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Impact of betaine diet on haematological parameters in postpartum Murrah buffaloes

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Abstract

Metabolic stress associated with postpartum period negatively impact the health status of animal. Betaine is a growth promoting nutritional additive widely used in livestock. Betaine contains three methyl groups and thus acts as a methyl donor in metabolism. Methyl donation is needed for DNA methylation, prevention of oxidative stress, prevention of apoptosis, energy metabolism and protein synthesis. The present study was carried out to investigate the effect of betaine on haematological parameters in postpartum lactating Murrah buffaloes. Eighteen postpartum Murrah buffaloes were randomly categorized into three groups for the experiment. T1 group was kept as control. T2, and T3 group was supplemented with betaine @ 50 g/animal/day and 100 g/animal/day respectively. Betaine was supplemented from day 5 postpartum and was continued upto 4 months postpartum. The mean values of TEC, Hb, PCV, MCV and MCHC differed non-significantly between all the treatment groups. The overall mean value of total leukocyte count of T1 group was higher than T2 and T3 group which differed ($p < 0.05$) significantly between groups.

Keywords: betaine, erythrocyte, buffalo, leukocyte, PCV

Introduction

India has the world's largest livestock population and having 57% of the world's buffalo population. Buffalo is the main milk producing animal contributing >50 % of the total milk production in India. During the transition and lactation period, buffaloes undergo large metabolic adaptations in glucose, fatty acid and mineral metabolism to support lactation and avoid metabolic dysfunction. Hematological and serum biochemical reference values are used to diagnose various systemic diseases in animals.

Reproductive efficiency and productivity loss during lactation period can be minimized by providing strategic feed supplement like betaine. The main physiological role of betaine is a methyl donor and an osmolyte. Betaine acts as an osmolyte to maintain the cellular water and ion balance to improve the capacity against heat stress. Betaine helps various intestinal microbes against osmotic variations and thus improves microbial fermentation activity. Betaine acts as molecular chaperone, as it repairs denatured proteins (Roth *et al.*, 2012) [5]. Dietary supplementation of betaine positively affects nutrient digestibility and health status of animal.

Materials and Methods

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.). 18 postpartum lactating Murrah buffaloes of 3rd to 5th parity were randomly divided into three groups. T1 group was kept as 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.

Experimental animals were fed according to their body weight and production (ICAR Feeding Standard, 2013) [2]. All the animals were offered identical basal ration consisting of green fodder, wheat straw and concentrate. The water was kept available to animals round the clock. Betaine (Betaine HCl, feed grade) supplementation was started day 5 postpartum and was continued up to 4 months postpartum.

For analysis of haematological parameters 5ml of blood sample was collected from animal on 7th, 25th, 50th, 75th, 100th and 125th day postpartum from the external jugular vein. Total erythrocyte count (TEC) ($10^6/\mu\text{l}$), Total leukocyte count (TLC) ($10^3/\mu\text{l}$), Hemoglobin (Hb) (g%), Packed cell volume (PCV) (%), Mean corpuscular volume (MCV) (fl) and Mean corpuscular hemoglobin concentration (MCHC) (g/dl) were analysed by using blood autoanalyzer.

Results and Discussion

Total Erythrocyte Count (TEC)

The mean value of TEC in T3 group was 5.15 ± 0.45 ($10^6/\mu\text{l}$) on day 7 postpartum which increases to 6.11 ± 0.19 ($10^6/\mu\text{l}$) on day 75 postpartum after betaine supplementation. Due to metabolic stress, there is reduction in red blood cell count and haemoglobin in animals causes iron deficiency anaemia. Betaine supplementation in diet may increase erythrocyte count in animals. Betaine as a methyl donor may contribute to methionine biosynthesis which required for normal erythropoiesis. Folate plays a vital role in the methionine cycle. It is involved as 5-methyl tetrahydrofolate methionine

in the methylation process where the methyl group is transferred to homocysteine to form methionine in the presence of methionine synthase enzyme.

The mean values of total erythrocyte count differed non-significantly between all the treatment groups. The overall mean values of total erythrocyte count of T1, T2 and T3 group were 5.63 ± 0.14 , 5.64 ± 0.07 and 5.68 ± 0.12 ($10^6/\mu\text{l}$) which differed non-significantly ($p > 0.05$) between treatment (Table 01 and Figure 01).

Present reports are in agreement with Abd El-Moniem *et al.* (2016) [1], they reported that mean values of RBCs differed significantly in all supplemented rabbits compared to those in the control one. Similarly, Mishra *et al.* (2019) [3] studied that group T0 was fed with basal diet (control) and betaine was supplemented in group T1 and T2 @ 3 g/kg basal diet during late pregnancy (day 76 onwards till parturition) and throughout the length of gestation, respectively. There was non-significant effect ($p > 0.05$) on RBC ($10^{12}/\text{l}$) following betaine supplementation in gestating sows.

Table 1: Mean total erythrocyte count ($10^6/\mu\text{l}$) in postpartum buffaloes at various interval

Treatment Days	T1	T2	T3
Day 7	5.49 ± 0.40	5.77 ± 0.16	5.15 ± 0.45
Day 25	5.46 ± 0.43	5.68 ± 0.22	5.38 ± 0.36
Day 50	5.54 ± 0.32	5.57 ± 0.20	5.93 ± 0.19
Day 75	5.81 ± 0.34	5.38 ± 0.19	6.11 ± 0.19
Day 75	5.70 ± 0.30	5.52 ± 0.14	5.70 ± 0.23
Day 125	5.76 ± 0.41	5.94 ± 0.10	5.79 ± 0.25
Average	5.63 ± 0.14	5.64 ± 0.07	5.68 ± 0.12

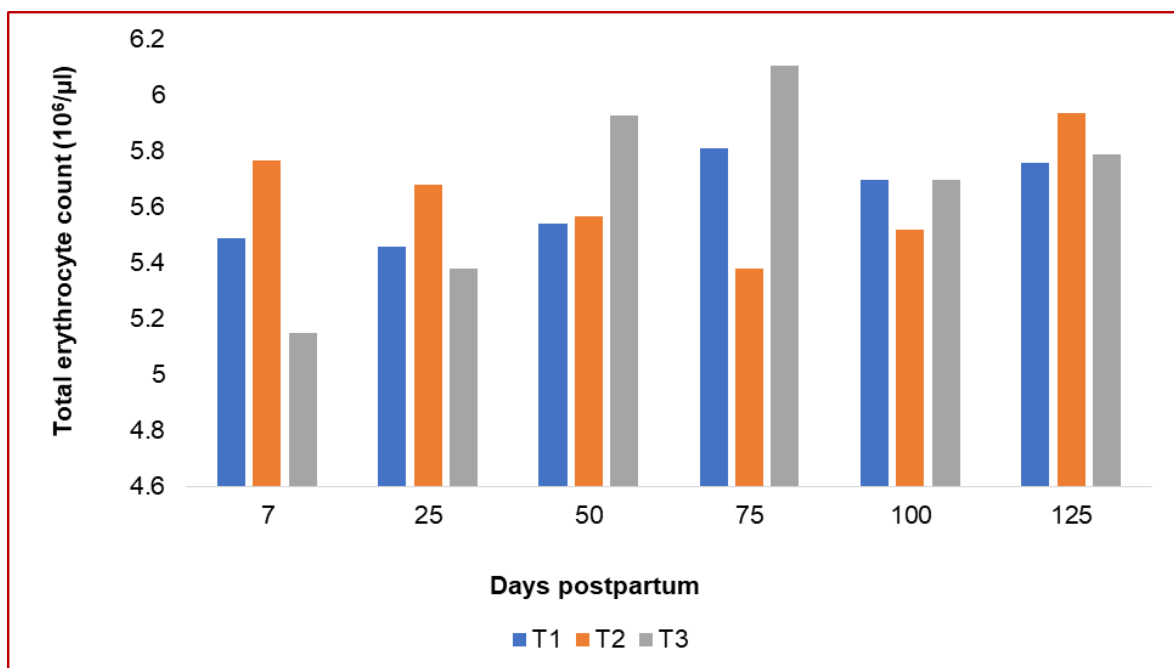


Fig 1: Mean total erythrocyte count ($10^6/\mu\text{l}$) in postpartum buffaloes at various interval

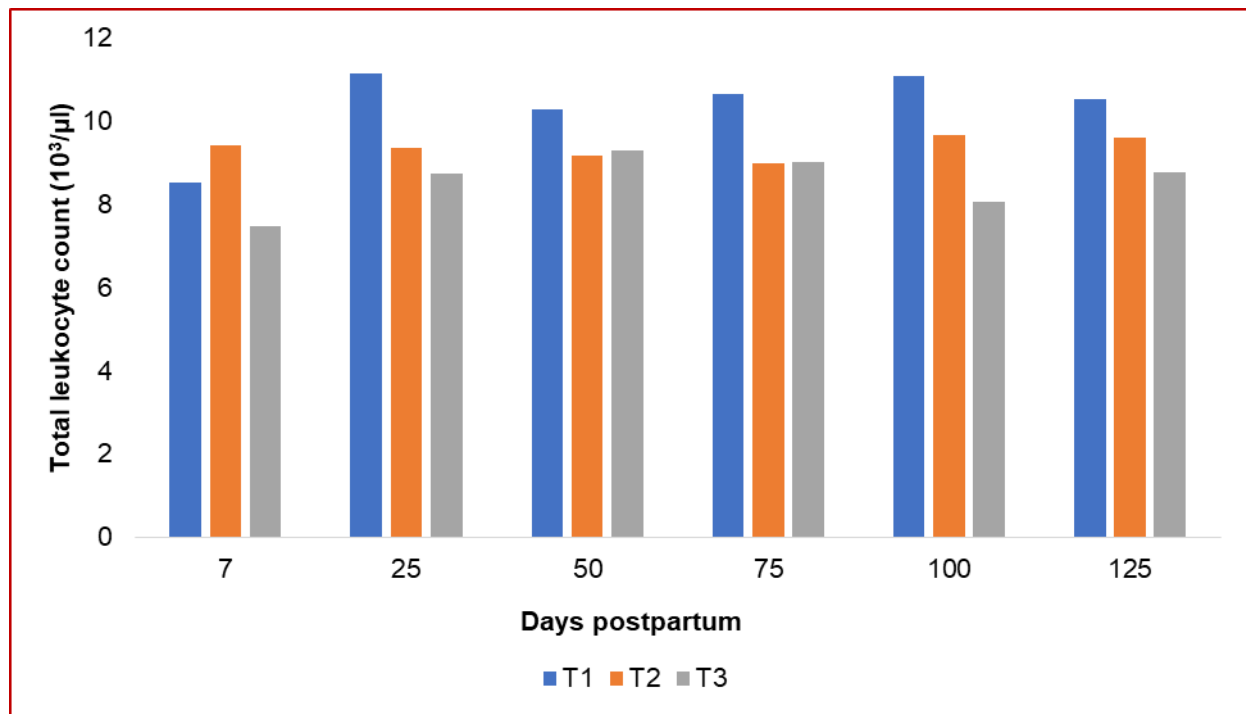


Fig 2: Mean total leukocyte count ($10^3/\mu\text{l}$) in postpartum buffaloes at various interval

Total Leukocyte Count (TLC)

The mean values of total leukocyte count of buffaloes differ non-significantly between all the groups on day 7, 25, 50, 100 and 125 postpartum. On day 75 postpartum the mean value of TLC (11.09 ± 0.43) of T1 was higher than T2 and T3 (9.68 ± 0.92 and 8.08 ± 0.84) group and differed ($p < 0.05$) significantly between groups. The overall mean value of total leukocyte count of T1 group (10.38 ± 0.33) ($10^3/\mu\text{l}$) was higher than T2 and T3 group (9.39 ± 0.31 and 8.58 ± 0.36) ($10^3/\mu\text{l}$) which differed ($p < 0.05$) significantly between groups. The mean value of TLC was 8.55 ± 1.04 ($10^3/\mu\text{l}$) on 7 postpartum which increases to 11.17 ± 0.95 and 11.09 ± 0.43 ($10^3/\mu\text{l}$) on 25 and 75 day postpartum in control group. Betaine has antioxidant and anti-inflammatory actions which decreases

TLC count in T2 and T3 group as compared to control (Table 02 and Figure 02).

The present results were in agreement with Nemmar *et al.* (2017) [4], they studied that water-pipe smoke (WPS) exposure for 2 weeks caused a slight but insignificant increase in WBC numbers as compared with the control group but betaine treatment reversed the WPS induced increase in WBC (slight reduction in WBC number) in mice.

In contrary to present report, Mishra *et al.* (2019b) reported that there was non-significant effect ($p > 0.05$) on WBC ($10^9/l$) following betaine supplementation in gestating sows. Whereas, Abd El-Moniem *et al.* (2016) [1] reported that Bet and Vit. C groups, both WBCs and lymphocytes values were significantly ($p < 0.01$) higher than in control one in rabbits.

Table 2: Mean total leukocyte count ($10^3/\mu\text{l}$) in postpartum buffaloes at various interval

Treatment Days	T1	T2	T3
Day 7	8.55 ± 1.04	9.45 ± 0.61	7.49 ± 0.90
Day 25	11.17 ± 0.95	9.37 ± 0.51	8.76 ± 0.89
Day 50	10.29 ± 0.55	9.18 ± 0.76	9.32 ± 0.93
Day 75	10.66 ± 0.58	9.00 ± 0.72	9.05 ± 0.99
Day 100	$11.09^A \pm 0.43$	$9.68^{AB} \pm 0.92$	$8.08^B \pm 0.84$
Day 125	10.54 ± 0.93	9.63 ± 1.18	8.80 ± 0.88
Average	$10.38^A \pm 0.33$	$9.39^B \pm 0.31$	$8.58^B \pm 0.36$

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

Haemoglobin (Hb)

The mean values of haemoglobin differed non-significantly between all the treatment groups. The overall mean values of haemoglobin of T1, T2 and T3 group were 11.54 ± 0.33 , 11.76 ± 0.20 and 11.84 ± 0.26 (g %) which differed non-significantly between treatment (Table 03 and Figure 03). Similar findings were reported by Abd El-Moniem *et al.*

(2016) [1], they concluded that the mean values of haemoglobin differed non-significantly in all groups (control, supplemented with 500 mg Vit. C (II), 200 mg Vit. E (III) or 750 mg Bet (IV) /kg diet for 8 weeks) in rabbits during heat stress. Mishra *et al.* (2019b) reported that there was non-significant effect ($p > 0.05$) on haemoglobin (g/dl) following betaine supplementation in gestating sows.

Table 3: Mean haemoglobin (g%) in postpartum buffaloes at various interval

Treatment Days	T1	T2	T3
Day 7	11.18± 0.85	11.43± 0.47	11.07± 0.95
Day 25	11.23± 0.93	11.75± 0.59	11.17± 0.84
Day 50	11.03± 0.56	11.53± 0.50	11.72± 0.65
Day 75	11.10± 0.58	11.20± 0.42	11.80± 0.72
Day 100	12.20± 0.83	11.68± 0.45	12.25± 0.56
Day 125	12.50± 1.07	12.98± 0.39	12.65± 0.58
Average	11.54± 0.33	11.76± 0.20	11.84± 0.26

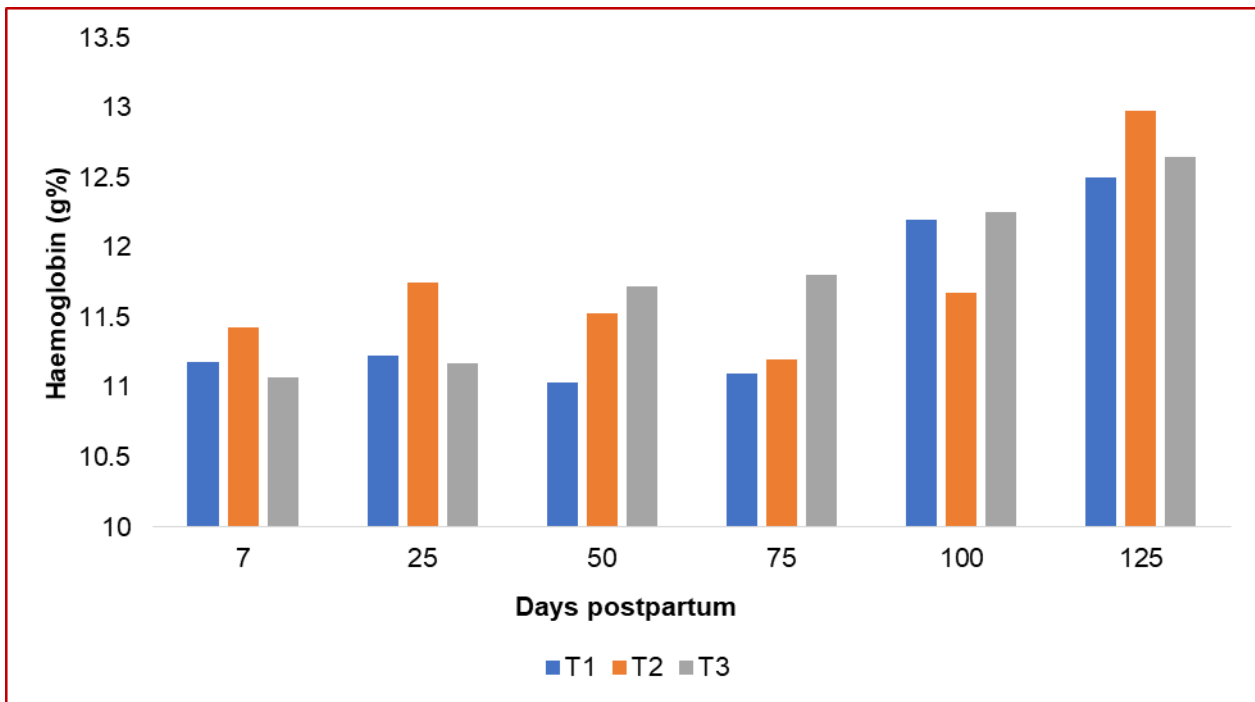


Fig 3: Mean haemoglobin (g%) in postpartum buffaloes at various interval

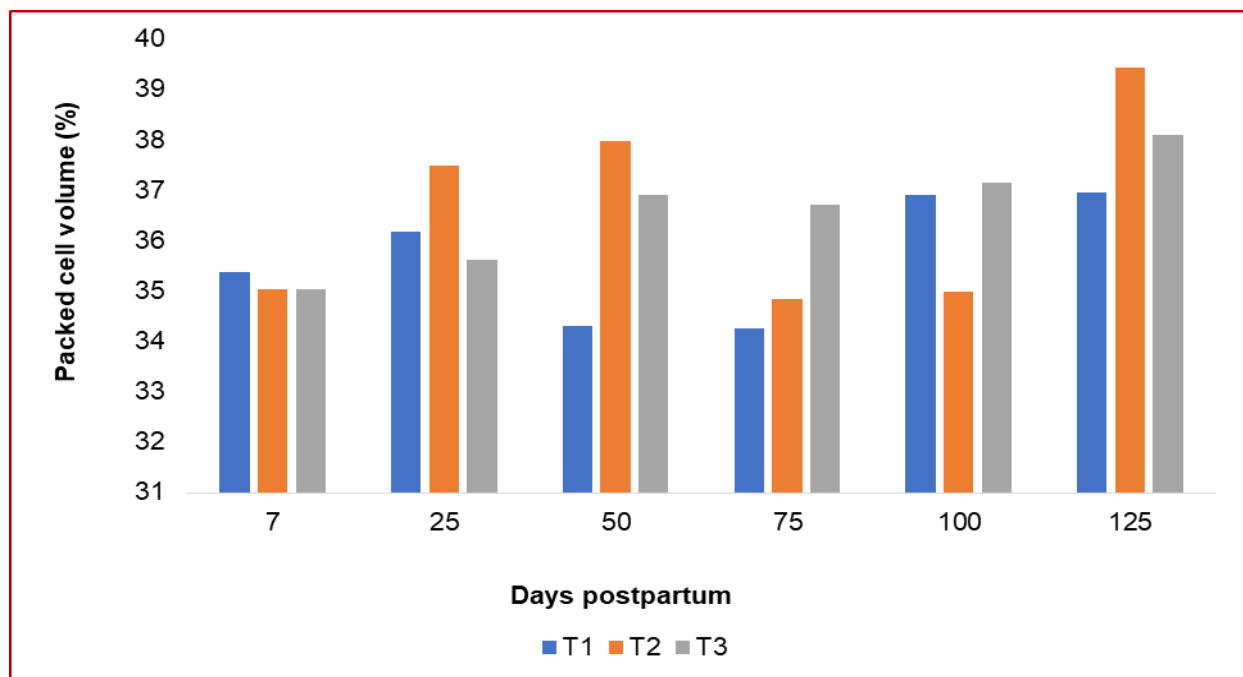


Fig 4: Mean packed cell volume (%) in postpartum buffaloes at various interval

Packed Cell Volume (PCV)

The mean values of packed cell volume differed non-significantly between all the treatment groups. The overall mean values of packed cell volume of T1, T2 and T3 group were 35.67±0.92, 36.64±0.67 and 36.60±0.92 (%) which

differed non-significantly between treatment (Table 04 and Figure 04). However, T2 and T3 group have numerically higher PCV as compared to control group. This might be due to the fact that betaine supplementation might increase the erythrocyte count in buffaloes.

Similar findings were reported by Mishra *et al.* (2019) [3], they studied that control group was fed with basal diet and betaine was supplemented in group T1 and T2 @ 3 g/kg basal diet during late pregnancy (day 76 onwards till parturition) and

throughout the length of gestation, respectively. There was non-significant effect ($p>0.05$) on PCV (%) following betaine supplementation in gestating sows.

Table 4: Mean packed cell volume (%) in postpartum buffaloes at various interval

Treatment Days	T1	T2	T3
Day 7	35.37± 2.68	35.05± 1.71	35.05± 3.04
Day 25	36.19± 2.81	37.50± 2.20	35.63± 2.75
Day 50	34.31± 1.57	37.98± 1.64	36.92± 2.29
Day 75	34.25± 1.57	34.84± 1.00	36.72± 2.56
Day 100	36.92± 2.11	35.00± 1.39	37.15± 1.65
Day 125	36.96± 3.11	39.45± 1.19	38.10± 1.79
Average	35.67± 0.92	36.64± 0.67	36.60± 0.92

Mean Corpuscular Volume (MCV)

The average values of mean corpuscular volume differ non-significantly between all the treatment groups. The overall average values of mean corpuscular volume of T1, T2 and T3 groups were 64.98±1.08, 65.44±0.99 and 65.68±1.05 (fl) which differed non-significantly between the treatment (Table 05 and Figure 05). However, T2 and T3 group have numerically higher mean corpuscular volume as compared to

control group. This might be due to the fact that betaine supplementation might increase the erythrocyte count in buffaloes.

Similar findings reported by Abd El-Moniem *et al.* (2016) [1], they concluded that the mean value of MCV differed non-significantly in all groups having control (I), supplemented with 500 mg Vit. C (II), 200 mg Vit. E (III) or 750 mg Bet (IV)/kg diet for 8 weeks in rabbits during heat stress.

Table 5: Average mean corpuscular volume (fl) in postpartum buffaloes at various interval

Treatment Days	T1	T2	T3
Day 7	67.63± 2.70	61.78± 3.21	69.02± 1.76
Day 25	67.40± 1.93	66.72± 2.97	67.51± 2.95
Day 50	66.16± 3.67	69.30± 1.64	63.40± 2.77
Day 75	59.95± 3.22	64.94± 2.10	61.39± 3.84
Day 100	65.00± 1.83	63.38± 1.73	65.74± 1.22
Day 125	63.71± 1.71	66.52± 2.17	66.99± 1.53
Average	64.98± 1.08	65.44± 0.99	65.68± 1.05

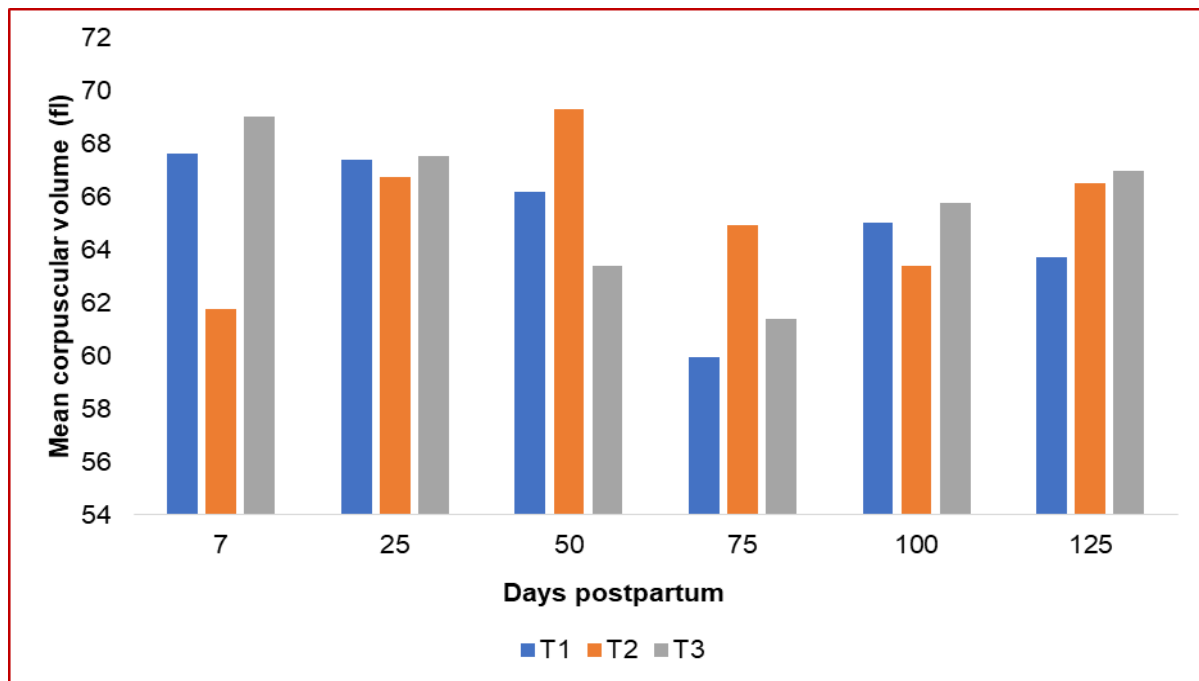


Fig 5: Average mean corpuscular volume (fl) in postpartum buffaloes at various interval

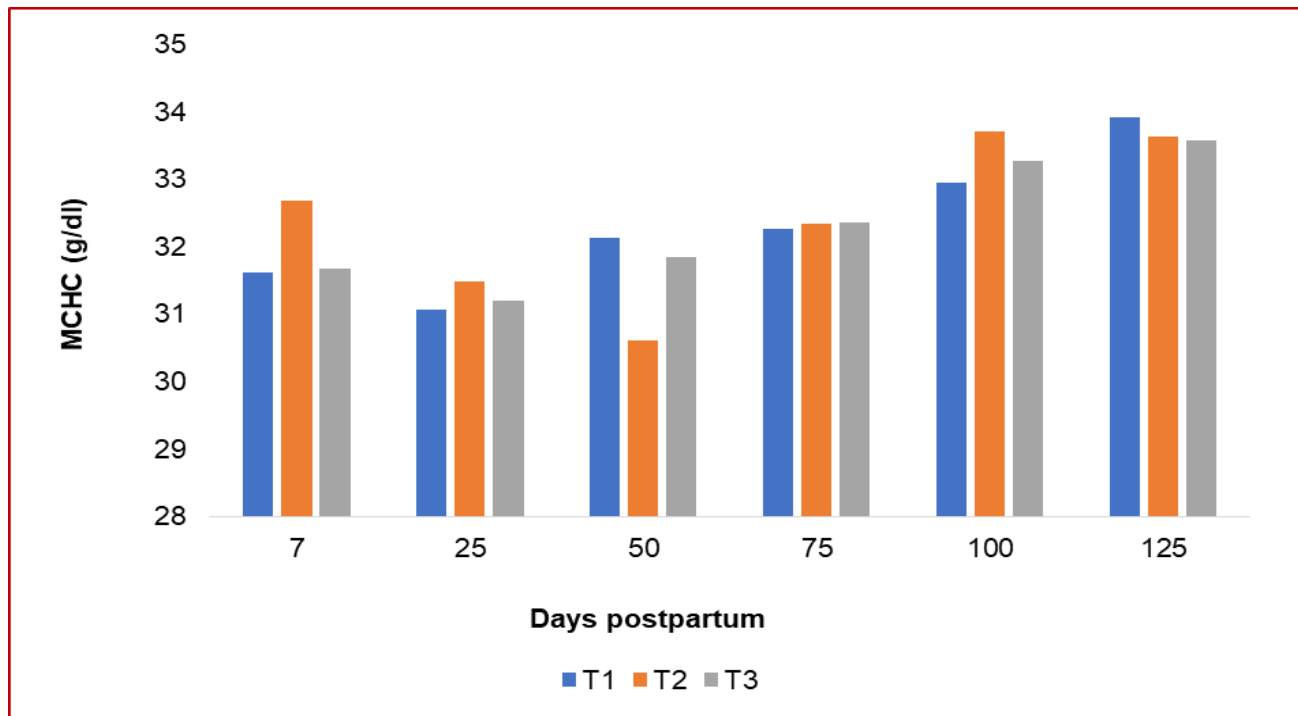
Mean Corpuscular Hemoglobin Concentration (MCHC)

The average values of mean corpuscular hemoglobin concentration differed non-significantly between all the treatment groups. The overall average of mean corpuscular hemoglobin concentration of T1, T2 and T3 group were 32.32±0.26, 32.41±0.49 and 32.32±0.22 (g/dl) which differed

non-significantly ($p>0.05$) between treatment (Table 06 and Figure 06). Similar findings were also reported by Abd El-Moniem *et al.* (2016) [1], they concluded that the mean value of MCHC was non-significant in betaine fed group and control groups in rabbits during heat stress.

Table 6: Average mean corpuscular hemoglobin concentration (g/dl) in postpartum buffaloes at various interval

Treatment Days	T1	T2	T3
Day 7	31.62± 0.42	32.69± 0.59	31.67± 0.52
Day 25	31.06± 0.74	31.48± 0.87	31.19± 0.33
Day 50	32.13± 0.54	30.61± 0.11	31.84± 0.51
Day 75	32.27± 0.55	32.34± 0.65	32.35± 0.51
Day 100	32.95± 0.67	33.71± 0.35	33.28± 0.47
Day 125	33.92± 0.36	33.63± 0.71	33.58± 0.17
Average	32.32± 0.26	32.41± 0.49	32.32± 0.22

**Fig 6:** Average mean corpuscular hemoglobin concentration (g/dl) in postpartum buffaloes at various interval

Conclusion

The present study was conducted to correlate the roles of hematological parameters in relation to estrus, reproductive stages in postpartum buffaloes. Total leukocyte count was higher in control group as compare to betaine supplemented Murrah buffaloes. Betaine supplementation did not influence TEC, PCV, Hb, MCV and MCHC in postpartum Murrah buffaloes.

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