

EFFECTS OF EXOGENOUS RECOMBINANT BOVINE SOMATOTROPIN (RBST) ON HEMATOLOGICAL INDICES OF KUNDHI BUFFALO MALE CALVES

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ABSTRACT: The aim of present research was assessment of the rbST effects on hematological indices and also its optimum safe dose in kundhi buffalo male calves for beef production. The calves were divided into three groups, with or without rbST treatment. The rbST administered intramuscularly during fortnight, for eleven weeks with an interval of two weeks. Then blood samples were collected at the end of eleven weeks for analysis. In comparison with group A and B, red blood cells count, hemoglobin, mean corpuscular hemoglobin concentration, platelets count and mean corpuscular volume indices were significantly ($P < 0.05$) higher. There was no significant effect on mean corpuscular hemoglobin, erythrocyte sedimentation rate and packed cell volume. The white blood cells in rbST treated groups ($P < 0.05$) increased, and this increase was attributed due to increase in neutrophil number. However, there was non-significant effect of rbST on eosinophils, basophils, monocytes and lymphocytes between all groups. It was concluded from outcomes that rbST produced dose dependent effect on hematological values in kundi buffaloe calves and no adversely higher values were observed that determine polycythemia or leukocytosis. It is therefore suggested that rbST can be used at the dose rate of 1mg/kg b.w. as growth promoter in Kundhi buffaloe calves.

Keywords: rbST, Hematology, Hematological, Polycythemia, Leukocytosis, Kundhi Buffalo, Calves.

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INTRODUCTION

In the developed countries such as USA, hormones are used as growth promoters for improving the efficiency of feed conversion thereby weight gain in beef animals as well as for milk production in dairy animals (Marounek et al., 2007). Somatotropin is a homeorhetic hormone secreted from the pituitary gland play major role in nutrient partitioning in the animal body, and employed as a growth promoter and also for milk production (Capper, 2010).

Now a days, instead of somatotropin genetically engineered synthetic recombinant bovine somatotropin hormone (rbST) used as a feed supplement or via injection, for production of milk yield in livestock animals and as growth promoter in beef animals (Collier and Bauman, 2014). Previously research suggested that rbST increased the milk production in various species cows (Eppard, 1997), goat and sheep (Baldi, 1999) and in buffalo (Moallem et al., 2000; Jorge et al., 2002; Gulay et al. 2004; Mishra and Shukla, 2004). In Pakistan, rbST is also used via injection or supplemented in the feed. In increasingly vulnerable environment, the rbST is regularly practiced in commercial dairy animals particularly in buffalo for augmentation of milk production. For that reason, it is important to monitoring the effect of rbST on health status of the animals. The blood hematological indices are important indicators of the health status of the animals.

The documented studies about effect of rbST on hematological profile in different species such as in cow (Dilbar et al., 2014), small ruminants (Azza et al., 2010), sheep (Sallam et al., 2005; El-Din et al., 2009) and in Nilli ravi buffalo (Jabbar, 2004; Jabbar et al., 2010; Khaliq and Rahman, 2010). The kundhi buffalo is a local breed of sindh province in Pakistan. Although there are some reports on lactating buffalo especially on kundhi breed. But very few researches so far has been carried on fattening buffalo calves of kundhi breed to see the effect of rbST on hematological profiles. The current study evaluated the effect of rbST with different dosage regimes in fattening kundhi buffalo male calves to monitoring the health status. The main objective was assessment of rbST regarding

its dose selection during the therapy, whether low or high doses could be used safely devoid of any side effect on the health status such as hematological indices.

MATERIAL AND METHODS

Experimental animals

A total of eighteen healthy Kundi buffalo male calves, 6 months of age, with average body weight of 60kg were selected for current study. The experiment was conducted at Livestock Experimental Station, Sindh Agriculture University, Tandojam Pakistan. The calves were housed in pens with free access to water and provided sufficient housing space and open space for exercise. The calves were fed twice a day add libitum with access to a total mixed ration. Animals were divided into three groups (A, B and C). Each group consisted of six animals. Before commencement of the study, the calves were allowed for at least three weeks of acclimatization period. During adaptation period the calves were ear tagged, drenched and vaccinated against some common infectious diseases.

Feeding management

An economical fattening ration (concentrate mixture) consisted of crushed maize and wheat bran as major energy ingredients were formulated (Table-1) according to previously described method (Sharma et al., 1995). The chemical composition and nutritive values of feed ingredients such as Fats, Crude Protein, TDN, Crude Fiber and Ash of the ration was determined as per standard methods described by AOAC (2000).

Table 1 - Formulation and chemical composition of experimental fattening ration (% on dry matter (DM) basis).

| INGREDIENTS | Quantity (Kg) | DM | CP | TDN | CF | Ca | P |
|--|---------------|-------|-------|--------|-------|------|------|
| Dry roughages | | | | | | | |
| Wheat Straw | 20 | 18 | 0.468 | 8.64 | 7.4 | 0.04 | 0.01 |
| Green roughages | | | | | | | |
| Berseem (<i>Trifolium alexandrinum</i>) | 40 | 7.2 | 1.152 | 4.608 | 1.61 | 0.26 | 0.02 |
| Concentrates | | | | | | | |
| Maize Crushed | 3 | 2.7 | 0.297 | 2.241 | 0.05 | — | 0.02 |
| Wheat Bran | 4 | 3.56 | 0.57 | 2.492 | 0.32 | 0.01 | 0.04 |
| Rice Polish | 10 | 9 | 1.08 | 7.29 | 0.29 | 0.02 | 0.12 |
| Cotton Seed Cake | 16 | 14.4 | 5.04 | 11.38 | 1.44 | 0.02 | 0.14 |
| Moong Kutta | 5 | 4.65 | 0.93 | 3.627 | 0.24 | 0.01 | 0.04 |
| Di-Calcium Phosphate | 0.25 | 0.25 | — | — | — | 0.09 | — |
| Molasses | 2 | 1.56 | 0.062 | 1.139 | — | 0.02 | — |
| Salt | 0.25 | 0.25 | — | — | — | 0.09 | — |
| Total | 100.5 | 61.66 | 9.599 | 41.417 | 11.43 | 0.57 | 0.41 |
| Chemical composition | | | 16 | 68 | 18.5 | 1.05 | 0.6 |

Treatment period

After 3 weeks of adaptation period, the treatment of rbST and fattening ration was started. All the calves in three groups were fed experimental ration. The group A served as control without treatment while B and C experimental groups. The rbST was administered intramuscularly to animals at dose rate of 0.5 and 1.0 mg/kg body weight (BW), respectively for group B and C, fortnightly for eleven weeks of period with interval of two weeks.

Collection of blood samples and complete blood count (CBC)

The blood samples from the calves collected weekly, (at last day of week) for the period of eleven weeks. The samples drawn in chilled EDTA vacutainers (BDH, Germany) and were brought to the Department of Veterinary Physiology and Biochemistry, Sindh Agriculture University tandojam, Pakistan for comprehensive examination. All the CBC parameters were done manually. All parameters were performed according to the standard hematological procedures as previously described (Sharma, 2005). Red blood cells (RBC's) and white blood cells (WBC's) or leukocytes quantified via haemocytometer method, platelets or thrombocytes through chamber counting system, neutrophils, eosoniphils, basophils, lymphocytes and monocytes through differential leukocytes count (DLC),

packed cell volume (PCV) measured via microhaematocrit method, erythrocyte sedimentation rate (ESR) via Westergren's procedure, hemoglobin (Hb) level was by acid-hematin, mean corpuscular volume (MCV) by $PCV/RBC \times 10$ formula, mean corpuscular hemoglobin concentration (MCHC) through $Hb/PCV \times 100$ equation and mean corpuscular hemoglobin (MCH) through $Hb/RBC \times 10$ formula.

Statistical analysis

The data was shown as means \pm S.E.M., and analyzed in student edition statistical software via one-tailed ANOVA. Least significant difference LSD was applied to indicate significant difference between and within the means of control and rbST treated groups. The significance level was set at $P < 0.05$.

RESULTS

Red blood cells (RBC) count and hemoglobin (Hb) level

The mean values of RBCs count and hemoglobin level treated with rbST for the period of eleven weeks depicted in Figure 1. The overall mean RBC concentration increased ($P < 0.05$) in calves of group C as compared to other groups (A and B). No difference was observed between A and B or between B and C. The increased RBCs count was observed within normal limits in group C. Concurrently, with increased RBCs count, the overall mean hemoglobin level also increased ($P < 0.05$) in calves of group C treated with rbST at dosage of 1.0 mg/kg BW in contrast to remaining groups. Similarly, no difference was noticed between A and B or B and C groups, and increased hemoglobin in groups C was within the limits.

Mean corpuscular hemoglobin concentration (MCHC)

The effect of rbST treatment on MCHC for eleven weeks showed in Figure 1. In comparison with groups A (control) and B, the indices of MCHC raised ($P < 0.05$) in group C animals received rbST with dose of 1.0 mg/kg BW. There was no significant effect of rbST between groups A and B or B and C. Moreover, the increase in MCHC concentration in C group was within normal values.

MCH, ESR and PCV

In comparison with control, no significance difference was observed for mean values of MCH, ESR and PCV values in buffalo calves fed fattening ration between and within the groups (A, B and C) as mentioned in Figure 1.

White blood cells (WBCs) count

The mean values of WBCs of animals treated through rbST for period of eleven weeks publicized in Figure 2. The rbST treatments produced pronounce effect on WBCs count. In comparison with control (group A), dose dependent significant effect of rbST was observed in B and C groups. The raised indices of WBCs were higher in group C than B. The increased in total WBC count were within normal limits.

Platelets count

The overall mean showed significant effect of rbST in groups B and C as compared to A in dose dependent manner as shown in Figure 2. The increase in platelets count was higher in calves of group C (rbST 1.0 mg/kg BW) as compared to that of group B (rbST 0.5 mg/kg BW). The increase in platelets count was within limits such as no thrombocytosis was seen in buffalo calves treated with rbST.

Mean corpuscular volume (MCV)

The effect of rbST MCV is depicted in Figure 2. The overall mean values showed that the MCV values increased in rbST treated calves groups (B and C) in contrast to non-treated group (A) in dose dependent fashion. The increased indices of MCV observed to be higher in group C than B group animals.

Differential leukocytes count (DLC)

Mean values of different WBCs observing through DLC mentioned in Figure 3. DLC analysis showed that rbST treatment produced significant effect on neutrophils counts. The overall mean values showed that the number of neutrophils increased in rbST treated groups as compared to control group. The increase in neutrophils was within normal ranges and no sign for neutrophilia was observed in calves treated with rbST. There was no significant effect of rbST on esionophils, basophils, lymphocytes and monocytes between and within the groups.

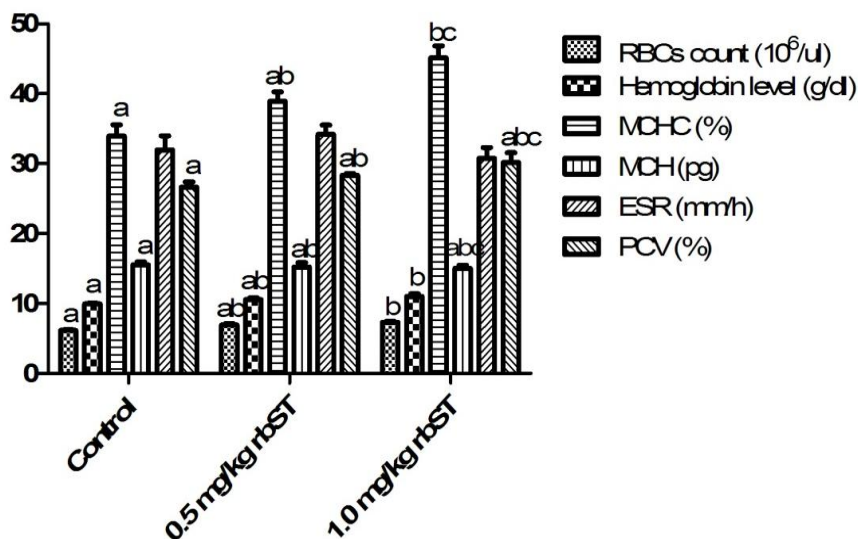


Figure 1 - Mean RBCs count, hemoglobin level, MCHC, MCH, ESR and PCV of Kundhi buffalo calves fed fattening ration during treatment period of 11 weeks with rbST. Significant difference level ($P < 0.05$) with different superscripts.

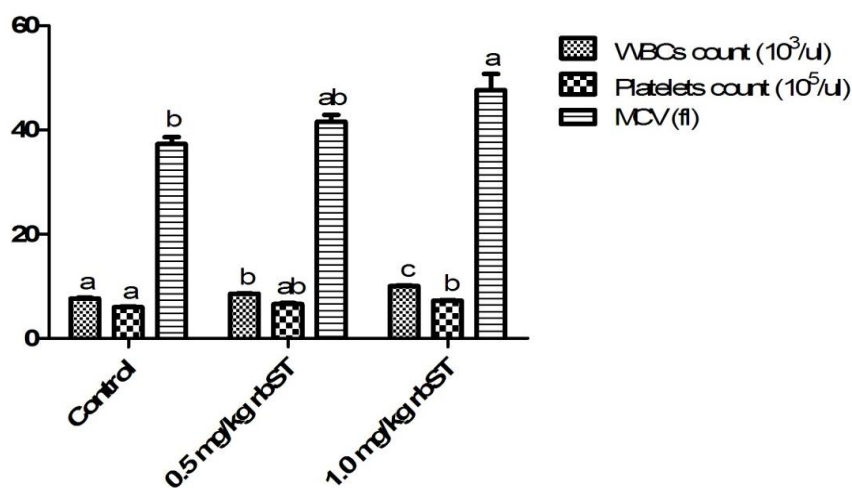


Figure 2 - Mean WBCs count, platelets count and MCV of Kundhi buffalo calves fed fattening ration during treatment period of 11 weeks with rbST. Significant difference level ($P < 0.05$) with different superscripts.

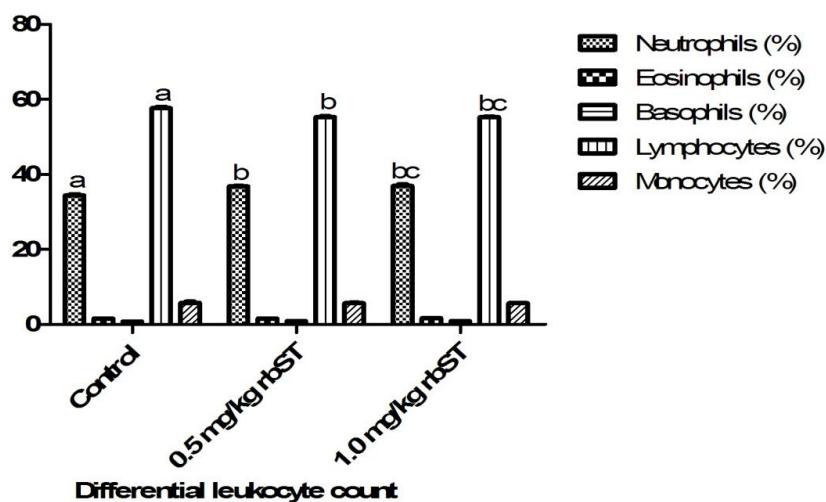


Figure 3 - Mean differential leukocytes or WBCs (neutrophils, eosinophils, basophils, lymphocytes and monocytes) of Kundhi buffalo calves fed fattening ration during treatment period of 11 weeks with rbST. Significant difference level ($P < 0.05$) with different superscripts.

DISCUSSION

Recombinant bovine somatotropin (rbST) is a synthetic hormone which has widely been studied in lactating animals for milk production (Azza et al., 2010; Chaiyabutr et al., 2011) and as growth promoter in young animals. But its effects on hematological indices have not been much studied particularly in fattening kundhi buffalo calves. The RBC count was significantly increased in rbST treated calves as compared to control in dose dependent fashion. Nasser et al. (2007) found non-significant increased indices of RBC in goats when medicated 50 mg rbST one time each week for the period of eight weeks. Though, Eppard et al. (1997) investigated significant decrease value of RBC in lactating cows following rbST treatment. Other studies in cow and buffalo publicized not significantly alteration in RBC values when treated with rbST (Azza et al., 2010; Khaliq and Rahman, 2010). Nour-El-Din et al. (2009) and Dilbar et al. (2014) did not observe any change in RBC count in lambs' and bull after treatment with rbST. The discrepancies in the effect of rbST treatment on RBC count suggest that, effect depend on species, physiological status of the animal, dose and duration of the treatment. Concurrently with increase in RBC count, the rbST also increased the hemoglobin level and MCHC of kundhi buffalo calves. However, somewhat declined in hemoglobin level was observed in contrast with control, when induced with 100 mg rbST for eight weeks once daily (Nasser et al., 2007). But, Nour-El-Din et al. (2009) mentioned raised level of Hb in lambs induced with 100 mg of rbST every week like compared to non-treated animals. But, Dilber et al. (2014) observed no effect on Hb and MCHC in rbST treated bulls with 500 mg dose every week for the period of ten weeks. The rbST treatment increased the body weight (Gavidia, 2001) and stimulation of erythropoiesis during growth is necessary to ensure proportionality between erythrocyte and body masses (Kurtz, 1988). So possibly in present study, RBC and its related indices also increased with proportionate with body mass during growth process. The rbST might have direct effect on hemopoietic stem cells. The rbST-induced erythropoiesis may be mediated by insulin-like growth factor (IGF-I). As IGF-I concentration increased in rbST treated animals (Schernhammer, 2006) and stimulate the erythropoiesis either directly or indirectly through release of erythropoietin (Kurtz, 1988). The rbST produced no effect on the MCH between the groups might be species difference and difference in the protocol of the experiment. The ESR values were not different between the groups, which coincide with preceding report (Dilbar, 2014). While Khaliq and Rahman (Khaliq and Rahman, 2010) noticed decrease indices of ESR in lactating buffaloes treated with rbST when injected 500 mg two times with an interval of sixteen days. No significant effect was observed on PCV percentage in rbST treated calves which coincide with previous study (Chaiyabutr, 2011). Khaliq and Rahman (2010) noticed decrease indices of PCV in rbST treated buffaloes. Alike outcomes were acquired in ovine (Sallam et al. 2005), caprine (Nasser, 2007) and bovine (Dilbar et al., 2014).

In our study no effect was observed probably due to low dose of rbST and many other factors such as species, age, sex and physiological conditions. The platelets count also increased in the present study was within normal ranges. However, increasing trends in platelets count had also been observed in lactating cows treated with rbST (Chaiyabutr et al., 2011; Burton et al., 1992). In our study MCV values increased which coincide with Burton et al. (1992) report. The increase in MCV might have been resulted from overall increase in blood cells. The total leukocyte count was raised in rbST medicated buffalo calves. The differential leukocytes count analysis further confirmed that increased WBC was mainly accompanied due to an increase in neutrophils. In current study, lymphocyte count also increased which is accordingly with previous report (Khaliq and Rahman, 2010). The previous studies also proved that leucocytosis is actually because of neutrophilia (Azza et al., 2010; Chaiyabutr et al., 2011; Burton et al., 1992; Hoeben et al., 1999), but Khaliq and Rahman (2010) noticed decrease in neutrophils in Nilli-ravi buffalo. The exact motive for higher indices of WBC count in rbST medicated animal still not clear, possibly neutrophil is the part of innate immunity probably rbST stimulate this immunity.

CONCLUSION

It was concluded from outcomes that rbST produced dose dependent effect on hematology in kundi buffaloe calves and no adversely higher values were observed that determine polycythemia or leukocytosis. It is therefore suggested that rbST can be used at the dose rate of 1mg/kg b.w. as growth promoter in Kundhi buffaloe calves. It is recommended that further toxicological studies conducted in the future about effect of different doses of rbST on various body systems of diverse species of livestock. The outcomes of toxicological studies in the future give guidance to the veterinary practitioner regarding dose management during therapy.

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Competing interests

The authors declare that they have no competing interests exist.

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