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RESEARCH ARTICLE

High Dose of Recombinant Bovine Somatotropin Do Alter Serum Biochemical and Hormonal Profiles of Nili Ravi Buffaloes

Tanweer Khaliq*, Zia-ur-Rahman and Ijaz Javed

[1]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: t_khaliqch@yahoo.com

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ABSTRACT

A research study was conducted to investigate the effect of high dose of recombinant bovine somatotropin (rbST) on the serum biochemical, liver enzymes and hormones of Nili Ravi buffaloes. Clinically healthy lactating buffaloes (n=16) were selected and divided into two equal groups. One served as experimental group (n=8) and the other as a control (n=8). Dose of 500 mg rbST/16 days/animals was injected subcutaneously to the experimental group subcutaneously, twice during the experimental period of 32 days after an interval of 16 days. Serum biochemical parameters (glucose, cholesterol, total proteins, urea and globulin), serum hormones (T_3, T_4 and cortisol) and liver enzymes (AST and ALT) were estimated. Glucose was significantly high while total proteins, globulin and urea were significantly low in the treated group. Significantly high ALT and low T_4 level were recorded in the bST treated group. It was concluded from this study that the studied parameters did alter in buffaloes with 500 mg rbST injection.

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INTRODUCTION

It is estimated that population of planet earth is increasing day by day and will reach nine billion in 2040-2050 (Anonymous, 2005a). Dairy products are considered as integral component of food sources (Anonymous, 2005b). Although tremendous improvement has been done in last century in milk productions but still more improvement is needed to meet ever increasing demands (Buzby *et al*., 2006; Bilal *et al.*, 2008; Pretty *et al*., 2008; Serbester *et al*., 2012). Economics of rearing buffaloes and cows is still being debated, and controversy continues in and against buffaloes without enough scientific data. According to the recent livestock census (Anonymous, 2011-12), the number of cattle in Pakistan has increased by about 4.2 million over that of buffaloes (cattle 36.9 and buffaloes 32.7 million). Several studies have shown that buffaloes can thrive well on poor-quality roughages and can comparatively better tide over scarcity and draught periods successfully. Being a multiple purpose animal, buffaloes play an important role in the economy of Pakistan. Buffalos are considered the most important milch animal in Pakistan and are also considered an important economic resource in many countries of world (Borghese and Mazzi, 2005). Bovine somatotropin (bST), a hormone produced from bovines that control and coordinates the metabolism of many tissues in dairy cattle (Bauman *et al.,* 1988; Batth *et al*., 2012). Recombinant bovine somatotropin (rbST) is a genetically engineered synthetic analogue of the natural hormone. Milk yield response to bST has been observed for all dairy cattle breeds and in animals of different genetic potential (Hartnell, 1995; Helal and Lasheen, 2008; Boonsanit *et al.,* 2012) and in goats as well (Chadio *et al.,* 2000). For many years, it was erroneously believed that bST only acted to promote general body growth; hence the name commonly used – bovine growth hormone. However, it is now known that bST is an important metabolic hormone and plays an important role in regulating the metabolism of proteins (MacRae *et al.,* 1991), fats (Etherton *et al.,* 1995) and carbohydrates (Knapp *et al.,* 1992).

The magnitude of animal's response to bST is greatly determined by environment, physiological stage and nutritional management. In Pakistan, as buffaloes contributes more than 58% of milk produced per annum, but scientific literature regarding the effect of bST on different parameters in buffaloes is scarce.

Ludri *et al*. (1989) reported that rbST injection to Indian buffaloes and Polidori *et al*. (1997) to Italian buffaloes to increase milk production without altering changes in milk fat contents. Likewise, Radcliff *et al*. (2000) and Van Baale (2005) using long and short term

treatment with rbST in cow did increase the milk production without altering the composition of milk. In all experiments normal recommended dose (320mg/head/14 days) was used. In the present study, double dose of rbST (500 mg/animal/16 days) was injected subcutaneously to find its effect on serum biochemical and hormonal profiles in Nili Ravi buffaloes.

MATERIALS AND METHODS

The experiment was conducted at Livestock Experiment Station, University of Agriculture Faisalabad. Sixteen Nili Ravi buffaloes, 7-10 years age, 3-4-months post calving and with 2nd to 4th lactation stage were used as experimental animal and kept under standard management conditions. All animals were examined thoroughly before the start of an experiment for physical and clinical health. Free stalls mixed ration as given in Table 1 (sugarcane and berseem) was offered to all animals. Maize oil cake (Rafhan Products Co. Ltd, Faisalabad) was offered to animals at least 7-10 days before the start of experiment according to milk yield (Table 2). Animals were divided into equal two groups. Group I served as control while animals of group II were injected 500 mg rbST s/c twice after 16 days intervals during the experimental period, i.e., 32 days.

Table 1: Chemical composition of fodders offered to buffaloes during experimental periods

	Sugar Cane (%)	Berseem (%)
Dry matter	31.8	13.9
Organic matter	94.9	88
Crude protein	6.2	20.4
Non-digestible fiber (NDF)	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

Table 2: Chemical composition of Rafhan Maize Oil Cake offered to buffaloes during the experimental period.

Dry matter %	93
Protein %	19
Digestible Protein %	16
Fat %	8
Crude Fibre %	10
Calcium %	0.06
Phosphorus %	0.56
TDN %	85
Metabolizeable Energy k.cal/kg Ruminants	2986
Net Energy Mcal/lb	0.81

Blood samples were collected on day 0, 1, 4, 8, 12, 16, 17, 20, 24, 28 and 32. Serum was separated and stored in aliquot at -20°C until further analysis. Glucose, total proteins, urea and cholesterol concentration was measured by using commercially available kits (Randox Laboratories, Ltd). Serum albumin concentration was determined by bromocresol-binding technique (Varley *et al.,* 1980). From total proteins, albumin was subtracted to get reading of globulin. Serum alanine transaminase (ALT; U/L) and aspartate transaminase (AST; U/L) were also performed using commercially available kit. Triiodothyronine (T_3) and thyroxin (T4) concentration was determined using BioCheck Triiodothyronine Enzyme immunoassay kit. For measurement of serum cortisol DRG solid phase enzyme immunoassay (Ref. No. EIA-1887; Lot No. 43K055) was used.

Data thus obtained were analyzed by applying one-way analysis of variance and significant results were further tested by using Duncan Multiple Range (DMR) test keeping significance level at $P<0.01$.

RESULTS

Overall serum glucose concentration was high ($P\leq0.01$) in rbST treated buffaloes as compared to control animals (Table 3). Mean serum glucose concentration did increase significantly in buffaloes after 2nd rbST injection on day 20 to 32 of the experimental period. The increase in serum glucose concentration was 12.12% after first injection while on the application of 2nd injection the increased amounted to 24.06%. In control group, overall serum glucose did increase to 15.11%. Very interestingly, the overall increase in the serum glucose was 19.18% between 17-24 days of 2nd injection of rbST in buffaloes.

Overall serum cholesterol was non-significantly high in rbST injected buffaloes (Table 3). Mean serum cholesterol concentration was high at different days in control animals as compared to rbST injected buffaloes. Treated buffaloes showed a significant decrease (4.02%) in serum urea concentration as compared to control animals. Overall serum globulin concentration was significantly low in rbST injected buffaloes. On day-12, globulin concentration did increase and then decreased on day 16, 17 and again increased from day 20 to 30 days. Significant decrease in overall serum total proteins was observed in rbST injected buffaloes as compared to control animals (Table 3).

On different days, mean serum ALT concentration was high in rbST injected buffaloes (Table 4). Overall ALT concentration increased 3.50% in rbST injected buffaloes. Mean overall concentration of serum AST did not differ between the groups. Means of serum T_3, T_4 and cortisol concentration are presented in Table 4. Non-significance difference was recorded in case of serum T_3 and cortisol overall mean and at different days of experimental period. However, overall mean serum T_4 did decrease significantly in rbST injected buffaloes as compared to control animals.

DISCUSSION

In the present study, serum glucose did increase significantly while globulin concentration did decrease significantly by two consecutive injections of rbST (500mg/16days/animal) in buffaloes. Similarly Aboin *et al.* (2013) reported significant increase in plasma glucose in non-lactating and non-pregnant dairy cows. Helal and Lasheen (2008) reported that plasma glucose concentration was significantly increased by rbST injection (500mg/animal/14days) to Egyptian Dairy Buffaloes. Gulay *et al.* (2004) reported that treatment with rbST during postpartum period stimulate glucose metabolism in cattle. Eisemann *et al.* (1986) did not observe any change in glucose after growth hormone injection. Variation in serum glucose concentration in rbST injected animals may be related to the dose, length of injection, number of samples being obtained after injection and above all timing of samples relative to feeding. Some of the inconsistency may also be related to

Table 3: Mean glucose, cholesterol, globulin, urea and proteins concentration of control and bovine somatotropin (500 mg s/c) injected buffaloes at various days.

Days	Glucose (mg/dL±SE)		Cholesterol (mg/dL±SE)		Globulin (g/dL±SE)		Proteins (g/dL±SE)		Urea (mg/dL±SE)	
	Control	bST	Control	bST	Control	bST	Control	bST	Control	bST
0	69.45±1.67^{ghi}	65.69±1.70^{i}	84.92±4.22	85.55±2.38	3.91±0.25	3.49±0.41	10.01±0.25	9.43±0.21	28.02±2.04	24.54±1.32
1st Injection										
1	65.42±1.65^{i}	66.53±1.32^{hi}	79.20±3.19	80.89±1.37	3.02±0.30	2.52±0.50	9.07±0.24	8.69±0.43	27.29±1.25	27.54±1.13
4	73.93±1.81^{defg}	73.10±1.90^{defg}	85.48±2.52	84.55±2.09	3.88±0.46	2.62±0.32	10.85±0.19	9.76±0.47	25.83±1.26	22.61±1.07
8	75.47±0.69^{cde}	76.19±1.14^{bcd}	78.59±1.52	84.82±2.43	3.05±0.23	2.58±0.13	9.28±0.24	8.87±0.28	24.21±1.78	24.14±1.38
12	74.20±1.19^{def}	75.06±1.27^{cde}	75.18±1.10	75.28±2.68	4.17±0.42	3.63±0.20	10.70±0.33	9.58±0.25	31.43±2.70	25.20±0.72
16	77.41±1.56^{bcd}	65.83±1.22^{i}	74.88±3.01	76.12±0.99	3.03±0.50	3.60±0.35	10.29±0.36	10.18±0.33	21.35±2.44	23.35±1.04
2nd Injection										
17	69.86±1.40^{fghi}	71.11±1.45^{efgh}	78.40±3.18	75.35±5.10	3.36±0.20	3.42±0.33	9.99±0.14	9.43±0.24	22.50±2.58	21.90±1.12
20	69.31±2.15^{ghi}	79.61±1.86^{bc}	78.59±2.35	83.75±2.72	3.09±0.27	2.47±0.20	9.25±0.37	9.34±0.08	25.95±1.81	24.01±0.53
24	79.17±1.03^{bc}	85.12±1.76^{a}	85.62±3.20	82.50±2.26	3.03±0.17	2.42±0.08	9.11±0.16	8.16±0.17	33.04±3.02	24.59±1.36
28	86.16±1.42^{a}	80.68±1.44^{b}	77.63±2.71	78.32±5.10	3.13±0.35	2.51±0.36	10.19±0.23	9.41±0.24	21.33±1.26	21.21±2.01
32	79.26±1.00^{bc}	77.13±1.13^{bcd}	77.71±2.51	85.07±2.51	3.11±0.27	3.98±0.33	10.75±0.25	10.90±0.25	22.20±2.24	20.98±1.26

AB, similar alphabets in a row do not differ significantly (P≤0.01). a-i, similar alphabets for interaction do not differ significantly (P≤0.01).

Table 4: Mean serum alanine transaminase, aspartate transaminase, triiodothyronine, thyroxine and cortisole concentration of control and bovine somatotropin (500 mg s/c) injected buffaloes at various days.

Days	Alanine transaminase (ALT; U/L)		Aspartate transaminase (AST; U/L)		Triiodothyronine (T_3; ng/mL±SE)		Thyroxine (T_4; µg/dL±SE)		Cortisole (ng/mL±SE)	
	Control	bST	Control	bST	Control	bST	Control	bST	Control	bST
0	36.02±2.59	38.35±1.18	59.86±0.67	58.56±3.11	0.891±0.098	1.089±0.089	8.535±0.926	7.814±1.190	31.49±3.49	34.59±3.13
1st Injection										
1	33.60±1.95	36.38±1.22	54.91±0.28	54.74±2.83	1.021±0.079	1.080±0.100	8.595±0.788	7.620±1.144	31.19±3.59	35.26±3.22
4	31.03±1.98	35.34±1.51	55.10±1.26	55.26±2.81	1.040±0.085	1.056±0.086	8.618±0.779	7.050±1.057	33.52±395	30.94±2.99
8	32.24±0.82	38.89±2.49	53.26±1.51	57.28±2.53	1.033±0.090	1.049±0.081	8.737±0.762	6.928±1.057	33.69±3.63	31.17±2.62
12	33.13±0.93	41.01±1.04	59.23±2.04	56.08±2.88	1.027±0.069	1.051±0.084	8.682±0.763	6.872±1.042	34.17±3.87	27.83±2.81
16	31.43±1.37	39.07±1.81	54.54±0.77	55.00±2.49	1.049±0.079	1.089±0.076	8.460±0.772	7.275±1.203	31.67±2.34	28.08±1.77
2nd Injection										
17	33.86±2.80	42.03±3.57	57.41±0.85	54.76±2.32	1.033±0.065	1.081±0.071	8.352±0.806	7.363±1.154	32.92±4.16	30.85±2.31
20	34.73±1.95	40.92±1.89	59.82±1.32	56.63±3.01	1.039±0.066	1.063±0.071	8.429±0.808	7.160±1.188	31.94±4.04	29.12±1.55
24	35.53±3.30	40.06±3.33	57.78±1.50	59.47±2.88	1.041±0.057	1.067±0.062	8.375±0.814	6.950±1.144	31.26±3.58	28.75±2.19
28	35.67±2.01	42.63±2.82	59.76±0.94	59.33±3.01	1.034±0.055	1.079±0.067	8.333±0.816	7.368±1.211	32.65±4.52	30.88±2.69
32	36.34±2.61	40.58±3.08	59.29±1.85	54.69±3.04	1.039±0.048	1.070±0.072	8.455±0.803	7.720±1.241	31.79±4.12	33.78±2.53

AB, similar alphabets in a row do not differ significantly (P≤0.01).

feeding. Some of the inconsistency may also be related to the preparation of rbST, by different companies and purity of hormones. Pituitary derived rbST by recombinant DNA technology resulted in an increase conversion of propionate to glucose in liver slices from lactating cow (McShane *et al.,* 1989). Alterations in serum glucose contents during pregnancy to lactation are the result of physiological changes in metabolic processes, which take place at the beginning of lactation (Zvorc *et al.,* 2006) therefore, regulating glucose delivery and uptake limiting step of milk synthesis. This may lead to the conclusion that insulin is independent of glucose uptake and decreases for other tissues except for the mammary gland following the onset of lactation. High serum cholesterol in rbST injected buffaloes may be due to breed and lactation in addition to high dose of rbST to cause stress in high milk production. Both lipogenesis and lipolysis are affected by rbST treatment, with effects on lipid synthesis being of major importance when animals are in positive energy balance, whereas effects on lipolysis predominate when animals are at an energy balance near zero or negative. In addition, the effects of rbST on lipid metabolism are chronic rather than acute. These chronic effects predominately involve alterations in the ability of acute homeostatic signals to alter rates of lipogenesis and lipolysis. These effects appear to be a direct action of rbST on adipose tissue (Bauman and Vernon, 1993; Etherton *et al.,* 1993).

A significant decrease in overall serum urea was observed in rbST treated buffaloes as compared to control at the end of the experiment. In another study, Sallam *et al.* (2004) reported a significant decrease in plasma urea concentration in sheep. Eisemann *et al.* (1986) observed a non-significant change in blood urea in rbST treated cow. In another study by McShane *et al.* (1989) a slight lowering of urea concentration at 2, 3 and 4th week of bST treatment was observed. In contrast, Annexstad *et al.* (1990) and McGuffey *et al.* (1990) determined slight higher serum urea in dairy cow after administration of rbST.

Limited information is available about the effects of rbST on proteins metabolism of domestic animals than for either lipid or carbohydrate metabolism. It is clear that rbST treatment increases muscle proteins accretion in growing animals (Crooker *et al.,* 1990) and reported an increase in milk protein synthesis by lactating cows (Laurent *et al.,* 1992). A decrease in serum protein and globulin in rbST injected buffaloes may be related to high dose of rbST injected to these animals. A possible mechanism to suggest for the decrease in proteins could be the utilization of amino acids as a gluconeogenic substrate for glucose synthesis to fulfill the demand of glucose for making up of lactose to increase milk production. However Sallam *et al.* (2004) didn't find any change in plasma protein in rbST treated sheep. On the other hand, Prasad and Singh (2010) reported that rbST treatment influenced milk proteins and lactose without affecting plasma glucose. They also mentioned that variation in milk yield, plasma glucose (P≤0.01) and fat between buffaloes was significant (P≤0.05).

Buffaloes injected with two consecutive injections of 500mg/16 days of rbST did show a significant increase in ALT while AST did not show any alteration as compared

to control animals. These results are similar to the finding of Reese *et al.* (1984) and in contrast to Kudlac *et al.* (1988) where they did observe a significant increase in AST activity during lactation. This may be due to increased metabolic activity of liver due to rbST injection, together with possible stress of lactation and energy loss, which leads liver cells to secrete ALT. However Sallam *et al.* (2004) didn't find any change in ALT as well AST in rbST trearted sheep. Triiodothyronine (T_3) concentration in serum of rbST-treated buffaloes did not change. Oldenbroek *et al.* (1993) find similar observations in cattle treated with rbST. In general, concentration of T_3 and T_4 in blood rises during late lactation (Baldi, 1999). A significant decrease in serum thyroxine was observed in the rbST-treated as compared to their normal buffaloes. Siget *et al.* (1995) did not notice significant changes in the levels of T_3 and T_4 under the influence of application of rbST fed various levels of crude proteins. Annexstad *et al.* (1990) reported that circulating concentration of T_4 was not affected by rbST. Likewise, Johnson *et al.* (1991) observed no changes in circulating levels of thyroxine when animals were injected with rbST. Bouda *et al.* (1991) and Graf *et al.* (1991) did observe similar changes in thyroxine, and values were found to be within the physiological range. Chadio *et al.* (2002) observed no change in thyroxin level after administering rbST however a highly significant increase in T_3 was observed. Schams *et al.* (1991) did observe a similar result for thyroid hormone and are in accordance to the present study where T_3 was not affected while a significant decrease in T_4 was observed in dual purpose German cattle showing a negative correlation with milk production. This decrease in T_4 might be due to the mechanism of animals to conserve energy by decreasing basal metabolic rate, which is typical of a dual-purpose cow (Schams *et al.*, 1991).

Serum cortisol concentration did not differ between experimental groups. The lack of negative effect of cortisol on milk production and on its composition suggests that at least 500 mg of somatotropin did not induce stress in experimental buffaloes. Environmental factors, water deprivation (Silanikove, 1992) psychological as well as the outcome of the diseases such as mastitis (Shuster *et al*. 1991) have been reported to affect negatively in terms of milk production in dairy cow. The common denominator in most of these situations is the stimulation and activation of the hormonal axis which induces the elevation in serum corticosteroids from adrenals and in turn decreases milk in production.

Conclusion: Most of the studied parameters do g*et al*ter with the high dose (500 mg rbST injection) in lactating buffaloes. Long term's studies are required to examine the health related biomarkers. Additional studies are underway in our laboratory to study the effect of rbST on the milk composition and serum biomarkers of lactating buffaloes in accordance with the present protocol.

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