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Impacts of Dietary L-Threonine Supplementation on Performance and Intestinal Morphology of Broiler Chickens during Summer Time

Research Article

K. Shirzadegan[1*], I. Nickkhah[2] and M.A. Jafari[2]

[1] Department of Animal Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
[2] Department of Animal Science, Faculty of Agriculture, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

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*Correspondence E-mail: k.shirzadegan@znu.ac.ir

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ABSTRACT

The present study evaluated effects of 0.0%, 0.5%, 0.75% and 1.0% L-threonine in diet on performance and gastrointestinal tract development in broiler chickens during summer. From 0 to 42 days old, body weight gain was greater in 0.75% L-threonine fed birds than in 0.0% and 0.5% L-threonine fed birds (P<0.05). Moreover, in jejunum and ileum both the 0.75% and 1.0% L-threonine intake caused longer villus height as compared to 0.0% and 0.5% L-threonine (P<0.05), but no other morphological parameters were affected by L-threonine supplementation (P>0.05). No significant differences were observed between the treatments for feed intake and feed conversion ratio during the starter, grower or whole experimental period (P>0.05). During the growth period, 0.75% L-threonine fed birds had the highest body weight gain (P<0.05). Carcass traits were not affected by treatments (P>0.05). In conclusion, supplementation of 0.75% L-threonine improved the intestinal morphology and body weight gain of broilers under hot conditions. As well as, the higher levels of L-threonine (1.0) percent in diet can will increased the villus height in the jejunum and ileum.

KEY WORDS broiler chickens, intestinal morphology, L-threonine, performance.

INTRODUCTION

Threonine is the third most limiting amino acid in broiler diets, especially in a low crude protein diet. Threonine is used in important metabolic processes such as protein synthesis and uric acid formation. Also, poultry are not capable of synthesizing threonine denovo, which makes it a nutritionally essential amino acid. L-threonine is added to the diet of poultry and pigs so as to match the dietary amino acid balance to the nutritional requirements of the animal. There are many reports on the threonine requirements of poultry (Rosa *et al.* 2001; Shan *et al.* 2003; Ahmadi and Golian, 2010), but less information is available on the effects of dietary L-threonine on the immune system and intestinal morphology of them in hot weather conditions. The dietary supplementation of L-threonine, similar to that of lysine, results in a further decrease in crude protein because most of the threonine in broiler diets comes from soybean meal and meat meals. Thus, adequate knowledge of the requirement of the next limiting amino acid beyond threonine or adequate knowledge of the crude protein level needed to maximize bird productivity is needed to take advantage of feed-grade amino acids at affordable prices (Kidd *et al.* 2002). Dozier *et al.* (2001) indicated that broilers fed inadequate threonine had decreased life performance, but no effect was apparent on carcass fat. It has been reported that threonine is an important component of mucus (40% of protein in mucus glycoproteins) in the digestive tract (Carlstedt *et al.* 1993). Most parts of mucins are not digestible and the associated threonine cannot be recovered

(Fuller, 1994). Therefore, it is necessary to balance threonine in broiler diets by adding L-threonine or by using soybean meal and meat meal as the most important ingredients, which supply threonine in the chick diet.Compared with other essential amino acids, threonine content in proteins of the intestine represents up to 30% (Neutra and Forstner, 1987), suggesting that threonine affects intestinal functionality and maintenance. In healthy rats, threonine restriction reduced small and large intestine mucin synthesis (Faure *et al.* 2005). Moreover, threonine is involved with amylase secretion in the digestive tract. Block *et al.* (1996) showed that the threonine requirement for amylase synthesis is approximately 11% of the protein. Adequate digestible threonine levels are needed to support optimum growth (Kidd *et al.* 1999) because it serves as important component of body protein and plays an important role as precursor of lysine and serine (Ojano-Diranin and Waldroup, 2002). Threonine is also needed for optimal immune response and gastrointestinal mucin production (Kidd, 2000); as well as to improve the livability of heat stressed broilers (Kidd, 2000). Increased dietary L-threonine concentration is known to improve nitrogen retention in broiler chicks; therefore changing the threonine concentration is an important tool to improve nitrogen utilization (Dozier *et al.* 2001). Waldroup *et al.* (2002) found that threonine supplementation of turkey diet can increase weight gain. Whereas, Lehman *et al.* (1997) reported that weight gain and feed conversion efficiency were not affected by an increase in threonine level from 0.69% to 0.91% in a wheat- corn- soybean diet fed to turkey. Thus this paper evaluates the influences of L-threonine on growth performance and intestinal morphology of broilers raised in hot conditions.

MATERIALS AND METHODS

Experimental design and management

The present study was conducted from August to September of 2013, during which the weather was relative hot and humid. A total of 300 commercial broiler chicks (Cobb 500, male) were divided into 4 groups of 75 birds each, which consisted of 5 replications of 15 birds each. They were arranged using a completely randomized design**.** The photoperiod was 23h light: 1h dark, throughout the experiment. The chickens were housed in a clean room, previously disinfected with formalin. The rearing room was provided with fans and coolers to adjust the temperature to 29 ± 1 ˚C throughout the experiment. Relative humidity in the rearing house was 42%. The temperature and humidity inside the barn were recorded twice daily at 08:00 and 14:00. Prophylactic measures against the most common infectious diseases were conducted. The chicks were vaccinated against Newcastle and Gumboro diseases as prescribed by a local vet. This study lasted 6 weeks. Broilers were fed the same basal diet based on wheat- barley- corn and soybean meal (Table 1). Feed in mash form and water were provided freely. Crystalline L-threonine (98.5% Thr, PT Cheil Jedang, Iran) was added to the basal diet at 0.0% (control), 0.5%, 0.75% and 1.0% dry matter. Dietary treatments were achieved by the addition of crystalline L-threonine at the expense of inert filler to give 0.47 (the current NRC (1994) requirement for threonine). The treatment diets were formulated to meet the requirements of broiler chickens according to NRC (1994). Feed intake (FI), average body weight gain (BWG) and feed conversion ratio (FCR) were determined during the starter (0 to 21 days of age), grower (22 to 42 days of age) and whole the experimental periods (0 to 42 days of age). On days 21 and 42 of age, one chick from each replicate pen was killed by cervical dislocation to collect the intestine samples. The intestine was removed and divided into three segments of duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileocecal junction). At the end of the experiment, two birds from each replicate were randomly selected and scarified. Afterward, the thigh, breast, pancreas, gizzard and liver weights and the weight and length of the different intestinal segments (duodenum, jejunum and ileum) and their relative lengths (intestinal segment lengths/small intestine length×100) and weights (organ weight/live body weight×100) were calculated. The empty weight of these parts was determined separately. Then, approximately 5 cm of the middle portion of the duodenum, jejunum and ileum was cut and digesta washed out using normal saline and fixed in 10% neutral buffered formalin. Following histological fixation, the tissues were processed through standard alcohol dehydration-xylen sequence and embedded in paraffin. From each segment, 5 sections of 5 µm thickness were made, stained with haematoxylin and eosin and periodic acid shift (PAS). Morphometric analyses of digital photos from light microscopy were performed by means of Image J analysis software (Abramoff *et al.* 2004). Villus height (VH), villus width (VW), crypt depth (CD) and goblet cell number (GN) were determined and the VH:CD (VC) ratio was calculated. Statistical analysis was carried out using a general linear model (GLM procedure) to evaluate the effects of treatments on performance and intestinal morphology of broilers, using SAS (2002). Statistical significance of differences among treatments was determined using the Duncan's multiple range test at the 5% level.

RESULTS AND DISCUSSION

Performance

Results of effects of dietary L-threonine on broilers' performance are presented in Table 2.

Table 1 Composition of broilers starter and grower diets

Ingredients (g/kg)	Diets[1] (starter)			
	A	B	C	D
Soybean meal-44	374	374	374	374
Wheat, white W-	150	150	150	150
Corn, grain	163.3	163.3	163.3	163.3
Barley, pacific	100	100	100	100
Bakery waste	100	100	100	100
Corn oil	50	50	50	50
Anchovy meal	20	20	20	20
Dical-phos	13.8	13.8	13.8	13.8
Oyster shells	11.7	11.7	11.7	11.7
Mineral-vitamin mix[4]	5.0	5.0	5.0	5.0
Salt	3.0	3.0	3.0	3.0
Filler[5]	10.0	5.0	2.5	0
DL-methionine	0.96	0.96	0.96	0.96
L-threonine	0.0	0.5	0.75	1.0
Total	1000	1000	1000	1000
Analyzed				
ME (kcal/kg)	3200	3200	3200	3200
CP (g/kg)	230.0	230.0	230.0	230.0
Ca (g/kg)	10.0	10.0	10.0	10.0
Available phosphorus (g/kg)	4.5	4.5	4.5	4.5
NaCl (g/kg)	2.0	2.0	2.0	2.0
(Grower diet)				
Soybean meal-44	332.2[3]	332.2	332.2	332.2
Wheat, white W-	150	150	150	150
Corn, grain	244.4	244.4	244.4	244.4
Barley, pacific	100	100	100	100
Bakery waste	100	100	100	100
Corn oil	40.5	40.5	40.5	40.5
Anchovy meal	10	10	10	10
Dical-phos	12.1	12.1	12.1	12.1
Oyster shells	13.8	13.8	13.8	13.8
Mineral-vitamin mix[4]	5.0	5.0	5.0	5.0
Salt	3.0	3.0	3.0	3.0
Filler[5]	10.0	5.0	2.5	0
DL-methionine	0.86	0.86	0.86	0.86
L-threonine	0.0	0.5	0.75	1.0
Total	1000	1000	1000	1000
Analyzed				
ME (kcal/kg)	3200	3200	3200	3200
CP (g/kg)	200.0	200.0	200.0	230.0
Ca (g/kg)	9.0	9.0	9.0	9.0
Available phosphorus (g/kg)	3.5	3.5	3.5	3.5
NaCl (g/kg)	1.5	1.5	1.5	1.5

[1] A: 0.0% L-threonine; B: 0.5% L-threonine; C: 0.75% L-threonine and D: 1.0% L-threonine.
[2] Starter diet (0 to 21 days old).
[3] Grower diet (22 to 42 days old).
[4] Provides per kg of diet: vitamin A: 9000 IU; vitamin D_3: 2000 IU; vitamin E: 19 IU; vitamin B_{12}: 15 µg; Menadion: 3 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Niacin: 30 mg; Pyridoxine: 3 mg; D-pantothenic acid: 110 mg; Folic acid: 1 mg; Biotin: 0.1 mg; Choline chloride: 500 mg; Manganese: 110 mg; Zinc: 84.7 mg; Iron: 55 mg; Copper: 10 mg; Iodine: 1 mg and Selenium: 0.22 mg.
[5] The dose titrations were achieved by addition of L-threonine at the expense of washed builder's sand.
ME: metabolizable energy and CP: crude protein.

These findings indicate that effect of different inclusion rate of dietary L-threonine on broilers' FI and feed conversion ratio was not statistically significant during the starter or grower periods or over the whole experiment (P>0.05). However, the best feed conversion ratio was observed in the chicks fed on ration C containing 0.75% L-threonine. Furthermore, no significant difference was determined between the groups for BWG during the starter period (P>0.05). During the grower period and over the whole experiment, BWG was affected by L-threonine (P<0.05). BWG of 0.75% fed birds was greater than that of other birds during the grower period (P<0.05), but there were no significant differences among the other groups.

Over the whole experiment, BWG of 0.75% fed birds was higher than that of 0.0% and 0.5% fed birds (P<0.05). Moreover, no significant difference was noticed between the 1.0% birds and other treatments for BWG over the whole experiment (P>0.05).

Rosa *et al.* (2001) found that threonine requirement of the classic strain broiler chick was 0.69% for body weight gain and 0.68% for feed conversion ratio. Use of L-threonine decreased the crude protein requirements of the birds and reduced dietary crude protein which will improve nitrogen efficiency utilization, reduce nitrogen excretion, improve poultry tolerance to high ambient temperatures and reduce the level of ammonia in litter (Kidd and Kerr, 1996).

Dozier *et al.* (2000) reported that an increase in the concentrations of the dietary threonine to 0.74% optimized life performance of male broilers. Moreover, some researchers presented the negative effects of hot conditions and threonine decline on gut morphology. Hence, the present experiment evaluated the possible positive effects of L-threonine on intestinal development and performance of broiler chickens. The extensive involvement of threonine in the intestinal mucosa and digestive enzymes may be related to its influence on AMEn and growth performance (Dozier *et al.* 2001). These findings are in agreement with our results in the current experiment.

Table 2 The effects of L-threonine on performance of broiler chickens during the starter (0 to 21 day of age) and grower (22 to 42 days of age) periods and over the whole experiment (0 to 42 days of age)

Items	0-21 days	22-42 days	0-42 days
	BWG (g)		
A	651.250	1340.15^b	1991.14^b
B	630.15	1401.59^b	2031.74^b
C	665.34	1536.11^a	2201.45^a
D	633.37	1412.09^b	2045.46^{ab}
Pooled SEM	8.19	29.02	33.11
P-value	0.21	0.01	0.03
	FI (g)		
A	1034.08	2947.38	3981.46
B	1008.92	2980.09	3989.01
C	1042.62	3112.73	4105.80
D	1023.39	3069.94	4155.35
Pooled SEM	0.06	37.88	36.52
P-value	0.34	0.42	0.38
	FCR (g:g)		
A	1.57	2.22	1.98
B	1.55	2.18	1.99
C	1.56	2.07	1.89
D	1.58	2.22	1.97
Pooled SEM	0.05	0.11	0.08
P-value	0.48	0.56	0.09

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.
SEM: standard error of means.
A: 0.0% L-threonine; B: 0.5% L-threonine; C: 0.75% L-threonine and D: 1.0% L-threonine.

The present study demonstrated that feed consumption and BWG were not improved by increasing the levels of L-threonine at ages 0 to 21 and 0 to 42 days old. These results are also in opposite with the findings of Koid and Ishibashi (1995) who reported increased feed consumption by chicks as dietary digestible threonine level was increased. Douglas *et al.* (1996) noted that an improvement in feed consumption due to threonine supplementation in the diet. They further demonstrated that threonine requirement should be 70% of the lysine requirement in chickens. The findings of Trindade *et al.* (1999) also supported our results who reported that threonine levels in the diets had significant effect on feed intake in both sexes of the broiler chicks.

Results of our study have been supported by Martinez *et al.* (1999) who reported that the threonine requirement is 70% of lysine in the diets for better-feed conversion ratio in broiler birds. However, Kidd *et al.* (2000) reported that threonine supplementation did not show any significant improvement in feed conversion ratio in broiler birds. The results of our study indicated that as threonine was increased in the diet, feed conversion ratio was not improved. In fact the results show that higher threonine supplementation in diet than NRC recommendation does no induce any significant improves in FCR or carcass weight of broilers.

Carcass characteristics

Influences of dietary L-threonine on carcass characteristics of broiler chicks are presented in Table 3. Results indicated that the effects of dietary L-threonine on carcass traits were not statistically significant at 42 days old (P>0.05). However, the chickens fed 0.75% and 0.5% L-threonine had the greatest thigh and breast weight respectively. Moreover, the highest liver and pancreas weight percentages were also related to 0.5% L-threonine, but the highest gizzard weight percent was achieved by 1.0% L-threonine.

Intestinal morphology

Effects of dietary treatments on intestinal morphology of broiler chicks are presented in Table 4. The experiment revealed that L-threonine supplementation had no effect on proportional intestinal part (duodenum, jejunum and ileum) lengths and weights at age 21 days (P>0.05). In duodenum, 0.75% fed birds had longer VH than other birds (P<0.05). The L-threonine consumption had no effects on VW, CD, GN and VH:CD (P>0.05). In jejunum and ileum both the C and D birds had a higher VH than B and A groups (P<0.05). Moreover, VH of B chickens were higher than that of A group (P<0.05). However, no significant differences were seen among the groups for other variables (P>0.05).

The current study indicated that dietary L-threonine improved the intestinal morphology of the broilers. The villus height in duodenum, jejunum and ileum were increased among supplemented groups. Reported mucus consists of high molecular weight glycoproteins and it is known to contain a large proportion of this protein (Carlstedt *et al.* 1993). Because threonine is highly concentrated in proteins directly associated with the gastrointestinal mucosa, as well as digestive enzymes in the lumen necessary for effective nutrient recovery, diets low in threonine may adversely affect the efficiency of nutrient recovery (Dozier *et al.* 2001). Mitchell and Carlisle (1995) indicated the villus height is positively related to villus surface area and the expansion of surface area that occurs with villus growth has been used to explain the increased absorptive capacity, whereas the decreased villus height lowered the absorptive

Table 3 The effects of L-threonine on carcass traits of broilers at age 42 days

Items (%)	A	B	C	D	Pooled SEM	P-value
Thigh	23.02	22.89	23.26	23.13	1.36	0.12
Breast	24.33	24.58	23.98	24.01	1.01	0.24
Liver	2.14	2.31	2.00	2.17	0.59	0.19
Pancreas	2.16	2.33	2.12	1.97	0.33	0.07
Gizzard	2.16	2.05	2.24	2.32	0.76	0.58

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
A: 0.0% L-threonine; B: 0.5% L-threonine; C: 0.75% L-threonine and D: 1.0% L-threonine.
SEM: standard error of the mean.

Table 4 Influence of dietary L-threonine supplementation on duodenum, jejunum and ileum morphology plus their lengths and weights (based live weight %) in broiler chickens on day 21 and reared under hot condition (summer)

Items		VH (µm)	VW (µm)	CD (µm)	GN (µm)	VC	W	L
Duodenum	A	1294.63^{b}	130.32	137.86	7083.36	9.40	1.43	18.17
	B	1310.08^{b}	128.65	131.37	7005.27	9.97	1.52	18.78
	C	1349.35^{a}	129.23	137.55	7073.51	9.80	1.38	17.55
	D	1290.87^{b}	133.66	134.12	7018.66	9.61	1.47	18.06
	SEM	0.004	0.86	2.83	0.65	0.34	0.27	0.56
	P-value	0.01	0.09	0.22	0.35	0.12	0.54	0.61
Jejunum	A	1009.01^{c}	76.68	85.98	8046.35	11.73	2.84	51.33
	B	1044.06^{b}	77.51	88.53	8162.36	11.80	2.77	52.03
	C	1081.19^{a}	77.23	94.27	8081.71	11.46	2.91	51.68
	D	1083.33^{a}	78.09	92.12	8097.55	11.75	3.01	52.19
	SEM	1.11	1.01	3.43	24.11	0.23	0.48	0.77
	P-value	0.03	0.21	0.38	0.46	0.72	0.28	0.37
Ileum	A	419.55^{b}	71.61	67.21	9098.24	6.23	2.12	36.41
	B	418.49^{b}	69.44	67.06	9059.63	6.24	2.02	35.98
	C	448.35^{a}	71.44	66.19	9074.50	6.76	1.84	37.53
	D	499.65^{a}	70.77	64.83	9045.36	7.69	1.95	38.11
	SEM	3.78	2.13	1.66	20.07	1.01	0.32	0.89
	P-value	0.02	0.41	0.30	0.11	0.27	0.09	0.15

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
VH: villi height; VW: villi wide; CD: crypt depth; MLT: muscular layer thickness; GN: goblet cells number; VH to CD ratio (VC): villi height to crypt depth ratio; W: intestinal weight and L: intestinal length.
SEM: standard error of the mean.
A: 0.0% L-threonine; B: 0.5% L-threonine; C: 0.75% L-threonine and D: 1.0% L-threonine.

capability of small intestine (Yamauchi *et al.* 1996). Therefore, a higher villus height increases the intestinal surface area and nutrient absorption consequently (Soltan, 2009) and results in better performance. We find out better body weight gain for supplemented groups, in particular 0.75% L-threonine fed chickens may be related to their longer VH at day 21 of age and therefore improved nutrient utilization.

CONCLUSION

Generally, our results indicate that 0.75% L-threonine in diet could improves the intestinal morphology and consequently BWG of broilers under hot and humid status, but don't has any significant affect on carcass traits. Furthermore, although the higher dietary L-threonine (1%) similar to 0.75% increased the villi height in jejunum and ileum, but did not change the performance of broilers.

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اثرات مکمل سازی جیره با ال-ترئونین روی عملکرد و مورفولوژی روده جوجه‌های گوشتی در طی تابستان

ک. شیرزادگان*، ا. نیکخواه و م.ع. جعفری

چکیده

مطالعه حاضر به منظور اثرات افزودن مقادیر ۰، ۰/۵، ۰/۷۵ و ۱ درصد ال-ترئونین در جیره روی عملکرد و توسعه روده جوجه‌های گوشتی در زمان تابستان صورت گرفت. از ۰ تا ۴۲ روزگی، میزان افزایش وزن بدن پرندگان تغذیه شده با ۰/۷۵ درصد ال-ترئونین بیشتر از پرندگان تغذیه شده با ۰ و ۰/۵ درصد بود (P<۰/۰۵). علاوه بر این، در ژئوژنوم و ایلئوم مصرف ۰/۷۵ و ۱ درصد ال-ترئونین نسبت به ۰ و ۰/۵ درصد، موجب افزایش بیشتر طول پرزها گردید (P<۰/۰۵)، اما سایر فاکتورهای مورفولوژیکی تحت تأثیر مکمل‌سازی ال-ترئونین قرار نگرفت (P>۰/۰۵). هیچ تفاوت معنی داری برای مصرف خوراک و ضریب تبدیل در طی دوران آغازین، رشد و کل دوره آزمایش در بین تیمارها مشاهده نشد (P>۰/۰۵). در طی دوره رشد، پرندگان تغذیه شده با ۰/۷۵ درصد ال-ترئونین بیشترین افزایش وزن بدن را نسبت به سایرین داشتند (P<۰/۰۵). در ضمن، صفات لاشه نیز تحت تأثیر تیمارها قرار نگرفت (P>۰/۰۵). به طور کل، افزودن ۰/۷۵ درصد ال-ترئونین موجب بهبود مورفولوژی روده و افزایش وزن بدن جوجه‌های گوشتی قرار گرفته در شرایط گرم آب و هوایی می‌شود. علاوه بر این، سطوح بالاتر ال-ترئونین (۱ درصد) در جیره می‌تواند موجب افزایش ارتفاع پرز ژئوژنوم و ایلئوم جوجه‌های گوشتی گردد.

کلمات کلیدی جوجه‌های گوشتی، مورفولوژی روده، ال-ترئونین، عملکرد.