

Effect of Extra Supplementation of Methionine and Threonine on Growth Performance and Immune Response in Broilers

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Abstract

Extra supplementation of amino acids is considered to be good for better immune response in broilers. The aim of present study was to investigate the effect of extra supplementation of methionine (Met) and threonine (Thr) on growth performance, carcass characteristics and immune response in broilers. Three hundred day-old boiler chicks (mixed sex) were distributed into five experimental groups with six replicates (10 chicks/replicate) in each group. Five (A, B, C, D and E) iso-caloric (ME 2850 kcal/kg) starter and five (A, B, C, D and E) iso-caloric (ME 2950 kcal/kg) finisher diets were formulated. Diet A was standard commercial diet with 100% NRC amino acids recommendations whereas diets B, C, D and E contained 110% methionine + cysteine (M+C) level, 120% M+C level, 110% threonine (Thr) and 120% Thr, respectively. Results showed that the feed intake, weight gain and feed conversion ratio were found non-significant ($P < 0.05$) between the treatment groups. Breast meat yield was improved ($P < 0.05$) in chicken fed diets (B, C, D and E) with extra supplementation of Met and Thr. Highest ($P < 0.001$) haemagglutination inhibition titer level against Newcastle disease was found in chicks (6.50) fed diet E after first vaccination and in chicks fed diet B (5.50) after second vaccination. Similarly, higher weight (5.00g) of bursa of fabricius was also found in chicks fed diet B. The ELISA titer level against infectious bursal disease was not affected ($P > 0.05$) by dietary treatments. It is concluded that supplementation of Met and Thr above the recommended levels improve breast meat yield and immune response in chicks. However, it is not economical to do extra supplementation of Met and Thr in broiler diet in terms of growth performance.

Keywords: Methionine+Cystine, threonine, growth performance, immune response, broilers

Introduction

Amino acids are the constituents of proteins and essential for proper immune function. Deficiency of amino acids can lead to less growth performance and stress on the immune system [29]. The stress on immune system means that chicks are more susceptible to diseases. Methionine (Met) is a sulfur-containing first limiting amino acid in poultry diets with multifunctional properties. Met has the property to convert into other amino acids through transamination. It is assumed that Met supplementation can fulfil the deficiency of nutritionally non-essential amino acids [8] Met increases the feed conversion ratio (FCR), breast meat yield and dressing percentage in broilers. It works as contributor of methyl group which is used in the methylation of DNA and protein so it is involved in the gene expression [3].

Glutathione (GSH) protects the cells from free radicals e.g. hydroxyl radicals, lipid peroxide radicals and H_2O_2 [21]. Met influences the synthesis of the GSH in the cell. It is proved that GSH plays a salient role in the acclimatization of metabolic pathway related to immunological challenges. Furthermore, antibody production in the body and T-helper cell functioning also regulated by intensification of GSH in antigen-processing cells. Thus, intracellular GSH and concentration of CD4 cells (a type of glycoprotein found on the surface of immune cells) decline by the inadequate amount of sulphur containing amino acids. It is used for signalling the other immune cells in response to infection. It also has a function in lymphocyte proliferation and increase antibody production and its deficiency leads to reduction in the propagation of lymphocytes and reduce cytotoxic T-cell activity [21]. Dietary supplementation of Met has been proved beneficial for broilers to strengthen their immune system against Newcastle virus through IgG secretion, T-cell proliferation, antibody titre and leucocyte migration [34]. Supplementary Met enhanced the response to total antibody production against SRBC a T-dependent antigen [34]. Met level required to adjust leukocyte migration inhibition was higher than the level required to enhance the growth performance in broilers chicks [9]. However, using a purified diet, Met need appeared higher for growth than for humoral immunity [6].

Threonine (Thr) is also very important for immune functions because it is involved in synthesis of mucin plasma γ -globulin in chicks. Thr is required for proper gastrointestinal and immune functioning of the broilers [11], because it is required for the synthesis of mucin [23]. Mucin is essential protein for proper intestinal immunity [21,19]. Any modification in the production of mucin can impair the function of gut protection and absorption [15]. On the basis of these findings, it can be hypothesized that Thr actively involved in modulating immune function. To reduce the economic loss in terms of mortality and morbidity in broilers and to lower down the use of antibiotics, it is hypothesized that amino acids will extensively become economical nutraceuticals for amelioration of health and restraining of contagious diseases in broilers. Animal feeding trails showed that different components of immune system showed different response at varying dietary levels of Thr [20]. Similarly, antibody titres levels increase in chickens by increasing dietary Thr levels infected with the Newcastle disease virus [7] Also, dietary supplementation with Thr increased serum levels of IgG in sows [17]. These findings provide support for a role of dietary threonine in modulating immune function in livestock and perhaps humans. There is discrepancy in the literature on extra supplementation of Met and Thr in broiler diet for immune response. Therefore, present study was designed to determine the effect of higher dietary levels of methionine+cysteine (M+C) and Thr in broiler diet on growth performance (feed intake, weight gain and FCR), carcass parameters (dressing percentage and breast meat yield) and immune response against Newcastle disease and infectious bursal disease.

Materials and Methods

Chicks and experimental diets

Three hundred day-old boiler chicks (mixed) of Hubbard strain were distributed into five experimental groups with six replicates (10 chicks/replicate) in each group. Fresh clean water and feed were provided *ad libitum* around the clock. Five (A, B, C, D and E) iso-caloric (ME 2850 Kcal/kg) starter and five (A, B, C, D and E) iso-caloric (ME 2950 Kcal/kg) finisher diets were formulated. Diet A was control diet with 100% amino acids according to NRC recommendations [25] whereas diets B, C, D and E contained 110% M+C, 120% M+C, 110% Thr and 120% Thr, respectively. Starter and finisher diets were fed from 1-21 and 22-35 days, respectively. Ingredient and nutrient composition of starter and finisher diets are presented in Tables 1 and 2.

Table 1. Ingredient and nutrient composition of starter diets

Parameter (%)	Diets ¹				
	A	B	C	D	E
Ingredients					
Maize	57.44	57.44	57.44	57.44	57.44
Rice polish	5.00	5.00	5.00	5.00	5.00
Canola meal	7.00	7.00	7.00	7.00	7.00
Soybean meal	26.26	26.26	26.26	26.26	26.26
Lime stone	1.23	1.23	1.23	1.23	1.23
Di-calcium phosphate	1.51	1.51	1.51	1.51	1.51
Salt	0.36	0.36	0.36	0.36	0.36
Soya oil	0.35	0.35	0.35	0.35	0.35
L-Lysine HCL	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.23	0.31	0.39	0.23	0.23
L-Threonine	0.07	0.07	0.07	0.14	0.21
Supplement ²	0.30	0.30	0.30	0.30	0.30
Nutrient composition (%)					
Dry matter	91.22	91.32	91.15	91.19	91.03
Ash	6.36	5.79	6.16	5.49	5.69
Crude Protein	20.01	19.14	19.41	20.23	19.41
Lysine	1.04	1.08	1.10	1.07	1.12
Methionine	0.48	0.53	0.57	0.50	0.55
Methionine + Cystine	0.80	0.90	0.98	0.84	0.86
Threonine	0.65	0.68	0.67	0.75	0.82
Tryptophan	0.22	0.24	0.26	0.24	0.25
Arginine	1.23	1.25	1.30	1.31	1.31
Valine	0.81	0.81	0.81	0.81	0.81
Isoleucine	0.73	0.76	0.84	0.82	0.83

¹Diet A without extra amino acid supplementation; diet B with 110% M+C supplementation; diet C with 120% M+C supplementation; diet D with 110% threonine supplementation; diet E with 120% threonine supplementation.

²Lincomycine 4.4% 0.100 g, Mineral-Mix 0.250 g, (Packing of 25 kg with: Iron 95940 mg, Copper 20250 mg, Zinc 123 mg, Manganese 153 mg, Selenium 401 mg, Iodine 2032 mg, Sepiolite (binder) 100000 mg, Calcium 6.3% and Magnesium 1.5%), Vitamix 0.250 g (Packing of 25kg with: Vit. A 20000000 U.I, Vit. D3 4000000 U.I, Vit. E 60000 mg, Vit. K3 8,640 mg, Vit. B1 4000 mg, Vit. B2 12000mg, Niacin 80290 mg, Vit. B5 20000 mg, Vit. B6 6000 mg, Vit. B8 200 mg, Vit. B9 2000 mg, Vit. B12 20 mg, Calcium 27.8%, Antioxidants (BHA 60 mg and Ethoxyquine 60 mg)) and M. Tox 0.500 g per 100 kg of feed.

Table 2. Ingredient and nutrient composition of finisher diets

Ingredients (%)	Diets ¹				
	A	B	C	D	E
Corn	63.14	63.14	63.14	63.14	63.14
Soybean meal	27.55	27.55	27.55	27.55	27.55
Soya oil	0.83	0.83	0.83	0.83	0.83
Lime stone	1.46	1.46	1.46	1.46	1.46
Salt	0.34	0.34	0.34	0.34	0.34
Rice Polishing	5.00	5.00	5.00	5.00	5.00
DL-Methionine	1.37	1.42	1.48	1.37	1.37
L-Threonine	0.00	0.00	0.00	0.06	0.11
Supplement ²	0.31	0.31	0.31	0.31	0.31
Nutrient composition (%)					
Dry matter	90.22	90.32	90.15	90.19	90.03
Ash	5.49	6.20	6.66	6.51	6.65
Crude Protein	19.14	19.41	18.59	17.50	18.87
Lysine	0.83	0.95	0.97	0.91	0.96
Methionine	0.27	0.35	0.39	0.31	0.33
Methionine + Cysteine	0.54	0.59	0.68	0.56	0.58
Threonine	0.56	0.56	0.56	0.62	0.67
Tryptophan	0.20	0.22	0.22	0.21	0.23
Arginine	1.08	1.18	1.20	1.19	1.21
Valine	0.84	0.86	0.89	0.86	0.87
Isoleucine	0.75	0.76	0.78	0.75	0.77

¹Diet A without extra amino acid supplementation; diet B with 110% M+C supplementation; diet C with 120% M+C supplementation; diet D with 110% threonine supplementation; diet E with 120% threonine supplementation.

²Lincomycine 4.4% 0.100 g, Mineral-Mix 0.250 g, (Packing of 25 kg with: Iron 95940 mg, Copper 20250 mg, Zinc 123 mg, Manganese 153 mg, Selenium 401 mg, Iodine 2032 mg, Sepiolite (binder) 100000 mg, Calcium 6.3% and Magnesium 1.5%), Vitamix 0.250 g (Packing of 25kg with: Vit. A 20000000 U.I, Vit. D3 4000000 U.I, Vit. E 60000 mg, Vit. K3 8,640 mg, Vit. B1 4000 mg, Vit. B2 12000mg, Niacin 80290 mg, Vit. B5 20000 mg, Vit. B6 6000 mg, Vit. B8 200 mg, Vit. B9 2000 mg, Vit. B12 20 mg, Calcium 27.8%, Antioxidants (BHA 60 mg and Ethoxyquine 60 mg)) and M. Tox 0.500 g per 100 kg of feed.

Data collection

Feed intake and weight gain were recorded weekly and used to calculate FCR. All chicks were vaccinated against Newcastle disease virus (NDV) on day 5 and 28 and Infectious Bursal disease virus (IBDV) on day 15 and 22, by using live vaccines. Blood samples were collected seven days after every vaccine from randomly selected six chicks per group. To check the maternal anti bodies level against IBDV, blood samples was taken before the vaccination against IBDV. Blood serum was separated by centrifugation of blood. Haemagglutination inhibition (HI) test was performed to check antibody titer level against NDV [2]. To check immunity against IBDV, ELISA was performed using IDEXX USA Elisa kit. All blood sera were analysed at Veterinary Diagnostic Laboratory, Department of Pathology, University of Agriculture Faisalabad, Pakistan. Weight of different lymphoid organs (thymus gland, spleen and bursa of Fabricius) of slaughtered chicks was also recorded. For this purpose six chicks from each group were slaughtered at 35th day of experiment. Dressing % and breast meat yield was also recorded.

Chemical analysis

Feed samples were analysed for dry matter (DM), ash and crude protein (CP). Feed samples were oven dried at 105°C for 4h and ash was determined by incinerating the weighted amount of feed in furnace at 550°C for 6 h. The nitrogen (N) was determined by Kjeldhal method and CP was calculated as N×6.25 [4].

Economics

Cost of all the experimental diets was calculated. Feeding cost per kg live weight gain was calculated to compare the economics.

Statistical analysis

Data collected for each parameter were analysed using PROC GLM procedure of Statistical Analysis System [30]. The means were compared using Tukey’s test [32] and the differences were checked for statistical significance (P<0.05).

Results

Growth Performance

Table 3 shows data on feed intake, weight gain, feed conversion ratio, dressing % and breast meat yield of chicks fed different experimental diets. Feed intake, weight gain and feed conversion ration were found non-significant (P>0.05) between the experimental groups. Highest weight gain (1735.60g) was observed in chicks fed diet C. The FCR was same (1.69) for chicks fed diets A (control) and E. Non-significant (P>0.05) effect of extra methionine and threonine supplementation was found on dressing percentage. Breast meat yield was high in broilers fed on diet C, D or E.

Table 3. Growth performance of broilers fed experimental diets

Variables	Diets ¹					SEM ²	Significance ³
	A	B	C	D	E		
Feed intake	2873.41	2863.18	2954.14	2904.98	2836.68	20.14	NS
Weight gain	1703.32	1653.16	1735.06	1679.43	1678.06	13.82	NS
Feed conversion ratio	1.69	1.73	1.71	1.73	1.69	0.01	NS
Dressing %	58.53	59.05	57.80	59.02	60.47	0.44	NS
Breast meat %	29.83 ^c	30.57 ^b	33.79 ^a	32.63 ^a	33.79 ^a	0.82	*

¹Diet A without extra amino acid supplementation; diet B with 110% M+C supplementation; diet C with 120% M+C supplementation; diet D with 110% threonine supplementation; diet E with 120% threonine supplementation.

²Standard error of mean.

³NS: Non-significant,*: 0.01<P < 0.05

Immune response

Data on immune response of broilers fed experimental diets is given in Table 4. Highest (P<0.001) HI titer level against NDV was observed in group E (6.50) and B (5.50) after first and second vaccination, respectively. However, Elisa titer level against IBDV was not different (P>0.05) within treatment groups. Weight of bursa of fabricius was effected (P<0.05) by feeding different levels of methionine and threonine. Highest weight (5.0g) was found in group B, the weight of other immune organs was not affected (P>0.05) by dietary treatments.

Table 4. Immune response of broilers fed experimental diets

Variables	Diets ¹					SEM ²	Significance ³
	A	B	C	D	E		
ND titer ⁴ after 1 st vaccine	4.8 ^c	6.2 ^{ab}	5.5 ^{bc}	4.8 ^c	6.5 ^a	0.34	***
ND titer after 2 nd vaccine	3.5 ^{ab}	5.5 ^a	5.0 ^{ab}	1.8 ^b	2.5 ^{ab}	0.70	***
IBD titer ⁵ before vaccine	712.0	305.0	1041.0	1961.0	5767.0	990.79	NS
IBD titer after 1 st vaccine	265.0	109.0	64.0	159.0	114.0	34.29	NS
IBD titer after 2 nd vaccine	128.0	169.0	75.0	36.0	15.0	28.47	NS
Weight (g) of lymphoid organs							
Spleen (g)	2.00	1.50	1.67	2.17	1.83	0.12	NS
Bursa of Fabricius (g)	4.50 ^a	5.00 ^a	3.00 ^b	2.83 ^c	3.33 ^b	0.43	*
Thymus (g)	5.83	6.00	6.00	6.17	4.67	0.27	NS

¹Diet A without extra amino acid supplementation; diet B with 110% M+C supplementation; diet C with 120% M+C supplementation; diet D with 110% threonine supplementation; diet E with 120% threonine supplementation.

² Standard error of mean.

³ NS: Non-significant; *: 0.01 < P < 0.05; ***: P < 0.001.

⁴ Log₂ HI titer level

⁵ Elisa titer level

Economics

Table 5 shows the economics of different experimental diets in terms of feed cost per kg weight gain. Feed cost/ kg weight gain was not effected (P>0.05) by using higher levels of methionine and threonine in diet.

Table 5. Effect of amino acids supplementation on feeding cost of boilers.

Parameters	Diet ¹					SEM ²	Significance ³
	A	B	C	D	E		
Weight gain (kg)	1.70	1.65	1.74	1.68	1.68	0.01	NS
Total feeding cost (PKR ⁴)	161.37	162.98	171.06	163.54	160.55	1.87	NS
Feeding cost/kg weight gain (PKR ⁴)	94.89	98.67	98.87	97.44	95.71	0.78	NS

¹Diet A without extra amino acid supplementation; diet B with 110% M+C supplementation; diet C with 120% M+C supplementation; diet D with 110% threonine supplementation; diet E with 120% threonine supplementation.

² Standard error of mean.

³ NS: Non-significant.

⁴ PKR, Pakistanis rupees

Discussion

Amino acids are the essential component of broilers diet. They not only involve in muscle accretion but also play many other important roles i.e. improve the development of lymphoid organs [17], as an anti-oxidant [31] and maintain the intestinal integrity [21]. Supplementation of amino acids over the top of recommended levels can improve the homogeneity of flock which has important economic impact at the slaughter level [9] In the present study, growth performance of chicks fed different high levels of M+C (110 and 120%) and Thr (110 and 120%)

diets was found non-significant (P>0.05). [33] also found non-significant difference in feed intake and FCR for extra methionine supplementation. [1] reported that the FCR was improved (P<0.05) by Thr supplementation (at least 110% recommended level of Ross), however it tended to improve it throughout the research period due to significant increase (P<0.05) in body weight gain. Dressing percentage was also not improved significantly (P>0.05) by supplementation of amino acids as results were obtained by other experiments [1,16,28,22] Results of different studies on Met [24,14] and Thr supplementation [13,27,26,11] showed non-significant (P<0.05) improvement in dressing percentage and breast meat yield. Breast meat yield was improved (P<0.05) by amino acids supplementation but it was same (P>0.05) for higher levels of Thr (110 and 120%) and higher level of Met (120%). [1] also reported that Thr supplementation increased (P<0.05) the relative weight of breast. In this regards other studies [16,10,12,11] also confirm our finding that supplementation of Thr significantly increase breast meat yield. In the present study, the HI antibody titer level against NDV, after first and second ND vaccine was found significant (P<0.001) within the groups. Highest HI titer level after first vaccine was observed in serum collected from the bird fed 120% Thr supplemented diet. Similar trend in HI titer was observed by Bhargava [1,6] reported that Thr supplementation enhanced immune response in broilers. [20] also observed that the immune system is affected by level of Thr in diet. Dietary Thr strengthens the immune system in broilers [21,35]. In the present study, the HI antibody titer after second vaccination was found highest in serum collected for broilers of group B fed diet supplemented with 110% M+C. Similar trend in HI antibody titer after second vaccine was observed by Bhargava [7] But in another study Bhargava [6] found that inverse relationship exists between supplementation of Met and HI titer to NDV in chicks immunized at day 4. In the present study, antibody titer level against IBDV was not affected (P>0.05) by extra supplementation of Met and Thr. Among the lymphoid organs, the weight of Bursa was higher for experimental diet B (110% M+C) but the weight of spleen and thymus was not affected (P>0.05). These results are in agreement with those of Kidd [18] who reported that no effect of dietary threonine on the development of immune organs in young broilers.

Conclusions

From results of present study, it is concluded that breast meat yield and immunity of broilers improved by feeding high level of Met (10% more than NRC recommendations) and Thr (20% more than NRC recommendations). However the growth performance and economics are not affected by supplementing high levels of methionine and threonine.

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