Post-hatch L-Threonine Supplementation to Low Protein Diets Influences the Growth Performance and Immune Response of CARIBRO Vishal broiler Chickens

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Abstract
Two experiments were conducted to evaluate growth performance, immune responses and carcass traits of CARIBRO Vishal broilers fed dietary L-Thr in low protein diets without or with the balance of Lys and Met. Broilers fed 85% CP diet with Thr alone showed depressed daily weight gain (linear, p=0.004), daily feed intake (quadratic, p=0.031) and higher FCR (linear, P=0.008) at d 21 (exp. 1) whereas, birds fed low CP diets but supplemented with Thr and balance of Lys and Met exhibited similar (p>0.05) growth rate as that of control diet (exp. 2). Incremental Thr levels increased (p<0.05) the immune and digestive organs weights, and enhanced antibody responses against SRBC and PHA-P antigens in both experiments. Thr inclusion increased (p<0.001) total protein and globulin in both experiments. Dietary crude protein in broiler diets could be reduced up to 85% of fast-growing broilers' requirement with adequate supplementation of 3 limiting amino acids (Lys, Met, and Thr) for CARIBRO Vishal broiler chicks. Dietary L-Thr supplementation enhanced the immune status of broiler chickens fed low CP diets.

Keywords: Amino acids, Broilers, Globulin, Immunity, Lymphoid organs, Threonine

1. Introduction

Broiler meat contributes immensely to the supply of animal protein foods. Hence, sustainable broiler production at backyard, small or commercial scale will impact positively on attaining food security. Sustainability entails increasing broiler meat yield with efficient input-use in an eco-friendly manner and profit optimization. Feeding poultry birds on ideal protein and AA requirements, and not crude protein basis, is seen as one of the viable means of achieving this feat. This entails formulating low-protein diets and supplementation of appropriate crystalline limiting AA in broiler diets to match the muscle tissue accretion and maintenance needs because it has proven to optimize birds' growth and health status, enhance feed quality, lessen cost of feed, enhance nitrogen retention efficiency, and supports environmental sustainability [1, 2].

Threonine (Thr) is considered as the third limiting essential AA in poultry diets following Methionine (Met) and Lysine (Lys) especially when high dietary levels of Thr-limiting ingredients, particularly cereals, soybean meal, and meat and bone meals are used in feed formulation [1, 2]. Thr (2-amino-3-hydroxybutyric acid, $C_4H_9NO_3$) constitutes about 11 % amino acid sequence of mucin, a major glycoprotein which confers protection from injury to the intestinal epithelium. Thus, Thr is actively involved in mucin protein synthesis which is required for maintaining intestinal immune function; inhibition

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of apoptosis; stimulation of lymphocyte proliferation; and enhancement of antibody production [1, 3]. Furthermore, a higher amount of dietary threonine is metabolized in the intestine and utilized for synthesis of intestinal-mucosal proteins which are essential for adequate gut functions, and digestive secretions for optimum nutrient digestion, uptake and utilization [1]. Being the major AA in the gamma serum immunoglobulin, Thr is highly efficient in immune response [4]. Previous studies have found that dietary Thr supplementation could enhance growth performance, carcass yield, and improve immune responsiveness of broiler chickens to different antigens [2, 5].

CARIBRO Vishal broiler strain was developed in Central Avian Research Institute, Izatnagar, India for better meat yield potential from indigenous chickens. Its performance features include better growth rate, improve feed efficiency, and higher livability due to high disease resistance and suitability to tropical climate. This broiler strain is gaining wider acceptability among poultry farmers, both at backyard and commercial scale, in India due to regular supply to day-old chicks. Few published studies have shown the promising effects of feeding Thr supplemented low CP diets without compromising the growth performance and immune status of fast-growing broiler chickens [6]. However, the extent to which CP can be reduced with supplementation of limiting critical AA to obtain optimal performance and enhance immune system function for CARIBRO Vishal broiler chickens needs to be ascertained. Moreover, in contrast to previous research which often used single sex of broiler chicks [1, 7], this study adopted the use of mixed sexes, a prevailing situation in commercial production, to explore response of broiler chicks to reduced protein diets supplemented without or with limiting Met and Lys. Therefore, the present investigation was designed to evaluate the response of broiler chickens to diets containing reduced protein supplemented with L-Thr and with or without balance of Met and Lys on growth performance and immune response of broiler chickens.

2. Materials and methods

Study Procedures

All birds used in these experiments were provided proper care and management without unnecessary discomfort, and the study protocols were in accordance with the Institutional Animal Ethics Committee guidelines of ICAR-Central Avian Research Institute, Izatnagar, India (Approval no: CARI/CPCSEA/2017/2). Two experiments were carried out to at the Poultry Housing and Management Section of the Institute. In each experiment, 320 one-day-old, mixed sexes broiler chicks (CARIBRO Vishal strain) with similar hatching weight (mean±SD; 42.15±2.14 g) were wing banded and randomly distributed in a completely randomized design to four treatment groups. Each group had ten replicate cages of eight birds each with equal number of chicks per each sex.

Diets and feeding management

Proximate composition of major feed ingredients (corn, soybean meal, guar meal, deoiled rice bran and fish meal) were analyzed before diets formulation [8]. Standardized ileal digestibility (SID) values for respective amino acids obtained from published digestible coefficients of feed ingredients (Evonik Nutrition & Care GmbH, Germany) were applied to formulate diets to meet the requirements of broiler chickens. Dietary CP levels were 100, 95, 90 and 85% of fast-growing broiler specifications which equal to 230, 218.5, 207, and 195.5 g CP/kg for starter phase, respectively while the respective values for finisher phase were 210, 199.5, 189, and 178.5 g CP/kg diet [9]. L-Thr (98.5% Feed grade) was supplemented at 0, 0.5, 1.0 and 1.5 g/kg diet in both experiments. However, in experiment 1, DL-Met were added to starter (1.2 g/kg) and finisher (1.0 g/kg) feeds across all treatments, without L-Lys supplementation thereby causing varying Thr/Lys ratio.

In experiment 2, graded levels of L-Lys (98.5% Feed grade) and DL-Met (99% Feed grade) were added in order to meet their requirements and to achieve similar Thr/Lys ratio of 0.66 for starter phase [1] and 0.68 for finisher phase [10]. Diet compositions and nutritional values for experiments 1 and 2 are presented in Tables 1 and 2, respectively. Chickens were reared in battery cage for 42 days of age and fed a starter ration from 1–21 days of age and a finisher ration from 22–42 days of age. No antimicrobial, anticoccidial drugs or feed enzymes were included in the experimental diets. All broilers had unrestricted access to feed (mash form) and water. The chicks were vaccinated against Newcastle disease (Ranikhet) and Infectious bursal disease as per the recommendation of the Institute.

Growth performance

The chickens were weighed individually on a weekly basis in order to compute average daily weight gain (ADWG). Feed intake was recorded weekly per replicate cage to estimate average daily feed intake (ADFI). Feed conversion ratio (FCR) was estimated as the ratio of feed consumed to weight gain and was adjusted for mortality.

In vivo cellular immune response

The in vivo cutaneous basophilic hypersensitivity response to the lectin phytohaemagglutinin from Phaseolus vulgaris (PHA-P; HiMedia Laboratories Pvt. Ltd., Mumbai, India) was studied at 28 days post-hatch in eight birds per treatment group, on gender equalization basis, as a measure of cell-mediated immune response using the standard procedures [11]. The toe web thicknesses between the 3rd and 4th inter-digital space of both left and right feet were measured using a micrometre caliper (Mitutoyo, Japan). Thereafter, 0.1 ml of PHA-P [100 μg PHA-P dissolved in 0.1 ml phosphate-buffered saline (PBS)] was injected at the right foot whereas the left foot which served as control was injected with 0.1 ml PBS. The inflammatory response was determined post 24 hours of injection by measuring the thickness of the respective toe webs. The foot web index was calculated as a difference between the swelling in the right and left feet before and after 24 hours of injection. The foot web/pad index was calculated as follows: Foot web index (mm)=(R2–R1)–(L2–L1) where: R2=thickness of right foot web after 24 hours of injection, R1=thickness of the right foot web before injection, L2=thickness of left foot web after 24 hours of injection, and L1=thickness of the left foot web before injection.

Table 1. Ingredient and nutrient composition of diets for experiment 1 (as fed basis)

Ingredients (g/kg)	Starter diets				Finisher diets			
	T1	T2	T3	T4	T1	T2	T3	T4
Maize	546.8	584.5	621.8	658.6	574.0	595.0	616.0	637.0
Soybean meal	329.0	289.0	249.0	209.0	287.0	241.0	193.5	147.5
Guar meal	48.0	59.3	71.0	83.2	48.0	64.5	82.5	99.0
Deoiled rice bran	0.0	0.0	0.0	0.0	40.0	50.0	60.0	70.0
Fish meal	20.0	16.0	12.0	8.0	0.0	0.0	0.0	0.0
Vegetable oil	22.5	17.0	11.5	6.0	20.0	18.0	16.0	14.0
Dicalcium phosphate	16.0	16.0	16.0	16.0	15.0	15.0	15.0	15.0
Limestone	10.5	10.5	10.5	10.5	9.0	9.0	9.0	9.0
Salt (NaCl)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
DL-Methionine	1.2	1.2	1.2	1.2	1.0	1.0	1.0	1.0
L-Threonine	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
Vitamin and mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient composition (g/kg, except ME)[#]								
ME (MJ kg^{-1})	12.60	12.60	12.60	12.60	12.61	12.61	12.61	12.60
CP	230.4	218.7	207.2	195.8	210.4	199.7	189.4	178.9
EE	49.4	45.4	41.3	37.3	47.0	46.5	45.5	44.7
CF	29.3	29.6	29.9	30.2	33.3	34.7	36.2	37.7
Ca	10.0	9.8	9.5	9.3	8.1	8.1	8.0	8.0
Av. P	4.5	4.3	4.2	4.0	3.8	3.8	3.7	3.7
Lys	10.7	10.0	9.3	8.6	9.4	8.9	8.3	7.8
Met	4.5	4.4	4.3	4.2	4.0	4.0	3.9	3.8
Thr	7.2	7.3	7.4	7.4	6.5	6.6	6.7	6.9
Thr/Lys	6.7	7.3	7.9	8.6	6.9	7.5	8.1	8.9
Met+Cys	7.4	7.2	7.0	6.8	6.8	6.6	6.5	6.3

T1=100% CP, T2=95% CP+0.5 g Thr, T3=90% CP+1.0 g Thr, and T4=85% CP+1.5 g Thr.

[1]Premix supplied (units /kg diets): Vit A: 12000 IU, Vit D3: 5000 IU, Vit E: 75 IU, Vit K3: 3 mg, Vit B1: 3 mg, VitB2: 8 mg, Vit B6: 5 mg, VitB12: 0.016 mg, Pantothenic acid: 13 mg, Niacin: 55 mg, Folic acid: 2 mg, Biotin: 0.2 mg, Cu: 16 mg, İ: 1.2mg, Se: 0.3 mg, Mn: 120 mg, Fe: 40 mg, Zn: 100 mg

[2]AA composition is on Standardized Ileal Digestible basis

In vivo humoral immune response

The in vivo humoral immune response to the sheep red blood cell (SRBC) antigen was studied at 28th day post-hatch according to the standard procedures [11]. Fresh sheep blood was collected and was suspended in Alsevar's solution. The blood was washed three times in isotonic PBS (pH 7.2) using centrifugation (3000 rpm; 10 mins), and 1% SRBC suspension was prepared. Thereafter, 8 chicks (4 male; 4 female) from each treatment group were injected intravenously with 1 ml of 1% SRBC suspension. After 5 days post-injection, blood samples (2 ml) were obtained from the jugular vein of each chick and allowed to clot for serum collection. The antibody response to SRBC was determined using a standard haemagglutination assay. The reciprocal of highest dilution in which there were complete haemagglutination was the end point of titre and the values were expressed as log2.

Relative weight of immune and digestive organs

At 21 and 42 d post-hatch, 8 birds per treatment were selected based on average weight and gender equalization. The birds were slaughtered by cervical dislocation and exsanguination. Thereafter, they were carefully excised to remove the immune and digestive organs (bursa of Fabricius, thymus, spleen, liver, empty gizzard, intestine, and pancreas). The organs were weighed using a sensitive microbalance (KERN/ABT 220-5DM; Kern & Sohn GmbH, Balingen, Germany) and the values were expressed as a percentage of live weight [11].

Statistics

Data were based on each replicate cage as the experimental unit. Data generated from this study were subjected to One-Way Analysis of Variance technique in a completely randomized design using General Linear Model procedure of SAS (9.1.3 windows portable version, Cary, NC, USA) according to the following model: $Y_{ij}=\mu+A_i+\varepsilon_{ij}$ where: Y_{ij}=observation, μ=overall mean; A_i=diet effect (i=1 to 4) and ε_{ij}=residual error. Means of significant results were compared by Tukey's HSD Test where necessary at p<0.05 or otherwise stated. Orthogonal polynomials were used to assess the linear and quadratic effects of varying supplemental levels of L-Thr.

3. Results and discussion

Growth performance

Body weight gain at day 21 showed similar (p>0.05) values between the control diet and low crude protein diet (up to 207 g CP/kg) supplemented with Thr only (exp 1; Table 3). However, 15% CP reduction elicited depression in daily weight gain (ADWG; linear, P=0.004), daily feed intake (ADFI; quadratic, P=0.031) and higher feed conversion ratio (FCR; linear, P=0.008). Depression effect was alleviated in broilers fed 85% CP supplemented with 1.5g Thr/kg by addition of Lys and Met (exp 2). This findings confirms the order of limiting amino acids in broiler nutrition. Being the third limiting amino acid implies that meeting Thr requirement becomes worthy of consideration after those of Lys and Met were met [2]. Similarly, Corzo et al. [12] found that body weight gain and FCR showed similar values between the control diet and the low crude protein diet supplemented with all amino acids in broiler chicks at day 21. Thus, increasing the supply of L-Thr in low-CP diets could improve bird's performance only if other essential AA are not limiting. During finisher phase, broilers fed Thr supplemented low CP diets exhibited similar (p>0.05) growth performance (ADWG, ADFI and FCR) to those given the control diet. This suggests that varying Thr/Lys ratio would have more pronounced effects on broiler chicks during starter phase when optimum amount of balanced AA is required and very essential for muscle tissue accretion and immune system build-up. This observation is consistent with the previous findings which reported decreased body weight gain and higher FCR in broiler chickens fed different dietary CP and Thr levels only during the starter phase but with no suppressive effect during remaining experimental period [6]. Similarly, Ospina-Rojas et al. [13] found no significant difference in weight gain, diet intake and FCR of male broilers (22-42 d) fed on low CP diets with different Thr/Lys ratio.

In vivo humoral and cell-mediated immune response

Incremental levels of Thr inclusion enhanced antibody responses against SRBC inoculation (p=0.029; linear, p=0.005). Also, the *in vivo* immune response to PHA-P was found to be increased (p<0.001; linear, p<0.001) in T3 (Thr

10%) in comparison with other dietary groups (exp 1; Table 4).

Table 2. Ingredient and nutrient composition of diets for experiment 2 (as fed basis)

Ingredients (g/kg)	Starter diets				Finisher diets			
	T1	T2	T3	T4	T1	T2	T3	T4
Maize	546.8	584.5	621.8	657.4	574.0	595.0	615.0	635.0
Soybean meal	329.0	288.0	245.0	205.0	287.0	241.0	193.5	147.5
Guar meal	48.0	57.0	71.4	82.0	48.0	61.0	76.5	90.0
Deoiled rice bran	0.0	3.0	3.0	6.5	40.0	52.3	64.6	77.4
Fish meal	20.0	15.0	10.0	5.0	0.0	0.0	0.0	0.0
Vegetable oil	22.5	17.0	11.5	5.0	20.0	18.0	16.0	14.0
Dicalcium phosphate	16.0	16.0	16.0	16.0	15.0	15.0	15.0	15.0
Limestone	10.5	10.5	10.5	10.5	9.0	9.0	9.0	9.0
Salt (NaCl)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-Lysine HCL	0.0	1.1	2.2	3.3	0.0	1.0	2.0	3.0
DL-Methionine	1.2	1.4	1.6	1.8	1.0	1.2	1.4	1.6
L-Threonine	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
Vitamin and mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient composition (g/kg, except ME)[2]								
ME (MJ kg^{-1})	12.60	12.61	12.62	12.60	12.61	12.62	12.62	12.62
CP	230.4	218.5	207.4	196.5	210.4	199.8	189.7	179.3
EE	49.4	45.2	41.2	36.1	47.0	46.1	45.2	44.3
CF	29.3	29.7	30.1	30.7	33.3	34.7	36.3	37.8
Ca	10.0	9.7	9.4	9.1	8.1	8.0	8.0	7.9
Av. P	4.5	4.3	4.1	4.0	3.8	3.8	3.7	3.7
Lys	10.7	10.8	10.9	11.1	9.4	9.6	9.8	10.0
Met	4.5	4.6	4.7	4.7	4.0	4.1	4.3	4.4
Thr	7.2	7.2	7.3	7.3	6.5	6.6	6.7	6.8
Thr/Lys	6.7	6.7	6.6	6.6	6.9	6.9	6.8	6.8
Met + Cys	7.4	7.3	7.3	7.3	6.8	6.8	6.8	6.8

Same footnote as in Table 1.

In exp 2, SRBC HA titer increased linearly (p=0.009) in broilers fed Thr supplemented low protein diets with balance of Lys and Met while the quadratic effect tended to be significant (p=0.053), whereas the PHA-P response increased quadratically (p=0.015) compared to the control group. Increasing levels of Thr in low protein diets, without or with Lys and Met, linearly enhanced immune-competence of broiler chickens against SRBC antigen. This confirms that Thr modulates immune functions and that the immune system is sensitive to dietary Thr intake [5,6]. Along with our findings, previous study observed that incremental Thr levels increased broiler immune responses against Newcastle disease virus and sheep red blood cells irrespective of dietary protein content [2]. Abbasi et al. [6] reported that Thr supplementation of diets enhanced primary immune responses and could preserve memory immune responses for a long time regardless of the dietary CP level.

The observed tissue swelling of experimental birds in response to PHA-P antigen signified enhanced immunomodulatory effects arising from increase in numbers of circulating lymphocytes [11]. In agreement, Khalaji et al. [7] reported that immune response to PHA-P increased linearly and quadratically by increasing dietary L-threonine levels in the diet of broiler chickens. Thr is known to play active roles in antibody production, being the major amino acid in the gamma serum immunoglobulin, and its involvement in synthesis of the mucin protein which is required for maintaining intestinal immune function [11].

Table 3. Growth performance of broiler chickens fed L-Thr supplemented low protein diets

Diet type	ADWG (g)	ADFI (g)	FCR	ADWG (g)	ADFI (g)	FCR
	Experiment 1			Experiment 2		
		1 - 21 d				
100% CP	27.70^{a}	41.84^{a}	1.51^{b}	28.37	40.81	1.44
95% CP, 0.5g Thr	25.91^{a}	41.55^{a}	1.62^{ab}	27.05	40.89	1.51
90% CP, 1.0g Thr	27.77^{a}	44.41^{a}	1.62^{ab}	27.38	42.30	1.55
85% CP, 1.5 g Thr	21.26^{b}	37.53^{b}	1.78^{a}	26.99	40.87	1.52
Pooled SEM	1.24	1.44	0.06	0.90	1.23	0.14
Significance level	0.003	0.021	0.043	0.690	0.789	0.210
Linear contrast	0.004	0.131	0.008	0.360	0.774	0.118
Quadratic contrast	0.068	0.031	0.692	0.617	0.545	0.154
		22 - 42 d				
100% CP	52.17	114.39	2.02	56.29	111.26	1.98
95% CP, 0.5g Thr	50.20	111.29	1.99	55.22	117.40	2.12
90% CP, 1.0g Thr	52.28	116.18	2.03	53.70	118.33	2.22
85% CP, 1.5 g Thr	43.61	101.41	2.13	52.04	110.95	2.14
Pooled SEM	4.66	8.22	0.06	2.11	4.65	0.17
Significance level	0.523	0.594	0.889	0.527	0.560	0.181
Linear contrast	0.268	0.363	0.545	0.149	1.000	0.101
Quadratic contrast	0.479	0.484	0.624	0.891	0.166	0.146

Means within the same column with different superscripts are significantly different (p<0.05).
ADWG, average daily weight gain; ADFI, average daily feed intake; FCR, feed conversion ratio

Table 4. Humoral and cell mediated immune response of broiler chickens fed L-Thr supplemented low protein diets

Diet type	SRBC HA Titer (Log_2)	Foot web index (mm)	SRBC HA Titer (Log_2)	Foot web index (mm)
	Experiment 1		Experiment 2[#]	
100% CP	3.24^{b}	0.29^{bc}	3.36^{b}	0.22
95% CP, 0.5g Thr	3.50^{ab}	0.15^{c}	3.58^{ab}	0.39
90% CP, 1.0g Thr	3.75^{a}	0.67^{a}	3.72^{a}	0.33
85% CP, 1.5 g Thr	3.75^{a}	0.41^{b}	3.85^{a}	0.23
Pooled SEM	0.07	0.04	0.07	0.05
Significance level	0.029	<0.001	0.032	0.091
Linear contrast	0.005	<0.001	0.009	0.851
Quadratic contrast	0.308	0.180	0.053	0.015

Means within the same column with different superscripts are significantly different (p<0.05).
[#]Diets contains additional Lys and Met

Relative weight of immune and digestive organs

Dietary treatments had no marked effect (p>0.05) on digestive and immune organs weight at d 21 (exp 1; Table 5). Thr supplemented low CP diets increased the relative weights of liver (linear, p=0.013), intestine (p=0.022) and spleen (quadratic, p=0.043) at d 42. In experiment 2, broilers fed 195.5 g CP/kg diet supplemented with 1.5g Thr/kg and balance of Lys and Met had higher (p<0.05) weights of liver and gizzard at d 21 (Table 6). Also, there were linear and quadratic effects on relative weights of pancreas (p=0.023) and bursa (p=0.014), respectively due to dietary treatment. At d 42, Thr supplemented diets increased (p<0.05) liver weight. There was quadratic increase in the weights of gizzard (p=0.029) and intestine (p=0.035). Meanwhile, there were both linear and quadratic effects (p<0.05) of Thr supplemented low protein diets with balance of Lys and Met on pancreas, bursa, thymus and spleen. According to our findings, Thr supplemented low protein diets increased the relative weight of bursa, thymus, and spleen. This confirms the crucial role of Thr in developing and boosting immune function of broiler chickens. Lymphoid organs form integral part of immune system which functions to confer immunity to poultry birds through production of lymphocytes and plasma cells as well as phagocytosis of invading pathogens or antigens [11]. Bursa of Fabricius is required for generation of antibody diversity through the amplification and differentiation of B lymphoid progenitors within its follicular microenvironment which ensure functional maturation of the B cell [11]. Thymus provides a unique microenvironment for differentiation and functional maturation of stem cells into T lymphocytes. Spleen is the site for antibody production, immune response against and clearance of antigens circulating in the bloodstream. It plays an important role in hematopoiesis, particularly with lymphopoiesis and granulopoiesis, and also eliminates defective or worn-out erythrocytes and platelets [11].

More so, Thr is actively involved in stimulation of lymphocyte proliferation, enhancement of antibody production, and inhibition of apoptosis required for immuno-competence responses [5].

Higher relative weights of liver, gizzard, intestine, and pancreas observed in this study also explains the crucial roles of Thr in metabolism of ingested dietary nutrients through enhanced digestive capacity with resultant higher feed utilization efficiency and nutrient uptake by broiler chickens. Also, synergy of Thr with aspartate and methionine exert positive liver and lipotropic function, enhance phospholipid synthesis and fatty acid oxidation thereby lessen the risk of fatty liver. The involvement of Thr in mucin synthesis, increased digestive enzymes activities, maintenance of intestinal mucosal integrity and barrier function have been well-documented [1, 2, 6, 7].

Table 5. Immune and digestive organs weight (% body weight) of broiler chickens fed L-Thr supplemented low protein diets (exp 1)

Diet type	Bursa	Thymus	Spleen	Liver	Gizzard	Intestine	Pancreas
			21 days				
100% CP	0.26	0.13	0.17	4.21	3.39	10.15	0.44
95% CP, 0.5g Thr	0.37	0.13	0.17	3.45	3.26	9.37	0.44
90% CP, 1.0g Thr	0.25	0.12	0.18	4.03	3.29	8.98	0.54
85% CP, 1.5 g Thr	0.33	0.14	0.14	3.94	2.87	10.58	0.48
Pooled SEM	0.09	0.06	0.06	0.70	0.48	0.35	0.03
Significance level	0.154	0.971	0.785	0.882	0.490	0.395	0.613
Linear contrast	0.604	0.883	0.529	0.943	0.193	0.777	0.406
Quadratic contrast	0.666	0.752	0.511	0.637	0.556	0.115	0.595
			42 days				
100% CP	0.25	0.07	0.18	2.17	2.17	5.10	0.26
95% CP, 0.5g Thr	0.27	0.08	0.14	2.33	2.50	5.10	0.30
90% CP, 1.0g Thr	0.29	0.08	0.16	2.50	2.33	5.52	0.28
85% CP, 1.5 g Thr	0.29	0.09	0.19	3.00	2.50	5.81	0.28
Pooled SEM	0.03	0.01	0.02	0.22	0.21	0.23	0.02
Significance level	0.804	0.556	0.164	0.070	0.628	0.108	0.308
Linear contrast	0.362	0.230	0.373	0.013	0.380	0.022	0.811
Quadratic contrast	0.785	0.841	0.043	0.452	0.692	0.538	0.131

Table 6. Immune and digestive organs weight (% body weight) of broiler chickens fed low protein diets supplemented with L-Thr balanced with L-Lys and DL-Met (exp 2)

Diet type	Bursa	Thymus	Spleen	Liver	Gizzard	Intestine	Pancreas
21 days							
100% CP	0.30	0.15	0.15	3.24^b	3.17^b	9.49	0.37
95% CP, 0.5g Thr, LM$^+$	0.28	0.17	0.14	2.95^b	3.12^b	8.86	0.41
90% CP, 1.0g Thr, LM$^+$	0.26	0.13	0.14	3.38^b	3.23^{ab}	9.66	0.50
85% CP, 1.5 g Thr, LM$^+$	0.27	0.10	0.19	4.42^a	3.41^a	11.05	0.48
Pooled SEM	0.03	0.02	0.02	0.26	0.26	0.53	0.03
Significance level	0.972	0.115	0.348	0.010	0.009	0.069	0.060
Linear contrast	0.604	0.883	0.529	0.885	0.189	0.777	0.023
Quadratic contrast	0.014	0.752	0.511	0.664	0.114	0.115	0.595
42 days							
100% CP	0.16	0.26	0.06	2.41^{ab}	2.30	5.19	0.26
95% CP, 0.5g Thr, LM$^+$	0.15	0.29	0.08	2.06^b	2.49	5.09	0.30
90% CP, 1.0g Thr, LM$^+$	0.17	0.26	0.08	2.53^{ab}	2.73	5.75	0.32
85% CP, 1.5 g Thr, LM$^+$	0.21	0.29	0.09	2.65^a	2.66	5.80	0.35
Pooled SEM	0.02	0.04	0.01	0.13	0.11	0.21	0.02
Significance level	0.269	0.883	0.194	0.029	0.061	0.085	0.082
Linear contrast	0.033	0.018	0.015	0.263	0.296	0.562	0.037
Quadratic contrast	0.028	0.001	0.006	0.237	0.029	0.035	0.005

Means within the same column with different superscripts are significantly different ($p<0.05$).
$^{\#}$LM$^+$, additional lysine and methionine

4. Conclusions

Dietary crude protein in CARIBRO Vishal broiler diets could be reduced up to 85% specified for fast-growing strain with the supplementation of three limiting amino acids (Lys, Met, and Thr) to maintain its growth performance. L-Thr feeding increased the development of organs associated with the immune function and digestive system, serum globulin and enhanced the immune response of broiler chickens fed low CP diets.

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References

1. Najafi, R., Ahmar, R., and Tazehkand, G. N., Effect of different dietary threonine levels on optimal growth performance and intestinal morphology in 1-14 days old Ross 308 Broilers, Braz J Poult Sci, 2017, 19, 59-66

2. Sigolo, S., Zohrabi, Z., Gallo, A., Seidavi, A., Prandini, A., 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, Poult Sci, 2017, 96, 2751–2760

3. Taghinejad-Roudbaneh, M., Babaee, M. J., Afrooziyeh, M., and Alizadeh, B., Estimation of dietary threonine requirement for growth and immune responses of broilers, J Appl Anim Res, 2013, 41, 474-483

4. Kidd, M. T., Kerr, B. J., L-Threonine for Poultry: A Review, J Appl Poult Res, 1996, 5, 358-367

5. Li, P., Yin, Y., Li, D., Woo Kim, S., and Wu, G., Amino acids and immune function: a review, Br J Nutr, 2007, 98, 237–252

6. Abbasi, M. A., Mahdavi, A. H., Samie, A. H., Jahanian, R., Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks, Braz J Poult Sci, 2014, 16, 35-44

7. Khalaji, S., Zaghari, M., Shivazad, M., Estimation of standardised ideal threonine equivalency values of a multi-enzyme and its effects on broiler chick's performance, Ital J Anim Sci, 2011, e10, 47-50

8. AOAC., Official Methods of Analysis, seventeenth ed., Association of Official Analytical Chemists, USA, Washington DC, 2000

9. Aviagen, Ross Broiler Nutrition Specifications. Aviagen, USA. Document No: 0419-AVNR-035, 2019, pp 1-10, Available on: https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/RossBroilerNutritionSpecs2019-EN.pdf.
10. Kidd, M. T., McDaniel, C. D., Branton, S. L., Miller, E. R., Boren, B. B., Fancher, B. I., Increasing amino acid density improves live performance and carcass yields of commercial broilers, J Appl Poult Res, 2004, 13, 593-604
11. Alabi, J. O., Bhanja, S. K., Fafiolu, A. O., Oluwatosin, O. O., Onagbesan, O. M., Mehra, M., Goel, A., Influence of in ovo Threonine on growth performance, immunocompetence and carcass characteristics in broiler chickens, Indian J Anim Sci, 2020, 90, 1628-1637
12. Corzo, A., Fritts, C. A., Kidd, M. T., Kerr, B. J., Response of broiler chicks to essential and non-essential amino acids supplementation of low crude protein diets, Anim Feed Sci Technol, 2005, 118, 319-327
13. Ospina-Rojas, I. C., Murakami, A. E., Eyng, C., Picoli, K. P., Scapinello, C., Regina do Amaral Duarte, C., Threonine-lysine ratio on the requirements of digestible lysine in diets for broilers, Acta Sci Anim Sci., 2014, 36, 157-162