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EFFECTS OF BOVINE SOMATOTROPIN ON LIBIDO, SERUM TESTOSTERONE, HAEMATOLOGY AND CERTAIN BIOCHEMICAL METABOLITES OF SAHIWAL BULLS

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In this study, effects of bovine somatotropin (bST) on libido, serum testosterone, haematological indices and serum biochemical metabolites in Sahiwal bulls were investigated. Bulls of the treatment group (n=3) were given bST @ 500 mg weekly, for 10 weeks, while control bulls (n=3) were injected with equal volume of normal saline. Reaction time of these bulls was recorded fortnightly. Blood samples collected fortnightly were analyzed for haematological parameters, while, serum samples were assayed for testosterone and biochemical metabolites. Results revealed that bST treatment of Sahiwal bulls significantly decreased reaction time (5.25±0.28 vs 7.50±0.42 sec) and increased serum testosterone concentrations (14.02±0.19 vs 12.55±0.44 ng/ml) compared to controls ($P<0.05$). Similarly, total leukocyte counts (10.49±0.83 vs 7.91±0.38 x 10^3/ul) were increased, while PCV (36.78±0.64 vs 40.78±1.28%), MCV (51.02±0.47 vs 54.90±0.68fl) and MCH (16.20±0.10 vs 16.86±0.14 pg) decreased. Serum globulin (5.43±0.11 vs 5.17±0.04 G/dl) was higher and serum urea (12±0.47 vs 15.33±0.82 mg/dl) lower in treated bulls ($P<0.05$). However, bST had no effect on blood Hb, RBC counts, MCHC, ESR, DLC, platelets counts, serum total proteins, albumin, glucose and SGPT activity. Thus, treatment of bulls with bST improved libido, increased testosterone, total leukocyte counts and serum globulin, and decreased PCV, MCV, MCH and serum urea.

Keywords: Bovine somatotropin, libido, serum testosterone, haematology, biochemical metabolites, Sahiwal bulls

INTRODUCTION

Somatotropic hormone is a polypeptide produced by somatotrophs of the anterior pituitary regulated by two hypothalamic peptides that either stimulate (growth hormone-releasing factor) or suppress (somatostatin) the secretion of somatotropin from the pituitary gland (Tuggle and Trenkle, 1996). However, the quantity of bovine somatotropin (bST) that could be extracted from the pituitary of the slaughtered animals was insufficient to have its production on commercial levels. In 1982s, recombinant bST was formed by DNA technology that allowed scientists to study diverse aspects of its biology (Bauman, 1992).

Somatotropic hormone plays an important role in body growth and metabolism, development of the mammary glands, milk production, and reproduction (Lucy *et al.*, 1999; Bhatti *et al.*, 2007; Khaliq *et al*., 2013). The effects of using bST on milk production and its composition, metabolites and plasma hormones in sheep (Sandles *et al.*, 1988), pituitary gland in dairy goats (Chadio *et al.*, 2002) and immunity in dairy cattle (Bauman, 1992) have been studied. Similarly, recombinant bST treatment at estrus improved the conception rate of the repeat breeding Holstein cows which was associated with an increase in the circulating concentration of progesterone (Morales-Roura *et al.,* 2001).

Recombinant bST increases mean concentrations of insulin and Insulin-like Growth Factor I (IGF-I) in plasma (Waites *et al*., 1985; Du *et al*., 2013; Riaz *et al*., 2013). There are some evidences signifying that bST plays an important role in the reproductive functions of the male (spermatogenesis and steroidogenesis). Somatotropin receptors have been found in Sertoli and Leydig cells, epididymis, prostate glands, vas deference and seminal vesicles (Lobie *et al.*, 1990; Abu-Seida, 2012). Recombinant bST improved growth performance, and physical parameters of semen in rams (Ibrahim, 2013) and the integrity of sperm membrane in bulls (Vieira *et al.,* 2010). In swine, porcine somatotropic hormone (pST), when given to neonatal boars, enhanced testicular development and spermatogenesis (Deaver and Bryan, 1999).

In Pakistan, bST (Boostin–S, LG Life Sciences, Korea: A synthetic somatotropin preparation) is commonly used with a view to increase growth rate and milk yield of animals (Qudus *et al.,* 2013). However, there is relatively little information regarding the effects of long-term treatment with exogenous somatotropin on reproductive functions in bulls. Recently, Waqas (2013) studied the effects of bST on

the semen quality in Sahiwal bulls. In the present study, the possible effects of bST (Boostin^{TM}-S) on libido and serum testosterone concentrations in adult Sahiwal breeding bulls were investigated. Moreover, the possible toxic effect of this treatment on liver and kidney functions (with reference to haematological and serum biochemical metabolites) in these bulls, if any, were also monitored.

MATERIALS AND METHODS

***Experimental animals*:** The present study was conducted at the Semen Production Unit (SPU), Qadirabad, Sahiwal, Pakistan. Elite breeding bulls of high genetic makeup are maintained at this unit. Semen collected from these bulls is processed, frozen and supplied to various artificial insemination (AI) centers throughout the Punjab province of Pakistan. A total of six adult Sahiwal bulls, aged 4-4.5 years, with clinically normal reproductive tracts, and regularly donating semen of acceptable quality were selected for this study. The bulls were kept under similar nutritional, hygienic and other management conditions. The details regarding management of these bulls have been described elsewhere (Rahman *et al.*, 2012).

***Experimental design*:** Among selected bulls, three bulls were used as treatment group and the rest three as control group. Animals of treatment group were subjected to bST (Boostin^{TM}–S, LG Life Sciences, Korea: A synthetic somatotropin preparation) administration- a subcutaneous dose of 500 mg per bull on weekly basis for 10 weeks, while to control group equal volume of normal saline was injected as placebo according to the same protocol as for treatment group.

Libido of each bull was monitored fortnightly in terms of reaction time. For this purpose, bulls were not sexually prepared. Each bull was led to a restrained teaser and freely permitted to mount and serve an artificial vagina. The time between the entry of the bull into the test area and the first semen ejaculation was taken as the reaction time (Henney *et al.,* 1990).

About 20 ml blood with and without anticoagulant (EDTA) was collected from each experimental bull fortnightly. The samples with anticoagulant were used for the determination of haematological parameters viz. haemoglobin (Hb) concentration, RBC count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), WBC count, differential leukocyte count (DLC) and platelets count, using standard procedures (Benjamin and Maxine, 1978; Coles, 1986).

Blood samples without anti-coagulant were used for separation of serum and analyzed for concentrations of biochemical metabolites viz; serum total proteins, albumin, globulin, glucose, urea and serum glutamic-pyruvic transaminase (SGPT) activity, using commercially available kits. For this purpose, samples and standards were processed as per instructions of the manufacturers of the respective kits. Absorbance of the samples and standards was measured using chemistry analyzer (BTS-330, Biosystems, Spain). The concentration of the respective biochemical metabolite was calculated through dividing absorbance of the sample by absorbance of the respective standard and multiplying by concentration of the standard.

The serum testosterone concentrations were determined using solid phase ELISA based on the principle of competitive binding between testosterone in the sample and testosterone-HRP conjugated for a constant amount of rabbit anti-testosterone. The detail of the assay has been described elsewhere (Ahmad *et al.*, 2012).

***Statistical analysis*:** Mean values (±SE) were computed for various parameters of bulls of each group. In order to see the magnitude of variation in these parameters between two groups, the data was analyzed through t-test (Steel *et al*., 1997).

RESULTS AND DISCUSSION

***Libido and serum testosterone*:** In the present study, mean reaction time was significantly lower in the treated group as compared to the control group ($P<0.01$; Table 1), indicating that rbST treatment increased libido of the Sahiwal treated bulls. Mean serum testosterone concentration in treated group (14.02±0.19 ng/ml) was significantly higher ($P<0.01$) as compared to control group (12.55±0.44 ng/ml; Table1). This agrees with El-Harairy (2000) and El-Gohary *et al.* (2011), who reported higher blood testosterone concentrations in rams treated with rbST compared to untreated rams. In bulls, Hafez *et al.* (2005) reported that bulls treated with rbST had 61% higher ($P<0.05$) plasma testosterone compared to untreated bulls.

According to Waites *et al.* (1985), bST and FSH stimulated secretion of IGF-I by the Sertoli cells of seminiferous tubules of testes. In turn, IGF-I increased the steroidogenic response of Leydig cells to LH and stimulated secretion of testosterone.

It is well established that libido in the male is mainly controlled by male sex hormone, testosterone (Hafez and Hafez, 2006), having a positive statistical correlation ($r=0.531$; $P<0.001$) (Bilskis *et al.*, 2012). In the present study, a significant increase in serum testosterone was recorded in bulls treated with bST compared to controls. This indicates that improvement in the libido of bST treated bulls was due to increased serum testosterone in these bulls.

***Haematological parameters*:** Mean blood haemoglobin (Hb) concentration did not differ between control and bST treated bulls (Table 1). However, Hb has been reported to decline slightly in treated goats as compared to control when goats were treated with 100 mg bST once every week for 8 weeks

Table 1. Mean (±SE) values of reaction time, serum testosterone, haematological parameters and serum biochemical metabolites in control and treated group of Sahiwal breeding bulls

Parameters	Control group	Treated group	t-value
Reaction time (Sec)	7.50 ± 0.42	5.25 ± 0.28	4.48**
Serum testosterone (ng/ml)	12.55 ± 0.44	14.02 ± 0.19	3.11**
Hb (G/dl)	12.43 ± 0.29	11.87 ± 0.14	1.75 NS
PCV (%)	40.78 ± 1.28	36.78 ± 0.64	2.80*
RBC (10^6/µl)	7.21 ± 0.11	7.25 ± 0.11	0.25 NS
MCV (fl)	54.90 ± 0.68	51.02 ± 0.47	4.70**
MCH (pg)	16.86 ± 0.14	16.20 ± 0.1	3.86**
MCHC (G/dl)	30.84 ± 0.26	31.78 ± 0.41	1.95 NS
ESR (mm/hr)	4.67 ± 0.17	5.22 ± 0.32	1.53 NS
WBC count (10^3/µl)	7.91 ± 0.38	10.49 ± 0.83	2.83*
Neutrophils (%)	56.78 ± 3.01	60.33 ± 2.24	0.95 NS
Lymphocytes (%)	40.00 ± 2.39	37.33 ± 2.24	0.62 NS
Monocytes (%)	1.89 ± 0.39	1.67 ± 0.33	0.43 NS
Eosinophils (%)	1.33 ± 0.69	0.67 ± 0.47	0.80 NS
Platelet count (10^3/µl)	149.44 ± 11.03	199.67 ± 22.53	2.00 NS
Total protein (G/dl)	8.21 ± 0.06	8.37 ± 0.17	0.87 NS
Serum albumin (G/dl)	3.00 ± 0.07	2.93 ± 0.11	0.51 NS
Serum globulin (G/dl)	5.17 ± 0.04	5.43 ± 0.11	2.33*
Serum glucose (mg/dl)	54.67 ± 1.52	57.67 ± 1.23	1.54 NS
Serum urea (mg/dl)	15.33 ± 0.82	12.00 ± 0.47	3.54**
SGPT (U/L)	36.00 ± 4.53	37.00 ± 1.93	0.20 NS

NS = Non significant; * = Significant at P<0.05; ** = Significant at P<0.01.

(Nasser *et al.,* 2007). When goats were treated with 50 mg bST, there was no effect on blood Hb. However, Nour-El-Din *et al.* (2009) reported an increase in Hb in lambs treated with 100 mg bST weekly as compared to control.

Significantly lower mean PCV was noticed in treated bulls as compared to control group (P<0.05; Table 1). Khaliq and Rahman (2010) also noted a decrease in PCV in bST treated buffaloes as compared to control (32.76±0.32 vs 33.36±0.41%) when a dose of 500 mg bST was injected to treated buffaloes twice at an interval of 16 days. Similar results were obtained in ewes (Sallam *et al.*, 2005) and goats (Nasser *et al.*, 2007). Physiological significance of this decreased PCV due to treatment of bulls with bST remains unclear.

There was non-significant difference in mean RBC counts in control and bST treated bulls (Table 1). Nasser *et al.* (2007) also recorded a slight but non-significant increase in RBC count in goats when 50 mg bST was given once every week for 8 weeks. However, when goats were injected with 100 mg rbST, there was a slight decrease in RBC count. However, Eppard *et al.* (1997) observed a significant decrease in RBC counts after bST treatment in lactating cows.

The MCV in bST treated bulls was significantly lower (P<0.01) than control bulls (Table 1). Low MCV is usually due to iron deficiency that results from parasitic infestation and chronic diseases (Benjamin and Maxine, 1978). However, in the present study the cause of significant reduction in MCV in Sahiwal bulls injected with bST remains unclear.

The MCH was lower in rbST treated group (P<0.05) compared to control (P<0.01; Table 1). The MCH is affected by variation in Hb synthesis (Benjamin and Maxine, 1978). The Hb concentration was slightly but non significantly lower in bST treated group, which might have been the cause of decreased MCH in bulls treated with bST.

The mean MCHC in rbST treated bulls was non significantly higher than controls (Table 1). MCHC varies due to Hb concentration (Benjamin and Maxine, 1978), which also differed non-significantly in treated and control bulls in this study.

There was a non significant difference in ESR between bulls of the two groups (Table 1). However, Khaliq and Rahman (2010) recorded a decrease in ESR when they administered 500 mg of rbST twice at an interval of 16 days to buffaloes. Besides species, differences in protocol between two studies can be blamed for this discrepancy.

Total leukocyte count was significantly higher in rbST treated bulls (P<0.05; Table 1). However, differential leukocytic count (DLC) revealed that percentages of none of the four leukocytes differed between bulls of the two groups. Sallam *et al.* (2005) reported no difference in the WBC counts in ewes given 50 mg rbST. However, a slight increase in WBC count was noted when 100 mg rbST was

used. The reason for significantly higher value of total leukocyte count in bST treated is still not clear.

Mean value of platelet count in control bulls was 149.44±11.03 x 10^3/µl, while in treated group it was 199.67±22.53 x $l0^3$/µl, the difference was non-significant (Table 1). Thus, treatment of Sahiwal bulls with bST had no effect on platelets count.

***Serum biochemical metabolites*:** Difference in the serum total proteins and albumin between bulls of two groups was non-significant (Table 1). These results are supported by those of Sallam *et al.* (2005) and Nasser *et al.* (2007), who recorded non significant effects of bST treatment on serum total proteins and albumin in ewes and goats, respectively. However, Nour-El-Din *et al.* (2009) reported that serum total proteins and albumin in growing lambs given 100 mg bST was higher ($P<0.05$) than control group.

Treatment of Sahiwal bulls with rbST significantly increased serum globulin compared to controls ($P<0.05$; Table 1). These results agree with those of Nour-El-Din *et al.* (2009), who reported higher serum globulin in lambs treated with 100 mg bST (4.03±0.05 vs 3.57±0.05 G/dL). However, Nasser *et al.* (2007) reported in 8 weeks study that there was non-significant difference in the mean values of serum globulin between control animals and those given 100 mg bST.

In the present study, difference in blood glucose between bulls of the two groups was non-significant (Table 1). Mellado *et al.* (2006) also recorded non-significant difference in serum glucose in male goats treated with 125 mg rbST for 99 days compared with controls. However, a significant increase in serum glucose was reported by Sauerwein *et al.* (2000), who treated bulls every two weeks with 640-mg rbGH for 14 weeks. This discrepancy can be attributed to differences in the dose of rbST and duration of treatment in the two studies.

Mean serum urea was significantly lower in bST treated bulls than controls ($P<0.01$; Table 1). This shows that treatment of Sahiwal bulls with bST resulted in decreased serum urea. Similarly, Nasser *et al.* (2007) reported a significant decrease in the urea concentration in goats treated with 50 or 100 mg bST for 8 weeks. However, Mellado *et al.* (2006) reported an increase in serum urea in goat bucks treated with 125 mg rbST for 99 days.

In the present study, there was no difference in SGPT activity between bulls of the two groups (Table 1). Similar results were reported by Sallam *et al.* (2005), who treated ewes for 8 weeks, with 50 or 100 mg rbST/2 weeks per ewe.

These results clearly indicated that libido and serum testosterone concentrations were significantly improved by bST treatment. Previous studies have revealed that bST treatment improves growth performance of dairy cows (Hodate *et al.,* 1991), Holstein steers (Bruckental *et al.,* 1997), lactating ewes (Brozos *et al.*, 1998), goats (Puchala *et al.,* 2001), lambs (Nour-El-Din *et al.,* 2009) and rams (Ibrahim, 2013). Moreover, improvement in semen quality parameters following treatment with bST has also been reported in Holstein males (MacDonald and Deaver, 1993), Zebu bulls (Santos *et al.,* 1999; Waqas, 2013), rams (Shakweer *et al.,* 2004; Ibrahim, 2013), stallion (De-Botton *et al.,*2008), buffalo calves (El-Aziz *et al.,* 2009) and lambs (El-Gohary *et al.,* 2011). Thus, besides improving milk yield in female animals (Sallam *et al.,* 2005; Vargas *et al.,* 2006; Nasser *et al.,* 2007; Qudus *et al.,* 2013), bST treatment has also beneficial effects on reproductive functions of male.

The results also showed that although bST treatment significantly affected a few of haematological and serum biochemical metabolite concentrations, the values of these affected parameters were within the normal reference values reported for bulls. Thus, it is quite fair to conclude that there was no adverse effect of bST treatment on liver and kidney functions and haematological indices in Sahiwal bulls.

***Conclusions*:** Based on the finding of the present study, it can be concluded that treatment of bulls with bST improved libido, increased serum testosterone, total leukocyte counts and serum concentration of globulin, and decreased PCV, MCV, MCH and serum urea. However, it had no effects on blood Hb, RBC count, MCHC, ESR, DLC, platelets count, serum total proteins, albumin, glucose and SGPT activity.

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