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# Effect of Betaine Supplementation on Growth Performance of Growing Murrah Buffalo Calves

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## ABSTRACT

This experiment was conducted to study the effect of betaine (BET) supplementation on growth performance of Murrah buffalo calves. Twenty-one Murrah buffalo calves with body weight (BW) of 98.70±1.31 kg and age of 8.12±0.55 months were selected and randomly distributed into three groups, 7 calves in each group. The diet of all three groups was the same except that the diet of respective group additionally supplemented with 0, 7, and 14 g/d BET during 90 d of the experiment period. The BW and average daily dry matter intake (DMI) were recorded at fortnightly intervals. Blood samples were taken on fortnightly intervals for the estimation of liver enzymes. There was an improvement ($P<0.05$) in the BW and average daily gain (ADG) in calves receiving 7 or 14 g/d BET. However, DMI was unaffected with the supplementation BET. Feed conversion ratio (FCR) was significantly ($P<0.05$) lower in group supplemented with betaine either at 7 or 14 g/d than in the control group. No effect of dietary BET addition in the diets of calves was observed on the alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The results of our study indicate that supplemental BET may play a positive role in regulating the growth of growing Murrah buffalo calves.

**Key words:** Betaine, Buffalo calves, Growth, Liver enzymes

## INTRODUCTION

In order to accelerate growth, improve immunity and liver function of the buffalo calves, various feed additives are used in diets of calves.- Betaine is a trimethyl derivative of the amino acid glycine and widely found in a variety of plants in nature. There is increasing evidence that it is a highly valuable feed additive and may produce positive effects on animal performance (DiGiacomo *et al.,* 2016; Dunshea *et al.,* 2019). Betaine acts as a methyl group donor, and an organic osmolyte, has the ability to improve growth performance in animals. Betaine protects cells from osmotic stressors and allows them to continue normal metabolic activities. Betaine plays an important function in the amino acid, lipid metabolism and antibody production in animals (Zulkifli *et al.,* 2004). Methyl groups are very important in the metabolism of all animals and these methyl groups cannot be synthesized in the body, and must be supplied through diets. Betaine acts as a methyl donor and thus may play an important role in protein synthesis (Sun *et al.,* 2008). Chand *et al.* (2017) found that dietary supplementation of 1.5% and 2% betaine increased the feed intake and body weight gain (BWG) and reduced the FCR of fast-growing broilers. Singh *et al.* (2015) observed a significant increase in feed intake and BWG of broilers fed with diet contained betaine. Mishra *et al.* (2019) reporterd that supplementation of betained reduced serum concentrations of cholesterol and triglycerides in pigs. However, response of supplementary betaine depends on various factors like species, life stage, basal diet and doses of betaine (Mishra *et al.,* 2020) and any blanket recommendation may be misleading. Hence, this experiment was conducted to evaluate the of effect of betaine supplementation on growth performance of buffalo calves.

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## 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 Livestock Research Center-1, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Twenty one Murrah buffalo calves of similar BW (98.70±1.31 kg) and age (8.12±0.55 months) were utilized in this experiment and randomly distributed into three groups of 7 each. The feeding schedule was same for all groups except that diets are supplemented with betaine at 0.0 (B0), 7 (B7), and 14 g/d/calf in respective groups for 90 d of the study period. The BET was supplemented in form of BET hydrochloride (purity 98%, Chemkart, Mumbai, India). The calves were offered diet in the form of total mixed ration (TMR) 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 concentrate mixture contained 53% yellow maize, 10% wheat bran, 16% mustard cake, 16% groundnut cake, and 3% vitamins and minerals premixes. TMR was prepared daily and offered two times a day at 08:00 h, and 18:00 h. Clean and fresh tap water was offered *ad libitum* to the animals. Experimental calves were kept in tie barn conditions with an arrangement of individual feeding and watering.

Bodyweight was recorded using digital electronic balance before the start of the experiment, and thereafter at fortnightly intervals at 7.00 AM before feeding and watering. Bodyweight gain (BWG) was calculated by subtracting the BW of the previous fortnight from current fortnights. DMI was calculated at fortnightly intervals by subtracting refused feed from offered feed. The FCR was calculated by dividing feed intake with BWG. The samples of TMR were collected fortnightly, composited at the end of the experiment, dried (65 °C for 48 h), and stored for further analysis. The dried samples of TMR were analyzed for DM, total ash, crude fiber, and crude protein using AOAC (1995) procedures. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated by the method of Van Soest *et al.* (1991).

Blood samples from jugular vein were collected on 0, 15, 30, 45, 60, 75, and 90 d of the experiment period in EDTA containing vials before feeding and watering at 7.30 AM. Thereafter, blood samples were centrifuged at 3000 rpm for 30 min. Plasma was collected and stored at -20°C in Eppendorf tubes till further analysis. Alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline

**Table1. Ingredient and chemical composition of TMR fed during the study period**

| Attributes | Content (mg/kg) |
|---|---|
| **Ingredient composition of TMR on DM basis** | |
| Wheat straw | 100 |
| Paddy straw | 90 |
| Berseem fodder | 140 |
| Oat fodder | 70 |
| Yellow maize (ground) | 320 |
| Wheat bran | 67 |
| Mustard cake | 100 |
| Groundnut cake | 100 |
| Mineral and vitamin premix[a] | 13 |
| Betaine hydrochloride[b] | Vaiable[c] |
| **Chemical composition of TMR (g/kg)** | |
| Dry matter | 784 |
| On dry matter basis | |
| Organic matter | 901 |
| Ash | 99 |
| Crude protein | 160 |
| Crude fiber | 30.0 |
| Neutral detergent fiber | 460 |
| Acid detergent fiber | 256 |

[a]Premix composition per kg: vitamin A 32 lac IU, vitamin $D_3$ 6 lac IU, vitamin E 300 IU, vitamin K 400 mg, vitamin $B_2$ 2 g, vitamin $B_{12}$ 6 mg, niacinamide 4 g, calcium pantothenate 1 g, choline chloride 120 g, Ca 300 g, P 36 g, Na 20 g, Cu 800 mg, I 800 mg, Fe 8 g, Mn 22 g, Zn 20 g, Co 40 mg; [b]Betaine hydrochloride (purity 98%, Chemkart, Mumbai, India); [s]upplemental betaine in diet 7 g and 14 g/day/calf

phosphatase (ALP) in the plasma samples were measured by commercial kits (ERBA diagnostics Mannheim Germany).

The data of different variables were analysed by MIXED Model of SPSS software (SPSS for Windows, V 19.0; SPSS, Inc., Chicago, IL, USA)The means were compared by applying 'Duncan's Multiple Range Test'.

## RESULTS AND DISCUSSION

Bodyweight on 3, 4, 5, and 6th fortnights of the study period was higher (P<0.05) in B14 than B7 and control groups. ADG was significantly (P<0.05) higher in B14 than both the groups on all fortnights of the study period. There was no significant effect of supplemental BET on mean DMI. The mean FCR was lower (P<0.05)

**Table 2. Effect of betaine supplementation on growth performance of Murrah buffalo calves**

| Variables | Fortnight | Treatment | | | SEM | P value | | |
|---|---|---|---|---|---|---|---|---|
| | | B0 | B7 | B14 | | T | F | T × F |
| BW, kg | 0 | 95.76 | 98.60 | 101.75 | 1.31 | 0.001 | 0.001 | 0.075 |
| | 1 | 100.38$^{a}$ | 104.84$^{ab}$ | 110.17$^{b}$ | 1.32 | | | |
| | 2 | 104.42$^{a}$ | 111.72$^{ab}$ | 118.37$^{b}$ | 1.34 | | | |
| | 3 | 110.34$^{a}$ | 118.46$^{b}$ | 126.85$^{c}$ | 1.31 | | | |
| | 4 | 113.60$^{a}$ | 124.68$^{b}$ | 135.00$^{c}$ | 1.32 | | | |
| | 5 | 119.69$^{a}$ | 130.49$^{b}$ | 143.20$^{c}$ | 1.26 | | | |
| | 6 | 123.81$^{a}$ | 137.00$^{b}$ | 151.50$^{c}$ | 1.28 | | | |
| | Mean | 109.71$^{a}$ | 117.97$^{b}$ | 126.69$^{c}$ | 1.31 | | | |
| DMI, kg/day | 1 | 2.68 | 2.62 | 2.68 | 0.04 | 0.411 | 0.075 | 0.895 |
| | 2 | 2.81 | 2.76 | 2.84 | 0.04 | | | |
| | 3 | 2.95 | 2.93 | 2.99 | 0.04 | | | |
| | 4 | 3.09 | 3.09 | 3.17 | 0.04 | | | |
| | 5 | 3.23 | 3.23 | 3.33 | 0.04 | | | |
| | 6 | 3.37 | 3.39 | 3.50 | 0.04 | | | |
| | Mean | 3.02 | 3.00 | 3.08 | 0.04 | | | |
| ADG, kg/day | 1 | 0.31$^{a}$ | 0.38$^{b}$ | 0.43$^{c}$ | 0.03 | 0.000 | 0.048 | 0.327 |
| | 2 | 0.33$^{a}$ | 0.42$^{b}$ | 0.42$^{b}$ | 0.03 | | | |
| | 3 | 0.34$^{a}$ | 0.40$^{b}$ | 0.47$^{c}$ | 0.03 | | | |
| | 4 | 0.33$^{a}$ | 0.38$^{b}$ | 0.43$^{c}$ | 0.03 | | | |
| | 5 | 0.33$^{a}$ | 0.39$^{b}$ | 0.45$^{c}$ | 0.03 | | | |
| | 6 | 0.34$^{a}$ | 0.40$^{b}$ | 0.44$^{c}$ | 0.03 | | | |
| | Mean | 0.33$^{a}$ | 0.40$^{b}$ | 0.44$^{c}$ | 0.03 | | | |
| FCR | 1 | 8.65$^{b}$ | 6.89$^{a}$ | 6.23$^{a}$ | 0.62 | 0.000 | 0.072 | 0.478 |
| | 2 | 8.52$^{b}$ | 6.57$^{a}$ | 6.76$^{a}$ | 0.68 | | | |
| | 3 | 8.68$^{c}$ | 7.33$^{b}$ | 6.36$^{a}$ | 0.80 | | | |
| | 4 | 9.36$^{c}$ | 8.13$^{b}$ | 7.37$^{a}$ | 0.72 | | | |
| | 5 | 9.79$^{c}$ | 8.28$^{b}$ | 7.40$^{a}$ | 0.63 | | | |
| | 6 | 9.91$^{c}$ | 8.48$^{b}$ | 7.95$^{a}$ | 0.75 | | | |
| | Mean | 9.15$^{b}$ | 7.61$^{a}$ | 7.01$^{a}$ | 0.70 | | | |

B0, control group; B7, betaine supplemented group (7 g/d/calf); B14, betaine supplemented group (14 g/d/calf); BW, body weight; DMI, dry matter intake; ADG, average daily gain; FCR, feed conversion ratio; SEM, standard error mean; Means bearing different superscript (a, b, and c) in a row differ significantly at P<0.05

in both B7 and B14 groups than the control Table 2).

BET supplementation with dose 7 g/d or 14 g/d in growing calves improved the BW, ADG, and feed efficiency. In accordance with the present study, Lakhani *et al*. (2020) reported the positive effect of BET supplementation on BW, ADG, and feed efficiency of crossbred heifers. The findings of the current study are concurring with the results of Nofal *et al.* (2015), they revealed that BW and WG increased in growing chicks that received supplemental BET. BET supplementation improved growth due to its osmo-protective properties that increase nutrient digestibility, supporting intestinal cells and the growth of intestinal microbes (Eklund *et al.,* 2005). Block *et al.* (2004) reported higher ADG, as well as subcutaneous fat thickness in steers fed on diets, supplemented with BET and it might be due to the lipotropic nature of BET. BET plays a potential role in the absorption of digested nutrients by protecting intestinal cells and intestinal microbes from osmotic variation (Ratriyanto *et al.,* 2009).

However, other studies (Wang *et al.,* 2010; Peterson *et al.,* 2012) reported no effect of BET supplementation on BW, ADG, and feed: gain ratio. The contrary effect of the dietary additive of BET on BW, ADG, and feed:

**Table 3. Effect of betaine supplementation on energy metabolites and liver function of Murrah buffalo calves**

| Variables | Fortnights | Treatment | | | SEM | P value | | |
|---|---|---|---|---|---|---|---|---|
| | | B0 | B7 | B14 | | T | F | T × F |
| ALT, IU/L | 0 | 17.41 | 17.02 | 17.12 | 0.38 | 0.123 | 0.090 | 0.179 |
| | 1 | 17.31 | 17.79 | 16.57 | 0.34 | | | |
| | 2 | 17.03 | 15.93 | 15.83 | 0.37 | | | |
| | 3 | 17.04 | 17.30 | 16.50 | 0.35 | | | |
| | 4 | 17.94 | 16.46 | 16.41 | 0.37 | | | |
| | 5 | 17.92 | 17.89 | 15.80 | 0.34 | | | |
| | 6 | 17.90 | 16.98 | 15.35 | 0.38 | | | |
| | Mean | 17.51 | 17.05 | 16.23 | 0.36 | | | |
| AST, IU/L | 0 | 127.31 | 127.21 | 127.11 | 1.70 | 0.868 | 0.306 | 0.696 |
| | 1 | 126.98 | 126.95 | 126.94 | 1.82 | | | |
| | 2 | 127.60 | 125.16 | 125.13 | 1.52 | | | |
| | 3 | 127.28 | 127.46 | 126.38 | 1.61 | | | |
| | 4 | 128.05 | 127.82 | 126.75 | 1.75 | | | |
| | 5 | 127.95 | 127.94 | 126.95 | 1.44 | | | |
| | 6 | 128.51 | 127.43 | 126.79 | 1.63 | | | |
| | Mean | 127.67 | 127.14 | 126.58 | 1.64 | | | |
| ALP, IU/L | 0 | 8.49 | 8.14 | 8.16 | 0.28 | 0.074 | 0.085 | 0.713 |
| | 1 | 8.70 | 8.93 | 8.55 | 0.26 | | | |
| | 2 | 8.78 | 8.89 | 8.96 | 0.28 | | | |
| | 3 | 9.35 | 9.30 | 8.74 | 0.17 | | | |
| | 4 | 9.83 | 8.63 | 8.77 | 0.21 | | | |
| | 5 | 9.48 | 8.74 | 8.67 | 0.24 | | | |
| | 6 | 9.18 | 9.12 | 8.82 | 0.27 | | | |
| | Mean | 9.12 | 8.82 | 8.67 | 0.25 | | | |

B0, control group; B7, betaine supplemented group (7 g/d/calf); B14, betaine supplemented group (14 g/d/calf); ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; SEM, standard error mean

gain ratio may be due to differences in form, ruminal availability, and crude protein content in basal diets (Fernandez-Figares *et al.,* 2002). The inclusion of BET at either 7 g/d or 14 g/d in the diets of growing calves did not affect DMI, which corroborates with the findings of Peterson *et al.* (2012) in lactating Holstein dairy cows. Similar to the present findings, Wang *et al.* (2019) reported that DMI was unaffected with BET supplementation in the diets of post-partum dairy cows. Plasma activity of ALP, ALT, and AST were not influence by supplementation of betaine (Table 3). In agreement with the present findings, Yusuf *et al.* (2018) reported no changes in the concentration of ALT and ALP in finishing male broilers fed diets supplemented with BET at 1.5 and 3.0 g/kg diet. Zhang *et al.* (2014) reported that ALT was unaffected in dairy cows fed on diets supplemented with BET at 10, 15, and 15 g/d. Consistent with the present findings, Mishra *et al.* (2019) reported no effect of BET on liver enzymes (ALT, AST, and ALP) of crossbred (*Landrace* × *Desi*) sows.

## CONCLUSION

This study suggests that dietary supplementation of betaine at 7 and 14 g/d enhanced BW, ADG, and gain: feed ratio but had no significant effect on DMI, ALT, AST, and ALP, which indicate that the growth performance of buffalo calves supplemented with BET might be boosted up.

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