lipoproteins (**HDL**), cholesterol linked to low density lipoproteins (**LDL**), glucose, aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), total protein, albumin, and globulin. The concentrations for these parameters were determined by using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran) as described by Golrokh et al. (2016).

Immune Response

The birds were vaccinated against Newcastle disease virus (**NDV**; strain *Viscerotropicvelogenic*) at 1 (by

Table 1. Ingredients, chemical composition and energy of the broiler diets for starter and grower periods.

Item	Starter[1] (from 1 to 21 d) CP 97.5%	Starter[1] (from 1 to 21 d) CP 100%	Grower[2] (from 22 to 42 d) CP 97.5%	Grower[2] (from 22 to 42 d) CP 100%
Ingredients, %				
Corn	54.86	54.26	59.32	57.93
Soybean meal	31.47	31.13	31.43	32.80
Gluten meal	3.15	4.38	0.00	0.00
Soybean oil	3.80	3.55	4.90	4.87
Fish meal	1.28	1.28	0.00	0.00
$NaHCO_3$	0.16	0.16	0.18	0.18
Mineral oysters	1.26	1.26	1.13	1.13
Dicalcium phosphate	1.88	1.88	1.35	1.35
NaCl	0.23	0.23	0.23	0.23
Vitamin premix[3]	0.50	0.50	0.50	0.50
Mineral premix[3]	0.50	0.50	0.50	0.50
DL-Met	0.12	0.11	0.21	0.21
L-Lys	0.41	0.41	0.02	0.07
L-Thr	0.08	0.08	–	–
Zeolite	0.29	0.29	0.23	0.23
Calculated chemical composition, % as fed				
DM	85.86	85.85	88.68	86.83
CP[2]	21.45	22.00	19.01	19.45
Crude fat	5.25	5.25	6.95	6.95
Linoleic acid, %	1.25	1.25	1.00	1.00
Crude fiber	2.70	2.70	2.66	2.66
Ca	1.05	1.05	0.90	0.90
P	0.74	0.74	0.69	0.69
Available P	0.50	0.50	0.45	0.45
K	0.90	0.90	0.84	0.84
Mg	120.00	120.00	120.00	120.00
Na	0.16	0.16	0.16	0.16
Gly	0.93	0.93	0.86	0.86
Ser	1.10	1.10	1.02	1.02
Gly+Ser	2.40	2.40	2.21	2.21
His	0.59	0.59	0.55	0.55
Ile	0.90	0.90	0.86	0.86
Leu	1.90	1.90	1.80	1.80
Lys	1.27	1.27	1.11	1.11
Met	0.47	0.47	0.42	0.42
Cys	0.36	0.36	0.34	0.34
Met+Cys	0.85	0.85	0.76	0.76
Phe	1.08	1.08	0.98	0.98
Tyr	0.90	0.90	0.74	0.74
Phe+Tyr	1.97	1.97	0.18	0.18
Thr[3]	0.94	0.94	0.74	0.74
Trp	0.30	0.30	0.28	0.28
Val	1.00	1.00	0.96	0.96
Arg	1.30	1.30	1.30	1.30
Choride	0.18	0.18	0.16	0.16
Choline	0.17	0.17	0.15	0.15
ME[4], Kcal/kg	3,025	3,025	3,150	3,150

[1]For starter period, the 4 Thr dietary levels (100, 110, 120, and 130% of Ross 308 recommendations) were respectively 0.94, 1.03, 1.13, and 1.22% of the diet.

[2]For grower period, the 4 Thr dietary levels (100, 110, 120, and 130% of Ross 308 recommendations) were respectively 0.74, 0.81, 0.89, and 0.96% of the diet.

[3]Supplied the following per kg of diet: vitamin A, 5,000 IU; vitamin D_3, 500 IU; vitamin E, 3 mg; vitamin K_3, 1.5 mg; vitamin B_2, 1 mg; calcium pantothenate, 4 mg; niacin, 15 mg; vitamin B_6, 13 mg; Cu, 3 mg; Zn, 15 mg; Mn, 20 mg; Fe, 10 mg; K, 0.3 mg.

[4]The ME of the diets was estimated using the Carpenter and Clegg's equation (Leeson and Summers, 2001).

oral-spray), 7 and 20 d of age (oral route by drinking water), against infectious bronchitis (strain H120, by eye-drop application) and avian influenza (**AI**; strain H9N2, by oral-spray) at 1 d of age, and against Gumboro disease (strain 52/70, oral route by drinking water) at 12 and 21 d of age. All the vaccines were supplied from Razi Co. (Karaj, Iran).

One bird per each replicate was chosen at random and blood samples were collected from the brachial vein. Antibody titers (IgG2) against NDV and AI were measured by hemagglutination-inhibition test according to Cunningham (1971) at 17 and 42 d of age for NDV and 33 and 42 d of age for AI. Serum was separated by centrifugation (1,300 rpm for 15 min) after 1 h incubation at room temperature and stored at −20°C until the analysis.

The magnitude of the immune response against sheep red blood cells (**SRBC**) was determined as described by Pourhossein et al. (2015). Briefly, at 10 and 28 d of age SRBC suspension 5% phosphate-buffered saline (**PBS**) was injected into the breast muscle of 6 birds per treatment. Fourteen days after each sensitization (24 and 42 d of age, respectively), total antibody titers (total Ig) to SRBC were determined by hemagglutination assay in serum 7. In U-bottom microtiter plates, 2-fold serial dilutions of heat-inactivated (at 56°C) serum were made with PBS (0.01 mol/L; pH 7.4) for total antibody or PBS with 1.4% 2-mercaptoethanol for IgG antibody. All antibody titers were recorded as $\log_2$ of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC suspension in PBS. The IgM titer was determined by the difference between total and IgG titer.

Statistical Analysis

Data were tested for normality with the Shapiro-Wilk test before statistical analysis. Data measured at the end of two growth periods (i.e., starter and grower periods) were analyzed according to a completely randomized block design using the GLM procedure of SAS (2003) according to the model reported below:

$$Y_{ijk} = \mu + CP_i + Thr_j + (CP \times Thr)_{ij} + Period_k + e_{ijk},$$

where Y_{ijk} = the response variable, μ = overall mean, CP_i = fixed effect of dietary CP (i = 2), Thr_j = fixed effect of Thr inclusion (j = 4), $(CP \times Thr)_{ij}$ = first order interaction b, $Period_k$ = fixed block effect of growth period (k = 2), and e_{ijk} = random residual error.

Data measured once during or at the end of the experiment were analyzed according to a completely randomized design using the GLM procedure of according to the model reported below:

$$Y_{ij} = \mu + CP_i + Thr_j + (CP \times Thr)_{ij} + e_{ij},$$

whereY_{ij} = the response variable, μ = overall mean, CP_i = fixed effect of dietary CP (i = 2), Thr_j = fixed effect of Thr inclusion (j = 4), $(CP\ x\ Thr)_{ij}$ = first order interaction, and e_{ij} = random residual error. The IML procedure of SAS (2003) was used to generate contrast

Table 2. Growth performance of Ross 308 broilers fed diets with different CP and Thr levels.

Treatment		Starter period (from 1 to 21 d)						
CP (%)	Thr (%)	ADFI (g)	ADG (g)	G:F	ADEI[1] (kcal)	G:ME[2] (g/kcal)	ADPI[3] (g)	G:P[4]
97.5	100	51.27	32.94	0.64	155.09	0.21	11.00	2.99
	110	54.66	36.93	0.67	165.34	0.22	11.72	3.15
	120	53.62	36.09	0.67	162.20	0.22	11.50	3.14
	130	50.64	30.58	0.60	153.20	0.20	10.86	2.82
100	100	56.65	36.77	0.65	171.37	0.21	12.46	2.95
	110	55.18	36.46	0.66	166.91	0.22	12.14	3.00
	120	53.58	34.51	0.64	162.10	0.21	11.79	2.93
	130	52.05	34.36	0.66	157.46	0.22	11.45	3.00
Treatment		Grower period (from 22 to 42 d)						
CP (%)	Thr (%)	ADFI (g)	ADG (g)	G:F	ADEI[1] (kcal)	G:ME[2] (g/kcal)	ADPI[3] (g)	G:P[4]
97.5	100	137.75	69.09	0.50	429.79	0.16	26.17	2.64
	110	157.72	81.82	0.52	492.08	0.17	29.97	2.73
	120	149.33	75.07	0.50	465.90	0.16	28.37	2.65
	130	137.14	68.83	0.50	427.89	0.16	26.06	2.64
100	100	162.37	84.58	0.52	506.61	0.17	31.66	2.67
	110	154.96	79.60	0.51	483.48	0.16	30.22	2.63
	120	149.00	73.45	0.49	464.88	0.16	29.05	2.53
	130	144.21	67.74	0.47	449.94	0.15	28.12	2.41
SEM		2.015	1.433	0.012	6.298	0.004	0.402	0.067
P-values								
Period		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CP		0.01	0.06	0.66	0.01	0.64	<0.01	0.06
Thr		<0.01	<0.01	0.09	<0.01	0.09	<0.01	0.18
CP × Thr		0.01	0.01	0.70	0.01	0.68	0.01	0.62
Contrasts								
Linear		0.01	<0.01	0.05	0.01	0.04	0.01	0.09
Quadratic		0.01	0.01	0.12	0.01	0.13	0.01	0.17

[1]ADEI = average daily energy intake.
[2]G:ME = gain to metabolizable energy intake ratio.
[3]ADPI = average daily protein intake.
[4]G:P = gain to protein intake ratio.

coefficients to evaluate linear or quadratic response of Thr inclusion. Significance was considered at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

RESULTS

Growth Performance

The effects of different levels of dietary CP and Thr on growth performance are shown in Table 2. A CP ×Thr interaction effect ($P = 0.01$) was observed on ADFI, ADG, ADEI, and ADPI. In particular, when the 100% CP diets were fed to the broilers, the ADFI, ADG, ADEI, and ADPI decreased (Thr, $P < 0.01$; linear, $P = 0.01$) by increasing Thr inclusion level. However, when the 97.5% CP diets were fed, the highest ADFI, ADG, ADEI, and ADPI were observed at 110% Thr inclusion level (Thr, $P < 0.01$; quadratic, $P = 0.01$). A similar trend was observed in starter and grower periods (period, $P < 0.01$). The G:F, G:ME, and G:P were higher in starter than grower period (period, $P < 0.01$). The G:F (linear, $P = 0.05$) and G:ME (linear, $P = 0.04$) tended to decreased (Thr, $P = 0.09$) by increasing Thr supplementation level. The reduction of the dietary CP level tended to increase (CP, $P = 0.06$) the G:P.

Carcass Traits and Relative Organ Weights

The effects of different levels of dietary CP and Thr on carcass traits and relative organ weights are shown in Table 3. When the 97.5% CP diets were fed to broilers, the highest eviscerated carcass weight was observed at 110% Thr inclusion level, whereas no differences due to Thr supplementation were observed for this parameter at 100% CP treatment (CP × Thr, $P = 0.03$). On average between the CP diets, the carcass yield tended to be greater (at 110%) than other Thr inclusion levels (Thr, $P = 0.08$; quadratic, $P = 0.05$).

There were no CP, Thr, and CP × Thr interaction effects on the relative weights of the different anatomical parts, entrails, intestinal tracts, and organs related to immune system. The broilers fed the 100% CP diets had lower (CP, $P = 0.05$) abdominal fat than the broilers fed the 97.5% CP diets. However, the abdominal fat was raised (Thr, $P = 0.01$; linear, $P = 0.01$) by increasing Thr inclusion at both CP levels.

Blood Serum Parameters

The effects of different levels of dietary CP and Thr on blood serum parameters are shown in Table 4. The 97.5% CP diets resulted in higher blood serum

Table 3. Carcass traits and relative weights (as a percentage of eviscerated carcass) of anatomical parts, abdominal fat, entrails, gut tracts, and organs related to immune system of Ross 308 broilers fed diets withdifferent CP and Thr levels.

Treatment		Carcass traits				
CP (%)	Thr (%)	Pre-slaughtered BW (g)	Defeathered carcass weight (g)	Full carcass weight[1] (g)	Eviscerated carcass weight (g)	Carcass yield (%)
97.5	100	1,916	1,683	1,550	1,087	70.31
	110	2,303	2,095	1,914	1,447	75.76
	120	2,181	2,007	1,849	1, 387	74.97
	130	2,091	1,841	1,761	1,191	67.72
100	100	2,250	2,070	1,880	1,422	76.01
	110	2,219	2,006	1,812	1,363	75.39
	120	2,127	1,938	1,815	1,330	73.12
	130	2,122	1,906	1,787	1,243	69.89
SEM		109.9	94.4	103.6	68.7	2.518
P-values						
CP		0.48	0.29	0.46	0.22	0.44
Thr		0.40	0.22	0.51	0.05	0.08
CP × Thr		0.25	0.08	0.21	0.03	0.48
Contrasts						
Linear		0.92	0.77	0.66	0.47	0.09
Quadratic		0.17	0.06	0.18	0.01	0.05

Treatment		Anatomical parts					
CP (%)	Thr (%)	Breast (%)	Drumsticks (%)	Wings (%)	Neck (%)	Head (%)	Notarium (%)
97.5	100	28.11	29.44	4.00	3.20	2.62	3.41
	110	31.06	25.47	3.84	2.65	2.34	3.88
	120	31.40	27.58	3.49	2.84	2.32	3.41
	130	29.06	28.20	4.26	2.92	2.57	3.21
100	100	31.40	27.30	3.54	2.76	2.49	3.98
	110	32.31	25.51	3.72	2.63	2.30	3.77
	120	28.15	27.56	3.91	2.56	2.34	3.66
	130	28.94	27.03	3.78	2.75	2.45	3.64
SEM		2.272	1.758	0.185	0.211	0.219	0.486
P-values							
CP		0.86	0.52	0.25	0.14	0.67	0.43
Thr		0.68	0.42	0.37	0.40	0.62	0.85
CP × Thr		0.55	0.91	0.09	0.79	0.99	0.91
Contrasts							
Linear		0.57	0.97	0.27	0.57	0.87	0.49
Quadratic		0.41	0.26	0.25	0.13	0.20	0.73

Treatment		Abdominal fat and entrails				
CP (%)	Thr (%)	Abdominal fat (%)	Ventriculus (%)	Heart (%)	Kidneys (%)	Pancreas (%)
97.5	100	1.90	3.06	0.46	2.10	2.62
	110	1.82	2.45	0.48	2.59	2.34
	120	2.14	2.80	0.46	2.20	2.32
	130	2.32	3.23	0.49	2.50	2.57
100	100	1.43	3.02	0.53	2.67	2.49
	110	1.68	2.75	0.49	2.38	2.30
	120	1.90	3.33	0.47	2.82	2.34
	130	2.26	2.55	0.43	2.24	2.45
SEM		0.151	0.220	0.033	0.207	0.219
P-values						
CP		0.05	0.86	0.68	0.23	0.96
Thr		0.01	0.17	0.74	0.87	0.65
CP × Thr		0.57	0.07	0.26	0.08	0.55
Contrasts						
Linear		0.01	0.98	0.29	1.00	0.30
Quadratic		0.42	0.41	0.94	0.42	0.58

Treatment		Gut						
CP (%)	Thr (%)	Duodenum (%)	Jejunum (%)	Ileum (%)	Colon (%)	Right cecum (%)	Left cecum (%)	Rectum (%)
97.5	100	0.92	2.88	0.45	0.20	0.26	0.27	0.13
	110	0.87	2.59	0.53	0.21	0.32	0.26	0.10
	120	0.77	2.57	0.45	0.21	0.26	0.26	0.10
	130	0.88	2.71	0.47	0.21	0.24	0.25	0.11
100	100	0.88	2.60	0.53	0.26	0.34	0.27	0.10
	110	0.86	2.55	0.50	0.18	0.28	0.26	0.09
	120	0.88	2.61	0.47	0.18	0.26	0.25	0.10
	130	0.88	2.61	0.44	0.18	0.24	0.26	0.10

Table 3. *continued*

Treatment		Carcass traits						
CP (%)	Thr (%)	Pre-slaughtered BW (g) / Defeathered carcass weight (g)	Full carcass weight[1] (g)		Eviscerated carcass weight (g)		Carcass yield (%)	
SEM		0.087	0.206	0.070	0.030	0.051	0.054	0.010
P-values								
CP		0.85	0.51	0.83	0.82	0.80	0.93	0.15
Thr		0.85	0.84	0.82	0.58	0.60	0.99	0.37
CP × Thr		0.85	0.88	0.85	0.31	0.69	1.00	0.48
Contrasts								
Linear		0.74	0.75	0.49	0.29	0.22	0.80	0.88
Quadratic		0.49	0.42	0.78	0.44	0.80	0.86	0.10

Treatment		Organs related to immune system			
CP (%)	Thr (%)	Thymus (%)	Liver (%)	Spleen (%)	Bursa of Fabricius (%)
97.5	100	0.23	2.54	0.11	0.10
	110	0.23	2.62	0.09	0.09
	120	0.25	2.55	0.11	0.09
	130	0.27	2.59	0.13	0.12
100	100	0.22	2.19	0.09	0.08
	110	0.22	2.20	0.09	0.08
	120	0.25	2.39	0.11	0.09
	130	0.25	2.37	0.11	0.11
SEM		0.051	0.259	0.013	0.021
P-values					
CP		0.84	0.13	0.30	0.59
Thr		0.88	0.96	0.08	0.63
CP × Thr		1.00	0.96	0.77	0.99
Contrasts					
Linear		0.44	0.62	0.03	0.29
Quadratic		0.98	0.91	0.18	0.45

[1]Full carcass deprived of head and drumsticks.

concentrations of uric acid (CP, $P = 0.02$), total cholesterol (CP, $P = 0.01$), HDL cholesterol (CP, $P < 0.01$), LDL cholesterol (CP, $P = 0.01$), LDL to HDL ratios (CP, $P = 0.01$), and ALT (CP, $P = 0.05$), and lower (CP, $P = 0.01$) concentrations of triglycerides and VLDL compared with the 100% CP diets. However, regardless of the dietary CP content, the Thr supplementation decreased the blood serum concentrations of total cholesterol (Thr, $P = 0.02$; quadratic, $P = 0.04$), triglycerides, VLDL and LDL cholesterol, and LDL to HDL ratios (Thr, $P = 0.01$; linear, $P = 0.01$), whereas increased the concentrations of uric acid (Thr, $P < 0.01$; linear, $P < 0.01$) and HDL cholesterol (Thr, $P = 0.01$; linear, $P = 0.01$).

Immune Response

As Table 5 shows, there were no CP, Thr, and CP × Thr interaction effects on the immune responses against AI. Nevertheless, the reduction of the dietary CP level at 97.5% increased immune responses against NDV (CP, $P = 0.02$) and SRBC (CP, $P = 0.01$) measured after the third vaccination. However, incremental levels of Thr up to 120% of the recommended value increased (Thr, $P = 0.01$) both antibody responses against NDV (after the first and second vaccination: quadratic, $P = 0.03$; after the third vaccination: quadratic tendency, $P = 0.06$) and antibody responses against SRBC measured after the first vaccination (Thr tendency, $P = 0.06$; quadratic tendency, $P = 0.10$). The immune responses against SRBC measured after the second vaccination raised (Thr, $P = 0.01$; linear, $P = 0.01$) by increasing Thr inclusion level.

DISCUSSION

Considering the strong interest for the use of low protein diets in poultry nutrition, to meet the Thr requirement for optimum performance of broilers is crucial because Thr becomes a critical amino acid in low-protein diets based on corn and soybean meal (Abbas, 2015). Our results showed that in both growth periods (starter and grower), maximum growth performance, in terms of ADFI, ADG, ADEI, and ADPI, is achieved when Ross broiler requirements for CP and Thr are 100% satisfied. Levels of Thr higher than the recommend value adversely affected growth performance when the CP requirement was 100% satisfied. On the other hand, it is known that many amino acids can have toxic effects when fed in excess to growing chickens. In agreement with our findings, the Thr excess has been reported to reduce broilers' feed intake and growth rate (Edmonds and Baker, 1987; Carew et al., 1998). In both growth periods, the reduction of the dietary CP content (97.5% of the recommended value), irrespective of the Thr

Table 4. Blood serum parameters of Ross 308 broilers fed diets with different CP and Thr levels.

Treatment		Blood parameters						
CP (%)	Thr (%)	Uric acid (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL[1] (mg/dl)	HDL cholesterol[2] (mg/dl)	LDL cholesterol[3] (mg/dl)	LDL/HDL
97.5	100	4.60	158.10	73.52	14.70	79.46	78.44	0.99
	110	5.18	169.30	74.38	14.88	76.39	81.68	1.07
	120	6.08	164.09	74.58	14.91	81.25	74.34	0.92
	130	7.08	160.59	67.83	13.57	84.06	70.75	0.84
100	100	4.87	161.41	79.94	15.99	64.96	65.97	1.02
	110	5.03	159.09	76.80	15.36	72.75	67.34	0.92
	120	5.32	152.38	76.87	15.38	74.84	55.75	0.75
	130	5.88	134.84	71.48	14.30	76.27	36.49	0.48
SEM		0.265	4.809	1.776	0.356	1.935	5.936	0.080
P-values								
CP		0.02	0.01	0.01	0.01	<0.01	0.01	0.01
Thr		<0.01	0.02	0.01	0.01	0.01	0.01	0.01
CP × Thr		0.06	0.06	0.63	0.64	0.07	0.28	0.14
Contrasts								
Linear		<0.01	0.01	0.01	0.01	0.01	0.01	0.01
Quadratic		0.29	0.04	0.07	0.07	0.93	0.12	0.17
CP (%)	Thr (%)	Glucose (mg/dl)	AST[4] (U/l)	ALT[5] (U/l)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	
97.5	100	129.56	15.57	362.35	5.04	2.71	2.09	
	110	144.63	15.75	347.97	5.03	2.94	2.04	
	120	127.75	16.63	329.58	5.21	2.85	2.02	
	130	115.01	16.73	405.62	4.57	2.99	1.85	
100	100	131.42	20.24	316.49	4.92	2.44	1.97	
	110	126.45	16.90	332.15	5.02	2.70	2.08	
	120	113.66	14.77	325.95	4.50	2.94	1.80	
	130	91.90	17.40	345.54	4.34	2.95	1.89	
SEM		12.086	1.366	21.045	0.554	0.259	0.100	
P-values								
CP		0.14	0.25	0.05	0.51	0.53	0.36	
Thr		0.08	0.43	0.16	0.73	0.47	0.22	
CP × Thr		0.75	0.16	0.53	0.92	0.88	0.48	
Contrasts								
Linear		0.02	0.48	0.17	0.33	0.14	0.07	
Quadratic		0.21	0.15	0.13	0.58	0.65	0.64	

[1]VLDL = very low density lipoproteins.
[2]HDL = cholesterol linked to high density lipoproteins.
[3]LDL = cholesterol linked to low density lipoproteins.
[4]AST = aspartate amino transferase.
[5]ALT = alanine amino transferase.

inclusion level, had a negative effect on broiler growth performance achieving lower ADFI, ADG, ADEI, and ADPI values compared with the standard diet. However, an improvement in these growth performance parameters was observed when Thr was included at 110% level as shown by CP x Thr interaction effects. However, regardless of the dietary CP content, Thr levels exceeding the recommended value for Ross tended to reduce the feed efficiency (G:F) and energetic efficiency (G:ME), whereas the protein efficiency (G:P) tended to increase by reducing the dietary CP level. The literature is controversial in regard to the biological efficiency of free crystalline amino acids. In agreement with Waldroup et al. (2005) and Namroud et al. (2008), our findings seem to support the hypothesis that free crystalline amino acids are not able to completely replace protein-bound amino acids. Nevertheless, our observations disagree with those reported by Ciftci and Ceylan (2004) and Abbasi et al. (2014) that supported the hypothesis that the availability of free crystalline amino acids is higher than that of amino acids in intact proteins. In order to reduce the dietary CP content, the composition of the free amino acids might play a role important to support optimal growth performance. As suggested by Waldroup et al. (2005), a balanced mixture of several crystalline amino acids may be more effective compared with supplying a single essential amino acid to a diet low in CP. Moreover, synthetic free amino acids, compared with protein-bound amino acids, are known to be sensitive to acidic conditions and are rapidly absorbed in the digestive tract (Batterham, 1979; Kondos and Adriaans, 1982; Sato et al., 1984; Prandini et al., 2013), and this might adversely affect their metabolic efficiency. The use of amino acid encapsulated in a matrix, capable of resisting stomach conditions and allowing an intestinal slow release, can help in overcoming the limitations mentioned before (Piva et al., 2007; Prandini et al., 2013).

Table 5. Effects of different levels of dietary CP and Thr on antibody titers against avian influenza (AI), Newcastle disease (NDV), and sheep red blood cell (SRBC).

Treatment		AI (IgG2)		NDV (IgG2)		SRBC (total Ig)	
CP (%)	Thr (%)	at 33 d of age	at 42 d of age	After the first and second injection	After the third injection	After the first injection	After the second injection
97.5	100	5.00	6.00	3.67	4.00	4.00	4.00
	110	5.67	6.00	5.00	5.33	4.67	4.33
	120	5.00	6.33	6.67	6.67	5.33	5.00
	130	7.33	6.33	6.00	6.67	4.67	6.33
100	100	5.33	6.33	4.33	4.33	3.33	4.33
	110	5.67	6.67	5.33	5.00	3.67	4.66
	120	6.67	6.67	5.67	5.33	4.00	5.00
	130	6.00	6.67	5.00	5.33	4.00	4.00
SEM		0.972	0.408	0.527	0.353	0.333	0.441
P-values							
CP		0.81	0.17	0.51	0.02	0.01	0.79
Thr		0.50	0.82	0.01	0.01	0.06	0.01
CP × Thr		0.51	0.97	0.28	0.08	0.72	0.41
Contrasts							
Linear		0.15	0.38	0.01	<0.01	0.03	0.01
Quadratic		0.81	0.78	0.03	0.06	0.10	0.43

In our study, the dietary CP content did not affect carcass traits. The Thr inclusion at 110% level in the low CP diet increased the eviscerated carcass weight and consequently the carcass yield. Supplemental Thr decreased the eviscerated carcass weight and carcass yield at both dietary CP levels. However, no differences due to the dietary CP content and/or Thr inclusion level were observed in the yield of different anatomical parts, entrails, intestinal tracts, and organs related to immune system. In agreement with previous studies (Aleator et al., 2000; Allemen et al., 2000; Ciftci and Ceylan, 2004; Abbasi et al., 2014), an increase in abdominal fat was observed by reducing the dietary CP content. Moreover, regardless of the dietary CP content, Thr supplementation had an increasing effect on abdominal fat. As suggested by Estalkhzir et al. (2013), this result supports the hypothesis that the excess amino acid is converted by body's metabolism into fat and stored in the body.

Broiler diet with increased ME to CP ratio increases the process of lipogenesis in the body (Rosebrough and Steele, 1985). Swennen et al. (2005) and Kamran et al. (2010) suggested that birds fed on low-CP diets preferentially use carbohydrates as an energy source rather than free fatty acids, resulting in higher plasma triglyceride levels. Instead, in our study, the reduction of the dietary CP content at constant ME decreased the blood serum concentrations of triglycerides and VLDL (i.e. lipoproteins responsible for the transport of triglycerides from the liver via the blood to extrahepatic tissues) but increased the concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, and LDL to HDL ratio. Nevertheless, the Thr inclusion, though at higher levels when feeding the 97.5% CP diet compared with the 100% CP diet, improved serum lipid profile. In particular, the Thr supplementation reduced the concentrations of triglycerides, VLDL, total cholesterol, detrimental LDL cholesterol, and LDL to HDL ratio, whereas increased healthy HDL cholesterol concentration. The reduction of the dietary CP content as well as the Thr inclusion, especially at the highest levels (i.e., 120 and 130%), increased the serum uric acid concentration. However, the number of replicates used in this study probably caused only a tendency of specific interaction between CP and Thr. Excess or imbalanced dietary amino acids split into C-skeletons and ammonia. The latter, being very toxic to living cells, is converted to uric acid by the birds. Moreover, our ADFI results supported the hypothesis that decreasing feed intake to limit the adsorption and catabolism of excess amino acids may be one of the animal defense mechanisms to reduce the intracellular concentration of ammonia (Namroud et al., 2008).

Our immune response results showed that reduction of the dietary CP content of 2.5 percent points compared with the recommended value did not adversely affect broiler immunocompetence. Moreover, regardless of the dietary CP content, the Thr supplementation increases broiler immune responses, in agreement with Abbasi et al. (2014). In particular, incremental Thr levels up to 120% of the recommended value enhanced antibody responses against NDV and SRBC. On the other hand, there are evidences that Thr modulates immune functions (Bhargava et al., 1971; Wang et al., 2006; Li et al., 2007) and that changes in components of the immune system are sensitive to dietary intake (Li et al., 1999). The key roles that Thr plays in immune responses regard to inhibition of apoptosis, stimulation of lymphocyte proliferation, enhancement of antibody production, and synthesis of mucin protein required for maintaining intestinal immune function (Li et al., 2007).

Summarizing, our findings showed that Ross nutritional indications for dietary CP and Thr contents in starter and grower periods are accurate. The reduction of the dietary CP content by 2.5 percentage points compared with the recommended value negatively affected broiler growth performance (ADG, ADFI, ADEI, and ADPI), promoted fat abdominal deposition, and increased blood serum levels of uric acid, and total and high and low lipoprotein-linked cholesterol. However, the Thr inclusion improved serum lipid profile. Moreover, the Thr inclusion at 110% of Ross recommendations in low protein diet raised ADG, ADFI, ADEI, ADPI (though to suboptimal levels) and eviscerated carcass weight, and consequently carcass yield. Overall, current findings provided support for a role of Thr as non-specific immunostimulant in poultry.

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