J. Animal and Poultry Prod., Mansoura Univ., Vol. 2 (3): 43 - 53*, 2011*

EFFECTS OF METHIONINE AND THREONINE SUPPLEMENTATION ON PERFORMANCE OF BROILER CHICKENS INFECTED BY BURSAL DISEASE UNDER HEAT STRESS

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ABSTRACT

This study was conducted to find the effect of methionine and threonine supplementations higher than the NRC recommendation on growth performance and white blood cell differentiation of broiler chickens challenged with infectious bursal disease. 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 till 42 days of age. On day 28, all birds were challenged with a commercial live-IBDV vaccine. Body weight gain and feed intake and feed conversion ratio were significantly influenced by the dietary treatments before challenge and either methionine or threonine at the highest levels significantly decreased productive performance parameters in broiler chickens. Birds were fed with M3T3 had the lowest body weight gain after challenge. Treatment of two times methionine and threonine (M2T2) to the diet decreased peripheral blood heterophils and increased lymphocytes and H/L ratio on day 28. On day 42, complete white blood cell tended to increase with increasing level of methionine supplementation. Threonine did not affect peripheral blood differential leukocyte count of broiler chickens. In conclusion, present 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 methionine and threonine higher than NRC requirements in tropical condition can ameliorate the negative effects of heat stress.

Keywords*:* Methionine; threonine; broiler; infectious bursal disease; white blood cell.

INTRODUCTION

Increase in poultry production over the last 15 years in tropical and sub-tropical regions is required to adjust the valuable formulation strategy under tropical climate. Nutrient requirement standards have been reported by the National Research Council (NRC, 1994) are usually based on the needs of healthy birds under ideal management, but birds in commercial systems are normally exposed to different kinds of stresses, diseases and also the combination of environmental condition. So, both the ambient and stress conditions ought to be well managed to avoid negative effects on poultry production. Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious viral infection of chickens that is seen worldwide in the last 30 years. When broilers are subjected to conditions of disease there is consistently consideration of altering diet formulation. Such changes may involve replacement of specific ingredients and/or alterations to nutrient levels in the diet. 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 performance (Quentin *et al.*, 2005) and to compensate for the depressed growth performance in hot conditions, various nutritional conditions have been investigated. Infections lead to several changes in amino acid plasma levels and dietary levels of certain individual amino acids has been shown to affect immune response (Jeevanandam *et al.* 1990; Paauw and Davis, 1990). Furthermore, the reduction in protein synthesis and depress in plasma amino acid concentrations at high ambient temperatures it has been reported (Geraert *et al.*, 1996; Temim *et al.*, 2000). Methionine, lysine and threonine are regarded as to be the first, second and third limiting amino acids in broilers fed practical corn-soybean meal diets (Ojano-Dirain and Waldroup, 2002). Many research studies in the past decade and the majority of amino acid requirement research has been established on lysine (Sibbald and Wolynetz, 1987; Han and Baker, 1991, Bilgili *et al.*, 1992; Holsheimer and Veerkamp, 1992). Unfortunately, research into the threonine and methionine requirements of broilers is sparse compared with that of lysine and valuable information on the effect of methionine and threonine supplementation on performance of IBD challenged broiler chickens is lacking. Therefore, the objective of present experiment was to evaluate the effects of dietary methionine and threonine supplementation on performance of broiler chickens challenged with infectious bursal disease under tropical condition.

MATERIALS AND METHODS

Birds and housing environment

This experiment was carried out in the poultry research farm of the Faculty of Agriculture, South Valley University. 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^{o}C; maximum, 34^{o}C). The relative humidity was between 70 to 80%. The area of each pen was 2m^{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 3 times NRC (M3)] and 3 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.

Table 1. Ingredients and nutrient composition of diets.

Item	Starter 1 to 21 d	Finisher 22 to 42 d
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 premix1	0.60	0.60
DL-Methionine	0.20	0.10
L-Threonine	0.00	0.00
Lysine	0.11	0.12
[2] **Calculated composition**		
Crude protein (%)	22	20
ME (Mcal/kg)	3050	3150
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	235	212
Ca/P	138	157

[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.

Challenge protocol

On day 28, all birds were challenged by oral route with a commercial live-IBDV vaccine (V877 strain, Egyptian Vaccines and Pharmaceuticals co.). 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 gavages, that finally each bird received a dose ten times greater than the standard IBD vaccine.

Performance parameters

The chickens were weighed individually at 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.

Differential Leukocyte Counts

At the end of 28 and 42 d, blood samples were collected into heparinized vials from the brachial vein of 8 birds per treatment (8X9). Complete white blood cell counts and differential leukocyte counts were performed manually to test for changes in absolute numbers of leukocytes, lymphocytes, heterophil, monocytes, eosinophils, and basophils.

Statistical analysis

The data were analyzed by two-way ANOVAS in a completely randomized design in a factorial scheme of 3×3 (methionine levels × threonine levels) with the PROC GLM procedure of the Statistical Analysis System (SAS Institute, 2005). Mortality data were subjected to chi-square analysis. The results were expressed as mean ± standard error of mean. Differences among means were tested using Duncan's multiple range test (Duncan, 1955). Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

The effects of methionine and threonine on body weight gain (BWG), feed intake (FI) and feed conversation ratio (FCR) in broiler chickens is shown in (Table 2). On day 28 (period before IBD challenge), BWG, FI and FCR were significantly influenced by the dietary treatments and either methionine or threonine at the highest levels significantly decreased BWG and FI whereas FCR was significantly improved. There were significant interactions between methionine and threonine on FCR (Table 3). Supplementation of the basal diet with the highest levels of methionine or threonine (M3T3) had an improvement in FCR compare to other group. On d 28-42 (challenge period), the highest level of threonine significantly decreased BWG in broiler chickens. In the M3 group, feed intake was significantly decreased, although, there were no significant differences on feed intake between M2 and M3 groups. BWG were significantly influenced by methionine and threonine interaction (Table 3). Birds were fed with M3T3 had the lowest body weight gain.

The effect of dietary methionine and threonine on peripheral blood differential leukocyte count in broiler chickens is shown in Table 4. On d 28 (pre-challenge), peripheral blood heterophils, lymphocytes numbers and heterophils to lymphocytes ratio (H/L) were influenced by methionine and threonine interaction. Supplementation of two times methionine and threonine (M2T2) to the diet decreased peripheral blood heterophils and increased lymphocytes and H/L ratio in differential leukocyte count as compared with the other diets (Table 5). Other leukocyte subpopulations were not affected by the dietary treatments. On 14 d post challenge (d 42), only complete white blood cell was altered by the dietary methionine. Complete white blood cell tended to increase with increasing level of methionine supplementation. Threonine did not affect peripheral blood differential leukocyte count of broiler chickens. No significant interactions were observed between methionine and threonine on d 42.

The low concentration of methionine and threonine in high-protein corn-soybean diets has lead to wide use of synthetic methionine and threonine supplementation in poultry feed. 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 (Zulkifli *et al.* 2003). The single most striking observation to emerge from the present study data on d 28 was supplementation of two times methionine and threonine (M2T2) to the diet decreased peripheral blood heterophils and increased lymphocytes resulted decreasing in H/L ratio. The same authors reported that the heterophil to lymphocyte ratio is a reliable indicator of avian stress. Broilers exposed to various forms of stress have clearly shown an increase in heterophils and a decrease in lymphocytes, which leads to an increase in the H/L ratio (Martrenchar *et al.*, 1997 and Feddes *et al.*, 2002). It has been showed stress condition could increase the demand for some amino acids, either due to synthesis of specific proteins, to selective catabolism, or to use in the synthesis of specific molecules (Obled *et al.*, 2002; Reeds and Jahoor, 2001).

Table 2: The effects of methionine and threonine on body weight gain (BWG), feed intake (FI) and feed conversation ratio (FCR) in broiler chickens (mean ± SEM).

Main effect	BWG (g) (d 1-28)	FI (g/bird) (d 1-28)	FCR (d 1-28)		BWG (g) (d 28-42)	FI (g/bird) (d 28-42)	FCR (d 28-42)
M1	1224±13[a]	2107±43[a]	1.56±0.03[a]		1064±19	2248±39[a]	2.3±0.07
M2	1253±13[a]	1987±34[b]	1.45±0.04[b]		1062±19	2199±90[ab]	2.3±0.06
M3	1133±15[b]	1709±48[c]	1.39±0.04[c]		1011±25	2013±62[b]	2.2±0.04
T1	1235±13[a]	2070±48[a]	1.54±0.03[a]		1111±18[a]	2279±36	2.2±0.06
T2	1229±13[a]	1953±45[b]	1.45±0.03[b]		1057±22[a]	2097±94	2.3±0.10
T3	1146±16[b]	1780±62[c]	1.41±0.04[b]		969±20[b]	2085±62	2.3±0.07
M	***	***	***		NS	*	NS
T	***	***	***		***	NS	NS
M × T	NS	NS	*		***	NS	NS

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

Table 3: Means (±SEM) feed conversion ratio (FCR) and body weight gain (BWG) of broiler chickens where methionine × threonine interactions were significant.

Main effects	FCR (1-28)				BWG (28-42)		
	M1	**M2**	**M3**		**M1**	**M2**	**M3**
T1	1.7±0.03[ax]	1.5±0.04[bx]	1.4±0.02[bx]		1021±36[by]	1146±32[ax]	1148±34[ax]
T2	1.5±0.03[aby]	1.5±0.04[ax]	1.4±0.02[bx]		1132±38[ax]	1039±29[aby]	999±45[by]
T3	1.6±0.04[ay]	1.4±0.03[by]	1.3±0.06[by]		1033±25[axy]	1000±30[ay]	884±39[bz]

[x-z] Means within a column-subgroup and [a-c] means within a row-subgroup with no common letters differ significantly (*P* < *0.05*).

Table4- 5

Decrease in H/L ratio in this study may be explained by the fact that our experiment performed in tropical condition and requirements to extra amino acid which probably reflects the synthesis of proteins or other specific compounds like hormones and Hsp70 can ameliorate the negative effects of heat stress. There are, however, other possible explanations.

The higher population of WBC in the peripheral blood by the dietary methionine after challenge was in agreement with those of Bhargava *et al.* (1971a) and Al-Mayah (2006) whom stated that manipulation of some nutrient resulted immunoregulatory consequences due to the participation of the nutrient or its products in communication within and between leukocytes. In chickens, it has been shown that shortage or excess of dietary protein (Payne *et al.*, 1990) or amino acids (Bhargava *et al.* 1971 b) alters immune responses. From the nutritional point view, amino acids are needed to trigger such a response, which consists in clonal proliferation of lymphocytes, establishment of germinative centers in the bursa of Fabricius to refine immunoglobulin affinity, recruitment of new bone marrow monocytes and heterocytes, and synthesis of effectors molecules (immunoglobulins, nitric oxide, lysozyme) and communication molecules such as cytokines and eicosanoids (Rubin *et al.*, 2007). Research has shown that methionine interferes in the immune system, improving both humoral and cellular response. It has also been observed that methionine requirements are higher when the purpose is to maintain optimal immunity levels, as compared to growth (Swain and Johri, 2000; Shini *et al.*, 2005), and that lower sulfur amino acid like methionine and cysteine levels result in a severe lymphocyte depletion in the intestine tissues (Peyer's patches) and in the lamina propria (Swain and Johri, 2000). Generally, the peripheral blood differential leukocyte count obtained in this present study and the preponderant values in the methionine and threonine supplement may justify the positive relationship between nutrients and health parameters of bird.

Furthermore, NRC requirements for amino acids and protein are designed to support maximum growth and production in healthy bird kept under ideal conditions. The recommended levels for methionine and threonin in poultry depend on species, stage, environment condition and level of feed energy. The present findings although indicated that there was a significant decline in BWG and feed intake in birds subjected to the highest level of threonine and methionine before challenge but birds fed M2T3 and M3T3 diets had significantly better FCR than those fed with NRC diet before challenge and the highest body weight gain was observed in broiler chickens fed M2T1, M3T1 and M2T2 diets after challenge. Fasuyl and Aletor, (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, 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. 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) estimate on threonine, for 0-3-week-old chicks are too high. Improvement of feed efficiency in highest level of threonine and methionine before challenge may be explained as follows: nowadays birds need to more energy and protein to meet their needs compare with commercial birds were available in last decade due to genetic selection as well as management practice and feed related changes (Chamruspollert *et al.*, 2002), nutrition recommended set by NRC are usually based on the need of healthy birds under ideal management, male broilers were used in our experiment and methionine requirement of male broilers is more than NRC (1994) recommendation (Hesabi *et al.*, 2006) and finally, 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). Additionally, 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).

Decreasing feed intake in our experiment at initial phase (1-28) may be related to influence of amino acid on appetite control. Hypothalamus is an area of the brain that plays a critical role to control appetite in poultry. This may that excess methionine and threonine result in stimulate and increased hypothalamus activity to decrease feed intake. This observed results are consistent with those, Harper *et al.*, 1970; Edmonds and Baker, 1987; Peng *et al.*, 1973 who found that excessive dietary amino tend to attenuate feed intake.

In conclusion, these results indicate that supplementing diets with synthetic methionine and threonine more than NRC recommendation could be a nutritional strategy to cope with the unfavorable stress conditions for improvement in broiler chickens performance and immune cells in tropical region.

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تأثير إضافة المثايونين و الثيرونين على الأداء الأنتاجى تحت ظروف الأصابة بالجامبورو فى الجو الحار

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أجريت هذه التجربة لدراسة تأثير اضافة كلا من الميثايونين و الثريونين بمستويات اعلى من الموصى بها فى NRC على الإنتاج و خلايا كرات الدم البيضاء لكتاكيت اللحم التى تم تحفيزها بالعدوى ب الجامبورو. استخدم 450 ذكر من كتاكيت اللحم و تم توزيعها على 9 معاملات تجريبية حيث تم التغذية على 3 مستويات متدرجة من الميثايونين (مستوى الموصى به فى NRC و ضعفه و ثلاث أضعاف) كذلك 3 مستويات متدرجة من الثريونين (مستوى الموصى به فى NRC و ضعفه و ثلاث أضعاف) من عمر يوم الى 42 يوم من العمر. عند عمر 28 يوم تم تحفيز الطيور باستخدام لقاح IBDV الحى. كلا من الزيادة فى وزن الجسم واستهلاك العلف و الكفاءة الغذائية تأثر معنويا بالمعاملات التى تم استخدامها قبل التحفيز باللقاح الحى الكتاكيت التى تم تغذيتها على 3 أضعاف مستوى الميثايونين ادى الى اقل وزن جسم بعد التحفيز باللقاح الحى. كذلك التغذية على ضعف المستوى لكلا من الميثايونين و الثريونين ادى الى نقص فى مستوى الدم من heterophils وزيادة الخلايا الليمفاوية و النسبة بينهما على عمر 28 يوم. فى حين انه على عمر 42 يوم ازداد صورة و مكونات الدم بزيادة مستويات الميثايونين المستخدمة فى حين لم يؤثر مستويات الثريونين على صفات الدم لكتاكيت اللحم محل الدراسة. نتائج هذه الدراسة توصى بأن مستويات كل من الميثايونين و الثريونين المطلوبة للحصول على معدلات نمو عالية لذكور كتاكيت اللحم اعلى من تللك الموصى بها فى NRC فى ظروف الجو الحار وان هذه المستويات من الممكن ان تحسن من الأداء المنخفض للطيور الناتج عن تأثيرات الإجهاد الحرارى او الطيور المصابة او المعرضة للجامبورو.

قام بتحكيم البحث

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Table 4: Effect of dietary methionine and threonine on peripheral blood differential leukocyte count in broiler chickens.

Main effect	WBC ($\times10^3$/ ml)	Eosinophils (%)	Basophils (%)	Monocytes (%)	Heterophils (%)	Lymphocytes (%)	H/L ratio
Pre-challenge (d 28)							
M1	1.6±0.23	2.4± 0.2	3.8±0.5	15.4±0.8	44.0±1.6	34.0±1.6	1.3±0.09
M2	1.0±0.13	3.1±0.3	5.5±0.6	14.6± 1.1	39.6±1.7	35.0±1.2	1.1±0.09
M3	1.4±0.14	2.9±0.4	6.2±0.7	12.9±1.0	41.2±2.3	32.9.±1.7	1.4±0.10
T1	1.2±0.17	2.6±0.2	5.4±0.6	16.4±0.9	38.7±1.7	31.6±1.5	1.2±0.09
T2	1.4±0.23	2.5±0.3	5.6±0.7	12.2±1.4	42.8±1.9	34.9±1.4	1.2±0.10
T3	1.3±0.12	3.3±0.4	4.5±0.6	14.4±0.9	43.5±2.0	36.0±1.4	1.2±0.09
ANOVA (P value)							
M	NS	NS	NS	NS	NS	NS	NS
T	NS	NS	NS	NS	NS	NS	Ns
M × T	NS	NS	NS	NS	*	*	**
14 d post challenge (d 42)							
M1	1.0±0.26[b]	4.9± 0.8	4.0± 0.7	9.8±2.3	38.8±2.4	36.8±2.4	1.1±0.1
M2	1.8±0.23[a]	3.5±0.7	4.8±0.5	10.3±2.8	46.0±3.1	37.6±3.4	1.5±0.2
M3	2.1±0.24[a]	4.8±0.6	5.3±0.8	9.3±2.4	43.9±2.2	36.5.±2.5	1.3±0.2
T1	1.4±0.29	4.5±0.9	5.8±0.6	9.4±1.6	39.1±2.6	39.6±2.8	1.1±0.1
T2	1.7±0.28	4.1±0.6	4.1±0.7	10.4±2.2	44.6±2.6	38.6±2.6	1.3±0.2
T3	1.7±0.21	4.6±0.5	4.4±0.6	9.4±3.3	44.9±2.2	33.4±2.7	1.5±0.2
ANOVA (*P value*)							
M	**	NS	NS	NS	NS	NS	NS
T	NS	NS	NS	NS	NS	NS	NS
M ×T	NS	NS	NS	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.WBC= White blood cell; H/L= Heterophils to lymphocytes ratio**

Table 5: Interaction effect (Met×Thr) on heterophils, lymphocytes and heterophils to lymphocytes ratio on d 28 (pre-challenge) in broiler chickens challenged with IBD.

Treatments	Heterophils (%)			Lymphocytes (%)			H/L		
	M1	**M2**	**M3**	**M1**	**M2**	**M3**	**M1**	**M2**	**M3**
T1	42.2±1.6[a]	41.4±1.8[a]	32.3±3.6[bx]	30.4±2.1[y]	32.2±0.9[y]	32.3±4.1	1.5±0.14[ax]	1.2±0.10[abx]	0.9±0.18[b]
T2	47.0±1.8[a]	35.7±3.5[b]	45.6±0.4[ax]	31.5±1.7[by]	40.3±1.1[ax]	32.5±1.4[b]	1.5±0.11[ax]	0.7±0.12[by]	1.4±0.06[a]
T3	42.9±4.3[y]	41.8±3.7[a]	45.7±2.8[y]	40.1±2.0[x]	33.9±2.0[y]	33.9±2.4	1.0±0.15[y]	1.3±0.16[x]	1.2±0.17

[x-z] Means within a column-subgroup and [a-c] means within a row-subgroup with no common letters differ significantly (*P < 0.05*).