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EFFECT OF RECOMBINANT BOVINE SOMATOTROPIN ON HEAMOTOLOGICAL VALUES IN KUNDHI BUFFALOES

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ABSTRACT

This study was performed to investigate the effect of recombinant bovine Somatotropin (rbST) treatment on some haemortological in primaparous Kundhi buffaloes. Sixteen primaparous Kundhi buffaloes were divided into two groups keeping 8 animals in each group. Group-I served as control and Group-II was treated with 250 mg of rbST fortnightly for 166 days of 1st lactation. The rbST treatment was started after 60 days of lactation. Blood samples were collected every week during pre-treatment (average 60 days) and post-treatment (average 166 days) of 1st lactation. The findings revealed a non significant variation in the erythrogram whereas, leucogram showed a leucocytosis accompanied by neutrophilia and lymphocytosis. They attributed the increase in total leukocytes and lymphocytes as positive response of immune system of buffaloes to rbST treatment. Haemotological values of Granulocytes (GR#), Lymphocytes (LY#), Mean corpuscular hemoglobin concentration (MCHC), Monocytes (MO#), Mean Platelet Volume (MPV), Platelets (P1t), Pct (%), Platelet distribution width (PDW), and White blood cells (WBC) in rbST treated and non-treated buffaloes. Mean values of granulocyte (GR#) was significantly decreased (P<0.01) during parturition and winter weather in rbST treated buffaloes as compare to control group. Mean values of Haemogglobin (Hb) was significantly declined (P<0.01) during parturition, lactation and dry period in rbST treated buffaloes as compare control group. Mean values of lymphocyte was significantly decreased (P<0.01) in rbST treated buffaloes during various physiological condition in rbST treated buffaloes as compare to control group. Mean values of MCV was significantly declined (P<0.05) during conception and parturition in buffaloes. Mean values of PCV was significantly (P<0.01) increased during open days, conception and winter in rbST treated buffaloes as compare to control group. Mean values of platelet distribution were significantly increased (P<0.01) during lactation, dry period, conception, summer and winter in rbST treated group as compare to control group. Red Blood cell values were significantly (P<0.01) increased during lactation and parturition in rbST treated buffaloes as compare to control group. Red blood distribution width (RDW) was significantly (P<0.05) increased after administration of rbST during summer as compare to control group. Whereas, rbST treatment did not significantly affected the values of RDW during other physiological conditions in buffaloes. White blood cells were significantly lower (P<0.01) during various physiological conditions in rbST treated buffaloes as compare to control group. In conclusion, application of rbST produced a non-significant variation in the blood profile in primaparous kundhi buffaloes. But erythrogram and leucogram values showed a leucocytosis accompanied by neutrophilia and lymphocytosis after rbST treatment. Haemotological values of both groups were affected during various physiological conditions and weather.

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INTRODUCTION

Recombinant Bovine Somatotropin (rbST) is first major livestock related product of biotechnology research which is used as a powerful tool to enhance cattle performance in terms of milk production in cows by Abdelrahman *et al.,* (2008), Prasad and Singh, (2010), Macrina *et. al.,* (2011) and Mellado *et. al.,* (2011), and in buffaloes by Helal and Lasheen, (2008), Khaliq and Rehman, (2010) Lim and Ahmed (2015) and

Kachiwal *et al.,* (2015). Mean volume injected have varied from 5 to 50 mg/day in cattle and buffaloes reported by Abdelrahman *et al.,* (2008), Prasad and Singh, (2010), Macrina *et. al.,* (2011), Mellado *et. al.,* (2011) and Kachiwal *et al.,* (2015). The use of rbST has been studied on pure breed cattle and crossbred animals by Azza *et al*., (2010) Collier and Bauman, (2014) and Kuhro *et al.,* (2016), Dilbar *et al*., (2014) and few studies are made on sheep by El-Gohary *et al.,* (2011), and El-Din *et al.,* (2009) and in goats by Sanjrani *et al*., (2016), Sallam *et al.,* (2005) and Baldi *et al.,* (2002). Hemato-biochemical parameters are very important indicators of the health condition and metabolic activity in lactating animals reported by Sobiech *et al.,* (2008) and Karapehlivan, *et al.,* (2007). Monitoring the blood profile in animals gives a clear picture of their nutritional and health status before the changes are visible on the animal reported by Antunovic *et al.,*(2009). Short and long term rbST treatment of cows revealed a non significant variation in the erythrogram whereas, leucogram also showed a non significant leucocytosis accompanied by neutrophilia and lymphocytosis by Abdelrahman *et al.,* (2010) and Sallam *et al.,* (2005). Haemotological tests are widely used for the diagnosis of serious animal diseases which can lead to economic losses in animals like milk. As limited information is available regarding the haemotological profile during various physiological statuses of primaparous Kundhi buffaloes in Pakistan, the aim of the present study was to investigate the effect of rbST treatment on some haemortological parameters in primaparous Kundhi buffaloes.

MATERIALS AND METHODS

The experiment was performed on sixteen Kundhi buffaloes (primaparous) of with mean age of 1464.75± 12.75 days and weighing 461 ± 35.24 kg to evaluate the effect of rbST on haemotological parameters for 166 days of 1st lactation. Buffaloes were purchased from surrounding markets of Tandojam and kept at Livestock Experimental Station, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam for this study. The herd was vaccinated against FMD and HS as pre schedule of vaccination and dewormed regularly during the experimental period. Balancing of rations was computed by the method of: National Research Council 1978 as described in the nutrient requirements of Dairy cattle, Academy of Sciences, National Research Council, Washington DC. Nutritive values of components of feed ingredients such as Fat, Crude Protein, TDN, Crude Fiber and Ash, were analyzed by the standard methods described in (AOAC, (2000). Buffaloes were housed in individual tied stalls with free access to water. Buffaloes were fed twice daily ad libitum with access to a total mixed ration consisting of 61% dry matter (Table-1). The data on daily feed intake and feed refused were weighed and recorded on the record book. Strict hygienic measures were followed during milking of buffaloes twice daily which was recorded on day to day basis. Sixteen Kundhi buffaloes were divided randomly in group-A and group-B, placing eight animals in each group (Table-2). Group-A was kept as control only on balance ration containing 16% crude protein (Table-1) and group-B was kept on balance ration with rbST 250 mg/buffalo/fortnightly treatment. Blood samples for hematological tests were collected from jugular vein in Marble bottom and heparinized vacutainer tubes between 14:30 hrs to 15:30 hrs on one day in each week and body weights were recorded. Samples were placed on ice immediately after collection, centrifuged within 2 h at 3,000 rpm for 30 minutes at 5 ^{0}C (Jouan GR 412 centrifuge, Winchester, VA) and plasma collected and stored in polypropylene tubes at -20 ^{0}C until analyzed. They were carried to the Postgraduate Research Laboratory in the Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, SAU Tandojam. Haemotological parameters: neutrophil granulocytes percentage (GR %), Neutrophil granulocytes (GR#), Hematocrit Percentage (Hct %), Hemoglobin (Hgb g/dL), Lymphocytes percentage (LY %), Lymphocytes (LY#), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Monocytes Percentage (MO %), Monocytes (MO#), Mean Platelet Volume (MPV), Platelets (P1t), Pct (%), Platelet distribution width (PDW), Red blood cells (RBC), Red blood cell distribution width (RDW %) and White blood cells (WBC) were analyzed using Beckman Coulter AcT Diff Hematology Analyzer (Beckman Coulter, Tokyo, Japan). Statistical package Mini Tab (version 16) was used for statistical analysis. All the values were expressed as mean ± standard error (SE). One way ANOVA was applied to compare various hematological parameters. These parameters were also calculated to determine the effect of rbST on the haemotolocal values in primaparous Kundhi buffaloes.

Table 1. Composition of balance protein energy rations

Ingredients	Balance protein energy ration
Barseem	8 kg
Wheat Straw	4 kg
Cotton Seed Cake	3.2 kg
Mustard Cake	0.2 kg
Moong Kutta	1.0 kg
Wheat Bran	0.8 kg
Maize Crushed	0.4 kg
Rice Polish	2.0 kg
Molasses	0.4 kg
Di-Calcium Phosphate / Limestone	0.05 kg
NUTRIENTS	
Dry Matter	61%
Crude Protein	16%
Total Digestible Nutrients	67%
Crude Fiber	19%
Calcium	0.78%
Phosphorus	0.66%

Table 2. Experimental design

Group A	Weight (kg)	Age (days)	Group B	Weight (kg)	Age (days)
Buffalo 1	509	1444	Buffalo 2	445	1460
Buffalo 8	440	1460	Buffalo 5	444	1488
Buffalo 9	448	1474	Buffalo 7	448	1460
Buffalo 11	442	1480	Buffalo 15	440	1450
Buffalo 3	432	1460	Buffalo 10	515	1480
Buffalo 4	440	1460	Buffalo 13	511	1470
Buffalo 6	524	1480	Buffalo 14	433	1460
Buffalo 12	453	1460	Buffalo 16	444	1450
Mean	461	1464.75		460	1464.75
SEM	35.03	12.37		33.03	13.65

RESULTS

Hematological parameters including Neutrophil granulocytes percentage (GR %), Neutrophil granulocytes (GR#), Hematocrit Percentage (Hct %), Hemoglobin (Hgb g/dL), Lymphocytes percentage (LY %), Lymphocytes (LY#), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Monocytes Percentage (MO %), Monocytes (MO#), Mean Platelet Volume (MPV), Platelets (P1t), Pct (%), Platelet distribution width (PDW), Red blood cells (RBC), Red blood cell distribution width (RDW %) and White blood

cells (WBC) were measured. The comparisons of these hematological parameters are shown in (Table-3 and 4).

Table 3.Pre-treatment Haemotological values of Primaparous Kundhi buffaloes

Pre-treatment	Group-A (Control)	Group-B (rbST)
	Mean ± SE	Mean ± SE
GR (%)	29.85 ± 12.04	27.05 ± 8.55
GR# (x10^3/uL)	2.75 ± 1.27	2.5 ± 0.98
Hct (%)	27.85 ± 4.18	27.25 ± 5.01
Hgb (g/dL)	9.75 ± 1.65	9.75 ± 1.37
LY (%)	34.3 ± 5.69	57.55 ± 6.88**
LY# (x10^3/uL)	5.35 ± 1.51	5.45 ± 1.11
MCH (pg)	18.4 ± 1.24	18.35 ± 1.58
MCHC (g/dL)	34.75 ± 2.49	34.2 ± 2.47
MCV (fL)	52.85 ± 1	53 ± 1.455
MO (%)	12.8 ± 6.06	14.35 ± 5.96
MO# (x10^3/uL)	1.2 ± 0.64	1.3 ± 0.59
MPV (fL)	7.25 ± 1.29	7.25 ± 1.06
P1t (x10^3/uL)	147.85 ± 76.68	144.7 ± 77.91
Pct (%)	0.1 ± 0.07	0.1 ± 0.06
PDW (%)	16.55 ± 0.98	16.6 ± 1.12
RBC (x10^6/uL)	5.3 ± 0.81	5.35 ± 0.72
RDW (%)	19.45 ± 1.2	18.65 ± 1.93
WBC (x10^3/uL)	9.3 ± 2.02	10.1 ± 4.07

Table 4. Effect of RbST treatment on Haemotological parameters in Primaparous Kundhi buffaloes

Post-treatment	Group-A (Control)	Group-B (rbST)
	Mean ± SE	Mean ± SE
GR (%)	27.05 ± 8.47	25.6 ± 9.05
GR# (x10^3/uL)	1.8 ± 0.65	1.45 ± 0.58
Hct (%)	23.9 ± 4.6	23.3 ± 4.23
Hgb (g/dL)	7.5 ± 1.66	7 ± 1.6
LY (%)	58.05 ± 6.37	55.8 ± 8.42
LY# (x10^3/uL)	3.9 ± 1.19	3.35 ± 1.24
MCH (pg)	17.7 ± 3.88	17.35 ± 3.65
MCHC (g/dL)	32.1 ± 5.72	32 ± 5.32
MCV (fL)	53.3 ± 1.75	52.75 ± 2.08
MO (%)	15.3 ± 4.93	18.65 ± 5.79
MO# x10^3/uL)	1.5 ± 2.76	1.35 ± 1.45
MPV (fL)	6.55 ± 1.34	6.4 ± 1.25
P1t (x10^3/uL)	153.15 ± 40.05	162.7 ± 49.65
Pct (%)	0.1 ± 0.06	0.1 ± 0.07
PDW (%)	16.3 ± 1.12	16.55 ± 0.95
RBC (x10^6/uL)	4.4 ± 0.87	4.25 ± 0.95
RDW (%)	18.3 ± 1.77	19.25 ± 2.43
WBC x10^3/uL)	6.65 ± 1.7	6 ± 1.815

** = Values were significantly low (P<0.01) than the corresponding control

Haemotological parameters were generally unaffected by treatment. All analyzed blood components were within normal physiological ranges and were not affected by rbST treatment (Table-3-4). Overall mean concentrations during pre-treatment and post-treatment rbST are shown in (Table-3-4). No abnormal health characteristics were noted during pre or post rbST treatment. Mean values of granulocyte (GR#) was significantly decreased (P<0.01) during parturition and withter in rbST treated buffaloes as compare to control group (Fig-1).

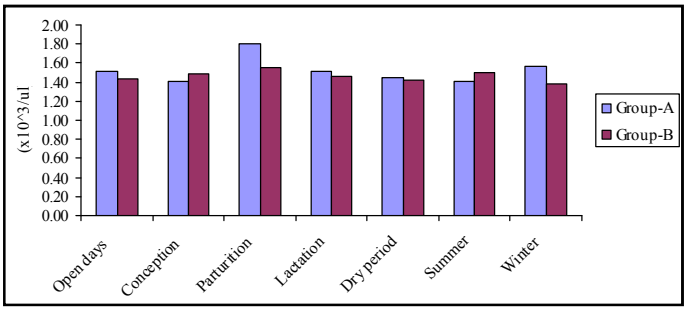

Figure 1. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of granulocyte (GR#) in Kundhi buffaloes

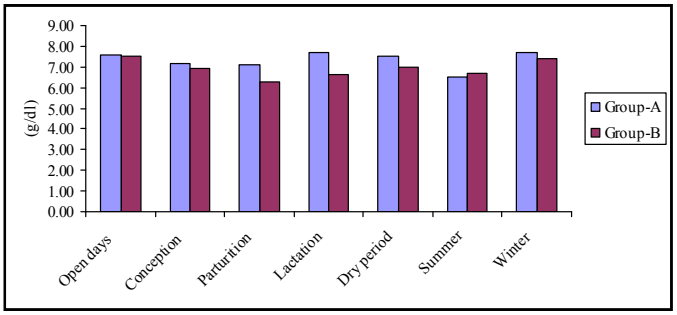

Figure 2. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of heamoglobin (Hct#) in Kundhi buffaloes

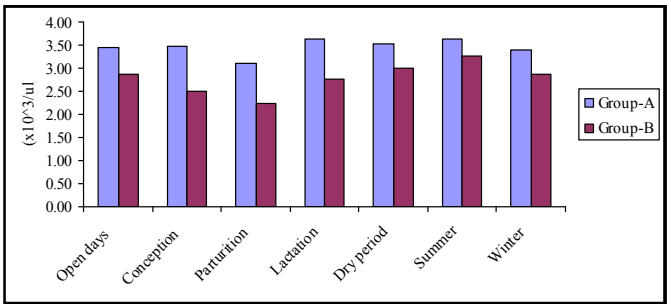

Figure 3. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of Lymphocytes (LY#) in Kundhi buffaloes

Mean values of Haemogglobin was significantly declined (P<0.01) during parturition, lactation and dry period in rbST treated buffaloes as compare control group (Fig-2). Mean values of Lymphocyte were significantly decreased (P<0.01) in rbST treated buffaloes during various physiological condition in rbST treated buffaloes as compare to control group (Fig-3). Mean values of carpulses volume (MCV) was significantly declined (P<0.05) during conception and parturition in buffaloes. Mean values of Packet cell volume was significantly (P<0.01) were increased during open days, conception and winter in rbST treated buffaloes as compare to control group (Fig-10). Mean values of platelet distribution were significantly increased (P<0.01) during lactation, dry period, conception, summer and winter in rbST treated group as compare to control group (Fig-11). Red Blood cell (RBC) values were significantly (P<0.01) increased during lactation and parturition in rbST treated buffaloes as compare to control group (Fig-12). Red blood distribution width (RDW) was significantly (P<0.05) increased after administration of rbST during summer as compare to control group (Fig-13). Whereas, rbST treatment did not significantly affected the values of RDW during other physiological conditions in buffaloes. White blood cells were significantly lower (P<0.01)

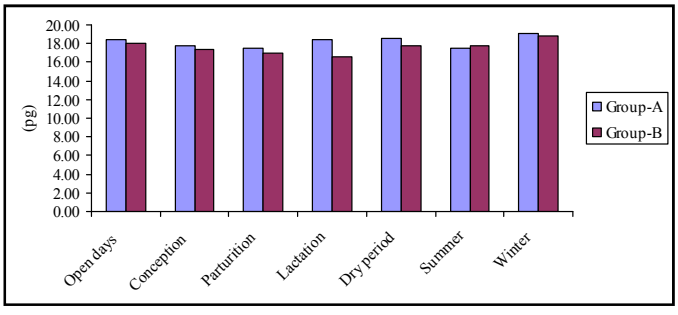

Figure 4. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of carpulses (MCH) in Kundhi buffaloes

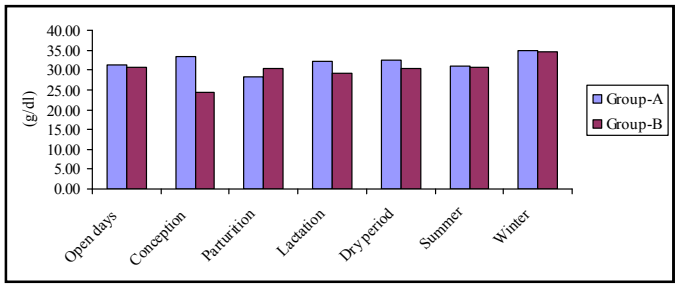

Figure 5. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of carpulses heamoglobin concentration (MCHC) in Kundhi buffaloes

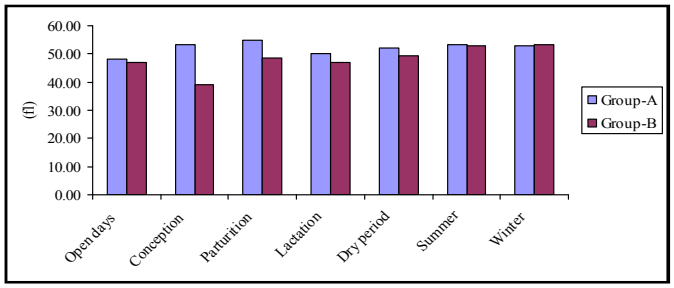

Figure 6. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of carpulses volume (MCV) in Kundhi buffaloes

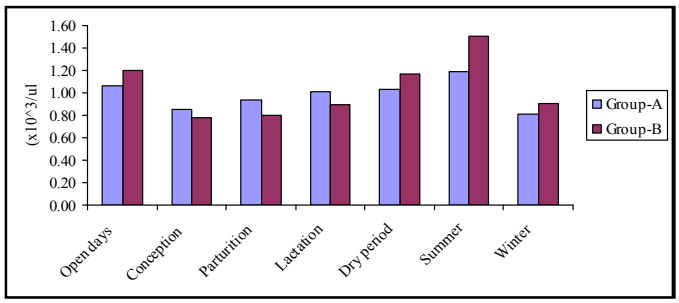

Figure 7. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of moncyte (MO#) in Kundhi buffaloes.

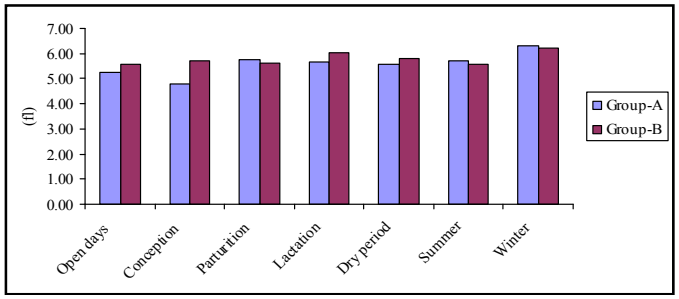

Figure 8. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of platelates volume (MPV) in Kundhi buffaloes

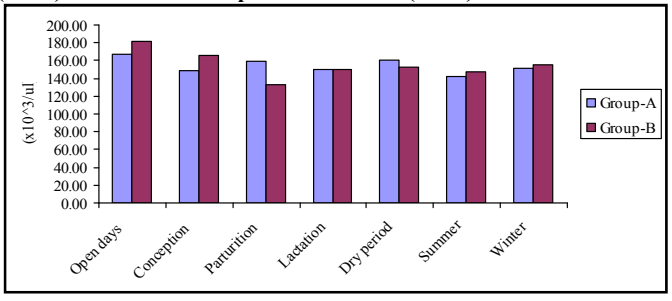

Figure 9. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of platelets (Plt) in Kundhi buffaloes

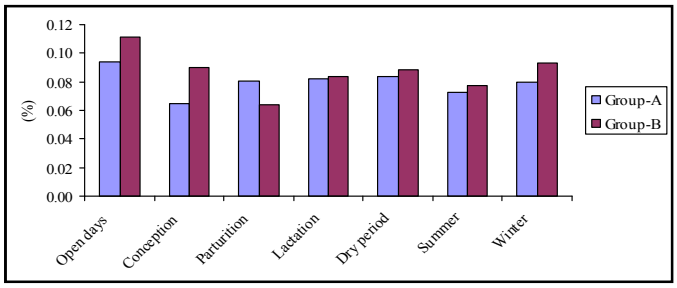

Figure 10. Effect of recombinant somatotropin hormone (rbST) on percentage of packet cell (Pct) in Kundhi buffaloes

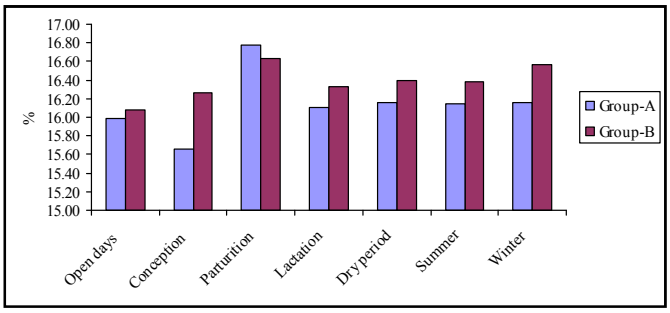

Figure 11. Effect of Recombinant bovine somatotropin hormone (rbST) on mean values of platelet distribuation width (PDW) in Kundhi buffaloes.

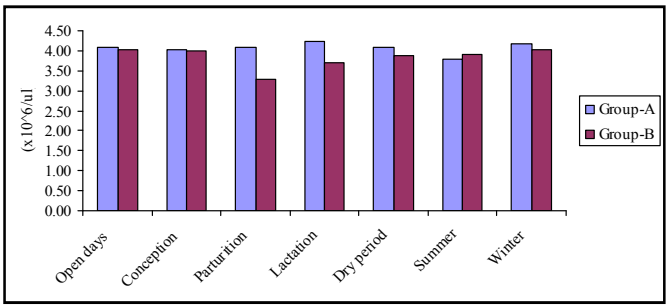

Figure 12. Effect of Recombinant bovine somatotropin hormone (rbST) on red blood cell (RBC) in Kundhi buffaloes

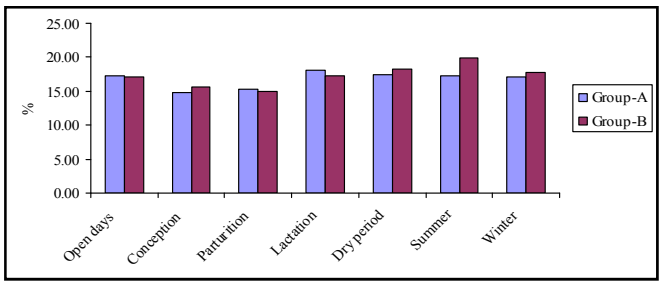

Figure 13. Effect of Recombinant bovine somatotropin hormone (rbST) on Red blood cell distribution width (RDW) in Kundhi buffaloes

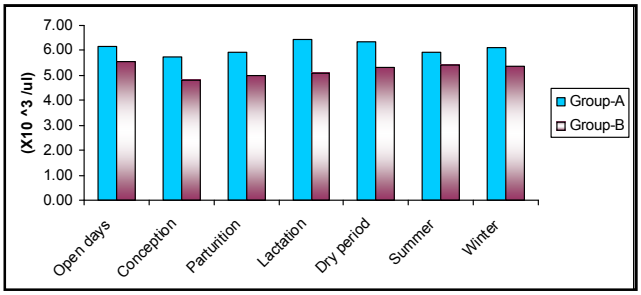

Figure 14. Effect of Recombinant bovine somatotropin hormone (rbST) on white blood cells (WBC) in Kundhi buffaloes

during various physiological conditions in rbST treated buffaloes as compare to control group Fig-14). Haemotological values during various physiological conditions and weather in rbST treated and non-treated buffaloes are demonstrated in (Fig-1 and Fig-14). Inspection of implantation site indicated occasional local and transient swelling.

DISCUSSION

Haemotological parameters were generally unaffected by rbST treatment. All analyzed blood components were within normal physiological ranges. Overall mean values of blood during pre-treatment and post-treatment rbST were remained unaffected. Haemotological values in rbST treated and non-treated buffaloes were affected by various physiological and weather conditions in Kundhi buffaloes as demonstrated in (Fig-1 to Fig-12). The studies have been carried out to investigate the effects of rbST treatment on blood parameters of large and small ruminants. Primiparous and multiparous Holstein-Friesian cows were treated with 250 and 500 mg of rbST respectively fortnightly for five months. Similar findings were reported by Abdelrahman *et al.,* (2010), Sallam *et al.,* (2005) and Eppard *et al.,* (1997) with non significant variation in the erythrogram whereas, leucogram showed leucocytosis accompanied by neutrophilia and lymphocytosis. Similar findings were also reported by Stevens *et al*., (1980) that rbST treatment did not change leucogram in cows. Burton *et al.,* (1990) in their studies determined the health and reproductive performance of dairy cows treated for up to two consecutive lactations with bovine somatotropin. They attributed the increase in total leukocytes and lymphocytes as positive response of immune system of cow to rbST treatment. Khaliq and Rehman, (2010) studied the effect of rbST on hematological parameters of lactating Nili-Ravi buffaloes treated with 500 mg of rbST subcutaneously twice at interval of 16 days. They reported a significant decrease in erythrocyte sedimentation rate (ESR) and neutrophils, whereas a significant increase in lymphocytes in rbST treated buffaloes as compare to control. However, studies showed no variation in packed cell volume, basophils, monocytes, and eosinophils counts between treated and control buffaloes as observed in our study. Studies on the effect of sustained release of recombinant bovine somatotropin (SR-rbST) on blood metabolites of Hanwoo and Holstein bull on the other hand revealed an increased quantity of each hematology variable such as red blood cells (RBCs), white blood cells (WBCs), Hemoglobin and MCHC, and decreased mean corpuscular volume in Hanwoo reported by Shin *et al.,* (Shin *et al.,* 1996). There were non-significant differences in all these haematological parameters among groups of lactating buffaloes presented by Hagawane, *et al*., (1999). During early stage of lactation, the mean haemoglobin concentration was 9.08±0.32 g% which showed lowered trend as compared to recorded means in other groups of lactating buffaloes by Tambare, (2005). Similarly, Flores, *et al.,* (1990) found non-significant difference in haematocrit values during late gestation and early lactation. The mean value of TLC in dry pregnant group of buffaloes was 10.05± 0.89 X 103 /cm which showed slightly higher trend than the normal healthy control group. Similar trend of increasing values was reported by Deshpande, *et al*., (1987). Hagawane, *et al.,* (1999) reported that non-significant differences in all these hematological parameters among group of pregnant and lactating buffaloes. Similar hematological values were reported for lactating Indian Murrah Buffaloes by Hirayama, *et al*., (2006) except hemoglobin, hematocrit and lymphocyte whose values were higher in Romanian buffaloes. Eppard *et al.,* (1997) also studied various haematochemical parameters which showed similar changes during growth, pregnancy and lactation in cows. Hematocrit and haemoglobin concentration were higher in non-lactating cows which then decreased during postpartum until 3rd or 4th month of lactation and increased afterward (Rowlands, (1975) and Stevens *et al.*, (1980). These changes were attributed to normal adjustment to change in the physiological state, nutrition, climatic conditions and rbST treated.

Conclusion

In conclusion application of rbST treatment produced a non-significant variation in the haemotological profile of primaparous kundhi buffaloes. But erythrogram and leucogram values showed a leucocytosis accompanied by neutrophilia and lymphocytosis after rbST treatment. Haemotological values of both groups were severely affected during various physiological conditions and weather. Further studies are needed to isolate other parameters related to hematological and hormonal variations.

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