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Effects of exogenous bovine somatotropin (rbST) on blood biochemistry of Kundhi buffalo calves

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Abstract
Recombinant Bovine somatotropin (rbST) is one of the hormones potentially used as growth promoters in feedlot animals; however, limited data is available regarding its effects on blood biochemistry of Kundhi buffalo calves (KBC). Therefore, the current study was conducted to analyze the effects of rbST on blood biochemistry of KBC. For this purpose, selected calves were randomly distributed into three groups (A, B and C) with four calves per group. Group A was kept as control, while group B and C were treated with rbST @ 0.5 mg- and 1.0 mg/kg body weight (BW) fortnightly, respectively. After eleven weeks of treatment, blood samples were analyzed for blood metabolites *i.e.* glucose, protein and lipids, which are major metabolic substrates in the blood required for the growth and fattening of calves and used as fattening indicators. Our data revealed that blood glucose was increased significantly in group C (74.45 ± 0.71 mg/dl) and B (70.50 ± 0.28 mg/dl) as compared to control group A (62.17 ± 1.30 mg/dl). Protein level showed significant difference between rbST treated groups C (7.92 ± 0.07 g/dl) and B (7.44 ± 0.08 g/dl) and control group A (6.44 ± 0.11g/dl). Consistent with the increase in blood metabolites, body weight of group C was significantly (P<0.05) increased, followed by group B and A, respectively. Moreover, feed intake was recorded significantly differently (P<0.005) between treated and untreated groups. Similarly, feed efficiency rate (FCR) was found significantly improved (P<0.005) in group C and B compared to group A. Furthermore, total lipid level was increased significantly in group C (521.39 ± 3.34 mg/dl) and B (516.07 ± 3.72 mg/dl) as compared to control group A (469.65 ± 7.03 mg/dl), but the difference was non-significant between group B and C. Calcium level was significantly different between rbST treated groups C (8.97 ± 0.13 mg/dl) and B (8.14 ± 0.15g/dl) and control group A (6.56 ± 0.11). Finally, non-significant difference was found in sodium and potassium levels between rbST treated groups and control group. Overall, our data revealed that fattening indicators have been significantly enhanced in calves treated with rbST@1 mg/kg BW and that this could be an effective dose for better outcomes.

Keywords: Adaptation period, blood biochemistry, fattening ration, recombinant bovine somatotropin

1. Introduction
Malnutrition and specifically the lack of protein-rich human diet have been considered as an emerging challenge for the growing population of the world. One of the sources of protein in the human diet is animal protein and unfortunate, Pakistan is however deficient in animal proteins. [1]. To cope with this challenge, proper management of fattening bulls can make the buffalo meat industry a valuable meat business across the country and, to a great extent can satisfy the demand for meat proteins. Indeed, the buffalo growth rate is compatible with other exotic cattle breeds. Studies have suggested that because of better digestibility, buffalos grow faster than cattle, even if fed on poor quality roughage and grasses. It is known that buffalo calves yield 50% of dressing meat when slaughtered at 18 months of age. Therefore, cost of fattening per kg body weight is much lower for buffalo calves than cattle, calves [2]. Secondly, the increase demand for animal protein has much pushed up researchers to boost up the growth and production of livestock through scientific interventions like feeding and application of growth promoters.

To increase livestock production, one of the areas that has been of much interest is the application several natural and synthetic hormones (growth promoters) to efficiently produce leaner meat [3]. Of these anabolic implants (both androgenic and estrogenic), bovine somatotropin and ionophores have been successfully administered either as implants or injections or supplementation in animal's routine diet [4].

Exogenous bovine somatotropin, known as recombinant bovine somatotropin (rbST) is a synthetically derived hormone that mirrored naturally occurring bovine somatotropin or slightly modified by recombinant means such as adding, reducing or altering any of amino acids. The mode of action of rbST, as like naturally occurring somatotropin, initiates upon binding of the hormone with the target receptor [5]. This binding step stimulates production of insulin-like growth factor-I (IGF-I) by the liver, which in turn stimulates the osteoblast and chondrocyte to promote bone growth. These events enhance calcium retention, concentration and thus increase bone mineralization. Consequently, this results into increase of muscular mass through the creation of new muscle cells and further promotes lipolysis, which results in the reduction of adipose tissue [6]. Current practices regarding the use of rbST indicate that it has been largely used for increased milk production in lactating cows, while, little attention has been given on its use as growth promoters in young calves. Moreover, in spite of being an expected promising candidate as growth promoters, a systemic and comprehensive data on use of rbST as growth promoters in buffalo calves is lacking. As a first step, the current study is therefore designed to observe the effects of rbST on growth performance and blood biochemical profile of Kundhibuffalo calves that were fed on fattening ration.

2. Materials and Methods

2.1 Experimental animals

The study was approved by the ethical committee of the Sindh Agriculture University, Tandojam and all the procedures were carried out according to the local committee norms. In the current study, twelve clinically healthy male Kundhi buffalo calves of 4 to 6 months of age, with an average body weight of 60 kg were randomly recruited at the Livestock Experiment Station (LES), Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. Throughout the experimental trial, they were kept on the same plan of nutrition and management. First, the calves were allowed for at least two weeks to acclimatize themselves to nutritious feed (basal diet) and environment during the adaptation period. During this period the calves were ear tagged for identification, drenched (deworming) and injected subcutaneously with Ivermectin (ICI, Pakistan) against helminths and other parasitic infestations, vaccinated against common infectious diseases such as foot and mouth disease, anthrax and hemorrhagic septicemia.

After 2 weeks of adaptation period, animals were distributed randomly into three groups, *i.e.* A, B *a*nd C with four animals per group. Group A was kept as control by keeping on fattening ration, while, group B and C were fed on fattening ration along with subcutaneous injection of rbST @ 0.5 mg/kg and 1 mg/kg body weight fortnightly (BW), respectively for a total period of 11 weeks.

2.2 Housing and feeding of experimental animals

Experimental calves were kept within standard space and with all-time access to drinking water. Experimental fattening ration (concentrate mixture) containing maize crush and wheat bran as major energy ingredients was formulated as described by [7-8]. The chemical composition and nutritive values of components of feed ingredients were determined as per standard methods described by the Association of Official Analytical Chemist sas presented in Table-1. Additionally, experimental animals were fed on burseem and wheat straw as main green and dry roughages. They was offered twice daily *ad libitum* with access to a total mixed ration consisting of 60% roughages.

2.3 Administration of rbST

Calculated amount as advised by the manufacturer was injected subcutaneously in the ischiorectal fossa (depression beside the tail head) or behind the shoulder (post scapular) with 14 day intervals.

2.4 Collection and analysis of blood samples

Blood samples were collected on day 7 and 14 after each injection of rbST and were processed for serum isolation. Samples for biochemical analysis were prepared as per instructions provided in the datasheet of commercially available test kits. The values of blood metabolites were determined by measuring absorbance of the standard and samples through UV-1800 Spectrophotometer (Hitachi, Japan) at a specific wavelength as recommended for each variable. The glucose in serum was determined by the "GOD-POP" enzymatic photometric test. The serum total protein was determined by the Biuret kit method. The serum total lipid level was determined by the sulfo-phospho-vanillin method. The serum calcium level was determined by the cresolphthalein-complexone (CPC) method. The sodium in serum was determined by the Uranylthioglycolate method with precipitation. The concentration of potassium in serum samples was determined by sodium tetraphenylborate method. To determine the body weight (for calculating daily body weight gain), animals were deprived of water and feed for 12 hrs after an adaptation period followed by determination of the initial body weight. After this, body weight was determined on a weekly basis just before feeding. To determine feed intake, the ration (Table-1) was weighed and offered to individual animals every morning. Next morning, the refusals were weighed and recorded to calculate the feed intake. In determining feed efficiency, the consumption of feed intake in kilograms for one kilogram weight gain of each calf was calculated that was expressed as FCR.

2.5 Statistical Analysis

Data obtained was analyzed statistically by using statistical software package MSTAT-C following complete randomized design (CRD) [9]. Further, Tukey's Honestly Significant Difference Test (THSDT) significant difference test was used to compare the difference between the means of treatment groups.

3. Results

3.1 Glucose (mg/dl)

Our results indicated that during treatment period the level of blood glucose was significantly ($p<0.05$) higher in group C (74.45 ± 0.71 mg/dl) followed by in group B (70.50 ± 0.28 mg/dl) as compared to control group A (62.17 ± 1.30 mg/dl) (Table-2). Moreover, our results indicated a significant increase of blood glucose level of calves belong to group B and C in contrast to control group A, respectively.

3.2 Protein (g/dl)

Our data showed that the calves of group C showed increased level (7.92 ± 0.07 g/dl)of blood protein followed by group B(7.44 ± 0.08 g/dl) as compared to control group A (6.44 ± 0.11 g/dl). For statistical analysis, the significant difference ($p<0.05$) in blood protein concentration of calves in group C and B compared to the control group A was observed (Table-2).

3.3 Lipids (mg/dl)
The results of our study revealed that the highest level of total lipids in the blood of calves was recorded in group C (521.39 ± 3.34 mg/dl) followed by in group B (516.07 ± 3.72 mg/dl) and A (469.65 ± 7.03 mg/dl) (Table-2). Statistical analysis showed significant difference ($p<0.05$) between rbST treated groups B and C as compared to control group A. Moreover, comparison of means showed significant increase in total blood lipids in group B and C in contrast to group A, while the differences in groups B and C was non-significant ($p > 0.05$) (Table 2).

3.4 Calcium (mg/dl)
Our study revealed that concentration of calcium in the blood of calves belonged to group C was found the highest (8.97 ± 0.13 mg/dl), followed by group B (8.14 ± 0.15 mg/dl) and control group A (6.56 ± 0.11 mg/dl), respectively. Blood calcium level showed significant difference ($p<0.05$) between rbST treated groups C and B compared to control group A. Furthermore comparison of means showed significant increase in blood calcium level of calves in group B and C in contrast to control group A, respectively, while, the difference was also significant in calves of group B and C (Table 2).

3.5 Sodium (mmol/L)
Our results indicated that sodium level was relatively similar in all the three groups of calves. The highest level of blood sodium was recorded in group B (135.61 ± 0.18 mmol/L) followed by control group A (134.49 ± 2.19mmol/L) and group C (133.35 ± 0.70 mmol/L). The results revealed non-significant difference ($p>0.05$) in concentration of sodium in blood of group B and C as compared to control group A. Furthermore, comparison of means also showed non-significant difference in blood sodium level of calves in group B and C in contrast to control group A respectively while, the difference was also non-significant in calves of group B and C (Table 2).

3.6 Potassium (mmol/L)
Our results showed non-significant difference ($p>0.05$) in potassium concentration in the blood of calves in group B (5.01 ± 0.05 mmol/L) and C(4.98 ± 0.05 mmol/L) as compared to control group A (4.97 ± 0.05 mmol/L) (Table-2). Moreover, comparison of means also showed non-significant difference in blood potassium level in group B and C in contrast to control group A respectively, while, the difference was also non-significant in calves of group B and C.

3.7 Physical parameters
In addition to the measurement of the key blood metabolites of fattening indicators as determined above, we also calculated physical parameters such as feed intake, weight gain and FCR, to correlate our findings. To this end, our results indicated that group C (mean: 5.39 ± 0.06) consumed the highest feed intake, followed by group B (Mean: 6.20 ± 0.08) and A (6.70 ± 0.11), respectively. Statistical analysis indicated a significant difference ($P<0.05$) between the rbST treated and untreated groups. Inconsistent with this, dose effects of the rbST on treatment groups showed a significant ($P<0.05$) increase in feed intake in group C and group B compared to group A. Similarly, our results indicated that group C that received higher doses of rbST (@1mg/kg bwt) revealed highest increase (0.78 ± 0.04) in average daily weight gain in contrast to group B (0.64 ± 0.00) and control group A (0.47 ± 0.00). Tukey's Honestly Significant Different Test of comparison of means of groups indicated significant ($P<0.05$) increase in average daily weight gain of calves in treatment groups of C and B in contrast to an untreated control group. Finally, the significant difference ($P<0.05$) was observed in FCR between the rbST treated and untreated groups. Moreover, FCR of group C calves was recorded 8.09 ± 0.28, of group B 8.64 ± 0.44 and of group A 11.14 ± 0.40 indicating highest FCR for group C, however, no significant difference ($P>0.050$ was observed between FCR of group C and group B, respectively.

4. Discussion
Use of rbST as growth promoter in Kundi buffalo calves (KBC) seems under reported, although, it has been successfully used for enhanced milk production in dairy cows. This is a matter of fact that buffalo calves grow faster than that of cow calves, despite very little attention has been given to them for fattening. The current report was therefore aimed to measure blood metabolites that are considered as fattening indicators after treatment with rbST for a considerable time period. Moreover, we have also determined physical parameters like daily feed intake, average weight gain and feed conversion ratio to confidently correlate our findings obtained from blood biochemistry. Thus, we determined blood glucose, protein, lipids, calcium, and sodium and potassium levels on a weekly basis after rbST treatment for a total period of 11 weeks. In parallel, we also determined daily feed intake, weight gain and FCR on weekly basis. We compared the mean values of above parameters and observed that rbST treatment has significantly increased, most of the blood metabolites as well feed intake, weight gain and FCR. Overall, based on our findings, we are confident that rbST can be a promising candidate for animal fattening.

Our results showed that the higher glucose level was observed in calves of group C that received rbST at the dose rate of 1.0 mg/ kg body weight, followed by group B that received rbST at the dose rate of 0.5 mg/ kg body weight. The findings of the present study are in agreement with those [10-13], who observed increased plasma glucose in calves treated with rbST. The higher glucose level in blood of calves in the rbST treated groups in the present study, and the overall diabetogenic effect of rbST in general, may be due to the fact that rbST more likely greatly increases liver gluconeogenesis as well as peripheral resistance to insulin. First, rbST inhibits the uptake of glucose by peripheral tissues (e.g., adipose) and reduces whole-body glucose oxidation to CO_2. In response, utilization of glucose by peripheral tissues is greatly reduced, thereby increasing glucose concentration in blood. Second, rbST treatment appears to increase gluconeogenic rates in liver, thereby directly increasing the supply of glucose via *de novo* synthesis. Furthermore, the rbST treatment group was higher than in part would contribute to increase the blood glucose level and ultimately increase weight gain. In contrast, our current observations are not in agreement with the findings [14-16],who reported that plasma glucose level was not different between rbST treated steers and controls. The similitude in plasma glucose levels between rbST-treated and the control calves were probably due to an adjustment to a new set point in maintenance of glucose homeostasis, because of effects of rbST on tissue metabolism of carbohydrates. The increased blood protein level in our study is due to the fact that rbST greatly reduced the protein catabolism in the body and maximized the absorption of amino acids from the gastrointestinal tract. These results are matching with those of [17-19]. Davis *et al*. also found that rbST treatment increased

serum total protein concentration in addition to increased milk production [20]. The increase in serum total protein by a short-term rbST treatment occurs due to reduction in oxidation of amino acids and increased mobilization of labile protein reserves, whereas in long-term studies increase in voluntary feed intake also contributes towards more plasma protein, While, the decrease in serum total protein by a short-term rbST treatment occurs because of decrease oxidation of amino acids, as rbST caused a concomitant increase in oxidation of none esterified fatty acids (NEFA) in growing heifers. The net effect is a decrease in serum total protein and an increase in whole body protein-synthesis and a decrease in lipid accretion, consistent with the production-effects of exogenous rbST in growing animals [21-23] The total lipids level observed in the blood of calves was significantly increased ($p<0.05$) in the rbST treated groups in comparison to control group but the difference was non-significant in group B and C ($p>0.05$) indicating that rbST at a specific concentration in the body exerts lipolytic activity beyond which the increase in dose not promote lipid catabolism. The findings of the present study are in accordance with those of [23, 24] who found an apparent increase in serum total lipids level in multifarious cows receiving 250 mg and 500 mg rbST, [25] also observed significant increase in polyunsaturated fatty acids in Holstein Friesian bull calves treated with rbST in his experiment on Holstein cows reported that plasma fatty acids were elevated in rbST treated cows also confirmed the present findings that non esterifies fatty acids (NEFA) were greatly increased by rbST treatment [27-28]. However, the present observations are not matching with the findings who reported that plasma total lipids were not affected by rbST treatment in cows and buffaloes [29-30]. Adipose tissue and lipid metabolism provide examples to illustrate the mechanisms that allow rbST to alter nutrient partitioning. Effects of rbST on adipose tissue are direct, and treatment alters both lipogenesis and lipolysis with the net effect being related to energy balance [31-32]. If treatment is initiated when animals are in positive energy balance, adipose tissue changes involve a reduction in lipogenesis, whereas rates of lipolysis are enhanced if treatment occurs when animals are in negative energy balance. The biologic mechanisms that allow for these adaptations include changes in amounts of key enzymes and alterations in the signal transduction system for the homeostatic signals that acutely regulate lipogenesis and lipolysis [33]. During the experimental period, high calcium concentration in group C and B as compared to control group A, revels that at a high dose rbST exerts more profound effect on the blood calcium level increasing it at a dose-dependent manner. This increase in serum calcium level in the present study may be due to excessive absorption of calcium from the gastrointestinal tract and its retention in the body of calves. The findings of the present study are not in coincidence with those who reported from their studies that serum electrolytes, particularly calcium were not affected by rbST treatment [34-36]. These contrasting results of increased serum calcium level in the present study under the influence of rbST are probably due to excessive absorption of calcium from the gastrointestinal tract and its retention in the body of calves. Besides these, many other factors like the differences in species, breed, sex, age, type of animal, nutritional status, stage of production and environmental condition in which the experiments have been conducted may be a source of variation. Sodium and potassium level in the blood were relatively similar in all groups of calves in the current study, showing that at a high dose of rbST, the concentration of these two electrolytes remains stable. Our current finding are in agreement with those who reported from their studies that serum electrolytes particularly sodium and potassium were not affected by rbST treatment. These findings are also in close relation with those who during their experiments on lactating buffaloes reported that sodium and potassium were not altered by rbST injections. Overall, our study indicated that rbST treatment @ 1mg/Kg BW is preferably better and remarkably effective for increasing the performance of young calves as compared to @ 0.5 mg/kg BW.

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Table 1: Formulation and Chemical composition of experimental fattening ration (% on DM basis).

Ingredients	Quantity (Kg)	DM	CP	TDN	CF	Ca	P
Dry roughages							
Wheat Straw	20	18	0.47	8.64	7.4	0.04	0.01
Green roughages							
Barseem	40	7.2	1.15	4.61	1.61	0.26	0.02
Concentrates							
Maize Crushed	3	2.7	0.30	2.24	0.05	-----	0.02
Wheat Bran	4	3.56	0.57	2.49	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	3.63	0.24	0.01	0.04
Di-Cal. Phosphate	0.25	0.25	------	------	------	0.09	-----
Molasses	2	1.56	0.06	1.14	------	0.02	-----
Salt	0.25	0.25	-----	-----	-----	0.09	------
Total	100.5	61.66	9.60	41.42	11.43	0.57	0.41
Chemical composition			16	68	18.5	1.05	0.6

Table 2: Effect of rbST on some blood biochemical variables of Kundhi buffalo calves fed fattening ration during treatment period of 11weeks.

Particular	Treatments		
	A (Control)	B	C
Glucose (mg/dl)	62.17 ±1.30[a]	70.50 ±0.28[b]	74.45 ±0.71[c]
Protein (g/dl)	6.44 ±0.11[a]	7.44 ±0.08[b]	7.92 ±0.07[c]
Lipids (mg/dl)	469.65 ±7.03[a]	516.07 ±3.72[b]	521.39 ±3.34[b]
Calcium (mg/dl)	6.56 ±0.11[a]	8.14 ±0.15[b]	8.97 ±0.13[c]
Sodium (mmol/l)	134.49 ±2.19[a]	135.61 ±0.18[a]	133.35 ±0.70[a]
Potassium (mmol/l)	4.97 ± 0.08[a]	5.01 ± 0.05[a]	4.98 ± 0.10[a]

Means with different superscripts in a row differ significantly ($P<0.05$)

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