Ann. For. Res. 65(1): 7033-7046, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

DIETARY BETAINE'S IMPACT ON NUTRITIONAL PARAMETERS IN POSTPARTUM MURRAH BUFFALOES

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ABSTRACT

Loss of productivity during lactation period due to negative energy balance can be reduced by providing strategic feed supplement such as betaine. The betaine acts as a methyl donor in metabolism and allowing cells to acts as an organic osmolyte used to protect against osmotic stress and high temperature. In this study, a total of 18 postpartum Murrah buffaloes were randomly divided into three groups for the experiment. T1 group was kept as a control. The T2, T3 groups were supplemented with betaine at 50 g/animal/day and 100 g/animal/day, respectively. Betaine was supplemented from day 5 postpartum and was continued until 4 months postpartum. The overall mean of body weight was significantly ($p<0.05$) higher in T3 group followed by T2 group and lowest in T1 group. The mean values of dry matter intake and feed conversion ratio of buffaloes differed non-significantly between all the groups.Based on these results, it is concluded that supplementation of diets with betaine improved body weight in lactating animals.

Keywords: Betaine, Buffalo, Bodyweight, Feed conversion ratio, Dry matter intake.

INTRODUCTION

Saliently, buffaloes enter a state of metabolic stress during lactation because of reduced dry matter intake and increased demand for nutrients to maintain milk synthesis. Hence, to combat this prominent challenge, buffaloes seek metabolic resources needed for reproduction and milk synthesis by mobilizing carbohydrate and fat reserves (Lean *et al*., 2013).A negative methyl donor balance occurs during early lactation in buffaloes because milk is high in methylated compounds

(Pinotti*et al*.,2002). During NEBAL the metabolism of buffalo gets disturbed that leads to reduced milk production.Proper understanding the relationship among climate, lactational stress and feeding regimens will give a firm base to ameliorate the health and welfare of buffaloes.

Betaine is a growth promoting nutritional additive extensively used in livestock. Betaine is naturally present in wheat and sugar beets, is an oxidative product of choline and a trimethylated derivative of glycine i.e., trimethyl glycine (De Zwart *et al*., 2003)**.**Betaine was isolated from sugar beet (*Beta vulgaris*) byScheibler in 1860s. Betaine (N, N, N-trimethylglycine) molecule contains three methyl groups and thus acts as a methyl donor in metabolism,thereby sparing methionine,reducing homocysteine concentrations via itsdetoxification and helps in synthesis of cellular macromolecules such as proteins, DNA, RNA and choline (Craig, 2004). Methyl donation is required for DNA methylation, prevention of oxidative stress, prevention of apoptosis, energy metabolism and protein synthesis. By providingmethyl groups betaine affects many important functions in the body such as growth, liver health and lactation. In buffaloes, betaine acts as a molecular chaperone that reduces the susceptibility of microbial populations to stress, being an antimicrobial agent to some bacteria. In ruminants, betaine also has a positive effecton fermentation, it increases total volatile fatty acid (VFA) production and increases acetate to propionate (A:P) ratio. It serves as a source of ruminal available nitrogen andincreases the fermentation rate of microorganisms.Betaine is converted to acetate in the rumen and can be used to synthesize milk fat.

Therefore, the present study was conducted to study the effect of dietary betaine on nutritional parameters in postpartum Murrah buffaloes.

MATERIALS AND METHODS

1. Location of work

The experiment was conducted at Livestock Farm Complex, Adhartal and Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.).

2. Selection of experimental animals

The animals were selected on the basis of similarity in body weight, parity, age and free from any anatomical, physiological and infectious disorders.18postpartumlactating Murrah buffaloes of 3rd to 5th parity were randomly divided into three groups. T1 group was kept as a control. T2 and T3 group was supplemented with betaine @ 50 g/animal/day and 100 g/animal/day respectively. The experiment was conducted as per the guidelines of Institutional Animal Ethics Committee (IAEC) vide order no. 06/IAEC/Vety./2019 dated 09/08/2019.

3. Management of animals:

Experimental buffaloes were stall fed and managed in an asbestos roof shelter with concrete floor and maintained in intensive system of housing. The shed of experimental buffaloes was properly cleaned with the help of broom, washed and cleaned by forced water. The walls and floor of the house were disinfected by using phenyl solution. Manger, waterer and other necessary equipments were properly washed and cleaned.

4. **Feeding**:

Experimental animals were fed according to their body weight and production (ICAR Feeding Standard, 2013). All the animals were offered identical basal ration consisting of green fodder, wheat straw and concentrate. The water was given to animals round the clock. Betaine (Betaine HCl, feed grade) supplementation was started day 5 postpartum and was continued up to 4 months postpartum.

Composition ofconcentrateration usedin theexperiment

Ingredients	Parts(%)
Yellowmaize	38.00
Cottonseedcake	13.00
Mustard cake	20.00
Wheatbran	9.00
Rice polish	8.00
Arharchuni	9.00
Mineral mixture	2.00
Commonsalt	1.00
Total	100

5. Nutritional Parameters

(a) Body weight of animal

The body weight of all animals was taken at fortnight interval up to 4 months with the help of electronic weighing balance.

(b) Dry matter intake (DMI)

The intake of green fodder, wheat straw and concentrate were recorded fortnightly by subtracting the amount of left over from the quantity offered. The sample of feed offered was subjected to overnight drying in oven for determination of dry matter. Representative samples were taken in previously weighed moisture cup/tin trays and kept in hot air oven at 100±2°C for 24 hr. Dry matter was calculated as follows:

$$\text{Dry matter (\%)} = \frac{b}{a} \times 100$$

Where, a = fresh weight of sample (g), b= dry weight of sample (g)

The values thus obtained were used in the computing dry matter intake by animals.

(c) Feed conversion ratio(FCR)

In animal husbandry, feed conversion ratio is a rate measuring of the efficiency with which the bodies of livestock convert animal feed into the desired output. For dairy animals, the output is milk so feed conversion ratio was calculated fortnightly using following formula.

1. FCR = Dry matter intake (kg/day) / 6% Fat Corrected Milk (FCM) yield
2. FCR = Dry matter intake (kg/day) / Energy Corrected Milk (ECM) yield
3. FCR = Dry matter intake (kg/day) / Solid Corrected Milk (SCM) yield

Statistical analysis

The data obtained during experiment were analyzed by IBM SPSS-24 statistical software program using one way analysis of variance (ANOVA) and two-way ANOVA in DMI. Various conditions and treatment groups were compared by using Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

1. Nutritional Parameters

(a) Body weight

Non-significant difference in mean body weight(kg) of buffaloes was observed among all the groups. Presentstudy revealsthattherewasdecrease inbody weightintheallgroupsaftercalvingfollowedby a gradualincreasein body weight.The overall mean values of body weight were found to be significantly ($p<0.05$) different between the groups. The overall mean of body weight was significantly ($p<0.05$) higher in T2(547.58±6.47) and T3(552.07±3.74) groups as compared to T1 group (539.23±4.22). Maximum body weight was observed in T3group (Table 01 and Figure 01).

The present findings were in agreement with Deshpande *et al*. (2020), they reported that supplementation of betaine @ 25 g/animal/day in buffalo heifers resulted in decreased mean body weight in control group (185.94±7.71 kg) as compared to treatment group (189.72±7.71 kg) during hot-humid season. Also, Lakhani *et al.* (2020) reported that the mean values of the final body weight in betaine-supplemented groups i.e. treatment I (237.16±1.20 kg) and treatment II (243.46±1.30 kg) were higher ($p<0.05$) as compared to the control group (221.13±1.80 kg) in Karan Fries heifers, which is similar to present findings.

Yu *et al*. (2004) conducted research work on growing pigs and concluded that 1,000 mg/kg and 1,500 mg/kg betaine treated groups had increased average daily body weight gain by 13.20% ($p<0.01$) and 9.28% ($p<0.05$) respectively as compared to the control group. Increase in body weight after betaine supplementation is due to increased cellular water content by osmolyte property of betaine and increased nutrients bioavailability and efficiency in animals. In ruminants, betaine also has a positive influence on fermentation which in turn increases the feed intake during lactation period and ultimately body weight of animals.

In contrast to present finding Peterson *et al*. (2012) reported that body weight was not affected (601, 605, 596 and 608±16.1 kg/day for 0, 25, 50 and 100 g of betaine/day, respectively; p= 0.08) by betaine supplementation in Holstein dairy cows. Davidson *et al*. (2008) reported that supplementation of 20 g/day RP-Met, 45 g/day RP-betaine and 40 g/day RP-choline had no treatment difference in mean body weight in Holstein lactating cows. Similar findings were reported by Raheja *et al*. (2019), they concluded that the overall mean body weight was more in betaine treated group (416.76±10.24 kg vs. 411.33±11.07 kg) as compared to control group. However, difference was non-significant in Karan Fries cows during hot-humid season.

(b) Dry matter intake (DMI)

The mean values of dry matter intake of buffaloes differ significantly (p<0.05) within treatment groups (T2 and T3). The mean values of dry matter intake of buffaloes differ non-significantly (p>0.05) within T1 group. The mean values of dry matter intake were lower (15.60±0.18,15.56±0.08,15.57±0.26 kg/day; T1, T2, T3) in all the groups on day 5postpartum and gradually increases after parturition. On day 125, the mean values of dry matter intake were 17.05±0.72, 17.46±0.53 and 18.41±1.32 kg/day for T1, T2 and T3respectively (Table 02 and Figure 02).

The mean values of dry matter intake of buffaloes differ non-significantly (p>0.05) between all the groups. However, the overall mean values of dry matter intake in control group (T1) were numerically lower (16.32±0.20 kg/day) than T2 (16.58±0.15 kg/day) and T3 (16.86±0.27 kg/day) groups of buffaloes. The probable reason may be the lactational stress which may have decreased the growth rate due to reduction in feed intake and decreased nutrients availability. Betaine act as a growth promoter by reducing the concentration of stress markers and increased nutrients bioavailability and efficiency.

In agreement to present reports, Raheja *et al*. (2019) concluded that the overall mean of DMI during 5 fortnights in control group was 1.79±0.12 kg/75 kg body weight and in treatment group was 1.86±0.12 kg/75 kg body weight with non-significant difference between control and treatment group during hot humid season. Similar findings were also reported by Deshpande *et al*. (2020), they concluded that the overall mean values of DMI in control group was numerically lower (5.89±0.24 kg) than treatment group (6.36±0.24 kg) of buffalo heifers during hot-humid season which differed (p>0.05) non-significantly between treatments.

In contrary to present reports, Zhang *et al*. (2014) recorded that feeding betaine to cows increased feed intake level (p<0.05) in group II and III (23.36±0.11 and 23.33±0.13 kg/day) as compared to control (22.76±0.21 kg/day) in lactating Holstein cows. Lakhani *et al.* (2020) enunciated that the mean values of the DMI (kg/day) in betaine-supplemented groups i.e. treatment I (6.21±0.09) and treatment II (6.28±0.17) were higher (p<0.05) as compared to the control group (5.92±0.13) in Karan Fries heifers supplemented with 0, 25 and 50 g/day/animal of betaine. Also, the DMI in betaine and bypass fat supplemented lactating buffaloes was significantly higher (p=0.011) by 4.51% as compared to control group during heat stress (Shankhpal*et al*., 2019). DMI in betaine supplemented crossbred lactating cows was higher by 8.79% as compared to control group during heat stress as reported by Shankhpal*et al*., 2018.

(c)Feed conversion ratio (FCR)

Themeanfeed conversion ratio (DMI/6% FCM, DMI / ECM and DMI / SCM) of buffaloes in all the groups during the experimental period has been presented in Table 03, 04, 05 and Figure 03, 04, 05 respectively.The mean values of FCR were higher in all the groups on day 5 postpartum. The mean values of FCR were lower on days 15, 30, 45, 60, 75, 90, 105and 120postpartum as compared to day 5.

The mean values of FCR (DMI / 6% FCM) of buffaloes differed non-significantly ($p>0.05$) between all the groups. However, the overall mean values of FCR in T1group (2.27±0.08) was numerically higher than T2 (2.18±0.13) and T3 (2.02±0.09) groups of buffaloes.

The mean values of FCR (DMI / ECM) of buffaloes differed non-significantly ($p>0.05$) between all the groups. However, the overall mean values of FCR in control group (1.67±0.06) was numerically higher than T2 (1.61±0.10) and T3 (1.50±0.07) groups of buffaloes.

The mean values of FCR (DMI / SCM) of buffaloes differed non-significantly ($p>0.05$) between all the groups. However, the overall mean values of FCR in T1 (1.78±0.06) group was numerically higher than T2 (1.73±0.12) and T3 (1.60±0.08) groups of buffaloes. Results showed that betaine supplemented groups require less expenses of energy (DMI) to produce desired output (milk).

In agreement to present reports Huang *et al*. (2008) reported that the mean values of feed conversion ratio (3.1, 3.03) did not differ significantly for control and betaine supplemented group in pigs. Wang *et al*. (2010) also recorded that feed efficiency (1.40, 1.41, 1.44, 1.42) forcontrol LB, MB and high betaine groups was not affected with increasing the betaine supplementation in cows.

In disagreement to present finding Yu *et al*. (2004) concluded that the 1,000 mg/kg and 1,500 mg/kg betaine treated groups had decreased feed conversion ratio by 7.93 % ($p<0.01$) and 6.55 % ($p<0.05$) respectively as compared to the control group in growing pigs. Similar reports were enunciated by Yang *et al*. (2009), they reported that the FCR of pigs fed (@ 0, 2, 4 and 6 % betaine) betaine diets (3.00±0.15, 2.80±0.19, 3.01±0.12) was significantly lower ($p<0.05$) than the control (3.45±0.15) and the control had highest FCR amongst all the treatments. The results indicates that betaine supplementation could improve growth performance in female pigs.

CONCLUSION

Supplementation of betaine enhanced body weightin postpartum Murrah buffaloes via ameliorating adverse impact of metabolic stress during lactation period.Dietary

supplementation withbetaine has non-significant effect on dry matter intake and feed conversion ratio in buffaloes.

AUTHOR CONTRIBUTIONS

All the authors contributed to the study conception and design as well as manuscript writing. JyotsanaShakkarpude had played main role in this research work.Aditya Mishrahad designed the research.Danveer S. Yadav, C.P.S. Solanki, Kapil Sharma, Shivank P. Singh and Akanksha Singhhelped in data collection.Deepika D. Caesar, Anand Kumar Jain and Sanju Mandal had helped in laboratory work.Rajesh Kumar Vandrehad played role in statistical analysis of data. Archana Jain, A.Bhimte& S. Rajoriyahelped in preparing manuscript according to author guidelines of this journal.

CONFLICT OF INTEREST

No conflict of interest was reported by the authors.

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Table 01: Mean body weight (kg)in postpartum buffaloes at fortnight interval

Treatment / Days	T1	T2	T3
Initial	553.00 ±13.02	553.50 ±20.70	555.66 ±16.60
day 15	542.92±12.59	547.55 ±20.04	552.00 ±16.52
day 30	536.17±12.75	542.67 ±21.33	545.17 ±15.51
day 45	526.67 ±12.42	540.00 ±21.14	539.83±15.40
day 60	520.50±12.99	538.00 ±20.83	535.00 ±15.77
day 75	529.17±12.30	544.00 ±20.37	549.17 ±14.09
day 90	537.50 ±12.88	549.33 ±20.86	557.83 ±13.35
day 105	549.67 ±12.48	554.00 ±21.12	563.83 ±13.30
day 120	557.50 ±12.72	559.17 ±21.36	570.17 ±13.47
Average	**$539.23^{B} \pm 4.22$**	**$547.58^{AB} \pm 6.47$**	**$552.07^{A} \pm 3.74$**

Means bearing different superscripts within row differ significantly ($p<0.05$).

Table 02:Mean dry matter intake (kg/day)in postpartum buffaloes at fortnight interval

Treatment / Days	T1	T2	T3
Initial	15.60 ± 0.18	$15.56^{b} \pm 0.08$	$15.57^{b} \pm 0.26$
day 15	15.67± 0.22	$15.60^{b} \pm 0.27$	$15.68^{b} \pm 0.26$
day 30	16.14± 0.54	$16.36^{ab} \pm 0.47$	$16.44^{ab} \pm 0.21$
day 45	16.34± 0.84	$16.68^{ab} \pm 0.69$	$16.65^{ab} \pm 0.68$
day 60	16.36± 0.24	$16.71^{ab} \pm 0.20$	$16.73^{ab} \pm 0.47$
day 75	16.49± 0.21	$16.74^{ab} \pm 0.26$	$16.87^{ab} \pm 0.76$
day 90	16.60± 1.01	$16.90^{ab} \pm 0.42$	$17.50^{ab} \pm 1.06$
day 105	16.66± 0.84	$17.23^{a} \pm 0.44$	$17.85^{ab} \pm 1.07$
day 120	17.05± 0.72	$17.46^{a} \pm 0.53$	$18.41^{a} \pm 1.32$
Average	**16.32± 0.20**	**16.58± 0.15**	**16.86± 0.27**

Means bearing different superscripts within column differ significantly ($p<0.05$).

Table 03:Mean feed conversion ratio (DMI /6%FCM)in postpartum buffaloes at fortnight interval

Treatment / Days	T1	T2	T3
Initial	2.77± 0.23	2.87± 0.50	2.63± 0.20
day 15	2.10± 0.22	1.80± 0.21	1.82± 0.13
day 30	2.09± 0.20	1.74± 0.13	1.72± 0.19
day 45	2.12± 0.28	1.66± 0.15	1.75± 0.07
day 60	2.30± 0.16	2.08± 0.24	1.93± 0.14
day 75	2.38± 0.25	2.29± 0.42	2.16± 0.20
day 90	2.02± 0.22	2.31± 0.46	2.10± 0.12
day 105	2.37± 0.28	2.35± 0.37	1.98± 0.12
day 120	2.23± 0.19	2.49± 0.26	2.10± 0.19
Average	**2.27± 0.08**	**2.18± 0.13**	**2.02± 0.09**

Table 04:Mean feed conversion ratio (DMI /ECM)in postpartum buffaloes at fortnight interval

Treatment / Days	T1	T2	T3
Initial	2.08± 0.17	2.13± 0.36	1.94± 0.15
day 15	1.56± 0.15	1.36± 0.17	1.36± 0.09
day 30	1.54± 0.13	1.30± 0.11	1.30± 0.14
day 45	1.59± 0.20	1.27± 0.11	1.32± 0.05
day 60	1.74± 0.12	1.57± 0.18	1.47± 0.09
day 75	1.81± 0.18	1.76± 0.32	1.69± 0.15
day 90	1.56± 0.17	1.82± 0.35	1.65± 0.11

day 105	1.83± 0.22	1.85± 0.30	1.56± 0.10
day 120	1.73± 0.15	1.95± 0.21	1.63± 0.16
Average	**1.67± 0.06**	**1.61± 0.10**	**1.50± 0.07**

Table 05:Mean feed conversion ratio (DMI /SCM)in postpartum buffaloes at fortnight interval

Treatment / **Days**	**T1**	**T2**	**T3**
Initial	2.18± 0.17	2.41± 0.46	2.08± 0.18
day 15	1.63± 0.16	1.48± 0.19	1.42± 0.10
day 30	1.65± 0.14	1.37± 0.12	1.36± 0.14
day 45	1.70± 0.22	1.34± 0.11	1.39± 0.05
day 60	1.84± 0.13	1.66± 0.20	1.56± 0.09
day 75	1.94± 0.21	1.90± 0.35	1.87± 0.20
day 90	1.68± 0.19	1.98± 0.39	1.77± 0.12
day 105	1.95± 0.24	1.99± 0.32	1.67± 0.11
day 120	1.89± 0.18	2.08± 0.23	1.76± 0.18
Average	**1.78± 0.06**	**1.73± 0.12**	**1.60± 0.08**

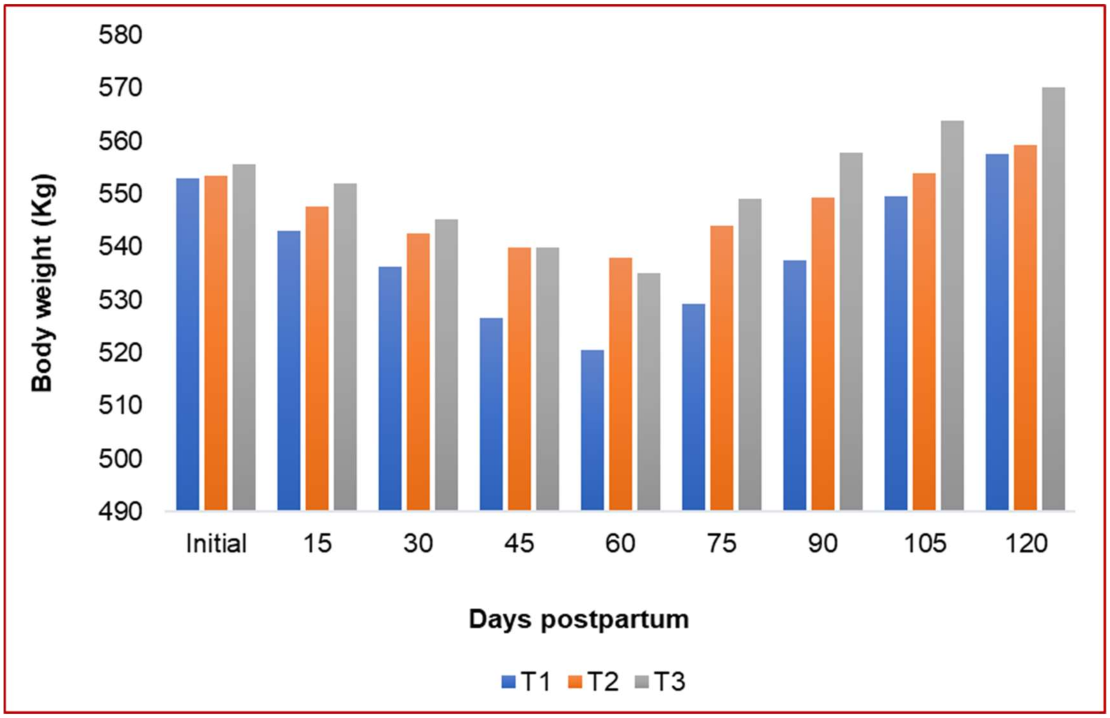

Figure 01: Mean body weight (kg)in postpartum buffaloes at fortnight interval

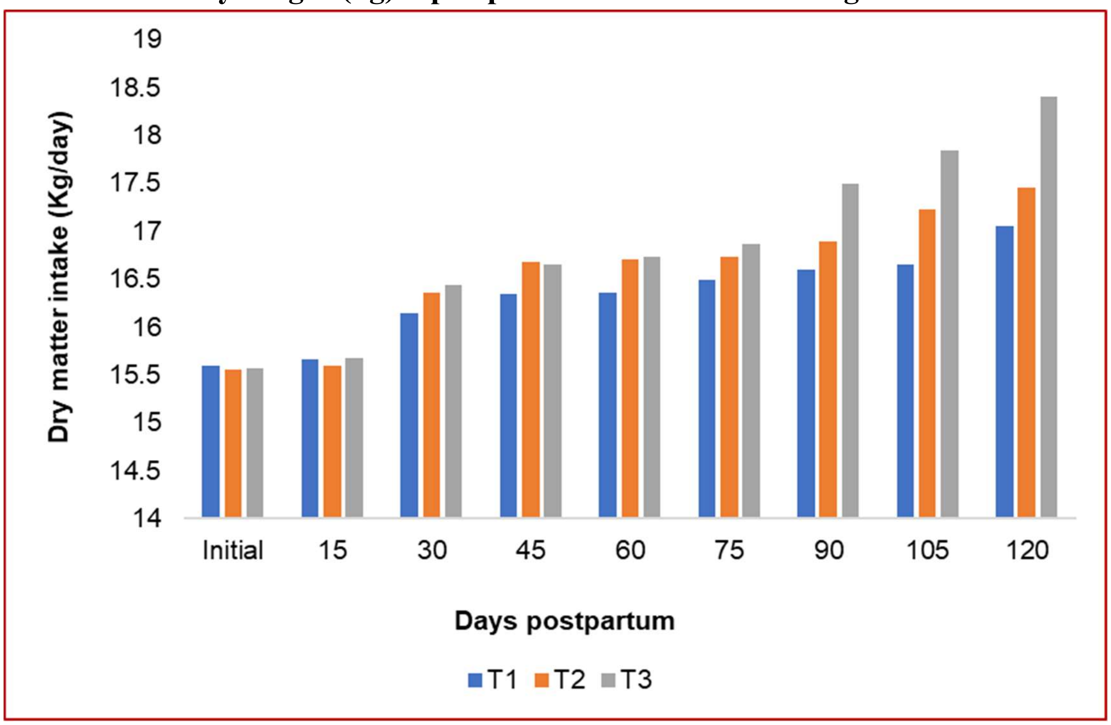

Figure 02: Mean dry matter intake (kg/day) in postpartum buffaloes at fortnight interval

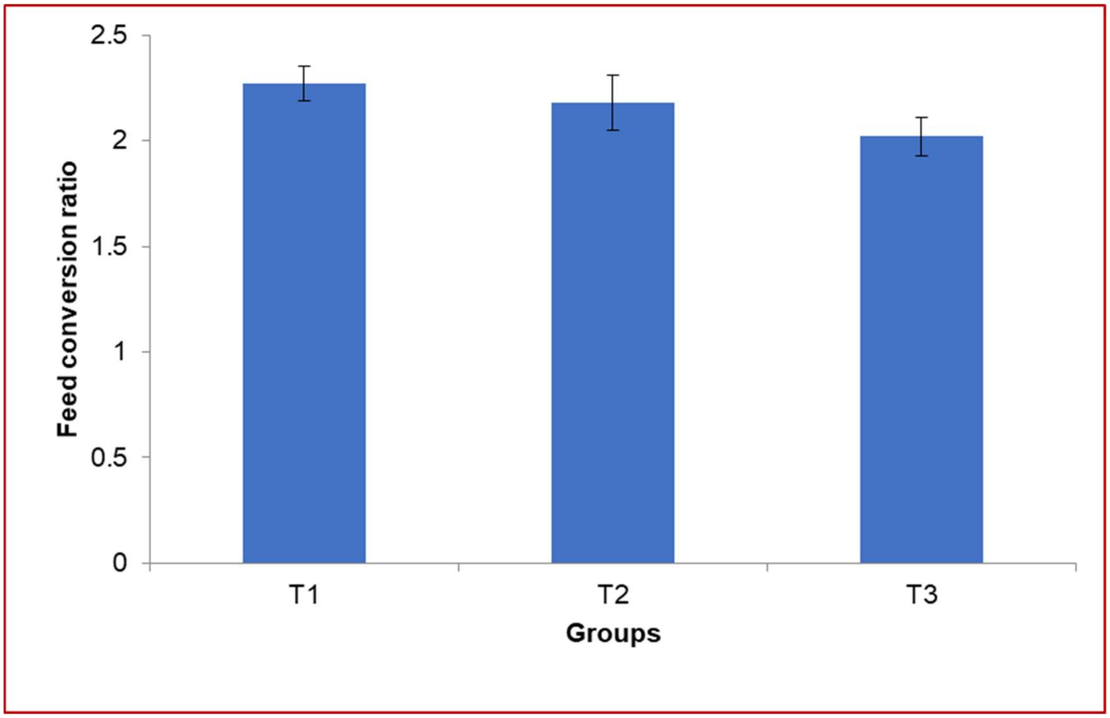

Figure 03: Average feed conversion ratio (DMI /6%FCM) in different groups of postpartum buffaloes

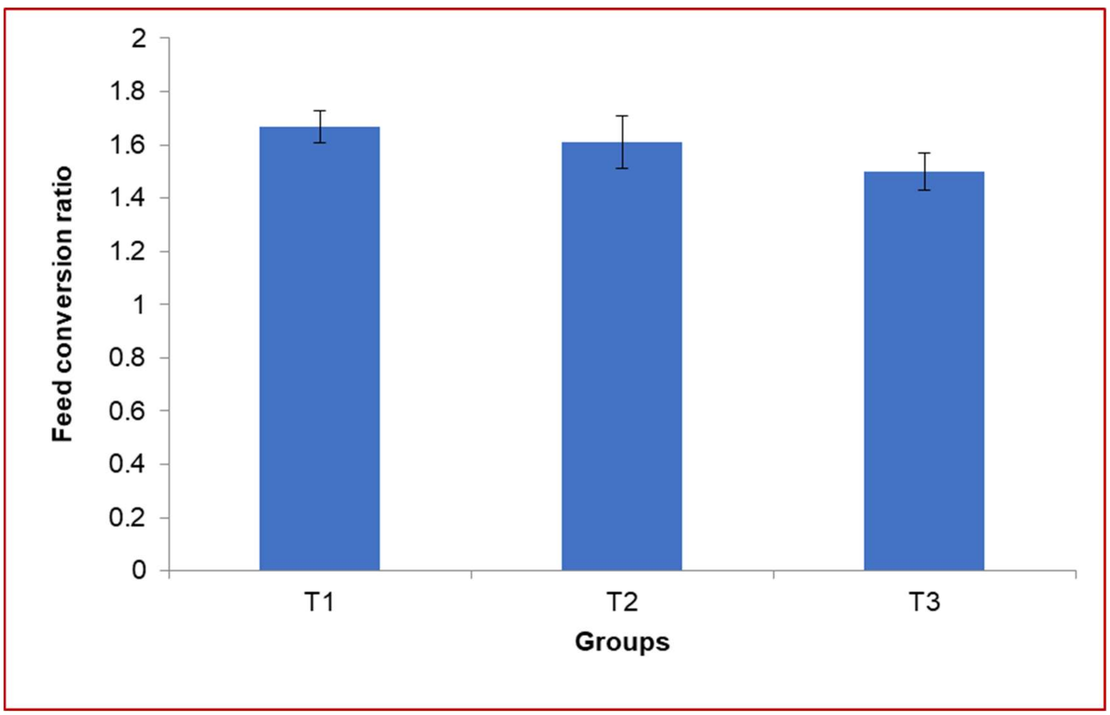

Figure 04: Average feed conversion ratio (DMI / ECM) in different groups of postpartum buffaloes

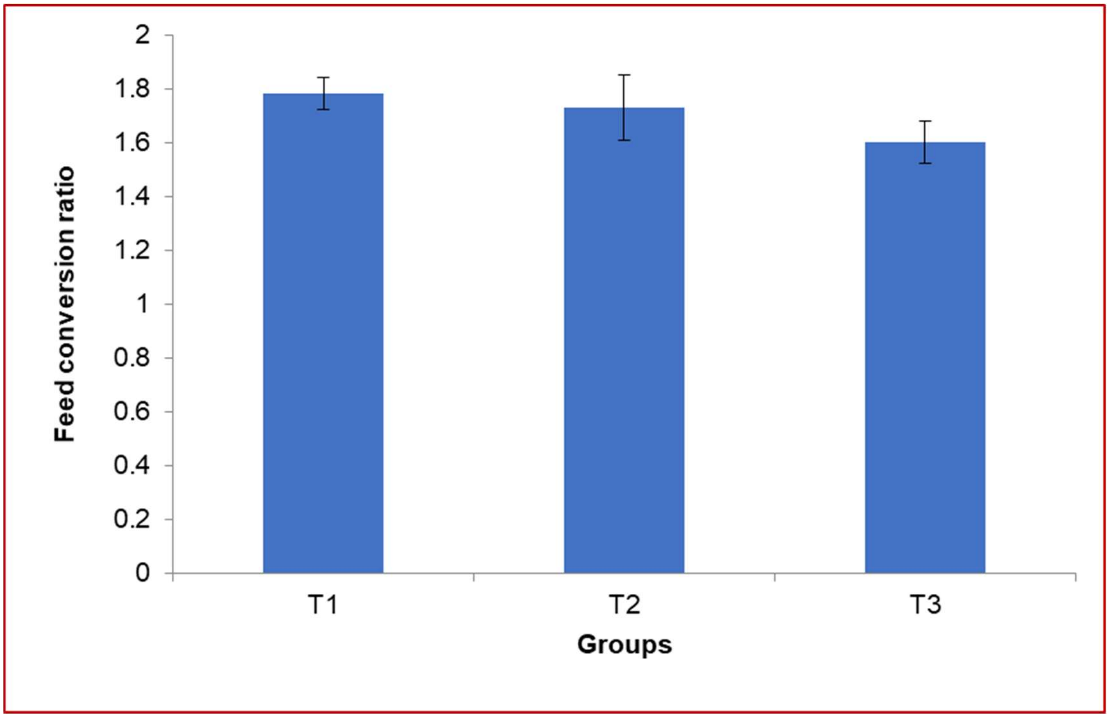

Figure 05: Average feed conversion ratio (DMI / SCM) in different groups of postpartum buffaloes