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Effect of Supplementing Betaine on Performance, Carcass Traits and Immune Responses in Broiler Chicken Fed Diets Containing Different Concentrations of Methionine

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ABSTRACT : An experiment was conducted with broiler (Cobb 400) male chicks (n = 480) to determine the effect of betaine (Bet) supplementation (0 and 800 mg/kg) to diets containing five concentrations (15, 18, 20, 22 and 24 g/kg crude protein, CP) of methionine (Met) in a 2×5 factorial study for performance, carcass traits, immune responses, and serum parameters. Each diet was fed *ad libitum* from 1 to 42 d of age to 8 replicates of 6 chicks. Birds were housed in battery brooders placed in an open-sided poultry shed. Body weight gain, feed intake, feed conversion efficiency and slaughter variables were recorded at 21 and 42 d of age. Serum biochemical profile, antibody production against Newcastle disease (ND) and lymphocyte proliferation ratio (LPR) were analysed at 42 d of age. Supplementing Bet to diets containing sub-optimal concentrations of Met (15 g/kg) improved weight gain and breast yield at 21 d of age ($p<0.01$), and feed conversion efficiency at 42 d of age ($p<0.05$). Feed efficiency at 21 d of age, body weight gain at 42 d of age, slaughter variables except breast yield at 21 d of age and ND antibody titres were not affected ($p>0.05$) by the interaction. LPR increased ($p<0.05$) with Bet supplementation at 20 g Met/kg CP equal to those broilers fed 24 g Met without Bet. Bet supplementation enhanced the concentrations of protein, globulin and cholesterol in serum of broilers fed sub-optimal concentrations of Met. Results suggested that Bet supplementation (800 mg/kg diet) enhanced growth (21 d), feed conversion efficiency (42 d), breast yield and lymphocyte proliferation in broilers fed a diet containing 15 g Met/kg CP. (**Key Words :** Betaine, Methionine, Performance, Carcass Traits, Immune Response, Broiler Chicken)

INTRODUCTION

Methionine (Met) is an important amino acid which is essential for proper development of muscle (Waldroup et al., 2006) and immune response (Rama Rao et al., 2003) in chickens. A maize-soybean meal based broiler diet is deficient in Met and is supplemented with synthetic DL-methionine (DLM) to meet the bird's requirement. Non-availability and higher cost of synthetic Met often forces poultry feed manufacturers to look for alternate sources for the amino acid. One of the major functions of Met in biological systems is methyl group sparing activity (Pesti et al., 1981; Chamruspollert et al., 2002). Reduction in quantity of supplemental DLM by fortifying the diet with methyl donors may reduce the dietary requirement of the amino acid. Betaine (Bet) is a natural methyl donor, which donates the methyl group to homocysteine for Met synthesis (Baker and Czarnecki, 1985; Emmert et al., 1996; Kidd et al., 1997). On a molecular weight basis, Bet contains about 3.75 times the methyl groups compared to Met. Virtanen and Rosi (1995) found that both DLM and Bet have similar efficacy in chickens for body weight at sub-optimal concentration of dietary Met. As Bet was reported to increase endogenous Met synthesis, it has been hypothesized that the relative requirement of Met may be reduced in broiler diets with Bet supplementation by sparing the Met from a methyl donor function (Virtanen et al., 1993; Virtanen and Rosi, 1995; Kidd et al., 1997).

The data of Florou-Panerri et al. (1997) showed that the quantity of supplemental Met could be reduced with Bet supplementation in a broiler diet without affecting performance. As reduction in the concentration of dietary Met is known to affect immunity in chickens (Takahashi et al., 1997; Rama Rao et al., 2003), sparing dietary Met with Bet may influence the immune responses. It is also clear that Bet cannot substitute Met for protein synthesis (Schutte et al., 1997), but can spare methyl donor activity, therefore carcass traits like breast yield and abdominal fat may be

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affected in broilers fed a diet with suboptimal concentrations of Met and supplemented with Bet. It would be ideal to optimize the quantity of supplemental DLM with Bet, which has a positive influence on carcass meat yield (McDevitt et al., 2000; Waldroup et al., 2006). The literature indicating the quantity of Met that can be spared with Bet supplementation in broiler diets is very limited (Florou-Panerri et al., 1997). Therefore, in the present study, an attempt was made to determine the effects of supplementing Bet in diets containing different concentrations of Met on performance, carcass traits and immune response in broiler chicks.

MATERIALS AND METHODS

Birds and management

Day-old commercial broiler (Cobb 400) male chicks (n = 480) were distributed randomly into 10 groups of 8 replicates with six chicks in each replicate. On day 1, chicks were wing banded and housed in raised wire-floored stainless steel battery brooders in an open-sided poultry shed. The brooder temperature was maintained at 33±1°C up to 7 d of age and gradually decreased to 27±1°C by 14 d of age, after which, chicks were maintained at room temperature (25-33°C). Heat was provided using incandescent bulbs during the initial 2 weeks. Birds were vaccinated against Newcastle and infectious bursal diseases as per normal vaccination schedule. The experiment was conducted following the guidelines of the Institute Animal Ethical Committee.

Diets

Maize and soybean meal-based basal diets were prepared containing 2,950, 3,050 and 3,150 kcal ME/kg and 23, 21 and 19% crude protein (CP), respectively, for pre-starter (1-11 d), starter (12-21) and finisher (22-42 d) phases. Analyzed (Llames and Fontaine, 1994) amino acid concentrations of feed ingredients, which were reported in our previous article (Rama Rao et al., 2006) were used in formulating diets. The basal diets were supplemented with synthetic DL-methionine (DLM) to yield 18, 20, 22 and 24 g Met/kg CP during all three phases indicated above. Concentration of Met in the basal diet was about 15 g Met/kg CP during all three phases (Table 1). Another set of five diets were prepared by adding (800 mg/kg) synthetic Bet (Betafin, Dansco Animal Nutrition, Denmark) to diets containing the above 5 concentrations of Met. The concentration of Bet used in this study was based on the literature (Zou et al., 1998; Wang, 2000; Zou, 2001), who reported optimum bird response at 800 mg Bet/kg diet. The concentrations of other amino acids, CP, calcium, phosphorus and ME were constant among diets in each phase. The levels of maize were altered while altering the supplemental concentrations of Met and Bet in the

Table 1. Ingredient and nutrient composition (%) of basal diets

	Pre-starter	Starter	Finisher
Age, d	1-11	12-21	22-42
Ingredient			
Maize	50.36	54.94	60.38
Soybean meal	42.21	36.73	31.10
Vegetable oil	3.58	4.36	4.85
Common salt	0.35	0.35	0.35
Sodium bi-carbonate	0.10	0.10	0.10
Dicalcium phosphate	1.93	1.97	1.71
Oyster shell grit	0.97	1.01	0.93
DL-methionine	*	*	*
L-lysine HCl	0	0.04	0.08
Premix[1]	0.50	0.50	0.50
Nutrients[2]			
Metabolizable energy, kcal/kg	2,950	3,050	3,150
Crude protein (%)	23.0	21.0	19.0
Lysine (%)	1.30	1.20	1.10
Methionine	0.34	0.31	0.29
Sulfur amino acid	0.72	0.66	0.61

* DL-methionine was added to yield the desired levels of methionine in diet.

[1] Premix provided (mg/kg diet): thiamin 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 15; pantothenic acid, 10; tocopherol, 10; riboflavin, 10; biotin, 0.08; menadione, 2; retinol acetate, 2.75; cholecalciferol, 0.03; choline, 650; copper, 8; iron, 45; manganese, 80; zinc, 60; selenium, 0.18; monensin sodium, 100; and hydrated sodium calcium alumino silicates, 800.

[2] Calculated based on analyzed values of individual feed ingredient except for energy which was based on published ME values.

experimental diets. A completely randomized design was followed while allotting the diets to different replicates.

Variables measured

Performance parameters : Body weight and feed intake per replicate were recorded at 21 and 42 d of age. Body weight gain was calculated as the difference between the body weight at 21/42 d of age and average weight of the day-old chicks of the respective replicate. The feed conversion efficiency was calculated as body weight gain per unit feed intake up to 21 and 42 d of age.

Carcass variables : At 43 d of age, one bird from each replicate weighing closest to the mean body weight of the respective treatment was slaughtered by cervical dislocation to study carcass variables. Ready-to-cook (RTC) carcass yield (including liver, gizzard and heart) and weights of abdominal fat and liver were recorded and expressed as g/kg pre-slaughter live weight of the respective bird.

Serum bio-chemical analysis : About 3 ml of blood was collected from the brachial vein of each bird (one bird per replicate) at 42 d of age to analyze concentrations of total protein, albumin, triglycerides and total cholesterol in serum utilizing diagnostic kits (Product No 72111, 72131, 72381, and 72181, respectively, M/S Qualigens India, Mumbai, India). Globulin concentration was calculated as the difference between the concentrations of total protein and albumin.

Immune response : The effect of concentration of Met without and with Bet supplementation on cell-mediated (*in vitro* lymphocyte proliferation ratio - LPR) and humoral immunity (antibody production against Newcastle disease vaccine - NDV) was studied.

Lymphocyte proliferation ratio (LPR) : The difference in *in vitro* proliferation of lymphocytes with and without stimulant (concanavalin A -Con A) was measured as a ratio. The LPR was assayed using MTT tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (Bounous et al., 1992). About 3 ml blood was collected from the brachial vein of one bird per replicate at 42 d of age in a centrifuge tube containing heparin disodium salt (5 mg). The un-clotted blood sample was layered gently over histopaque 1077 (Sigma, Mumbai, India) and centrifuged at 500×g for 20 minutes at 4°C. The cellular band at the interface was collected and transferred to another tube and washed 3 times with RPMI 1640 medium (AL 028A, Himedia, India). Viable cells were counted using the trypan blue dye exclusion method and cell concentration was adjusted to 1×10^7 cells/ ml of RPMI 1640 medium. These cells (10^5 purified lymphocytes) were used to measure lymphocyte proliferation by adding 10 μl of suspension to each well of a 96-well, flat-bottom, sterile tissue culture plate. Con A (0.9 μg in 150 μl RPMI/well) was used as stimulant for lymphocyte proliferation. The plate was incubated at 37°C and 5% CO_2 concentration for 69 h in a humid atmosphere, then 20 μl MTT (10 mg/ml) was added to each well and the plate was re-incubated for 3 h. At 72 h, 100 μl of 4% 1 N HCl - isopropanol was added to each well and mixed thoroughly with a micropipette to dissolve the formazin crystals, which gave a deep purple color. The color intensity was measured in an ELISA reader (V.200.1, μ Quant, Biotek Instruments, Inc., USA) at 550 nm. The LPR was calculated as follows

$$\frac{\text{OD of well with Con A - OD of well without Con A}}{\text{OD of well without Con A}}$$

Antibody titre to ND vaccine : Broilers were vaccinated against ND by ocular route at 7 and 28 d of age with Lasota strain (ND Lasota Vac-500, Indivax Pvt., Ltd., Hyderabad, India). At 37 d of age, about 2 ml blood was collected from one bird per replicate. Antibody titres in sera against ND vaccine were measured (Reynolds and Maraqa, 2000) using ELISA (96-6547, Synbiotics, San Diego, CA, USA).

Statistical analysis

Factorial analysis was carried out following the completely randomized design (Snedecor and Cochran, 1980) with levels of Met and Bet as the main factors. The treatment means were compared using Duncan's multiple range test (Duncan, 1955). The data of different production parameters were subjected to regression analysis ($y = a+bx+cx^2$) considering Met as the independent variable and performance parameters as dependent variables to find out the relationship (linear and non-linear) between concentration of Met and dependent variables.

RESULTS

Performance

Interaction : Interaction between dietary Met concentration and Bet supplementation significantly influenced body weight gain at 21 d of age ($p<0.01$) and feed conversion efficiency at 42 d of age ($p<0.05$) (Table 2). The feed conversion efficiency at 21 d of age and body weight gain at 42 d of age were not affected ($p>0.05$) by the interaction. At 21 d of age, the body weight gain at 20 g Met/kg CP was significantly higher than for those broilers fed 15 g Met/kg, and further increase in the amino acid concentration (>20 g/kg) did not show any beneficial effects on weight gain. At 15 g Met/kg CP, supplementation of Bet significantly increased weight gain compared to those fed 15 g Met without Bet supplementation. The body weight gains at 15 and 18 g Met/kg CP with Bet supplementation were statistically similar to those in broilers fed 18 and 24 g

Table 2. Body weight gain and feed conversion efficiency in broiler chickens fed different concentrations of methionine (Met) and betaine (Bet)

Met (g/kg)	Bet. (mg/kg)	21 d		42 d	
		Body weight gain (g)	Weight gain /feed intake	Body weight gain (g)	Weight gain /feed intake
Interaction					
15	0	545[d]	0.66	1,954	0.56[c]
18	0	676[bc]	0.68	2,128	0.57[bc]
20	0	710[ab]	0.70	2,186	0.58[abc]
22	0	725[ab]	0.71	2,228	0.60[ab]
24	0	738[a]	0.70	2,258	0.59[ab]
15	800	659[c]	0.67	2,069	0.60[ab]
18	800	708[ab]	0.70	2,161	0.57[bc]
20	800	732[a]	0.71	2,273	0.60[ab]
22	800	740[a]	0.72	2,324	0.61[a]
24	800	737[a]	0.73	2,244	0.60[ab]
N		8	8	8	8
SEM		12.18	0.002	47.2	0.003
Met. (g/kg)					
15		602	0.66	2,012	0.58
18		692	0.69	2,144	0.57
20		721	0.70	2,229	0.59
22		732	0.71	2,276	0.60
24		738	0.71	2,251	0.59
	Bet. (mg/kg)				
	0	679[b]	0.69[b]	2,151[b]	0.58
	800	715[a]	0.70[a]	2,214[a]	0.60
p values					
Interaction		0.001	NS	NS	0.011
Met. (g/kg)					
Linear		0.001	0.001	0.001	0.001
Non-linear		0.014	0.017	NS	NS
Bet. (mg/kg)		0.001	0.001	0.037	NS

[a, b, c, d] Means having a common superscript in a sub-column do not vary significantly (p>0.05).

Met/kg CP, respectively, without Bet supplementation.

At 42 d of age, feed conversion efficiency improved significantly with Bet supplementation at the lowest concentration of Met in the diet (15 g/kg CP). At higher concentrations of Met, the feed conversion efficiency was not affected by Bet supplementation.

Main factors : The body weight gain and feed conversion efficiency increased non-linearly (p<0.05) at 21 d of age and linearly (p<0.01) at 42 d of age with Met concentration in the diet (Table 2). Body weight gain and feed conversion efficiency at 21 d of age (p<0.001) and body weight gain at 42 d (p = 0.037) of age improved significantly with Bet supplementation compared to broilers fed Bet un-supplemented diets. The feed conversion efficiency was not influenced by Bet supplementation at 42 d of age.

Carcass traits

Interaction : The relative weights of RTC yield, liver and abdominal fat at 21 and 42 d of age and breast weight at 42 d of age were not affected (p>0.05) by the interaction between the concentration of Met and Bet supplementation (Table 3). Breast weight at 21 d of age was significantly (p<0.01) influenced by the interaction. The breast weight at 21 d of age increased (p<0.01) with each increment of Met up to 20 g/kg CP, and further increase in Met level did not show any additional improvement. Bet supplementation at lower concentrations (15 and 18 g/kg) of Met significantly increased the relative weight of breast compared to those broilers fed the same levels of Met without Bet supplementation. Such beneficial effects were not observed with Bet supplementation at higher concentrations of the amino acid in the diet (≥20 g/kg).

Main factors : The relative weights of breast and liver at

Table 3. Carcass traits (g/kg live weight) in broiler chickens fed different concentrations of methionine (Met) and betaine (Bet)

Met (g/kg)	Bet. (mg/kg)	RTC		Breast		Liver		Abdominal fat	
Age (d)		21	42	21	42	21	42	21	42
Interaction									
15	0	590	707	113.2[d]	170	39.4	21.4	13.1	16.6
18	0	607	704	131.9[c]	182	31.8	19.5	10.2	15.2
20	0	612	732	148.1[ab]	194	34.0	19.5	10.2	13.1
22	0	618	727	143.2[abc]	193	35.1	18.9	8.8	11.8
24	0	614	736	145.6[abc]	202	33.2	18.2	10.2	13.8
15	800	618	733	140.8[abc]	186	36.4	19.8	12.7	18.1
18	800	558	730	153.1[a]	194	31.8	17.5	10.6	15.6
20	800	629	728	150.3[ab]	204	33.2	18.0	10.1	30.9
22	800	605	726	133.9[bc]	204	34.9	17.9	11.5	12.5
24	800	612	743	149.6[ab]	205	32.1	17.8	8.8	11.6
N		8	8	8	8	8	8	8	8
SEM		7.5	8.0	2.02	1.8	0.50	0.32	0.41	19.59
Met. (g/kg CP)									
15		604	720	127.0	178	37.9	20.6	12.9	17.4
18		583	717	142.6	188	31.8	18.5	10.4	15.4
20		621	730	149.2	199	33.6	18.8	10.2	22.0
22		612	727	138.5	199	35.0	18.4	10.2	12.2
24		613	740	147.6	204	32.7	18.0	9.5	12.7
	Bet. (mg/kg)								
	0	608	721	135.9[b]	188[b]	34.7	19.5[a]	10.5	14.1
	800	604	732	145.2[a]	199[a]	33.7	18.2[b]	10.7	17.7
p values									
Interaction		NS	NS	0.003	NS	NS	NS	NS	NS
Met. (g/kg)									
Linear		NS	0.006	NS	0.001	0.015	0.016	0.010	NS
Non-linear		NS	NS	0.0016	NS	0.005	NS	NS	NS
Bet. (mg/kg)		NS	NS	0.007	0.001	NS	0.045	NS	NS

RTC = Ready to cook.

21 d of age increased non-linearly (p<0.01) with increased dietary Met concentration (Table 3). The weights of breast, liver, RTC yield at 42 d of age, and abdominal fat at 21 d of age increased linearly (p<0.05) with increase in concentration of Met in the diet. However, the RTC yield at 21 d and abdominal fat at 42 d of age were not affected by the Met concentration. Bet supplementation did not influence the carcass traits recorded, except for breast meat at 21 and 42 d and liver weight at 42 d of age. During these periods, breast yield increased and liver weight decreased with Bet supplementation.

Serum biochemical variables

Interaction : Interaction between the levels of Met and Bet supplementation in the diet significantly influenced the concentrations of total protein (p<0.001), albumin (p<0.001), globulin (p<0.05), triglyceride (p<0.001) and cholesterol (p<0.001) in serum (Table 4). Supplementation of Bet at lower levels of Met (15 and 18 g/kg CP) significantly increased the concentrations of total protein, triglycerides and total cholesterol in serum. At higher concentrations (22 and 24 g/kg CP), the protein variables did not increase with Bet supplementation. The serum triglyceride and cholesterol concentrations decreased significantly with Bet supplementation at 22 and 24 g Met/kg CP in diet.

Main factors : The concentrations of total protein, albumin, and cholesterol increased linearly (p<0.01) while the globulin concentration increased non-linearly with increase in dietary Met concentration (Table 4). Serum triglyceride concentration was not affected by the levels of Met in the diet. Similarly, the albumin and triglyceride concentrations were not affected by supplementation of Bet in the broiler diet. On the other hand, Bet supplementation significantly increased the concentrations of total protein (p<0.01) and globulin (p<0.01) and decreased cholesterol

Table 4. Serum biochemical profile and immune response in broiler chickens fed different concentrations of methionine (Met) and betaine (Bet)

Met (g/kg)	Bet. (mg/kg)	Total protein	Albumin	Globulin	TG	Chol	ND titre,	LPR
		--- g/dl ---			--- mg/dl ---		log 2	
Integration								
15	0	2.62[d]	0.97[c]	1.64[f]	59.0[b]	93[e]	8.25	0.50[b]
18	0	3.40[c]	1.56[ab]	1.84[ef]	69.2[b]	92[e]	8.13	0.52[b]
20	0	3.98[ab]	1.81[a]	2.16[cde]	69.8[b]	114[b]	7.38	0.42[c]
22	0	4.48[a]	1.81[a]	2.72[a]	87.3[a]	128[a]	7.88	0.52[b]
24	0	4.10[ab]	1.76[ab]	2.23[bcd]	96.0[a]	130[a]	7.75	0.65[a]
15	800	3.71[bc]	1.74[ab]	2.07[de]	94.2[a]	112[bc]	7.38	0.46[bc]
18	800	4.05[ab]	1.73[ab]	2.24[bcd]	89.7[a]	110[bcd]	7.50	0.53[b]
20	800	4.16[ab]	1.53[b]	2.64[a]	69.1[b]	97d[e]	8.00	0.62[a]
22	800	4.03[ab]	1.57[ab]	2.46[abc]	57.4[b]	99[cde]	8.50	0.52[b]
24	800	4.29[a]	1.72[ab]	2.57[ab]	67.6[b]	103[bcde]	7.63	0.47[bc]
N		8	8	8	8	8	8	8
SEM		0.077	0.038	0.053	2.06	2.09	0.013	0.011
Met. (g/kg)								
15		3.16	1.36	1.86	76.6	102	7.82	0.48
18		3.72	1.65	2.04	79.5	101	7.82	0.52
20		4.07	1.70	2.40	69.5	105	7.69	0.52
22		4.26	1.70	2.59	72.3	113	8.19	0.52
24		4.20	1.74	2.40	81.8	117	7.69	0.56
	Bet. (mg/kg)							
	0	3.72[b]	1.58	2.12[b]	76.3	111[a]	7.88	0.52
	800	4.05[a]	1.66	2.40[a]	75.6	104[b]	7.80	0.52
p values								
Interaction		0.001	0.001	0.013	0.001	0.001	NS	0.041
Met. (g/kg)								
Linear		0.001	0.001	0.001	NS	0.001	NS	0.045
Non-linear		NS	NS	0.037	NS	NS	NS	NS
Bet. (mg/kg)		0.002	NS	0.001	NS	0.021	NS	NS

[a, b, c, d, e] Means having a common superscript in a sub-column do not vary significantly (p>0.05).

TG = Triglycerides; Chol = Cholesterol. ND titre = Newcastle disease titre. LPR = Lymphocyte proliferation ratio.

(p<0.05) concentrations in serum.

Immune responses

Interaction : The antibody titres against NDV were not affected (p>0.05) by the interaction between concentrations of Met and Bet supplementation in diet (Table 4). However, the interaction significantly (p<0.05) influenced the LPR. Maximum LPR was observed at the highest concentration of Met (24 g/kg) without Bet, whereas with Bet supplementation such a higher response in LPR was observed at 20 g Met/kg CP. The LPR decreased with further increase in Met concentration in Bet supplemented groups.

Main factors : Variation in concentration of Met did not influence the antibody response to NDV. However, the LPR increased linearly (p<0.05) with concentration of Met in the diet. The immune response variables studied were not affected by Bet supplementation.

DISCUSSION

The data (weight gain at 21 d and feed conversion efficiency at 42 d of age) of the present study indicated that Bet supplementation was effective in improving broiler performance when the diet contained sub-optimal/lower concentrations of Met (15 and 18 g/kg CP). Met sparing activity of Bet (Virtanen et al., 1993; Virtanen and Rosi, 1995; Kidd et al., 1997) at sub-optimal concentrations of the amino acid in the diet might be a reason for improved broiler performance with Bet supplementation. Through its methyl donating property (Kidd et al., 1997), Bet might have spared the dietary Met from methyl donor function and the dietary Met was available for other vital functions like protein synthesis and immune modulation. Similar to the present observations, Florou-Panerri et al. (1997) found improved broiler performance with Bet supplementation to diets with lower concentrations of Met. At higher concentrations of dietary Met (30.1, 27.6 and 28.2 g/kg CP), Waldroup et al. (2006) observed no improvement in body weight and other performance variables with Bet supplementation. In the present study also, beneficial affects of Bet supplementation on performance and carcass variables were not observed in groups fed higher concentrations of Met (22 and 24 g/kg CP) in the diet.

Contrary to the present findings, Rostagno and Pack (1996) and Schutte et al. (1997) reported lack of improvement in broiler performance with Bet supplementation. The lower level (400 mg/kg diet) of Bet (Schutte et al., 1997) or Met (13.4 and 13.6 g/kg CP, respectively, in starter and grower) in the basal diet (Rostagno and Pack, 1996) used by the respective authors might be responsible for absence of response to Bet supplementation. However, improvement in body weight gain with Bet supplementation at 16 and 17 g Met/kg was observed by Rostagno and Pack (1996). The data thus suggested the importance of maintaining a minimum level of Met in the basal diet to elicit any response to Bet supplementation.

Performance, breast yield (21 d) and certain protein and lipid fractions in serum improved with Bet supplementation, particularly at the lower concentrations of Met (15 and 18 g/kg). The increased nutrient utilization due to Bet supplementation as reported in the literature (Remus et al., 1995; Augustine and Danforth, 1999) might have resulted in improved feed conversion efficiency (Table 2), particularly at sub-optimal concentrations of Met (Emmert et al., 1996; Schutte et al., 1997). Increased concentrations of total protein and globulin in serum of broilers fed the Bet supplemented diet (Table 4) also suggested a favorable role of Bet on nutrient utilization.

Improved weight gain and breast yield with Bet supplementation at 21 d, but not at 42 d of age suggested higher requirements of Met and methyl donors during the starter phase compared to the finisher phase. Reduced dietary Met requirement with age of the bird has been reported (NRC, 1994; Waldroup et al., 2006). Enhanced muscle protein accretion (Saunderson and McKinlay, 1990) due to improved utilization of dietary Met with Bet supplementation (Augustine and Danforth, 1999) might have contributed to higher breast yields with Bet supplementation (Table 3). Similar to the present study, increased breast yield with Bet supplementation was reported in broilers (Virtanen and Rosi, 1995; Firman et al., 1999; McDevitt et al., 2000; Wang, 2000; Waldroup et al., 2006).

Higher LPR was observed at 24 g Met/kg CP (Table 4), which was considerably higher than the level observed (<20 g/kg) for maximum body weight gain and performance (Table 2). The data thus suggested higher requirement of Met for LPR compared to performance, which is in line with the findings of earlier studies (Tsiagbe et al., 1987; Swain and Johri, 2000; Rama Rao et al., 2003). However, the maximum LPR response was observed at lower Met concentration (i.e. 20 g) with Bet supplementation in the diet. Increased LPR at lower Met concentrations (20 g/kg CP) with Bet supplementation might be due to enhanced digestibility and utilization of Met (Augustine and Danforth, 1999) and other nutrients like carotenoids, lysine, protein and fat (Remus et al., 1995), which are known to influence immune responses (Latshaw, 1991). Increased LPR with Bet supplementation might also be due to its role in enhancing phagocytosis, release of inflammatory cytokines (Zhang et al., 1996) and nitric oxide by heterophils and macrophages and increasing chemotactic effects of monocytes (Klasing et al., 2002).

Increased intestinal integrity (Kettunen et al., 2001a) and surface area (Hochachka and Somero, 1984; Klasing et al., 2002) and water retention capacity of intestinal mucosal cells (Kettunen et al., 2001b) was reported with Bet supplementation, which might be responsible for better performance of broilers with Bet supplementation in the present study. Though some of these references dealt with coccidiosis-challenged broilers (Kettunen et al., 2001a; Klasing et al., 2002), benefits of Bet supplementation were also observed in normal chickens without coccidia challenge (Hochachka and Somero, 1984; Kettunen et al., 2001b). The present experiment was conducted in stainless steel battery brooders with raised wire floors, where there was much less chance of faecal contamination and no bird exhibited signs of coccidial infection; therefore, it is presumed that the results of the present study were not confounded by coccidiosis.

Based on the data, it is concluded that Bet supplementation (800 mg/kg diet) was effective in enhancing growth (starter phase), feed conversion efficiency, breast yield and lymphocyte proliferation in broilers fed a diet containing sub-optimal concentrations of Met (15 g/kg CP). Bet supplementation was found ineffective at the higher concentrations of Met tested in the present study.

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