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EFFECT OF DIETARY BETAINE ON ENDOCRINE PROFILE IN POSTPARTUM LACTATING MURRAH BUFFALOES DURING HOT-HUMID SEASON

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ABSTRACT

Extreme heat along with metabolic stress associated with the postpartum period negatively affects the endocrine status of animals. Betaine is a feed additive that promotes animal growth. Since betaine contains three methyl groups, it acts as a methyl donor in animal metabolism. Eighteen postpartum Murrah buffaloes were randomly divided into T1 Group (Control), T2 and T3 Group was supplemented with betaine 50 g/animal/day and 100 g/animal/day respectively from 5th day postpartum and was continued up to 4 months postpartum. On day 50 postpartum the mean value of plasma cortisol of T1 Group was maximum followed by T2 Group and T3 Group had minimum plasma cortisol mean value which differed (P<0.05) significantly between groups. The mean concentration of plasma T_3 total, T_4 total, progesterone and estrogen showed (P>0.05) non-significant difference between all the groups. However, betaine fed T2 and T3 Group had higher plasma T_3, T_4, progesterone and estrogen as compared to control, it might be due to the effect of betaine, which reduces metabolic stress and activate hypothalmo-pituitary and gonadal axis after parturition and lactation period during heat stress.

Keywords: *Bubalus bubalis*, buffaloes, betaine, thyroxine, estrogen, progesterone, cortisol

INTRODUCTION

India is the highest milk producing country in the world. Milk output from dairy cows has enhanced extensively over the many decades (FAO, 2011) due to major advances in management-related aspects but the reproductive performance of high yeilding dairy animals has deteriorated gravely (Patton *et al*., 2006). With

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the development of high yielding milch animals, reproductive overall performance of dairy animals is declining, which is further aggravated by the changing climatic conditions, which are manifesting in the form of increasing ambient temperature around the world, especially in tropical countries like India. Heat stress results in lower fertility among buffaloes. Hyperthermia directly affects the cellular functions of different tissues of the reproductive system (Wolfenson *et al*., 2000). Buffalo infertility due to heat stress is multifactorial, as high ambient temperature causes a decrease in appetite, which directly enhances a negative energy balance (NEBAL). Energy balance is believed to be the main stimulator and regulator of postpartum ovarian rebound in buffaloes. Stress from productivity diseases in high-yielding dairy cows and buffaloes also contributes to infertility in animals.

During lactation, buffaloes are under a state of great metabolic stress due to an increased need for nutrients to maintain milk synthesis. During NEBAL, the animal's metabolism is disturbed and affects milk production. Reproduction of buffaloes is negatively affected by extreme climatic conditions and also by different feeding administrations. A reduction in feed input and milk production is a common sign of heat stress. A proper understanding of the relationship between climatic factors and feeding protocols will provide a solid base for augmenting buffalo health and welfare.

Reproductive effectiveness and low production during lactation can be lessened by furnishing a strategic feed supplement similar as betaine. Betaine is an oxidation product of choline and present in wheat and sugar beets like plants. Physiologically betaine is as a methyl patron and osmolyte. Betaine acts as an osmolyte to perpetuate cellular water and ionic balance to ameliorate capacity against heat stress. Betaine improves the activity of microbial fermentation via helping various intestinal microbes against osmotic pressure alterations. Betaine supplementation in the diet appreciatively affects the digestibility of nutrients. Its addition in the diet imparts essential amino acids similar as choline and methionine through betaine metabolism. This study was conducted to investigate the effect of betaine on the endocrine profile of postpartum lactating Murrah buffaloes during the hot and humid season.

MATERIALS AND METHODS

Experimental animals

The trial was performed at Department of Veterinary Physiology and Biochemistry and Livestock Farm Complex, Adhartal, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.). Eighteen postpartum lactating Murrah buffaloes were randomly divided into three groups. Group T1 was left as control. Group T2 and T3 were supplemented with betaine 50 g/animal/day and 100 g/animal/day respectively. The trial was organised according to Institutional Animal Ethics Committee (IAEC) guidelines.

Management of animals

Experimental buffaloes were stall fed and housed in shelters with asbestos roofs, concrete floors 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. Experimental buffaloes were fed according to their body weight and production (ICAR Feeding Standard, 2013). All buffaloes were given an identical basic portion comprised of green grass, wheat straw and concentrate. Water was offered to the animals for 24 h. Betaine supplementation (betaine HCl, feed grade) was commenced on 5th day postpartum and continued until four months postpartum. Blood sample was taken from buffaloes on day 7, 25, 50, 75, 100 and 125 after parturition.

Temperature humidity index (THI)

It was calculated by recording the dry and wet bulb temperature daily in the morning and evening during the experiment period (NRC, 1971).

$$THI=0.72\ (Tdb + Twb) + 40.6$$

Where, Tdb = dry bulb temperature,
Twb = wet bulb temperature

Quantitative competitive inhibition enzyme immunoassay for the determination of plasma Cortisol

Cortisol was estimated in plasma samples using cortisol ELISA kit (DiaMetra SRL, Italy). The standard curve range was 0 ng/ml to 500 ng/ml and sensitivity range was 2.42 ng/ml.

Quantitative competitive inhibition enzyme immunoassay for the determination of plasma Triiodothyronine (T_3) total

Triiodothyronine (T_3) total was estimated in plasma samples using Triiodothyronine (T_3) total ELISA kit (AB Diagnostic Systems GmbH, Germany). The standard curve range was 0 ng/ml to 9 ng/ml and sensitivity range = 0.20 ng/ml

Quantitative competitive inhibition enzyme immunoassay for the determination of plasma thyroxine (T_4) total

Thyroxine (T_4) total was estimated in plasma samples using thyroxine (T_4) total ELISA kit (AB Diagnostic Systems GmbH, Germany). The standard curve range was 0 nmol/L to 400 nmol/L and sensitivity range = 5.00 nmol/L.

Quantitative competitive inhibition enzyme immunoassay for the determination of plasma progesterone

Progesterone was estimated in plasma samples using 17α-Hydroxyprogesterone ELISA kit (Diagnostics Biochem, Canada). The standard curve range was 0 ng/ml to 20 ng/ml and sensitivity range = 0.11 ng/ml.

Quantitative sandwich enzyme immunoassay for the determination of plasma estrogen

Estrogen was estimated in plasma samples using bovine estrogen ELISA kit (KINESISDx, Los Angeles, USA). The standard curve range was 15 pg/ml to 240 pg/ml and sensitivity range was 1.362 pg/ml.

Statistical analysis

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

RESULTS AND DISCUSSION

Temperature Humidity Index (THI)

The average THI values recorded during the month of July, August, September, and

October were 79.41±0.42, 77.80±0.28, 77.65±0.29, 74.98±0.32. A substantial THI was observed, which lead to heat stress in experimental buffaloes during July, August, September, and October months, as it surpassed the upper critical limit (72 THI units) for buffaloes. The mean temperature humidity index during the experimental period has been presented in Figure 1.

Plasma cortisol

On day 7, 25, 75, 100 and 125 postpartum, the mean concentration of plasma cortisol showed (P>0.05) non-significant difference between all the groups. On day 50 postpartum the mean value of plasma cortisol of T1 Group (5.38±0.89 ng/ml) was maximum followed by T2 Group (4.15±0.38 ng/ml) and T3 Group had minimum plasma cortisol mean value (2.76±0.62 ng/ml) which differed (P<0.05) significantly among groups (Table 1).

The overall mean value of plasma cortisol of T1 Group (4.38±0.35 ng/ml) was maximum followed by T2 Group (3.85±0.23 ng/ml) and T3 Group (3.34±0.28 ng/ml) had minimum plasma cortisol mean value which differed (P<0.05) significantly between groups (Table 1 and Figure 2).

Present results were in agreement with Raheja *et al*. (2019), they concluded that the overall mean plasma cortisol concentrations were significantly (P<0.05) lower in cows of betaine treated group (4.91±0.68 ng/ml) as compared to Control group (6.89±0.49 ng/ml) in Karan Fries cows during hot-humid season. Deshpande *et al.* (2020) reported that the mean value of cortisol levels in Control group (7.18±0.45 ng/ml) was higher (P<0.05) than Treatment group fed betaine 25 g/animal/day (04.92±0.48 ng/ml) in buffalo heifers during hot humid season. Lakhani *et al.* (2019) reported numerically higher values of cortisol in control (10.36±0.16 ng/ml) than betaine Supplemented group (9.82±0.25 ng/ml) of Karan Fries heifers during hot humid season. In contrary to present findings, Zhang *et al*. (2014) reported that betaine supplementation 0, 10, 15, 20 g/day in diet exhibited non-significant effects on plasma cortisol of dairy cows during heat stress.

During heat and metabolic (lactation) stress cortisol is released from adrenal cortex. Cortisol induces physiological adaptations that enable animals to bear stressful conditions (Christison and Johnson, 1972). In the liver, the catabolism of betaine through the enzyme betaine homocysteine methyl-transferase (BHMT) induces the transmethylation of homocysteine to methionine. It has been consistently demonstrated that ingesting betaine lowers plasma homocysteine levels (Fridman *et al*., 2012). Increased homocysteine caused by stress is a stimulus for enhanced corticosteroid secretion, or in order to activate BHMT and lower homocysteine levels, corticosteroid secretion increases. Betaine may contribute to the maintenance of decreased resting cortisol concentrations by lowering plasma homocysteine.

Plasma Triiodo-thyronine (T_3)

On Day 7, 25, 50, 75, 100 and 125 postpartum, the mean concentration of plasma triiodo-thyronine (T_3) showed (P>0.05) non-significant difference between all the groups. The overall mean value of plasma triiodo-thyronine of T2 Group (3.20±0.20 ng/ml) was maximum followed by T3 (3.03±0.22 ng/ml) Group and T1 Group (2.76±0.14 ng/ml) had minimum mean value of plasma triiodo-thyronine which differed (P>0.05) non-significantly between groups (Table 2 and Figure 3).

The present results were in agreement with Zhang *et al*. (2014), they reported non-significant difference in serum level of triiodothyronine ($P>0.05$) in betaine fed cows during heat stress. Similarly, Mishra *et al*. (2019) reported that the serum concentrations of T_3 hormone (ng/ml) differed non-significantly ($P>0.05$) on 114 day post insemination in sows supplemented with betaine.

Plasma Thyroxine total (T_4)

On Day 7, 25, 50, 75, 100 and 125 postpartum, the mean concentration of plasma thyroxine total (T_4) showed ($P>0.05$) non-significant difference between all the groups. The overall mean value of plasma thyroxine in T2 Group (52.06±1.66 ng/ml) was maximum followed by T3 (51.43±1.23 ng/ml) Group and T1 Group (50.05±1.65 ng/ml) had minimum mean value of plasma thyroxine which differed non-significantly ($P>0.05$) between the groups (Table 3 and Figure 4).

The present findings were in agreement with Zhang *et al*. (2014), they reported non-significant difference in serum level of tetraiodothyronine (T_4) ($P>0.05$) in betaine fed cows during heat stress. Mishra *et al*. (2019) reported that the serum concentrations of T_4 hormone (ng/ml) differed non-significantly ($P>0.05$) on 114-day post insemination in sows supplemented with betaine.

The drop in serum thyroid hormone levels during postpartum period is likely a result of both the mammary gland's high demand for these hormones and the lower hormone secretion rate caused by an energy deficit. Decreased serum concentrations of thyroid hormones have been narrated as an adaptation to negative energy balance (Tiiratz, 1997). Betaine reduces negative energy balance by providing the methyl group and influences many key functions in the body such as growth, liver health and lactation. Betaine lowers the maintenance demand and metabolic heat production of growing pigs (Schrama *et al*., 2003). This might be the reason for slight and insignificant increase in thyroxine hormone in betaine treated T2 Group followed by T3 Group as compared to Control group.

Plasma progesterone

On Day 7 the levels of plasma progesterone were 0.75±0.02, 0.72±0.03 and 0.70±0.16 ng/ml in T1, T2 and T3 Groups respectively because of non-ovulatory phase post-partum in buffaloes (Table 4). On Day 25 the levels of plasma progesterone (1.48±0.12, 1.65±0.08, 1.79±0.13 ng/ml) were higher than day 7 concentration in all groups which may be due to the first ovulation and development of first corpus luteum (CL) after calving. The first ovulation is generally silent i.e., no behavioral estrus in both dairy and beef cows (Crowe *et al*., 2008).

The overall mean value of plasma progesterone of T3 Group (2.55±0.47 ng/ml) was maximum followed by T2 Group (2.44±0.47 ng/ml) and T1 Group (2.30±0.44 ng/ml) had minimum mean value of plasma progesterone which differed non-significantly ($P>0.05$) between groups (Figure 5). On Day 7, 25, 50, 75, 100 and 125 postpartum, the mean concentration of plasma progesterone showed ($P>0.05$) non-significant difference between all the groups (Table 4). However, the levels of plasma progesterone were higher in the buffaloes of betaine supplemented groups (T2 and T3) as compared to Control group (T1) which may be due to effect of lactational stress and negative energy balance in Control group. Betaine supplementations play important in improving reproduction in dairy animals particularly during stressful situation of hot-humid season.

Present results were in agreement with the

Table 1. Mean plasma cortisol (ng/ml) in postpartum buffaloes at various interval.

Days	Treatment		
	T1	T2	T3
Day 7	4.08±0.94	4.09±0.65	4.07±0.72
Day 25	3.82±0.71	3.51±0.54	3.17±1.10
Day 50	5.38^{A}±0.89	4.15^{AB}±0.38	2.76^{B}±0.62
Day 75	4.17±0.93	3.09±0.47	3.15±0.73
Day 100	3.98±0.98	3.68±0.70	3.44±0.53
Day 125	4.86±0.90	4.56±0.62	3.44±0.38
Average	4.38^{A}±0.35	3.85^{AB}±0.23	3.34^{B}±0.28

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

Table 2. Mean plasma triiodothyronine total (ng/ml) in postpartum buffaloes at various interval.

Days	Treatment		
	T1	T2	T3
Day 7	2.90±0.41	2.60±0.60	2.84±0.55
Day 25	2.67±0.27	3.26±0.63	2.81±0.76
Day 50	2.78±0.46	3.73±0.39	3.11±0.65
Day 75	2.75±0.30	3.89±0.33	2.87±0.46
Day 100	2.75±0.38	2.93±0.49	3.29±0.50
Day 125	2.72±0.43	2.82±0.36	3.27±0.51

Table 3. Mean plasma thyroxine total (ng/ml) in postpartum buffaloes at various interval.

Days	Treatment		
	T1	T2	T3
Day 7	51.96±3.59	51.98±4.21	51.99±1.69
Day 25	45.73±1.01	47.94±5.90	49.81±1.47
Day 50	49.96±5.06	53.92±4.19	50.19±2.18
Day 75	49.69±3.82	53.98±3.04	50.58±2.68
Day 100	51.67±5.61	53.03±5.29	54.22±5.35
Day 125	51.29±4.90	51.54±1.75	51.77±3.88

Table 4. Mean plasma progesterone (ng/ml) in postpartum buffaloes at various interval.

Days	**Treatment**		
	T1	**T2**	**T3**
Day 7	0.75±0.02	0.72±0.03	0.70±0.16
Day 25	1.48±0.12	1.65±0.08	1.79±0.13
Day 50	2.19±0.37	2.34±0.39	2.63±0.30
Day 75	2.64±0.27	2.75±0.13	2.82±0.32
Day 100	2.93±0.12	3.31±0.22	3.47±0.49
Day 125	3.82±0.48	3.88±0.30	3.90±0.45

Table 5. Mean plasma estrogen (pg/ml) in postpartum buffaloes at various interval.

Days	**Treatment**		
	T1	**T2**	**T3**
Day 7	41.05±2.82	41.06±5.65	41.29± 5.16
Day 25	32.56±2.51	33.84±4.84	36.83± 4.62
Day 50	25.13±3.16	28.56±3.56	29.76± 4.17
Day 75	30.61±2.44	32.77±3.16	33.84± 3.37
Day 100	25.88±2.60	26.04±2.53	28.71± 3.15
Day 125	16.85±1.70	17.65±1.36	18.27± 0.94

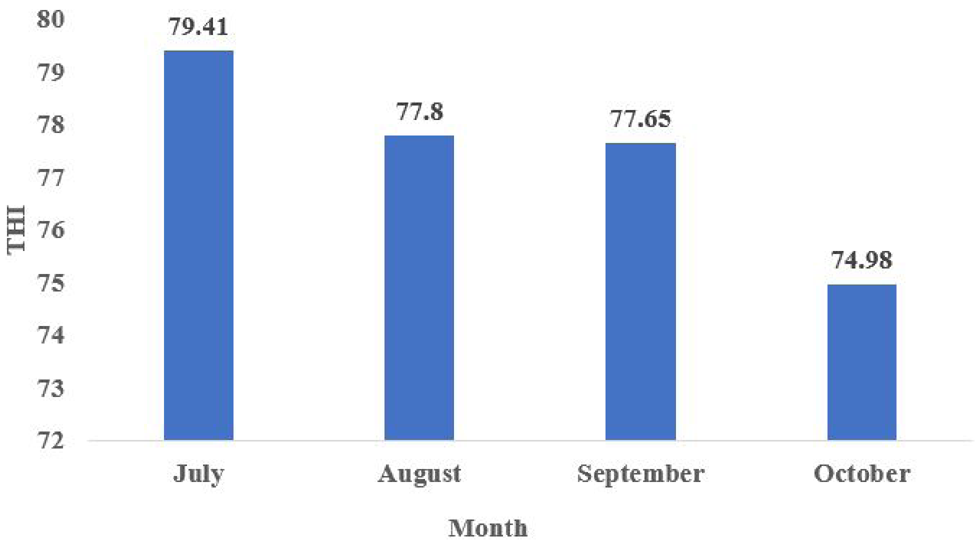

Figure 1. Monthly temperature humidity index (THI) during the experimental period.

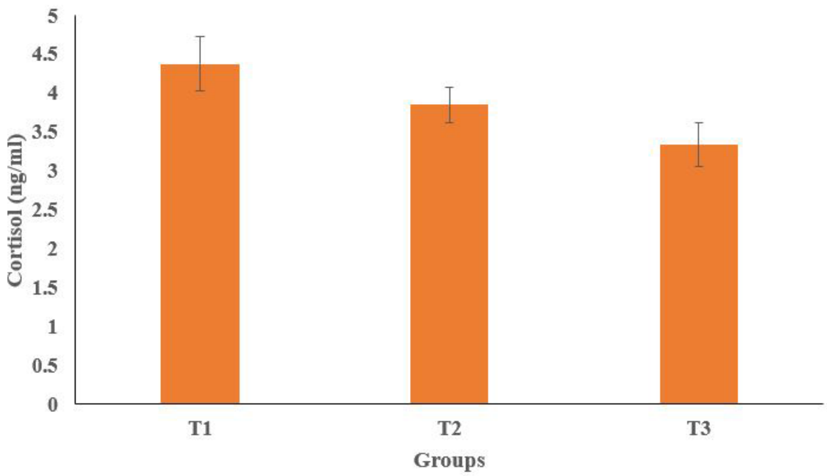

Figure 2. Effect of betaine on overall mean plasma cortisol (ng/ml) in postpartum Murrah buffaloes.

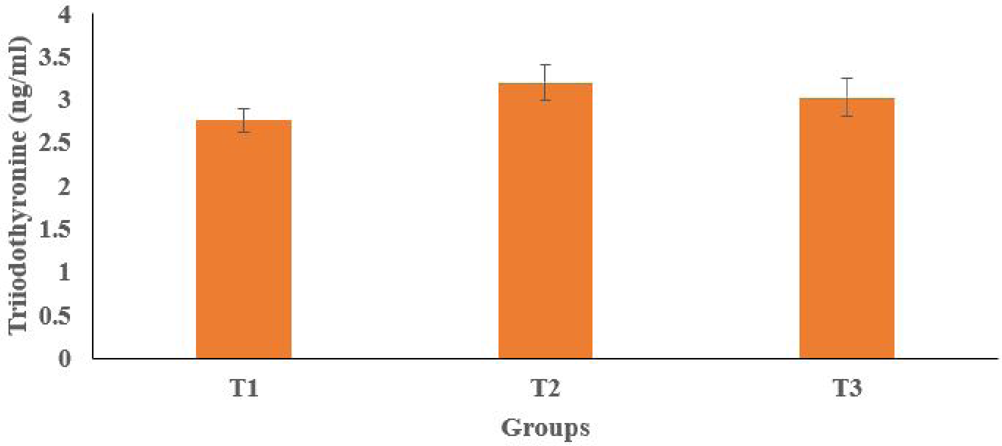

Figure 3. Effect of betaine on overall mean plasma triiodothyronine total (ng/ml) in postpartum Murrah buffaloes.

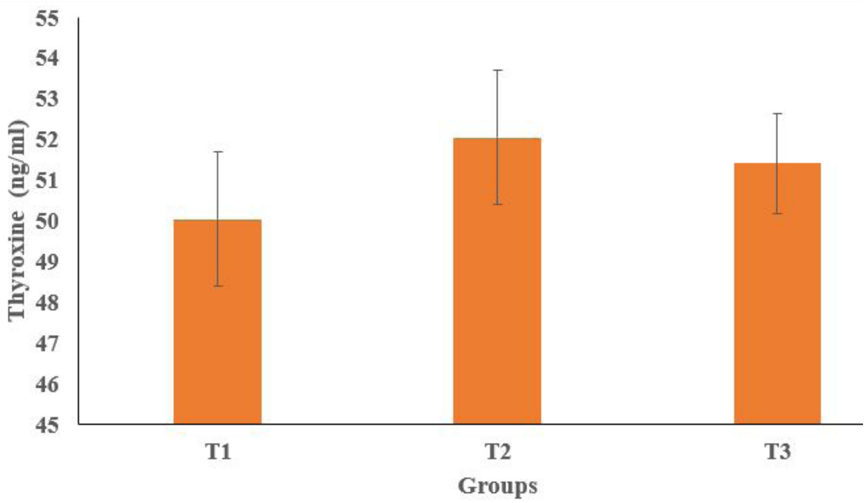

Figure 4. Effect of betaine on overall mean plasma thyroxine total (ng/ml) in postpartum Murrah buffaloes.

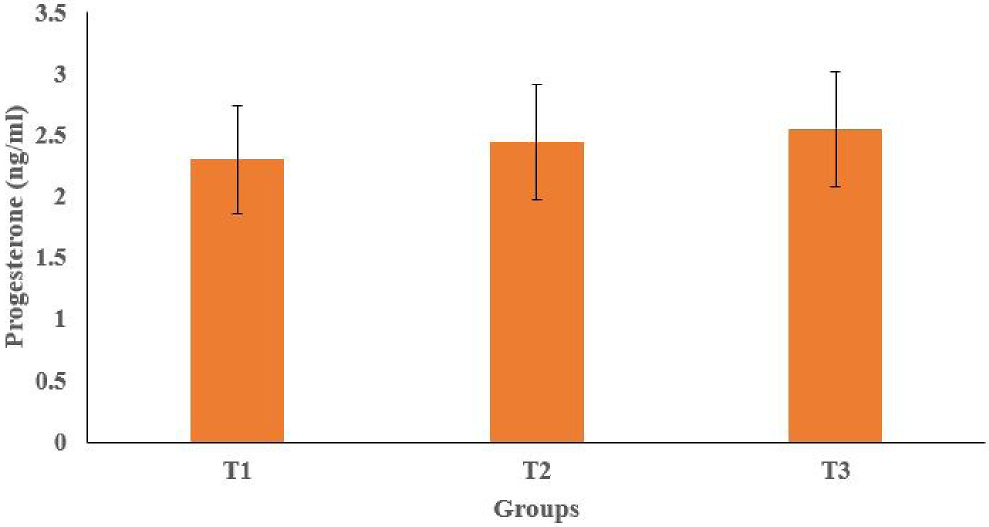

Figure 5. Effect of betaine on overall mean plasma progesterone (ng/ml) in postpartum Murrah buffaloes.

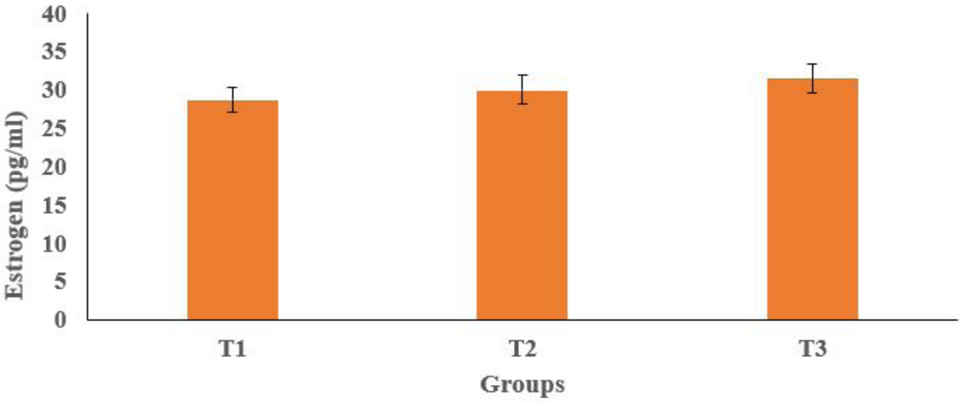

Figure 6. Effect of betaine on overall mean plasma estrogen (pg/ml) in postpartum Murrah buffaloes.

Acosta *et al*. (2017), they reported that choline and methionine supplementation during the transition period did not affect plasma and follicular fluid progesterone concentrations among treatment groups in multiparous Holstein cows.

Plasma estrogen

The plasma estrogen level of buffaloes was 41.05±2.82, 41.06±5.65 and 41.29±5.16 pg/ml in T1, T2 and T3 Groups on day 7 postpartum. On Day 25 the levels of plasma estrogen were lower than Day 7 postpartum mean values in all the groups (Table 5). Cyclic postpartum buffaloes may have two, three or four follicular waves during the oestrus cycles that occurs during the puerperal period. Eissa *et al*. (1995) reported maximum concentrations of estradiol-17β was 82.8±3.6 pg/ml at the day of parturition in buffalo cows. The results pertaining to plasma estrogen concentration of buffaloes in present investigation were within physiological range of 5 to 110 pg/ml (Savio *et al*., 1990), as also reported by Hussein *et al*., 2013 (48.8±15.39 pg/ml) and Mondal *et al*., 2010 (22.48±0.32 pg/ml) respectively in postpartum buffaloes.

The overall mean value of plasma estrogen of T3 Group (31.45±1.89 pg/ml) was maximum followed by T2 Group (29.99±1.89 pg/ml) and T1 Group (28.68±1.59 pg/ml) had minimum mean value of plasma estrogen which differed (P>0.05) non-significantly between groups (Figure 6). On Day 7, 25, 50, 75, 100 and 125 postpartum, the mean concentration of plasma estrogen showed (P>0.05) non-significant difference among all the groups (Table 5). However, betaine fed i.e., T2 and T3 Group had higher plasma estrogen as compared to control (Figure 6), it might be due to the effect of betaine, which reduces heat stress and activate hypothalmo-pituitary and gonadal axis after parturition and lactation period.

Present results were in agreement with Acosta *et al*. (2017), they reported that choline and methionine supplementation during the transition period did not affect plasma and follicular fluid estradiol concentrations among treatment groups in multiparous Holstein cows.

CONCLUSION

Betaine acted as a chaperon and decrease the susceptibility of animals to stress by reducing the concentration of cortisol in betaine supplemented buffaloes than control group. Betaine supplementation slightly improved postpartum thyroid, estrogen and progesterone profile it might be due to the effect of betaine, which reduces metabolic stress and activate hypothalmo-pituitary and gonadal axis after parturition and lactation period during heat stress and helps in early postpartum commencement of cyclicity in Murrah buffaloes.

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