

EFFECT OF BETAINE SUPPLEMENTATION ON IMMUNITY, ENERGY,
AND LIPID METABOLITES OF GROWING MURRAH BUFFALO CALVES

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ABSTRACT

This study aimed to observe the effect of betaine (BET) supplementation on the growth, immunity, energy, and lipid metabolites of Murrah buffalo calves. Twenty-one Murrah buffalo calves were taken and randomly distributed into three groups (n=3) after blocking by body weight (98.70±1.31 kg) and age (8.12±0.55 months). The feeding regimen was the same in all the groups except that the Treatment groups were supplemented additionally with 0.0 (B0), 7.0 (B7), and 14.0 (B14) g/d BET in three respective groups for 90 d of the experiment period. Total leukocytes (TLC), neutrophils, lymphocytes, total immunoglobulin (TIG), total antioxidant activity (TAA), energy, and lipid metabolites were observed at fortnightly intervals during 90 d experimental periods. Adding BET up to 14 g/d did not exert (P>0.05) any effect on total leukocytes and neutrophils concentration. Lymphocytes, TIG,

and TAA were increased significantly (P<0.05) in BET received groups either dose 7 or 14 g/d than in the control. No effect of dietary addition of BET was observed on the glucose, non-esterified fatty acid (NEFA), total cholesterol (TCH), and high-density lipoprotein cholesterol (HDL-CH). The results of our study indicate that supplemental BET may play a role in regulating the immunity of growing Murrah buffalo calves.

Keywords: *Bubalus bubalis*, buffaloes, total immunoglobulin, total antioxidant activity, non-esterified fatty acid, betaine, buffalo calves

INTRODUCTION

Betaine is a naturally occurring amino acid derivative and is produced either by choline oxidation in the body or naturally in the sugar beet (Cholewa *et al.*, 2014). A small concentration

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of BET is also reported in wheat germ, wheat bran, and spinach (Zeisel *et al.*, 2003). BET has osmoprotective properties, which are essential in stabilization membrane structure, enzyme function, immunity, liver, and kidney function (Sakomura *et al.*, 2013). Furthermore, BET (N, N, N-trimethylglycine) molecule has three methyl groups hence it acts as a methyl donor, the key component in one-carbon metabolism, and involves the synthesis of proteins, DNA, RNA, and choline (Bertolo and McBreaity, 2013). Supplementing dairy cows with BET reduces their blood levels of β -hydroxybutyrate and non-esterified fatty acids (NEFA) (Wang *et al.*, 2010). Moreover, BET plays a vital role in the development of the fetus and enhances the growth and immunity of newborns (Van *et al.*, 2016). Additionally, a positive impact of BET on immune status was reported in broiler chickens (Klasing *et al.*, 2002). Whether it boosts immune function in growing Murrah buffalo calves remains unexplored. As a result, our study examined the effect of dietary supplementation of BET on immunity, energy, and lipid metabolites of growing Murrah buffalo calves.

MATERIALS AND METHODS

The experimental procedures and protocols were approved by the University Animal Ethics Committee (Reg.No.2058/GO/Re/SL/19/CPCSEA) and CPCSEA, Ministry of Fisheries, Animal Husbandry, and Dairying, Government of India. The experiment was conducted on Instructional Livestock Research Complex-I, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. For this experiment, 21 Murrah buffalo calves of BW 98.70 ± 1.31 kg and age 8.12 ± 0.55 months were sorted out and randomly

distributed into three groups ($n=7$). Group I was acted as the control without BET supplementation (B0) and Group 2 and Group 3 were supplemented with BET at the rate of 7 (B7) and 14 g/day/calf (B14), respectively, for 90 days experiment period. The BET was supplemented in form of BET hydrochloride (purity 98%, Chemkart, Mumbai, India). The calves were fed a total mixed ration (TMR) based diet containing concentrate, fodder (berseem and oat), and straw (wheat and paddy) in a ratio of 45:35:20, respectively to meet their nutrient requirement (Table 1) as per the recommendation of NRC (2001). The total mixed ration (TMR) was prepared daily and fed two times a day at 08:00 h, and 18:00 h. Clean and fresh tap water was offered *ad libitum* to the animals.

Jugular vein blood was sampled at fortnightly intervals in EDTA containing vials before feeding and watering at 7.30 am for 90 days of the study period. The fraction of blood sample was used for the counting of total leukocytes (TLC), lymphocytes, and neutrophils. The remaining blood samples were then centrifuged at 3000 rpm for 30 minutes. Plasma was collected in Eppendorf tubes and stored at 20°C for further analysis of TIG, TAA, glucose, NEFA, total cholesterol (TCH), and HDL cholesterol (HDL-CH).

The dried samples of TMR were analyzed for DM (method, 973.18c), total ash (method, 942.05), crude fiber (method, 920.39), and crude protein (method, 988.05) using AOAC (1995) procedures. Acid detergent fiber and neutral detergent fiber were determined according to the procedure explained by Van Soest *et al.* (1991). The TLC was counted using the procedure described by Feldman *et al.* (2000). Differential leukocyte count was carried out using the cross-sectional technique (Schalm and Jain, 1986). The zinc turbidity method (McEvan and Fisher, 1970) was used to measure the

plasma immunoglobulin. The TAA was measured by the Ferric Reducing Antioxidant Power assay of Benzie and Strain (Benzie and Strain, 1999). The NEFA was measured according to the procedure of Shipe *et al.* (1980) utilizing the modified copper soap extraction method. Glucose, TCH, and HDL-CH were measured by kits (ERBA diagnostics Mannheim Germany).

The data of different variables were analyzed by MIXED Model using the statistical software package SPSS version 20 (SPSS for Windows, V 19.0; SPSS, Inc., Chicago, IL, USA). The animal within the group was considered the random effect, whereas treatment, fortnight, and their interaction were considered as the fixed effect. The means were compared by applying 'Tukey's Multiple Range Test'.

RESULTS AND DISCUSSIONS

Immune status

The biomarkers of immune status are presented in Table 3. Both the doses 7 g and 14 g/d BET supplementation did not affect the mean TLC, mean neutrophils, and TLC and neutrophils on different fortnights of the study period. Our results are agreeing with the findings of Park and Kim (2019) who recorded that BET supplementation in broilers chicks did not affect TLC and neutrophils. Neutrophil concentration unaffected with the supplementation of BET in broiler chicks is agreeing with the findings of Chand *et al.* (2017). The mean lymphocyte concentration was showed a significant difference among groups and was observed higher in the 14 g/d BET group than the 7 g/d BET and Control groups. On 3, 4, 5, and 6 fortnights of the study period, the lymphocyte concentration

was higher ($P < 0.05$) in the 14 g/d BET receiving group than in both groups. In agreement with the present findings of Chand *et al.* (2017) reported higher blood lymphocytes in broiler chicks fed BET at 1.5 and 2.0 g/kg feed. Similarly, Nofal *et al.* (2015) observed an increase in lymphocytes % and a decrease in neutrophils % in growing chicks received BET. BET supplementation improved the circulatory lymphocyte % might be due to the BET stabilizing the protein structure and protects the cell membrane from osmotic stressors (Zhou *et al.*, 2017). The mean TIG was showed a significant ($P < 0.01$) difference among the group and reported higher ($P < 0.05$) in the 14 g/d BET supplemented group as compared to 7 g/d BET and control groups. The TIG on 2, 3, 4, 5, and 6 fortnights of the study period was observed significant ($P < 0.05$) to each other and reported higher in calves receiving 14 g/d supplemental BET. Plasma concentration of TIG increased linearly as dietary BET level increased. It seems that BET increases the blood concentration of lymphocytes and their production of immunoglobulin. Inconsistent with the present findings, Salamat and Ghasemi (2016) reported that TIG and IgG were unaffected in broilers fed on diets supplemented with BET. TAA was significantly higher ($P < 0.05$) in 7 g and 14 g of BET /d groups in comparison to control. On 2, 3, 4, 5, and 6 fortnights, TAA was observed higher ($P < 0.05$) in the 14 g/d BET group as compared to 7 g/d BET and control groups. TAA is a consolidated index that reflects the total antioxidant level in the body (Zhang *et al.*, 2014). Congruent with the present finding, Zhang *et al.* (2014) reported higher TAA in dairy cows that received 15 g/d and 20 g/d BET. Lu *et al.* (2008) documented that BET acts as an antioxidant and protects cells from oxidative damage by scavenging free radicles in rats.

Table 1. Ingredient and chemical composition of TMR fed during the study period.

Attributes	Content
Ingredient composition of TMR, % on DM basis	
Wheat straw	10.0
Paddy straw	9.0
Berseem fodder	14.0
Oat fodder	7.0
Yellow maize (ground)	32.0
Wheat bran	6.7
Mustard cake	10.0
Groundnut cake	10.0
Mineral and vitamin premix ^a	1.3
Betaine hydrochloride ^b	Variable ^c
Chemical composition of TMR, %	
Dry matter	78.4
Organic matter	90.1
Ash	9.9
Crude protein	18.0
Crude fiber	30.0
Neutral detergent fiber	38.5
Acid detergent fiber	25.6

^aPremix composition per 2.5 kg: vitamin A 80 lac IU; vitamin D₃ 16 lac IU; vitamin E 750 IU; vitamin K 1 g; vitamin B₂ 5 g; vitamin B₁₂ 15 mg; niacinamide 10 g; calcium pantothenate 2.5 g; choline chloride 300 g; Ca 750 g; P 90 g; Na 50 g; Cu 2 g; I 2 g; Fe 20 g; Mn 55 g; Zn 52 g; Co 100 mg.

^bBetaine hydrochloride (purity 98%, Chemkart, Mumbai, India).

^cSupplemental betaine in diet 7 g and 14 g/day/calf.

Table 2. Effect of betaine supplementation on the immunity of Murrah buffalo calves.

Variables	Fortnight	Treatment				P-value		
		B0	B7	B14	SEM	T	F	T×F
TLC, 10 ³ /μl	0	9.57	9.52	9.56	0.13	0.649	0.331	0.755
	1	9.44	9.54	9.98	0.12			
	2	9.45	9.50	9.52	0.12			
	3	9.35	9.38	9.47	0.15			
	4	9.34	9.45	9.67	0.19			
	5	9.66	9.69	9.71	0.16			
	6	9.33	9.67	9.69	0.18			
	Mean	9.45	9.54	9.66	0.15			
Lymphocyte, %	0	61.14	60.71	61.00	0.62	0.000	0.024	0.010
	1	58.60 ^a	60.86 ^b	63.14 ^c	0.56			
	2	61.00 ^a	61.43 ^{ab}	62.14 ^b	0.64			
	3	60.51 ^a	61.43 ^{ab}	61.57 ^b	0.69			
	4	60.05 ^a	60.72 ^a	63.00 ^b	0.62			
	5	59.12 ^a	61.57 ^b	61.71 ^b	0.63			
	6	60.12 ^a	60.97 ^a	62.98 ^b	0.65			
	Mean	60.08 ^a	61.10 ^b	62.22 ^c	0.63			
Neutrophil, %	0	28.11	28.08	28.04	0.51	0.754	0.348	0.485
	1	28.26	28.29	28.01	0.51			
	2	28.29	28.15	28.15	0.47			
	3	28.65	28.46	27.45	0.48			
	4	29.42	28.04	28.47	0.34			
	5	28.75	28.60	28.30	0.40			
	6	27.35	27.27	27.20	0.41			
	Mean	28.40	28.13	27.95	0.05			
TIG, mg/ml	0	30.87	31.92	31.76	2.48	0.000	0.000	0.000
	1	28.75 ^a	30.25 ^a	35.68 ^b	2.43			
	2	29.17 ^a	31.82 ^b	37.81 ^c	2.48			
	3	29.85 ^a	33.54 ^b	38.69 ^c	2.48			
	4	30.84 ^a	35.48 ^b	37.65 ^c	2.44			
	5	29.75 ^a	36.83 ^b	40.12 ^c	2.49			
	6	30.13 ^a	38.63 ^b	41.89 ^c	2.35			
	Mean	29.91 ^a	34.07 ^b	37.66 ^c	2.45			

B0: Control group; B7: Betaine supplemented group (7 g/d/calf); B14: Betaine supplemented group (14 g/d/calf); TLC: Total leukocyte count; TIG: Total immunoglobulin; TAA: Total antioxidant activity; SEM: Standard error mean.

Means bearing different superscripts (a, b, and c) in a row differ significantly at P<0.05.

Table 2. Effect of betaine supplementation on the immunity of Murrah buffalo calves. (Continue).

Variables	Fortnight	Treatment				P-value		
		B0	B7	B14	SEM	T	F	T×F
TAA, $\mu\text{mol/L}$	0	830.48	830.75	830.92	16.86	0.000	0.000	0.034
	1	836.21 ^a	845.63 ^b	867.98 ^c	16.50			
	2	841.83 ^a	844.77 ^a	855.37 ^b	16.74			
	3	838.83 ^a	848.78 ^b	865.00 ^c	16.64			
	4	830.74 ^a	846.85 ^b	851.28 ^b	17.00			
	5	835.56 ^a	855.52 ^b	862.95 ^b	16.67			
	6	837.52 ^a	849.28 ^b	853.68 ^b	16.23			
	Mean	835.88 ^a	845.94 ^b	855.31 ^c	16.66			

B0: Control group; B7: Betaine supplemented group (7 g/d/calf); B14: Betaine supplemented group (14 g/d/calf); TLC: Total leukocyte count; TIG: Total immunoglobulin; TAA: Total antioxidant activity; SEM: Standard error mean.

Means bearing different superscripts (a, b, and c) in a row differ significantly at $P < 0.05$.

Table 3. Effect of betaine supplementation on energy and lipid metabolites of Murrah buffalo calves.

Variables	Fortnights	Treatment			SEM	P-value		
		B0	B7	B14		T	F	T×F
Glucose, mg/dl	0	54.29	54.91	54.83	0.58	0.788	0.806	0.982
	1	55.13	56.01	55.99	0.59			
	2	55.77	55.88	56.14	0.59			
	3	55.19	56.14	56.57	0.60			
	4	54.30	55.60	55.66	0.58			
	5	55.72	55.43	55.74	0.58			
	6	55.93	55.83	55.68	0.60			
	Mean	55.19	55.69	55.80	0.59			
NEFA, μ mol/L	0	142.12	142.17	142.38	2.78	0.425	0.113	0.906
	1	140.65	135.37	133.28	3.33			
	2	143.75	141.13	139.67	3.13			
	3	144.43	141.29	138.49	2.72			
	4	146.84	142.47	140.92	2.47			
	5	147.87	146.96	139.45	3.33			
	6	145.94	144.55	139.70	3.35			
	Mean	144.51	141.99	139.13	3.02			
Cholesterol, mg/dl	0	156.14	156.07	156.65	1.71	0.341	0.130	0.307
	1	153.23	152.45	148.35	2.04			
	2	152.48	148.35	145.98	1.66			
	3	152.32	151.00	150.45	1.65			
	4	150.81	150.78	149.98	1.98			
	5	152.78	150.98	149.86	2.00			
	6	153.00	152.00	151.00	2.05			
	Mean	152.97	151.66	150.32	1.87			
HDL cholesterol, mg/dl	0	50.82	50.22	50.16	0.77	0.871	0.130	0.859
	1	48.46	49.72	48.58	0.82			
	2	49.96	49.67	47.24	0.84			
	3	50.38	49.71	48.11	0.80			
	4	50.70	48.58	47.40	0.93			
	5	49.15	48.45	48.30	0.74			
	6	50.16	49.12	48.45	0.80			
	Mean	49.95	49.35	48.32	0.82			

B0: Control group; B7: Betaine supplemented group (7 g/d/calf); B14: Betaine supplemented group (14 g/d/calf); NEFA: non-esterified fatty acid; SEM: Standard error mean.

Energy and lipid metabolites

Consistent with previous studies in lactating dairy cows (Wang *et al.*, 2010) and sheep (DiGiacomo *et al.*, 2016), plasma glucose concentration did not differ between control and BET groups. However, Shah *et al.* (2020) reported an increase in the glucose concentration in BET-received dairy cows.

Circulating concentrations of plasma NEFA can act as indicators of energy balance and energy utilization (Wang *et al.*, 2010). In the current study, similar NEFA concentrations in the plasma of different groups suggest that body fat mobilization and the availability of energy were not affected by BET. This is conflicting with conclusions where plasma NEFA concentrations declined with enhancing BET levels in the diet of dairy cows (Shah *et al.*, 2020) and sheep (DiGiacomo *et al.*, 2016). In the current study, BET did not influence the TCH, and HDL-CH levels indicate that BET did not have an impact on lipid metabolism in growing Murrah buffalo calves. In accord with other studies in dairy cows (Wang *et al.*, 2010; Wang *et al.*, 2019) and broilers (Nasiroeslami *et al.*, 2018), TCH level was observed similar in control and BET fed groups.

CONCLUSION

This study suggests that dietary supplementation of both the doses 7 and 14 g/d of BET had no detectable effect on DMI, TLC, neutrophils, glucose, NEFA, TCH, and HDL-CH. Furthermore, BET calves had higher blood lymphocytes %, plasma TIG, and TAA concentrations, which represent that the immunity of calves fed diets with BET might be boosted up.

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