Change in Growth Performance and Liver Function Enzymes of Broiler Chickens Challenged with Infectious Bursal Disease Virus to Dietary Supplementation of Methionine and Threonine

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Abstract: Problem statement: The aim of this study was to verify the effects of methionine and threonine supplementations higher than the NRC recommendation on growth performance, liver function enzymes, blood parameters and immune tissues of broiler chickens challenged with infectious bursal disease. **Approach:** A total of 450 day-old male broiler chicks were assigned to nine groups. Chickens were fed by three graded levels of DL-methionine [NRC (M1), 2 times NRC (M2) and 3 times NRC (M3)] and three graded levels of L-threonine [NRC (T1), 2 times NRC (T2) and 3 times NRC (T3)] from day 1-42 of age. On day 28, all birds were challenged with a commercial live-IBDV vaccine. **Results:** Body Weight Gain (BWG) and Feed Intake (FI) and Feed Conversion Ratio (FCR) were significantly influenced by the dietary treatments in starter phase and either methionine or threonine at the highest levels significantly decreased BWG, FI and FCR in broiler chickens. A similar trend by methionine and threonine at the highest levels was noted on BWG and FI in grower phase. The highest level of threonine significantly increased spleen weight on day 28 (pre-challenge) and 14 days post challenge. At 14 days after challenge, dietary supplementation of methionine at NRC recommended level (M1) significantly increased the serum concentrations of Alanine Aminotransferase (ALT), aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH). In the T3 group, the serum concentrations of AST and LDH were significantly decreased, whereas the serum uric acid concentration was significantly increased. **Conclusion/Recommendations:** In conclusion, our data suggest that the methionine and threonine requirement of male broiler chicks is higher for growth performance than was suggested by the last NRC committee and liver function enzymes results showed that methionine and threonine supplementation three times higher than NRC requirements in broiler chickens has not toxic potential.

Key words: Methionine, threonine, broiler, infectious bursal disease, performance, liver enzyme

INTRODUCTION

Methionine and threonine are regarded as to be the first and third limiting amino acids in broilers fed practical corn-soybean meal diets (Ojano-Dirain and Waldroup, 2002). Optimal levels of these amino acids are needed to support optimum growth and health of broilers. Nutrient requirement standards have been reported by the National Research Council (NRC, 1994) and through more than 25 years due to genetic selection, management practice and feed formulation changes birds have more rapid growth and better performance than previous years (Havenstein *et al*., 1994; Williams *et al*., 2000). On the other hand, NRC

recommendation are usually based on the needs of healthy birds under ideal condition, but birds in commercial systems are normally exposed to different kinds of stresses, diseases and also the combination of environmental condition. Infectious Bursal Disease (IBD), also known as Gumboro Disease, is one of the most important poultry diseases in the last 30 years. It is a highly contagious viral disease, affecting the immune system of poultry. However, it is often not recognized due to a subclinical form. Affected chickens have reduced antibody response to vaccinations, strong postvaccinal reactions and increased susceptibility to concurrent or secondary infections. It has been reported that amino acids requirements increased in disease and

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infectious and infections lead to several changes in amino acid plasma levels (Bhargava *et al*., 1971). Amino acids are required for the synthesis of a variety of specific proteins (including cytokines and antibodies) and regulate key metabolic pathways of the immune response to infectious pathogens. Dietary levels of certain individual amino acids have been shown to affect immune response (Jeevanandam *et al*. 1990; Paaw and Davis, 1990). Methionine (Swain and Johri, 2000; Rama Rao *et al*., 2003; Shini *et al*., 2005) and threonine (Duval *et al*. 1991; Ball *et al*., 1999) are two amino acids that have proven immune regulatory action. There is some evidence that essential amino acid level in the feed higher than of NRC specifications needed to achieve optimal immune system and growth performances (Quentin *et al*., 2005). Response of liver function enzymes to excessive methionine and threonine more than NRC recommendation have not been reported in broiler chickens challenged with IBD. Therefore, the possibility that excess of methionine and threonine may exert parts of its toxic effects by altering the liver function enzymes should be considered. Hence, it may be necessary for new research to conduct multiple focus and attention to the actual broiler requirements. The present experiment was conducted to determine the effects of dietary methionine and threonine supplementation on performance, liver function enzymes and serum metabolites of broiler chickens challenged with infectious bursal disease.

MATERIALS AND METHODS

Birds and housing environment: A total of four hundred fifty day-old male broiler chicks (Cobb 500) were obtained from a local hatchery. The chicks were wing-banded, individually weighed and housed in floor pens with wood shavings as litter material. The pens were in a conventional open-sided house with cyclic temperatures (minimum, 24°C; maximum, 32°C). The relative humidity was between 80-90%. The area of each pen was 1.5 m^2. Feed and water were provided *ad libitium* and lighting was continuous.

Experimental design: Commencing from day one, five replicate pens of 10 chicks each were assigned to one of the nine dietary treatments, giving a total of 45 pens. There were 3 levels of methionine in the form of DL-methionine (NRC (M1), 2 times NRC (M2) and three times NRC (M3)) and three levels of threonine in the form of L-threonine (NRC (T1), 2 times NRC (T2) and 3 times NRC (T3)). Birds aged 0-21 days fed diets containing graded concentrations of methionine (NRC, 0.75 and 1.27% of diet) and threonine (NRC, 0.82 and

1.62% of diet) and diets with similarly graded concentrations of methionine (NRC, 0.49 and 0.88% of diet) and threonine (NRC, 0.76 and 1.51% of diet) to birds aged 22-42 days. The basal diets (mash form) were formulated to meet or exceed requirements by the NRC (1994) for broiler chickens (Table 1). No antimicrobial, anticoccidial drugs or feed enzymes were included in the basal diets. The chicks were vaccinated against Newcastle disease (Animal Health, Fort Dodge, Iowa, USA) on day 7 (Intraocular) and on day 21(intranasal).

Challenge protocol: On day 28, all birds were challenged by oral route with a commercial live-IBDV vaccine (V877 strain, Malaysian Vaccines and Pharmaceuticals Sdn. Bhd). The strain was characterized as an intermediate virulent classical strain. The content of the 1000 dose of IBD vaccine vial was reconstituted in 100 mL distilled water and each bird was inoculated with 1 mL IBD virus into the lumen of the crop by oral gavage, that finally each bird received a dose ten times greater than the standard IBD vaccine.

Table 1: Ingredients and nutrient composition of diets

Item	Starter 1-21 days	Finisher 22-42 days
Ingredient (%)		
Corn	45.35	50.95
Soybean meal	43.97	38.22
Palm oil	6.22	6.89
Di calcium phosphate	1.91	1.76
Limestone	1.20	1.05
Salt	0.44	0.31
Vitamin and mineral premix[1]	0.60	0.60
DL-methionine	0.20	0.10
L-threonine	0.00	0.00
Lysine	0.11	0.12
[2]Nutrition composition (calculated)		
Crude protein (%)	22.00	20.00
ME (Mcal kg^{-1})	3050.00	3150.00
Available phosphorus (%)	0.45	0.42
Calcium (%)	1.00	0.90
Methionine	0.50	0.38
Lysine	1.10	1.00
Argenine	1.40	1.33
Tryptophane	0.28	0.26
Threonine	0.80	0.74
Na	0.20	0.74
Cl	0.39	0.25
K	1.02	0.92
Crude Fiber	4.20	3.92
DEB meg Kg^{-1}	235.00	212.00
Ca/P	138.00	157.00

[1]: Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 μg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.18 mg; copper, 8 mg; selenium, 0.15 mg; [2]: Based on NRC (1994) feed composition table

Performance parameters: The chickens were weighed individually on d 1, 7, 14, 21, 28, 35 and 42. Feed intake was recorded weekly and Feed Conversion Ratios (FCR) were calculated. Mortality was recorded daily in each subgroup.

Organ weight: At 28, 35 and 42 days of age, eight birds from each treatment were chosen at random, weighed and then slaughtered according to the Islamic method (Halal). The weights of spleen and bursa of Fabricius were recorded. Organ weights were expressed on a relative body weight basis.

Serum metabolites and liver function test: On d 28 and 42 (14 days after challenge), eight birds from each group were chosen at random and blood samples (4.0 mL) were collected from the brachial vein using a 23-ga needle. Serum was separated by centrifugation at 3000 g for 10 min and stored at -20°C until analysis. Serum creatine, Alkaline Phosphatase (ALP), Alanine Aminotransferase (SGPT or ALT), Aspartate Aminotransferase (SGOT or AST) and Lactate Dehydrogenase (LDH) were measured by specific commercial kits (Roche Diagnostica, Basel, Switzerland) using an autoanalyzer (HITACHI 902 automatic autoanalyzer).

Statistical analysis: The data were analyzed using a two-way ANOVA in a completely randomized design (CRD) in a factorial scheme of 3×3 (methionine levels × threonine levels) with the PROC GLM procedure of the Statistical Analysis System (SAS Institute, 2005).

The results were expressed as mean ± standard error of mean. Statistical significance was considered at $p \leq 0.05$.

RESULTS

The effects of methionine and threonine on body weight gain (BWG), feed intake (FI) and feed conversation ratio (FCR) in broiler chicken are presented in Table 2. BWG, FI and FCR were significantly influenced by the dietary treatments in starter phase (0-21) and either methionine or threonine at the highest levels significantly decreased BWG, FI and FCR in broiler chickens. A similar trend by methionine and threonine at the highest levels was noted on BWG and FI in grower phase (22-42). No significant difference was seen between treatment groups in grower phase on FCR. There were significant interactions between methionine and threonine on FCR on day 21 and BWG on day 42 (Table 3). Birds were fed with M2T3 M3T2 and M3T3 had an improvement in FCR compare with other groups. The highest BWG was observed in broiler chickens fed M2T1. However, there were no significant differences on BWG between M2T1 and M3T1 birds.

Effect of methionine and threonine on means of relative organ weight of broiler chickens is shown in Table 4. The highest level of threonine significantly increased spleen weight before challenge and 14 days post challenge. There were not statistically significant differences on spleen weight between T2 and T3 groups 14 days post challenge. The bursa of Fabricius weights of birds were not affected by methionine and methionine × threonine interaction.

Table 2: The effects of methionine and threonine on Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversation Ratio (FCR) in broiler chickens challenged with IBD on d 28

Main effect	BWG (0-21)	FI (0-21)	FCR (0-21)	BWG (22-42)	FI (22-42)	FCR (22-42)
M1	751±7.9[a]	1179±34[a]	1.42±0.02[a]	1541±23	3159±46[a]	2.1±0.04
M2	762±6.8[a]	1139±24[a]	1.33±0.02[b]	1565±22	3138±43[a]	2.0±0.14
M3	663±8.1[b]	950±29[b]	1.29±0.02[b]	1496±30	2741±78[b]	2.0±0.14
T1	733± 7.0[b]	1175±35[a]	1.45±0.02[a]	1634±20[a]	3175±44[a]	2.0±0.10
T2	756±7.7[a]	1107±28[b]	1.31±0.01[a]	1547±25[b]	3005±76[b]	2.0±0.09
T3	686±9.6[c]	985±37[c]	1.30±0.02[b]	1421±26[c]	2858±82[c]	2.1±0.12
ANOVA (p-value)						
M	***	***	***	NS	*	NS
T	***	***	***	***	*	NS
M × T	NS	NS	**	**	NS	NS

[a-c]: Means within a column with different letters differ significantly (p<0.05); *: p<0.05; **: p<0.01; ***: p<0.001; NS: Non-Significant

Table 3: Means (±SEM) Feed Conversion Ratio (FCR) and Body Weight Gain (BWG) of broiler chickens where methionine × threonine interactions were significant

Treatments	FCR (0-21)			BWG (22-42)		
	M1	M2	M3	M1	M2	M3
T1	1.55±0.02[ax]	1.41±0.02[bx]	1.36±0.02[bx]	1551±28[bxy]	1680±35[ax]	1669±39[ax]
T2	1.30±0.02[abz]	1.34±0.03[ay]	1.27±0.02 [by]	1623±43[x]	1538±33[y]	1481±51[y]
T3	1.40±0.02 [ay]	1.24±0.02[bz]	1.23±0.03[by]	1447±47[y]	1478±39[y]	1337±50[z]

[x-z]: Means within a column-subgroup; [a-c]: Means within a row-subgroup with no common subscripts differ significantly (p<0.05)

Table 4: Effect of methionine and threonine on means of relative organ weight of broiler chickens challenged with IBD on day 28

Main effect	Spleen			Bursa of Fabricius		
	28 days pre-challenge	7 days post challenge	14 days post challenge	28 days pre-challenge	7 days post challenge	14 days post challenge
M1	0.15±0.021	0.19±0.027	0.16±0.016	0.18±0.018	0.090±0.011	0.058±0.007
M2	0.19±0.031	0.20±0.027	0.17±0.013	0.17±0.013	0.090±0.006	0.048±0.002
M3	0.17±0.025	0.18±0.027	0.14±0.013	0.17±0.016	0.086±0.009	0.044±0.003
T1	0.13±0.005[b]	0.21±0.027	0.12±0.010[b]	0.20±0.015	0.083±0.004	0.049±0.007
T2	0.13±0.015[b]	0.20±0.027	0.16±0.012[ab]	0.17±0.015	0.096±0.011	0.059±0.003
T3	0.25±0.033[a]	0.17±0.027	0.18±0.015[a]	0.16±0.015	0.088±0.011	0.049±0.003
ANOVA (p-value)						
M	NS	NS	NS	NS	NS	NS
T	***	NS	*	NS	NS	NS
M ×T	NS	NS	NS	NS	NS	NS

Organ weight data, expressed as percentages of body weight; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; NS: Non-Significant

Table 5: Effect of methionine and threonine on liver function enzymes and blood parameters in broiler chickens challenged with IBD on day 28

Main effect	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	LDH (U L^{-1})	Creatinine (mmol L^{-1})	Uric Acid (umol L^{-1})
28 day of experiment (before challenge)						
M1	7.5±0.56	275±12	3885±488	2866±166	26±1.1	225±17
M2	8.9±0.53	295±21	3147±266	2504±190	33±1.7	279±31
M3	6.6±0.72	255±9	3405±340	2660±184	29±1.5	260±25
T1	6.7±0.72	274±20	3668±344	2654±215	30±1.7	269±24
T2	7.6±0.66	270±13	3737±522	2740±131	30±1.8	286±24
T3	8.6±0.46	282±11	3031±232	2637±196	29±1.5	210±19
ANOVA (p-value)						
M	Ns	Ns	Ns	Ns	Ns	Ns
T	Ns	Ns	Ns	Ns	Ns	Ns
M × T	Ns	Ns	Ns	Ns	Ns	Ns
42 day of experiment (14 days after challenge)						
M1	7.4±0.3[a]	390±27[a]	2479±472	3604±312[a]	30±1.5	356±22
M2	6.2±0.6[b]	304±11[b]	2572±321	2577±218[b]	28±0.9	367±31
M3	6.0±0.2[b]	309±8[b]	2082±284	2900±196[b]	30±1.0	344±31
T1	6.7±0.3	362±33a	2303±244	2959±328[a]	29±1.0	300±23[b]
T2	6.9±0.4	344±13[a]	2348±462	3470±219[ab]	29±0.7	346±23[b]
T3	6.1±0.6	296±9[b]	2482±335	2649±187[b]	30±1.6	421±26[a]
ANOVA (p-value)						
M	**	***	NS	**	NS	NS
T	Ns	**	NS	*	NS	**
M × T	***	**	NS	*	NS	NS

[a-c]: Means within a column with different subscripts differ significantly ($p<0.05$). *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; NS: Non-Significant

Table 6: Interaction effect of methionine and threonine on ALT, AST and LDH at 14 days post challenge in broiler chickens challenged with IBD on d 28

Treatments	ALT (U L^{-1})			AST (U L^{-1})			LDH (U L^{-1})		
	M1	M2	M3	M1	M2	M3	M1	M2	M3
T1	6.8±0.5	7.0±0.7[x]	6.0±0.4	491±54[ax]	314±33[b]	286±10[b]	4385±524[ax]	2268±186[by]	2226±150[yb]
T2	7.0±0.4	7.7±0.9[x]	6.0±0.6[b]	386±25[ay]	311±8[b]	334±12[ab]	3788±280[xy]	3481±393[x]	3461±462[x]
T3	8.4±0.6[a]	4.0±0.6[cy]	6.1±0.1[b]	294± 17[y]	286±20	307±15	2641± 440[y]	2292±356[y]	3015±146[xy]

[x-z] Means within a column-subgroup and [a-c] Means within a row-subgroup with no common subscripts differ Significantly ($P < 0.05$).

The effect of methionine and threonine on liver function enzymes and blood parameters in broiler chickens challenged with infectious bursal disease virus are shown in Table 5. There was no significant difference between the treatments for blood parameters and liver enzymes on d 28. At 14 days after challenge, dietary supplementation of methionine at NRC recommended level (M1) significantly increased the serum concentrations of ALT, AST and LDH. In the T3 group, the serum concentrations of AST and LDH were significantly decreased, whereas the serum uric acid concentration was significantly increased. There were no significant differences between T2 and T3 groups on LDH. Methionine and threonine interaction had significant effect on serum level of ALT, AST and LDH (Table 6).

DISCUSSION

Dietary characteristics can modulate a bird's susceptibility to infectious challenges and subtle influences due to the level of nutrients or the types of ingredients may at times be of critical importance (Klasing, 1998). NRC requirements for amino acids and protein are designed to support maximum growth and production under ideal condition and well maintenance. The recommended levels for methionine and threonin in poultry depend on species, stage, environment condition and level of feed consumption. They are already known that most nutrients are involved in the development of the immune system and its responses. The present findings indicated that birds were fed at the highest level of methionine and threonine had significantly better FCR than those were fed with NRC diet in starter phase and the highest body weight gain was observed in broiler chickens were fed M2T1 and M3T1 diets in grower phase. Improvement of feed efficiency at the highest level of threonine and methionine in initial phase may be explained by several possibilities. First, nutrient requirements of broiler based on recommendation of NRC 1994 and these information related to previous NRC finding but, the present commercial bird is very different from commercial birds were available in last decade so birds have more rapid growth and better performance than previous years due to genetic selection as well as management practice and feed related changes (Chamruspollert *et al.*, 2002). Therefore birds need to more energy and protein to meet their needs. Second, amino acid and protein requirements of broiler chickens are higher in the starter period than grower period (NRC, 1994). Third, nutrition recommended set by NRC are usually based on the need of healthy birds under ideal management but the combination of environment condition and disease increase the requirement for essential nutrient. Fourth, methionine requirement of male broilers is more than NRC (1994) recommendation (Hesabi *et al.*, 2006). Finaly, amino acid interactions such as arginine and methionine in chicks raised at high temperatures likely differ from those raised at ambient temperature (Chamruspollert *et al.*, 2004). In addition, threonine is procures glycin amino acid that was a vital amino acid for maximal chick growth (Almquist and Grau, 1944). It should be noted that glycin affects protein synthesis not only as a building blocks for protein itself, but also is required for the creation of DNA, RNA and uric acid (Ngo and Coon, 1976). It should be noted that although threonine requirement of young chicks has been studied extensively but there is evidence that threonine

requirement of the broiler chick affected by protein, amino acid level and source (Robbins, 1987; Koide and Ishibashi, 1995).

Fasuyl and Alertor (2005) reported that better performance can still be obtained with adequate supplementation of essential amino acids especially methionine which has been identified to be in marginal quantities in most poultry diets. Garlich (1985) found that feed conversion was better when methionine was supplemented. A improve in broiler performance when methionine was added to a corn-soybean diet has been reported (Virtanen and Rosi, 1995; Hesabi *et al.*, 2006). In contrary to our result, no significant effects were seen for methionine levels on feed conversion ratio by Meirelles *et al.* (2003). They, however, observed numerical improvement with the increasing ratio. Furthermore, one report (Thomas *et al.*, 1979) suggests that the requirement of a commercial strain of broiler chicks is near 0.81% threonine. They studies demonstrate that 0.64% dietary threonine was insufficient for maximum growth and efficiency of feed utilization by Leghorn chicks. This level of threonine occasionally permitted maximum growth and feed utilization, but usually resulted in significantly poorer performance than the diet containing 0.72% or more threonine. However, the findings of the current study do not support the previous research by Kidd *et al.* (1997), Smith and Waldroup (1988) that the NRC (1994) and NRC (1984) estimate on threonine, for 0-3-week-old chicks are too high.

In birds, the spleen is primarily concerned with the immune reactivity belonging to cell-mediated immunity, delayed type of hypersensitivity as well as humoral immunity. Our Results are in disagreement with previous findings by Kidd *et al.* (2001) and Corzo *et al.* (2007) in that dietary threonine not influenced spleen organ weights of broilers chickens. These differences might be explained by the threonine concentration were used.

On the other hand, adverse nutritional effects or toxicity have been reported when the diet of an animal, usually a growing animal, is supplemented with a single amino acid, a mixture of amino acids, or a protein of poor nutritional quality (Munro and Goldberg, 1964). The present study showed that uric acid levels increased at the highest level of threonine before challenge. Increase in uric acid excretion on protein ingestion is due to the nuclear disintegration occasioned by the work of digestion. Another theory ascribes the increase to stimulation of purine synthesis. It has been suggested that part of the endogenous uric acid arises by synthesis from protein and carbohydrate and part from the metabolism of cell nuclei (Zwarenstein, 1926). The

amino acid threonine is an important compound for the production of purines and uric acid is an extremely insoluble purine compound.

It has been demonstrated that the activities of many liver enzymes involved in the catabolism of amino acids decrease when a low protein diet is fed, but increase with high protein intakes (Muramatsu *et al*., 1971). In our experiment, liver enzymes were not affected by treatment diets on d 28 whereas 14 days post challenge, methionine at NRC recommended level had significant increase in the concentration of ALT, AST and LDH. Furthermore, the highest elevation in serum ALT was observed in M1T3 and also the highest increase in serum AST and LDH levels was noted in M1T1. The findings may also explain that current NRC recommendations for methionine and threonine in broiler are probably not appropriate for broiler health under stress and diseases condition. Although, it should be note that the concentration of ALT, AST and LDH in M1T1 also were in normal range (Chattopadhyay *et al*., 2006; Marjanovic *et al*., 1975).

CONCLUSION

Hence, it can be concluded that the methionine and threonine requirements of broiler breeders would be expected to change substantially as they approach maximum performance under actual farm conditions and they supplementation higher than NRC recommendation in broiler chickens have not toxic potential.

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