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Effect of Tryptophan Supplementation in Protein Deficient Diets on Performance, Gut Development and Immune Responses in Broiler Chickens

Research Article

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ABSTRACT

The current trial was conducted to investigate effects of tryptophan (Trp) supplementation in crude protein (CP) deficient diets on performance, gut development and immune responses in broiler chickens. A total of 420 day-old broiler chicks (Ross 308) were assigned to one of 6 dietary treatments, comprising 5 replicates in a completely randomized design including: 1) control diet (CTL) (diet based on Ross 2014 recommendation), 2) CTL + 0.15% Trp (+Trp), 3) low CP diet 1 (LCP1) (10% CP lower than Ross 2014 recommendation), 4) LCP1 + 0.15% Trp, 5) low CP diet 2 (LCP2) (20% lower CP than Ross 2014 recommendation), and 6) LCP2 + 0.15% Trp. Body weight (BW), daily feed intake and feed conversion ratios were evaluated in different phases of the experiment. Digestive organs were measured on day 28 and at the end of the rearing period. Morphology of small intestine was evaluated on day 28 of age. The tonic immobility test was applied at the end of the experiment. Birds receiving low CP diets had lower BW and daily weight gain (DWG) during the starter period ($P<0.05$). Supplementation of Trp to LCP1 diets ameliorated reduced growth performance ($P<0.05$), with no effect when added to LCP2 diets. Abdominal fat deposition tended to be lower in birds consuming + Trp diets. Chickens fed on LCP2 had increased crypt depth and lower villus height to crypt depth ratio than birds in CTL group in jejunum and ileum ($P<0.05$). Feeding LCP2 and LCP2 + Trp diets increased heterophil to lymphocyte ratio (H/L) in broiler chicks while had no effect on tonic immobility duration. In conclusion, Trp. supplementation to LCP1 diets could ameliorate the loss of performance in broilers received low CP diets.

KEY WORDS broilers, gastrointestinal tract, immunity, performance, tryptophan.

INTRODUCTION

The utilization of synthetic amino acids is optimal, when all amino acids are provided adequately for protein accretion and maintenance. In addition, supplementing synthetic amino acids in the diet contributes to meeting the need but prevents excessive consumption of amino acids in broilers. Tryptophan (Trp) is an indispensable amino acid in poultry which is necessary for various metabolic functions. Since the concentration of Trp in body of organisms is among the lowest of all amino acids, it is considered rate-limiting in protein synthesis. Furthermore, Trp is found to influence the behavioral status of birds via the synthesis of two hormones; serotonin and melatonin (Emadi *et al*. 2010). Also, Trp is a major constituent of membrane proteins (Schiffer *et al*. 1992), and could replace the niacin demand of broiler chickens (Baker *et al*. 1973). Feeding diets with vegetable protein sources make Trp limiting for broilers after sulphur amino acids, lysine, threonine and isoleucine (Corzo *et al*. 2005a). Therefore, application of this amino acid in broiler

diets is necessary. However, there is still uncertainty about the use of synthetic amino acids in low crude protein (CP) diets. Some researchers believe that amino acid supplementation can completely replace CP in diets (Han *et al*. 1992; Parr and Summers, 1991), whereas others have shown that the use of low CP-amino acid supplemented diets had negative effects on productivity of broiler chickens (Bregendahl *et al*. 2002; Namroud *et al*. 2008). Dietary Trp recommendations for male chicks are contradict; 1.4 to 1.5 g/kg (Shan *et al*. 2003), 1.6 g/kg (Smith and Waldroup, 1988), 1.6 to 1.7 g/kg (Rosa *et al*. 2001), 1.9 to 2.2 g/kg (Steinhart and Kirchgessner, 1984) and 2.2 g/kg (Han *et al*. 1991). These contradictory results emphasis the need for further investigation in this regard.

Results of previous study have shown that deficiencies or excesses of protein, or of some specific essential amino acids, can result in changes of certain immune responses in birds (Abdukalykova and Ruiz-Feria, 2006). The effect of Trp supplementation on the immune system response against infectious bursal disease virus has been demonstrated by Emadi *et al*. (2010). However, more research is needed to study the impact of Trp on humoral immune responses. Furthermore, data on the effect of Trp supplementation on morphology of small intestine and behavioral indices are scarce. We hypothesized that Trp supplementation to each of basal or low CP diets may affect humoral immunity, intestinal morphology and tonic immobility of broiler chickens. Therefore, the objective of current experiment was to evaluate the effect of 0.15% Trp supplementation on growth performance, gut development and immune responses in broiler chickens.

MATERIALS AND METHODS

Experimental diets, animals and housing

Four hundred and twenty mixed sex broiler chicks (Ross 308; 1 d old chicks with equal numbers of males and females) were purchased from a local hatchery, weighted individually on arrival and randomly allocated to 6 treatments with 5 replicates of 14 chicks each (7 males and 7 females) in a completely randomized design. The effect of Trp supplementation was evaluated through feeding; 1) control diet (CTL) (diet based on Ross 2014 recommendation), 2) CTL + 0.15% Trp, 3) low CP diet 1 (LCP1) (10% CP lower than Ross 2014 recommendation), 4) LCP1 + 0.15% Trp, 5) low CP diet 2 (LCP2) (20% lower CP than Ross 2014 recommendation) and 6) LCP2 + 0.15% Trp. Chicks were housed in 1.2 × 1.2 m wire floor pens covered with paper roll and had free access to mash feed and water throughout the trial. Experimental diets were formulated to meet or exceed nutritional requirements of broiler chickens based on Ross Broiler Manual Guide recommendation (Aviagen, 2009). Broilers were kept in a temperature controlled house at 32 ˚C from days 1 to 7, 29 ˚C for days 8 to 14, 26 ˚C for days 15 to 21, and 22 ˚C from day 22 to the end of the trial. Ingredients and nutrient specifications of experimental diets are shown in Table 1. All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch.

Data collection and sampling

Broilers were weighed on days 1, 14, 28 and 42 of age. Daily feed intake (DFI) and daily weight gain (DWG) of chicks in each pen were recorded in different phases of the experiment. On days 28 and 42 of the experiment, two birds close to the mean body weight (BW) of each pen were selected, individually weighed and slaughtered through cutting of jugular veins and carotid arteries. Carcass yield, abdominal fat, liver and heart were collected, weighed and expressed as a percentage of live BW. Proportions of digestive organs including pancreas, proventriculus, gizzard, small intestine and cecum were measured at 28 and 42 days of age. The lengths of intestinal segments consisted of duodenum, jejunum, ileum and cecum were also measured and recorded.

Morphology of small intestine

On day 28 of age, two birds of each pen were slaughtered and intestinal samples were taken immediately from the jejunum: midway between the point of entry of the bile ducts and Meckel's diverticulum. Ileum, 10 cm proximal to the ileo-cecal junction, were taken to evaluate the villus height, crypt depth and villus height: crypt depth ratio (V/C). Segments with 1.5 cm in length were flushed with saline and fixed in 100 gL^{-1} buffered formalin (pH=7.0). The fixed intestinal samples were embedded in paraffin then sectioned (5 µm) and stained with hematoxylin-eosin and examined by light microscope (Olympus CX31, Tokyo, Japan). Villus height (µm) was measured from the tip of the villus to the villus crypt junction and crypt depth was measured from the base upward to the region of transition between the crypt and V/C was then calculated.

Immune responses, serum differential counts and tonic immobility

On day 9 of age, Newcastle and influenza antigens were injected into chickens at 0.2 mL per chick with dual vaccine of Newcastle-influenza. Also, chicks were orally vaccinated against Newcastle Disease (Lasota) on day 19 of age. Two chickens per pen were selected randomly for intraperitoneal injection with a 1.0 mL of sheep red blood cells (SRBC) suspension diluted with phosphate buffer saline (PBS) on day 24.

Table 1 Dietary ingredients and nutrient composition during different periods

Ingredients	Starter (1-14)			Grower (14-28)			Finisher (28-42)		
	Control	LCP1	LCP2	Control	LCP1	LCP2	Control	LCP1	LCP2
Corn grain	53.47	59.53	63.08	58.09	64.27	67.59	69.45	69.92	72.72
SBM[1]	40	34.40	31.10	36.00	30.30	27.20	25.30	25.00	22.40
Soybean oil	2.43	1.60	1.16	2.20	1.36	0.96	1.58	1.36	1.00
Dicalcium phosphate	1.74	1.80	1.83	1.50	1.56	1.59	1.39	1.36	1.39
Calcium carbonate	1.05	1.06	1.06	0.96	0.97	0.97	0.89	0.89	0.89
DL-methionine	0.30	0.35	0.38	0.24	0.29	0.32	0.33	0.26	0.28
L-lysine	0.16	0.33	0.42	0.10	0.27	0.35	0.15	0.25	0.33
L-threonine	0.10	0.18	0.22	0.06	0.13	0.17	0.06	0.11	0.14
Vitamin premix[2]	0.25	0.25	0.25	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix[3]	0.25	0.25	0.25	0.30	0.30	0.30	0.30	0.30	0.30
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100	100
Calculated nutrient level									
Metabolizable energy (kcal/kg)	2900	2900	2900	2950	2950	2950	3020	3020	3020
Crude protein (%)	22.2	19.8	18.7	20.45	18.45	17.35	17.6	16.6	15.6
Lysine (%)	1.24	1.24	1.24	1.10	1.10	1.10	0.96	0.96	0.96
Met + Cys (%)	0.92	0.92	0.92	0.83	0.83	0.83	0.75	0.75	0.75
Threonine (%)	0.83	0.83	0.83	0.73	0.73	0.73	0.63	0.63	0.63
Tryptophan (%)	0.40	0.38	0.36	0.39	0.37	0.36	0.38	0.35	0.35
Calcium (%)	0.93	0.93	0.93	0.83	0.83	0.83	0.74	0.74	0.74
Available phosphorous (%)	0.46	0.46	0.46	0.41	0.41	0.41	0.37	0.37	0.37

[1] SBM: soybean meal.
[2] Vitamin premix provided per kg of diet: vitamin A (retinol): 2.7 mg; vitamin D_3 (cholecalciferol): 0.05 mg; vitamin E (tocopheryl acetate): 18 mg; vitamin K_3: 2 mg; thiamine 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg.
[3] Mineral premix provided per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu ($CuSO_4.5H_2O$): 10 mg; I (KI, 58% I): 1 mg and Se ($NaSeO_3$, 45.56% Se): 0.2 mg.
LCP1: basal diet with 10% lower CP than Ross recommendation and LCP2: basal diet with 20% lower CP than Ross recommendation.

Five days later, the same wing-banded birds were bled to determine antibody titer against SRBC as well as influenza (IDV) and Newcastle disease viruses (NDV). Subsequently antibody titer against SRBC was measured by the haemagglutination assay method. Antibody titer against influenza and Newcastle, separately, was measured by haemagglutination inhibition method. Haemagglutination inhibition antibodies were then converted to $\log_2$. Antibody titers against SRBC were measured by the microtitre procedure described by Wegmann and Smithies (1966). On day 28 and at the end of the experiment, two birds were slaughtered.

Then, spleen and bursa of fabricius were weighed to evaluate the immune system development. The heterophil to lymphocyte ratio (H/L) was determined by blood sampling on day 28. Blood samples were taken from the wing vein using syringes containing heparin to avoid blood clot formation.

Blood smears were stained by May–Greenwald–Giemsa stain (Lucas and Jamroz, 1961). One hundred leukocytes per samples including granular (heterophils and eosinophils) and non-granular (lymphocytes and monocytes) cells were counted under an optical microscope (Nikon, Japan) with 100 × oil immersion lens, and H/L was calculated and recorded (Gross and Siegel, 1983).

Tonic immobility was tested on 6 chicks from each pen on day 42 according to procedure of Campo and Redondo (1996). Tonic immobility was induced as soon as a bird was caught by placing the bird on the back with the head hanging in a U-shaped wooden cradle (Jones and Faure, 1981). The bird was restrained for 15 seconds. The observer sat in full view of the chicken and at a distance of about 2 m from the bird. If the bird remained immobile for 10 seconds after the experimenter removed his hands, a stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 10 seconds, then it was considered that tonic immobility had not been induced and the restraint procedure was repeated. If tonic immobility was not induced after three attempts, the duration of tonic immobility was considered to be 0 second. If the bird did not show a righting response over the 10-min test period, a maximum score of 600 second was given for righting time.

Statistical analysis

Data were subjected to the analysis of variance appropriate for a completely randomized design using general linear model (GLM) procedure of SAS 9.2 (SAS, 2004). If a significant effect was detected, differences between treatments were separated using LSD test. Statements of statistical significance are based on a probability of $P < 0.05$.

RESULTS AND DISCUSSION

Performance, carcass measurements and digestive organ weights

According to Table 2, chickens that received LCP1 or LCP2 had lower BW and DWG than the CTL group during the starter period (P<0.05). Dietary inclusion of Trp to LCP1 diets reversed the impaired DWG and BW of broilers during the starter period of the experiment (P<0.05). However, addition of Trp to the LCP2 diet failed to reverse BW or DWG loss of broiler chicks in comparison with those in the CTL group (P<0.05).

Despite variations in DWG across the starter phase, neither DFI nor FCR were affected by dietary treatments. The greatest abdominal fat deposition was observed when broilers fed LCP1 diet which was significantly higher than + Trp on day 42 of age (Table 3; P<0.05). Supplementing Trp to the LCP1 diet decreased jejunal length compared with CTL and other dietary treatments except LCP1 on day 28 of age (Table 4; P<0.05).

Moreover, Trp inclusion to all dietary treatments significantly reduced duodenal proportional weight compared with CTL, LCP1 and LCP2 on day 42 of age (Table 5; P<0.05).

Table 2 Effects of dietary treatments on performance of broilers at different ages

Parameters	Dietary treatments						SEM	P-value
	CTL	+ Trp	LCP1	LCP1 + Trp	LCP2	LCP2 + Trp		
BW (g)								
14 d	401.3^{a}	403.3^{a}	371.3^{c}	411.9^{a}	360.5^{d}	388.3^{b}	5.8	0.037
28 d	1290.9	1331.5	1219.9	1273.1	1213.3	1265.1	16.6	0.904
42 d	2462.8	2560.5	2224.5	2287.3	2225.5	2377.1	55.8	0.421
DWG (g/d)								
1-14 d	25.8^{a}	25.9^{a}	23.3^{c}	26.2^{a}	22.5^{d}	24.8^{b}	0.44	0.047
14-28 d	59.9	62.7	57.8	62.7	57.4	59.9	0.90	0.386
28-42 d	85.8	89.6	75.1	81.8	70.7	75.8	3.77	0.535
1-42 d	57.2	59.4	52.7	54.3	52	53.8	1.4	0.599
DFI (g/d)								
1-14 d	29.7	30.4	28.7	28.7	29.3	28	0.26	0.071
14-28 d	95.6	95.7	90.2	95.2	96.9	95.9	1.02	0.067
28-42 d	149.4	153.9	145.8	145.9	147.5	134.6	2.16	0.738
1-42 d	91.6	93.4	86.9	89.9	88.8	89.9	0.95	0.277
FCR								
1-14 d	1.15	1.17	1.23	1.09	1.29	1.13	0.02	0.081
14-28 d	1.60	1.52	1.56	1.59	1.69	1.60	0.05	0.624
28-42 d	1.74	1.72	1.94	1.78	2.09	1.79	0.09	0.619
1-42 d	1.60	1.57	1.65	1.65	1.71	1.67	0.03	0.723

CTL: Control diet; + Trp: Control diet supplemented with 0.15% tryptophan; LCP1: 10% low-crude protein diet; LCP1 + Trp: 10% low-crude protein diet supplemented with 0.15% tryptophan; LCP2: 20% low-crude protein diet and LCP2 + Trp: 20% low-crude protein diet supplemented with 0.15% tryptophan.
BW: body weight; DWG: daily weight gain; DFI: daily feed intake and FCR: feed conversion ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of dietary treatments on carcass measurements

Parameters	Dietary treatments						SEM	P-value
Carcass contents*	CTL	+ Trp	LCP1	LCP1 + Trp	LCP2	LCP2 + Trp		
Carcass yield (%)								
Day 42	72.49	73.12	73.53	73.54	72.04	74.48	1.29	0.110
Abdominal fat (%)								
Day 42	1.57^{ab}	1.41^{b}	2.00^{a}	1.93^{a}	2.03^{a}	1.98^{a}	0.07	0.647
Heart (%)								
Day 28	0.39	0.44	0.49	0.49	0.44	0.45	0.01	0.827
Day 42	0.49	0.58	0.51	0.53	0.50	0.52	0.01	0.961
Liver (%)								
Day 28	2.11	2.13	2.10	2.27	2.25	2.18	0.04	0.189
Day 42	2.10	2.19	1.98	2.28	2.12	2.16	0.07	0.795

* Carcass measurements are expressed as a percentage of live body weight.
CTL: Control diet; + Trp: Control diet supplemented with 0.15% tryptophan; LCP1: 10% low-crude protein diet; LCP1 + Trp: 10% low-crude protein diet supplemented with 0.15% tryptophan; LCP2: 20% low-crude protein diet and LCP2 + Trp: 20% low-crude protein diet supplemented with 0.15% tryptophan.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Morphology of small intestine

According to Table 6, the greatest mean jejunal crypt depth was observed in broilers fed on LCP2 which was higher than broilers in CTL group (P<0.05).

Also, it resulted in lower V/C than broilers in CTL group (P<0.05). Moreover, dietary addition of Trp to LCP2 diet resulted in decreased V/C compared with chickens in CTL group (P<0.05).

Similarly, feeding LCP2 significantly increased crypt depth in the ileum but reduced the V/C (P<0.05). Otherwise, broilers receiving + Trp diet possessed higher V/C than the CTL (Table 6; P<0.05).

Immune responses, serum differential counts and tonic immobility

Results of the present study indicated that protein reduction of diet (LCP2) increased H/L (Table 7; P<0.05) but had no impact on the antibody production against Newcastle or influenza disease viruses as well as anti- SRBC response. Additionally, duration of the tonic immobility test was not affected by dietary treatments. In the present trial, Trp supplementation to the LCP1 diet ameliorated reduction in BW and DWG of broiler chicks but failed to reverse the growth performance after addition to LCP2 diets during the starter period.

Table 4 Effects of dietary treatments on relative weights of digestive organs and length of intestine on day 28

Parameters	Dietary treatments						SEM	P-value
	CTL	+ Trp	LCP1	LCP1 + Trp	LCP2	LCP2 + Trp		
Digestive organs*								
Gizzard (%)	1.81	1.64	2.31	1.78	1.72	1.73	0.11	0.403
Pancreas (%)	0.29	0.28	0.40	0.30	0.27	0.30	0.02	0.150
Proventriculus (%)	0.46	0.48	0.51	0.51	0.46	0.45	0.01	0.608
Duodenum (%)	0.81	0.78	0.77	0.68	0.73	0.83	0.02	0.115
Jejunum (%)	1.35	1.45	1.58	1.55	1.34	1.49	0.04	0.525
Ileum (%)	1.20	1.13	1.26	1.07	1.13	1.28	0.03	0.391
Cecum (%)	0.54	0.55	0.61	0.45	0.49	0.41	0.03	0.811
Length of intestine								
Duodenum (cm)	26	25	25	23	24	25	0.50	0.704
Jejunum (cm)	58^{ab}	58^{ab}	55^{bc}	53^{c}	63^{a}	59^{ab}	0.92	0.036
Ileum (cm)	59	60	57	57	65	57	1.03	0.068
Cecum (cm)	29	28	30	30	29	26	0.52	0.873

* Digestive organs expressed as percentage of live body weight.
CTL: Control diet; + Trp: Control diet supplemented with 0.15% tryptophan; LCP1: 10% low-crude protein diet; LCP1 + Trp: 10% low-crude protein diet supplemented with 0.15% tryptophan; LCP2: 20% low-crude protein diet and LCP2 + Trp: 20% low-crude protein diet supplemented with 0.15% tryptophan.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of dietary treatments on relative weights of digestive organs and length of intestine on day 42

Parameters	Dietary treatments						SEM	P-value
	CTL	+ Trp	LCP1	LCP1 + Trp	LCP2	LCP2 + Trp		
Digestive organs*								
Gizzard (%)	1.53	1.47	1.48	1.46	1.65	1.47	0.03	0.523
Pancreas (%)	0.22	0.21	0.22	0.22	0.24	0.22	0.005	0.251
Proventriculus (%)	0.45	0.45	0.45	0.43	0.51	0.43	0.01	0.720
Duodenum (%)	0.74^{a}	0.54^{b}	0.64^{ab}	0.56^{b}	0.63^{ab}	0.58^{b}	0.02	0.045
Jejunum (%)	1.32	1.18	1.29	1.31	1.35	1.19	0.04	0.425
Ileum (%)	1.08	1.10	1.07	1.37	1.24	1.14	0.05	0.546
Cecum (%)	0.46	0.48	0.47	0.51	0.52	0.52	0.02	0.799
Length of intestine								
Duodenum (cm)	34	27	28	28	28	32	1.09	0.805
Jejunum (cm)	66	65	66	66	67	65	0.91	0.065
Ileum (cm)	68	75	70	69	72	70	1.06	0.731
Cecum (cm)	37	35	33	33	34	34	0.47	0.675

* Digestive organs expressed as percentage of live body weight.
CTL: Control diet; + Trp: Control diet supplemented with 0.15% tryptophan; LCP1: 10% low-crude protein diet; LCP1 + Trp: 10% low-crude protein diet supplemented with 0.15% tryptophan; LCP2: 20% low-crude protein diet and LCP2 + Trp: 20% low-crude protein diet supplemented with 0.15% tryptophan.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of dietary treatments on intestinal morphology

Parameters	Dietary treatments						SEM	P-value
	CTL	+ Trp	LCP1	LCP1 + Trp	LCP2	LCP2 + Trp		
Jejunum								
Villus height (µm)	1310	1377	1267	1271	1212	1215	20.48	0.395
Crypt depth (µm)	258^{bc}	248^{cd}	292^{ab}	225^{c}	320^{a}	311^{ab}	30.07	< 0.0001
V/C*	5.08^{ab}	5.55^{a}	4.91^{b}	5.55^{a}	3.79^{c}	3.90^{c}	0.30	0.001
Ileum								
Villus height (µm)	921	991	893	945	842	945	19.65	0.063
Crypt depth (µm)	178^{b}	161^{b}	186^{ab}	166^{b}	221^{a}	185^{ab}	20.5	< 0.0001
V/C	5.16^{bc}	6.16^{a}	4.78^{c}	5.69^{ab}	3.80^{d}	5.11^{bc}	0.40	< 0.0001

* Villus height / crypt depth.
CTL: Control diet; + Trp: Control diet supplemented with 0.15% tryptophan; LCP1: 10% low-crude protein diet; LCP1 + Trp: 10% low-crude protein diet supplemented with 0.15% tryptophan; LCP2: 20% low-crude protein diet and LCP2 + Trp: 20% low-crude protein diet supplemented with 0.15% tryptophan.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of treatments on immune related parameters, heterophil to lymphocyte ratio and tonic immobility

Parameters	Dietary treatments						SEM	P-value
	CTL	+ Trp	LCP1	LCP1 + Trp	LCP2	LCP2 + Trp		
IDV (Log_2)	4.30	4.50	4.18	4.71	4.40	4.56	0.07	0.337
NDV (Log_2)	3.80	4	3.86	4	3.80	3.90	0.11	0.468
SRBC (Log_2)	8.70	8.70	8.36	8.57	8.50	8.55	0.08	0.286
H/L[5]	0.34^{cd}	0.37^{c}	0.34^{cd}	0.32^{d}	0.48^{a}	0.42^{b}	0.25	0.049
Spleen								
Day 28 (%)	0.10	0.10	0.09	0.10	0.08	0.10	0.003	0.782
Day 42 (%)	0.12	0.14	0.11	0.12	0.11	0.12	0.004	0.802
Bursa of fabricius								
Day 28 (%)	0.14	0.11	0.11	0.15	0.10	0.12	0.02	0.990
Day 42 (%)	0.07	0.07	0.06	0.08	0.06	0.06	0.003	0.465
Tonic immobility								
Attempts	1.50	1.55	1.75	1.58	1.85	1.40	0.08	0.127
Duration (sec)	256	249	280	150	197	263	17.10	0.230

CTL: Control diet; + Trp: Control diet supplemented with 0.15% tryptophan; LCP1: 10% low-crude protein diet; LCP1 + Trp: 10% low-crude protein diet supplemented with 0.15% tryptophan; LCP2: 20% low-crude protein diet and LCP2 + Trp: 20% low-crude protein diet supplemented with 0.15% tryptophan.
IDV: Influenza disease virus; NDV: Newcastle disease virus; SRBC: sheep red blood cells and H/L: heterophil to lymphocyte ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The positive impact of Trp on growth of chicks receiving low CP diets is due to the fact that Trp is an essential and limiting amino acid in poultry, and is required for various metabolic functions (Corzo *et al*. 2005a). Researchers observed the beneficial effect of Trp supplementation on growth performance of chickens fed on Trp deficient (Corzo *et al*. 2005a; Rosa *et al*. 2001) or Trp adequate (Emadi *et al*. 2010) diets. The results of this study are also in agreement with many others suggesting that synthetic amino acids could not completely replace the CP in broiler diets (Ferguson *et al*. 1998; Si *et al*. 2004; Waldroup *et al*. 2005; Yamazaki *et al*. 2006). In contrast, some researchers believed that meeting the need for essential amino acids can result in optimal growth performance in broiler chicks (Han *et al*. 1992; Parr and Summers, 1991). It seems that there is a limiting level for CP below which reduction may affect the growth performance of broilers.

In the current experiment, the numerically lower abdominal fat deposition was observed in + Trp fed groups compared with CTL birds on day 42.

This is in line with other reports (Akiba *et al*. 1988; Rogers and Pesti, 1990). It has been demonstrated that Trp supplementation may affect carcass lipid content (Akiba *et al*. 1988).

However, Trp level required to decrease lipid levels was higher than the level needed to maximize body growth of broilers (Rogers and Pesti, 1990). In contrast, Rosa *et al*. (2001) reported no effect for Trp on carcass lipid contents of broiler chickens.

Feeding low CP diets was observed to increase the intestinal length or weight in broiler chicks (Incharoen *et al*. 2010). Although gross anatomy of the gastrointestinal tract (GIT) was not altered following consumption of low CP diets in our study, Trp inclusion to LCP1 decreased the weight and length of duodenum and jejunum, respectively. There is a dearth of reports on the effect of Trp on the GIT of broiler chicks. Namroud *et al*. (2008) indicated that feeding low CP diets along with amino acid supplementation had no significant effects on proportional weight of the small intestine in broiler chickens, therefore, it is difficult to

come to a certain conclusion on the role of Trp in the GIT development.

Villus cells are produced in crypt so that a deeper crypt shows rapid tissue turnover and a high demand for new tissue (Choct, 2009). The greatest crypt depth in the jejunum was observed, when broilers received LCP2 and LCP2 + Trp diets which led to significant decrease of V/C compared with the CTL group. The effect of diet type on the anatomical variations in the small intestine has been reported previously (Ale Saheb *et al*. 2016; Laudadio *et al*. 2012). Further, feeding a low CP diet was reported to reduce the rate of protein synthesis in the intestine (Wykes *et al*. 1996). Protein is also necessary as a dietary component for development of morphological features (Incharoen *et al*. 2010). It is likely that the impaired V/C in birds supplemented with LCP2 is due to reduced CP of diet. Similar results were observed in the ileum after feeding chickens with LCP2 diet. Also, Trp addition to LCP1 diet increased V/C compared with the CTL group. It also shows the role of Trp on protein accretion in the body.

Supplementation of LCP2 increased H/L in the present study, although Corzo *et al*. (2005a) reported that feeding Trp deficient diets had no significant effect on H/L in broiler chickens. Duration of tonic immobility in this study was not affected by consumption of low CP diets or dietary Trp supplementation which is contradict with previous studies in which feeding low Trp diets were shown to result in abnormal behavior such as feed spillage (Corzo *et al*. 2005b; Shea-Moore *et al*. 1996). High duration of tonic immobility and H/L are considered to be indicators of chronic stress. High H/L ratio despite no sign of fearfulness in this study is in line with the work of Ale Saheb Fosoul *et al*. (2016), who indicated that alternating energy or protein contents of diets has led to fearfulness but no change in H/L ratio of broiler chickens. It might reflect that no solid relationship exist between tonic immobility and H/L.

CONCLUSION

In conclusion, feeding low CP diets (10 and 20% lower than the Ross manual recommendation) caused deterioration in growth performance of chickens. Although supplementation of Trp ameliorated the loss of growth during starter period, it could not show this effect on growth after feeding LCP2. It seems that there is a limiting level for CP reduction below which may affect the growth performance in broiler chicks. Broilers received + Trp diet possessed lower abdominal fat deposition than the CTL which might show the reducing impact of Trp on carcass lipid contents. As results indicated, feeding low CP diets impaired V/C and Trp addition to the CTL diet could increase the V/C. It shows the beneficial impact of protein and Trp on morphological features of the small intestine. Although broilers subjected to LCP2 diets had higher H/L, the duration of tonic immobility remained unaffected.

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