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Haematological Studies of Nili-Ravi Buffaloes Injected with Recombinant Bovine Somatotropin

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ABSTRACT

Sixteen lactating Nili-Ravi buffaloes were used to observe the effect of recombinant bovine somatotropin (rbST) on haematological profile. These buffaloes were divided into two groups, with eight buffaloes in each group. Buffaloes of group I were injected with sterilized normal saline to serve as control. Animals in group II were administered 500 mg of rbST subcutaneously, twice at an interval of 16 days. Blood with anticoagulant was collected to determine DLC, ESR and PCV. Overall mean ESR decreased significantly (5.74%) in rbST injected buffaloes as compared to control at the end of experimental period. Neutrophils percent decreased and lymphocytes percent increased significantly in rbST treated buffaloes compared to control. Packed cell volume, basophils, monocytes and eosinophils did not differ significantly between the two groups. In conclusion, high dose of rbST did not result in alteration of certain haematological parameters in buffaloes.

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INTRODUCTION

Nili-Ravi is the most popular breed of buffaloes which constitutes about 76.7% of the total buffalo population in Pakistan (Otto *et al.*, 2003). Nili-Ravi animals are highly important asset for the country, having a great potential for milk produced. Buffaloes contribute approximately 67% of total milk production in the country (Bhatti *et al*., 2009). However, much more needs to be done to exploit their potential for enhanced milk yield, using present day industry practices. Over the years, an overall increase in milk production in the country has taken place, but this increase is not a reflection of increase in per animal milk yield, rather it is merely due to increase in the number of animals (Anonymous, 2007). Some increase in total annual milk production in the country is because of crossbreds and a bit of it can be attributed to the use of artificial insemination services in buffaloes and cows but this is due to limited impact of breeding schemes through selection and artificial insemination etc. (Otto *et al.,* 2003).

The first major agriculture related product of biotechnology research was bovine somatotropin (bST). Naturally produced by a cow's pituitary gland, bST is one of the hormones involved in normal body growth, development of mammary glands and normal milk production (Murphy, 1998; Bhatti *et al*., 2007). Injections of crude pituitary extracts increased milk production in dairy cows (Lee and Schaffer, 1934), but its limited supply made field application impractical. Later in 1982, synthetic bST was produced by DNA biotechnology by Monsanto (US based corporation) that allowed scientists around the world to examine various aspects of its biology.

Use of recombinant bovine somatotropin (rbST) as a powerful tool to enhance cattle performance in terms of milk production has been reviewed by Peel and Bauman, (1987). While most studies either pertain to cattle, crossbred animals and to some extent with sheep (Fernandez et al. 1995) and goats (Disenhaus et al. 1995), only single study on buffaloes was reported by Ludri et al. (1989). These studies are either short term (14 days) or long term, mostly using the recommended dose (250 mg/14 days) of rbST. A dose dependence was also conceived by Chilliard (1988b), who reported 2.8 Kg/day increase in milk with 31-50 mg/day of rbST. He further emphasized that response to rbST was very rapid and was maximum after one week or less. McGuffey *et al. (*1987) and Verite *et al.* (1988) reported that increasing the injection frequency with the same total amount of bST did not change the milk response. However, feeding and management factors could contribute and bring some alteration in the composition of milk. As bST injection is time dependent (Chilliard, 1988a), maximal milk yield was obtained when animals had already passed one month of lactation period. However, milk response to bST was found to be of the same magnitude as compared to first lactation of these animals. The purpose of the present

study was to evaluate the effect of high dose (500 mg) of rbST on certain haematological parameters of Nili-Ravi buffaloes.

MATERIALS AND METHODS

The present study was carried out at the Livestock Experiment Station, University of Agriculture Faisalabad, Pakistan and included 16 clinically healthy Nili-Ravi buffaloes aged 7-10 years at 3-4 months post calving and in their second to fourth lactation season. During the experimental period, the minimum and maximum environmental temperatures were 12 and 33^0C and average relative humidity was recorded to be 71 percent. Temperature humidity index was calculated to be 67.31. All buffaloes were housed under the same experimental conditions and were on free stalls, fed mixed ration (sugarcane and Barseem fodder and maize oil cake; Rafhan Products Co. Limited, Faisalabad; Table 1) according to their milk yield. Experimental buffaloes were divided into group I (n=8) to serve as control, while buffaloes in group II (n=8) were injected with 500 mg rbST subcutaneously twice during the experimental period at an interval of 16 days.

Table 1: Chemical composition of sugarcane and Berseem offered to each buffalo during experimental period

	Sugarcane (%)	Berseem (%)
Dry matter	31.8	13.9
Organic matter	94.9	88
Crude protein	6.2	20.4
No-digestible fiber	50.5	54.0
Acid detergent fiber (ADF)	28	27
Soluble carbonate	46.7	18.4
Hemicellulose	22.5	27.4
Ash	5.1	12.0

Buffaloes were properly restrained for collection of blood samples on day 0, 1, 4, 8, 12, 16, 17, 20, 24, 28 and 32 with a sterile hypodermic needle (18 gauge, 5 cm long). Blood was collected in test tubes containing anticoagulant (heparin; as 1% for 10 ml blood) for haematological studies. All tests were performed in duplicate. Thin blood films were made immediately after the blood collection, fixed with methanol and stained with Modified Giemsa's stain solution (Benjamin, 1985). The stained blood films were examined under the oil immersion lens by using the battlement technique of counting. The leukocytes were classified as lymphocytes, monocytes, neutrophils, eosinophils and basophils. Three persons were allotted these slides without identification of groups and animals for counting. Erythrocyte sedimentation rate (ESR) was recorded by using Westergeren tube (Benjamin, 1985). The packed cell volume (PCV) was noted as percentage with the help of haematocrit scale as described by Bush (1991).

Means and standard error of means were calculated. Two way analysis of variance was performed to determine the difference between days of experiment, between groups as well as days x groups interaction (Steel *et al*., 1997). Duncan's Multiple Range (DMR) test was applied to see the differences between means (Duncan, 1955). Linear correlation coefficients were calculated between haematological profiles of normal and rbST treated buffaloes with that of milk and serum biochemical parameters recorded during the experiment.

RESULTS

Overall mean erythrocyte sedimentation rate (ESR) decreased significantly ($P<0.01$) in rbST treated buffaloes as compared to control buffaloes (Table 2). A decrease of 5.74 and 2.86% in ESR was observed in control and rbST treated group, respectively after 1st injection. Also, a significant decrease of ESR (6.85%) in control and an increase of 0.45% in rbST injected buffaloes was observed after 2nd injection. However, between days of injection, ESR did not differ significantly (Table 2).

Overall mean PCV in control and treated buffaloes was 33.36 ± 0.41 and 32.76 ± 0.32 percent respectively (Table 2). The PCV did not differ between days of treatment, or between the two groups at any stage of treatment.

Table 2: Mean erythrocyte sedimentation rate and pack cell volume of control and bovine somatotropin injected buffaloes at various days

	Erythrocyte sedimentation rate (ESR; mm/hr)			Pack cell volume (PCV; %)		
Days	**Control**	**bST**	**Overall mean**	**Control**	**bST**	**Overall mean**
0	92.63 ± 4.07	82.13 ± 3.19	87.38 ± 2.84	34.19 ± 0.95	33.38 ± 0.92	33.78 ± 0.65
1st Injection						
1	85.63 ± 4.38	79.50 ± 2.05	82.56 ± 2.47	34.06 ± 1.10	33.31 ± 0.95	33.69 ± 0.71
4	90.38 ± 4.23	77.50 ± 2.02	83.94 ± 2.81	34.19 ± 1.18	33.25 ± 0.94	33.72 ± 0.74
8	86.63 ± 4.23	79.88 ± 2.45	83.25 ± 2.52	35.19 ± 1.19	32.75 ± 0.97	33.97 ± 0.81
12	85.25 ± 4.31	82.13 ± 3.62	83.69 ± 2.75	33.88 ± 1.39	33.50 ± 0.81	33.69 ± 0.78
16	88.63 ± 5.13	79.88 ± 2.34	84.25 ± 2.95	31.06 ± 2.62	32.75 ± 0.93	31.91 ± 1.36
2nd Injection						
17	87.50 ± 3.65	80.13 ± 2.89	83.81 ± 2.44	32.31 ± 1.25	31.81 ± 1.16	32.06 ± 0.83
20	89.38 ± 3.44	82.00 ± 3.49	85.69 ± 2.55	32.88 ± 1.11	32.69 ± 1.22	32.78 ± 0.79
24	85.25 ± 4.63	85.13 ± 3.87	85.19 ± 2.92	34.06 ± 1.18	33.45 ± 1.13	33.76 ± 0.79
28	86.88 ± 6.48	84.63 ± 4.42	85.75 ± 3.80	33.19 ± 0.98	32.01 ± 1.26	32.60 ± 0.79
32	82.38 ± 4.31	80.63 ± 4.21	81.60 ± 2.92	31.94 ± 1.20	31.45 ± 1.49	31.69 ± 0.93
Overall means	87.32 ± 1.31^{A}	81.23 ± 0.95^{B}	84.27 ± 0.84	33.36 ± 0.41	32.76 ± 0.32	33.06 ± 0.26

Values with similar alphabets in a row do not differ significantly ($P \geq 0.01$).

Table 3: Mean values (± SE) of lymphocytes, neutrophils, eosinophils, monocytes and basophils of control and bovine somatotropin injected buffaloes at various days

Days	Lymphocytes (%)			Neutrophils (%)			Eosinophils (%)			Monocytes (%)			Basophils (%)		
	Control	bST	Overall mean	Control	bST	Overall mean	Control	bST	Overall mean	Control	bST	Overall mean	Control	bST	Overall mean
0	57.62 ± 0.75	57.13 ± 0.74	57.38 ± 0.52^{C}	34.13 ± 0.69	34.38 ± 0.63	34.25 ± 0.45^{A}	2.63 ± 0.26	2.63 ± 0.38	2.63 ± 0.22	5.13 ± 0.23	5.25 ± 0.37	5.19 ± 0.21^{B}	0.50 ± 0.19	0.63 ± 0.18	0.56 ± 0.13
1st Injection															
1	57.75 ± 0.88	57.63 ± 0.53	57.69 ± 0.50^{C}	33.38 ± 0.80	33.13 ± 0.64	33.25 ± 0.50^{AB}	2.00 ± 0.27	3.00 ± 0.33	2.50 ± 0.24	6.38 ± 0.26	5.63 ± 0.42	6.00 ± 0.59^{A}	0.50 ± 0.19	0.75 ± 0.16	0.63 ± 0.13
4	57.75 ± 0.75	58.75 ± 0.62	58.25 ± 0.49^{BC}	33.00 ± 1.15	32.13 ± 0.72	32.56 ± 0.66^{BC}	2.38 ± 0.38	3.25 ± 0.25	2.81 ± 0.25	6.25 ± 0.41	5.13 ± 0.40	5.69 ± 0.31^{AB}	0.63 ± 0.18	0.75 ± 0.16	0.69 ± 0.12
8	58.13 ± 0.88	59.63 ± 0.65	58.88 ± 0.55^{ABC}	33.50 ± 0.91	31.00 ± 1.04	32.25 ± 0.74^{BCD}	2.00 ± 0.27	2.63 ± 0.32	2.31 ± 0.22	5.75 ± 0.25	6.13 ± 0.35	5.94 ± 0.21^{AB}	0.635 ± 0.18	0.63 ± 0.18	0.63 ± 0.13
12	57.86 ± 0.79	59.25 ± 0.37	58.56 ± 0.46^{ABC}	32.38 ± 0.65	30.88 ± 0.88	31.63 ± 0.56^{BCDE}	2.88 ± 0.30	2.88 ± 0.23	2.88 ± 0.18	6.13 ± 0.35	6.25 ± 0.37	6.38 ± 0.24^{A}	0.63 ± 0.18	0.50 ± 0.18	0.56 ± 0.13
16	57.50 ± 0.59	57.75 ± 0.84	57.63 ± 0.45^{C}	33.50 ± 0.50	32.75 ± 0.62	33.13 ± 0.40^{AB}	2.63 ± 0.32	2.50 ± 0.25	2.56 ± 0.22	6.50 ± 0.33	5.50 ± 0.50	5.88 ± 0.32^{AB}	0.82 ± 0.13	0.75 ± 0.16	0.81 ± 0.10
2nd Injection															
17	59.38 ± 0.38	59.38 ± 0.60	58.56 ± 0.54^{ABC}	33.25 ± 0.80	31.38 ± 0.42	32.31 ± 0.50^{BCD}	2.75 ± 0.25	2.63 ± 0.32	2.69 ± 0.20	6.25 ± 0.37	5.63 ± 0.32	5.88 ± 0.20^{AB}	0.63 ± 0.18	0.50 ± 0.19	0.56 ± 0.13
20	58.50 ± 0.76	60.75 ± 0.70	59.63 ± 0.58^{AB}	33.00 ± 0.87	29.75 ± 0.77	31.38 ± 0.70^{CDE}	2.50 ± 0.33	2.50 ± 0.33	2.50 ± 0.22	6.13 ± 0.23	5.50 ± 0.20	5.88 ± 0.18^{AB}	0.50 ± 0.19	0.75 ± 0.16	0.63 ± 0.13
24	59.25 ± 0.88	61.00 ± 0.66	60.13 ± 0.54^{A}	31.25 ± 0.65	29.63 ± 0.87	30.44 ± 0.56^{E}	2.88 ± 0.23	2.63 ± 0.32	2.76 ± 0.19	6.25 ± 0.25	5.88 ± 0.23	5.94 ± 0.17^{AB}	0.75 ± 0.16	0.75 ± 0.16	0.75 ± 0.11
28	59.00 ± 0.95	60.25 ± 0.53	59.63 ± 0.55^{AB}	31.38 ± 0.80	29.88 ± 0.87	30.63 ± 0.54^{DE}	2.75 ± 0.25	3.13 ± 0.25	2.94 ± 0.17	6.00 ± 0.27	6.13 ± 0.30	6.13 ± 0.20^{A}	0.75 ± 0.16	0.63 ± 0.18	0.69 ± 0.12
32	59.38 ± 0.42	58.50 ± 0.46	58.94 ± 0.32^{ABC}	30.00 ± 0.85	31.50 ± 0.71	30.75 ± 0.57^{DE}	3.76 ± 0.32	3.25 ± 0.25	3.31 ± 0.20	6.75 ± 0.45	6.00 ± 0.27	6.38 ± 0.27^{A}	0.50 ± 0.19	0.75 ± 0.16	0.63 ± 0.13
Overall mean	58.23 ± 0.23^{B}	59.09 ± 0.22^{A}	58.66 ± 0.16	32.61 ± 0.26^{A}	31.49 ± 0.26^{B}	32.05 ± 0.19	2.61 ± 0.09	2.82 ± 0.09	2.72 ± 0.07	5.92 ± 0.11	5.94 ± 0.10	5.93 ± 0.07	0.63 ± 0.05	0.091 ± 0.05	0.65 ± 0.04

AB: Values with similar alphabets in a row do not differ significantly (P≥0.01). A-E: Values with similar alphabets in a column do not differ significantly (P≥0.05).

Overall highest lymphocytes (%) was observed on day 24 after 2nd injection, while lowest was on days 1 and 16 of first injection of rbST. Overall mean lymphocytes were significantly higher in rbST treated as compared to control buffaloes (Table 3). Overall neutrophils percentage decreased significantly on day 24 (Table 3) of 2nd injection compared to pretreatment values. Throughout the experimental period, buffaloes injected with rbST showed significantly lower percentage of neutrophils as compared to control animals and on overall basis, treated buffaloes had lower neutrophils compared to control ($P<0.01$). Mean eosinophils count of control and rbST injected buffaloes did not differ at any stage of the experimental period. Overall highest counts of monocytes were observed on day 1 and 12 after 1st injection and on 28 and 32 after 2nd injection (Table 3). Overall mean basophils count of control and rbST injected buffaloes were within normal ranges throughout the experimental period and did not differ among days of treatment or between treated and control groups.

DISCUSSION

A significant decrease in erythrocyte sedimentation rate was observed in rbST treated buffaloes compared to control. Haemoglobin and hematocrit have been reported to decline slightly with increasing dose of bST as well as milk yield (Staple *et al.*, 1988). Likewise, a significant reduction in packed cell volume has been reported by Bolt (1980) and Bines and Hart (1982). This decrease was attributed to the dilution effect of the blood as bST treatment caused increase in the moisture contents in the carcass as well as in some organs. Hematocrit was significantly lower in cows given 41.2 mg rbST/day as compared to cows receiving 0, 10.3 and 26.6 mg rbST/day (Soderholm *et al.*, 1988). Therefore, it seems that significant decrease in ESR and a decreasing pattern of packed cell volume were related to bST treatment rather than milk yield or may be in addition to milk yield. Zvorc *et al.* (2006) observed a decrease in haemoglobin and MCH during normal lactation in animals.

Various haematochemical parameters show changes during growth, pregnancy and lactation (Eppard *et al*., 1997). Hematocrit and haemoglobin concentration are high in non-lactating cows which then decrease post-partum until 3rd or 4th month of lactation and increase afterward (Rowlands, 1980; Stevens *et al.*, 1980). It has been reported that cows with higher milk production have lower hematocrit as compared to low yielding cows (Whitlock *et al*., 1974; Stevens *et al*., 1980).

Eppard *et al*. (1997) reported that during bST treatment a decrease in RBC count and hematocrit was observed, in addition to concomitant decrease in haemoglobin and RBC distribution. These changes were attributed to normal adjustment to change in the physiological state. As speculated by Eppard *et al.* (1997), clinically depressed hematocrit is associated with one of three general disorders; 1) increased hemalysis or shortened RBC survival, 2) alteration of the fluid phase of circulating volume and 3) decreased erythropoisis. Additionally, bST did not affect the appearance of immature red blood cells which tended to decrease rather than to increase mean cell volume and marginally affected serum bilirubin concentration.

In the present study, lympocytes increased, while neutrophils decreased significantly in rbST injected buffaloes as compared to controls. Eosinophils, monocytes and basophils did not show any change due to rbST treatment. Oldenbroek and Gassen (1993) observed a slight increase in lymphocytes following rbST treatment in dairy cows that was within physiological limits. Burton *et al.* (1992) reported that lympocytes fraction decreased at week 26 and 35 of bST treatment and these values increased to control value as the treatment was terminated. On the other hand, Annexstad *et al.* (1990) observed no effect of rbST treatment on lymphocyte percent after bST treatment. The present study in buffaloes indicated that lymphocytes increased significantly on day 20th and were highest on day 24th and 28th after 2nd injection of bST. In vitro studies conducted by Burton *et al.* (1992) speculated that peripheral blood lymphocytes from treated cows displayed an elevated proliferated responsiveness to mitogens and a significant decrease in neutrophils percent in culture. Lymphocytes in the present study were significantly correlated ($r = 0.740$) with milk production, milk fat and milk lactose concentrations, while a negative correlation was observed with milk protein in rbST injected buffaloes.

A significant decrease in neutrophils in bST treated buffaloes compared to controls in the present study is supported by Burton et al. (1992), who indicated that neutrophils fraction for 20.6 mg/d of rbST injected cows dropped dramatically and then reached control values by last week of treatment i.e. 46 weeks. Neutrophils were negatively correlated with milk production, milk fat and lactose, and positively correlated with milk protein in bST treated buffaloes. These results are also consistent with the notion that bST is granulopoietic (Kelley, 1989).

In the present study, the values of lymphocytes and neutrophils remained within physiological limits which suggest that rbST treatment with 500 mg/15 day had no adverse effects on animal health. No significant differences were observed in eosinophils, monocytes and basophils after rbST treatment in buffaloes, which is according to the observations recorded by Annexstad *et al.* (1990), McGuffey *et al.* (1991) and Oldenbroek and Gassen (1993). Overall mean monocytes increased significantly on day 1 and 12 of first rbST injection and on day 28 and 32 of 2nd injection. Variation in monocytes percentage was also observed by Burton *et al.* (1992) in rbST injected cows and was suggestive of the presence of IGF-1 concentration in the blood plasma. Burton *et al.* (1992) further observed that neutrophils and lymphocytes possess cellular receptors for IGF-I and that IGF-I can be produced by the leukocytes.

In conclusion, our study is suggestive of a significant increase in lymphocytes, while neutrophils and ESR decreased but were within physiological limits after two connective injections of 500 mg of rbST at an interval of 15 days in buffaloes.

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