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Article

Effect of Recombinant Bovine Somatotropin (rbST) on Somatic Cell Count and Mineral Contents in Milk of Kundhi Buffalo

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Abstract: The objective of present study to evaluate the effect of rbST on somatic cell count (SCC) and mineral contents in milk of kundhi buffalo. A total of 12 multiparous kundhi buffaloes were managed at livestock experimental farm, Sindh Agriculture University, Tandojam Pakistan. The animals were divided into four groups assigned with A, B, C and D letters (3animals/group). The experimental ration was designed according to the 2 x 2 factorial designs. The A and B groups were given low protein-energy diet, while C and D groups were given high protein-energy diet. Then rbST was administered at the dose rate of 3.0 mg/kg body weight subcutaneously to group B and D animals. SCC were increased ($P<0.001$) in milk of LPE+rbST treated group vs. LPE or HPE diet groups, while it was decreased ($P<0.001$) in HPE+rbST treated group vs. LPE+rbST treated group. The calcium level in milk of LPE+rbST treated and HPE groups altered ($P<0.001$) as compared to LPE group, while non-significant effect was observed in HPE+rbST treated group. A very slight increase in ash content was observed in rbST treated groups vs. untreated groups. The chloride contents were increased ($P<0.001$) in milk of HPE+rbST treated group, while non-

significantly increased in milk of LPE, HPE and LPE+rbST treated groups. The increase ($P<0.001$) sodium level was seen in milk of HPE vs. LPE groups, while non-significant effect was observed in sodium indexes of milk from rbST treated groups vs. LPE group. The concentration of ash and sodium contents was unaffected in milk through rbST treatment. The SCC, calcium and chloride contents were elevated in milk of buffaloes given rbST treatment, but within normal ranges. Thus, it is expected to be no adverse effects on the quality of milk and milk products.

Key words: rbST, Somatic Cell Count, Minerals Contents, Buffalo Milk

1. Introduction

Modern dairy farming has become a highly complex agribusiness and an intensive business now days. Globally livestock production growing faster than any other sector in terms of added values products. The developing countries were tried to cope with the increase in demand mainly by expanding livestock population. To produce maximum milk yield, the animals are pushed to their physiological limits through a combination of selective breeding, balanced ration and application of latest technologies. Recently, however, technologies have emerged or further evaluated that allow increase in milk production through the manipulation of metabolism (Lean *et al.,* 1991). In order to achieve or enhance the maximum milk production, introduction of new tools nowadays is getting importance. Amongst those emerging technologies the use of hormones have significant impact on the dairy industry for improvisation of milk production in dairy animals (Castro-Solla *et al.,* 2007), and bovine somatotropin is vital one. The bovine somatotropin is a hormone which naturally produced from the pituitary gland of cattle and buffaloes, and play vital role in nutrient partitioning and milk production (Capper, 2010). During the recent days, synthetic recombinant bovine somatotropin hormone employed for the production of milk yield in livestock animals especially in cattle and buffaloes (Collier and Bauman, 2014). The published literature recommended that rbST boosted up the milk production in diverse species such as in cow (Eppard, 1997), buffalo (Moallam *et al.,* 2000; Jorge *et al.,* 2002; Gulay *et al.,* 2004; Mishra and Shukla, 2004), sheep (Baldi, 1999) and in goat (Baldi, 1999). Somatic cell counts (SCC) are being used as an index of the inflammatory condition of the udder. These are secreted during normal course of lactation in milk and there are a valid indication of abnormal milk secretion, milk composition (fat, protein, lactose, ash, macro and micro minerals) and mammary disease in dairy animals (Haenlein and Hinckley, 1995). A number of factors like genetics, environmental, management, stage of lactation, parity and season influences secretion of SCC in milk of cattle, buffaloes and goat (Lin and Chang, 1994; Kehrli and Shuster, 1994; Wilson, 1994; Muggli, 1995; Singh and Ludri, 2001). Increased

milk yield may have chances to increase the incidence of mastitis and more somatic cell count then lower yielding animals (Eppard et al. 1992). The somatic cell count usually from part payment structure to farmers with defined threshold of concentration determining the qualification for bonus payment or penalty charges (Garcia-Cordero *et al.,* 2010). The high somatic cell count in milk reduced the shelf life and produces off flavors, also affect the udder health, quality of milk and milk products such as cheese and yoghurt (Ma *et al.,* 2000). The milk contains small quantity of chlorides and other minerals (calcium, sodium, potassium and magnesium) that influence on the physico-chemical properties and nutritive value of milk. The salty taste of cow milk is because of increase chlorides and decrease in lactose content especially in some conditions such as mastitis and late lactation.

In our conditions, the rbST is extensively used for milk production from buffalo local breed (kundhi). That milk is also used for manufacturing of different dairy products such as cheese and yoghurt. The milk and dairy products from rbST treated animals used by the human beings, possible cause public health issues. Fewer studies have been conducted to evaluate the effect of rbST on milk composition of buffaloes importantly on milk composition in kundhi breed of buffalo.

Keeping all these view in mind, the present study was conducted to see the effect of rbST supplemented diet on the somatic cell count and mineral contents in milk of kundhi buffalo. It was hypothesized that alteration in the chemical composition of milk, whether suitable for processing dairy products or for public health issues.

2. Materials and Methods

2.1. Experimental Design

A total of 12 multiparous kundhi buffaloes were managed at livestock experimental farm, Sindh Agriculture University, Tandojam Pakistan. The animals were given free access of water, vaccinated and de-wormed as per schedule. The animals were divided into four groups and ear tagged with A, B, C and D alphabets (3/group). The experimental ration was designed according to the 2 x 2 factorial designs. The A and B groups were given low protein-energy diet, while C and D groups were given high protein-energy diet as shown in table 1. Then rbST (Bostin® LG chemical Ltd. Pakistan) was administered at the dose rate of 3.0 mg/kg body weight subcutaneously to group B and D animals. The rbST was administered twice in a week with an interval of $3^{1/2}$ days for one month.

2.2. Collection of Samples

The milk samples (200 ml) were collected into screw capped sterilized glass bottles from each buffalo once at the end of week. The samples were brought to the laboratory of Dairy Technology, Sindh Agriculture University, Tandojam Pakistan for further analysis.

Table 1. Formulation and composition of ration. LPE- low-protein energy and HPE- high-protein energy.

Ingredients (kg)	**LPE diet**	**HPE diet**
Berseem	8.00	8.00
Wheat straw	4.00	4.00
Cotton seed cake	1.40	3.20
Linseed cake	0.20	0.20
Mung kuta	1.00	1.00
Wheat bran	2.00	0.80
Wheat crushes	1.00	0.40
Rice polish	0.80	2.00
Molasses	0.80	0.40
Calcium phosphate, limestone	0.05	0.05
Chemical composition (%)		
Dry matter	61	61
Crude protein	13	16
Total digestible nutrients	66	67
Crude fiber	18.00	19.00
Calcium	0.79	0.78
Phosphorus	0.63	0.66

2.3. Analysis Procedure

Somatic cell count (SCC/ml) of milk was analyzed by a Coulter counter (Coulter counter Inc., Hialeah, Florida USA) as previously described with little modification (Miller *et al.,* 1993). The calcium content was determined according to previous method (Bird *et al.,* 1981). The ash and chloride contents were determined according to the AOAC (AOAC, 2000). The values were calculated with given formulas:

Ash %= weight of ashed sample/weight of sample taken x 100,

Total chlorides %= titrated value x normality of acid x 0.0355/ sample weight.

Sodium concentration of buffalo milk samples was calculated from molar ration of sodium to chloride content (1:1.3) as suggested by Packard (1996). Firstly, the milk sample was analyzed for chloride content as mentioned above. Afterward, the sodium was calculated using following formula. However, molar excess value (30%) of chloride was taken into consideration to obtain an appropriate result. Thus,

it was deducted from the given value obtained after calculating the molar ratio. Sodium concentration mg/100 ml= chloride content in milk/1.3 x 1000

2.4. Statistical Analysis

The results were analyzed by employing one-tailed ANOVA, and least significance difference (LSD) was observed amongst the mean values. The data was analyzed through SPSS statistical software (Version 16.0). The significance point set as $P<0.05$.

3. Results

3.1. SCC in rbST Treated and Untreated Milk

The SCC indices in rbST treated and untreated milk of kundhi buffalo are mentioned in Table 2. The recorded SCC mean values were as 3522.6±334.89, 6426.6±418.48, 4744.2±524.03 and 4257.5±514.26 counts/ml in milk of LPE diet, LPE diet+rbST, HPE diet and HPE diet+rbST, respectively. As compared to LPE diet group, the SCC indices were increased ($P<0.001$) in rbST plus LPE diet treated group. As compared to HPE diet group, the SCC indices were decreased ($P<0.05$) in rbST plus HPE diet treated group.

Table 2. Somatic cell count (SCC/ml) in rbST treated and untreated milk

	Maximum	**Minimum**	**Mean**	**S.E**
LPE diet	7386	1369	3522.6	334.89
LPE diet+rbST	11391	2274	6426.6**	418.48
HPE diet	10911	1879	4744.2	524.03
HPE diet+rbST	11345	1457	4257.5*	514.26
P-value	P<0.001, <0.05			

3.2. Calcium level in rbST Treated and Untreated Milk

The calcium levels in rbST treated and untreated milk of kundhi buffalo are mentioned in Table 3. The observed calcium averaged values were as 157.89±2.80, 172.65±2.61, 178.01±4.20 and 177.51±4.20 mg/100 ml in milk of LPE diet, LPE diet+rbST, HPE diet and HPE diet+rbST, respectively. The high significance difference was observed between all the groups. The increase ($P<0.001$) in calcium concentration was seen in milk of buffaloes given LPE diet plus rbST treatment as compared to LPE diet. A non-significant difference was observed in milk of HPE diet given animals as compared to rbST treated groups.

Table 3. Calcium level (mg/100ml) in rbST treated and untreated milk

	Maximum	**Minimum**	**Mean**	**S.E**
LPE diet	120.12	190.00	157.89	2.80
LPE diet+rbST	150.02	195.02	172.65**	2.61
HPE diet	150.00	255.03	178.01	4.20
HPE diet+rbST	150.02	215.00	177.51	4.20
P-value	P<0.001			

3.3. Ash Content in rbST Treated and Untreated Milk

The ash content in rbST treated and untreated milk of kundhi buffalo are mentioned in Table 4. The recorded ash content mean values were as 0.69±0.01, 0.71±0.01, 0.71±0.02 and 0.73±0.02 (%) in milk of LPE diet, LPE diet+rbST, HPE diet and HPE diet+rbST, respectively. Ash content was relatively similar in all groups. A slightly increased in ash content was observed in rbST treated groups. The non-significance difference was observed between all the groups.

Table 4. Ash content (%) in rbST treated and untreated

	Maximum	**Minimum**	**Mean**	**S.E**
LPE diet	0.60	0.95	0.69	0.01
LPE diet+rbST	0.63	0.91	0.71	0.01
HPE diet	0.46	0.95	0.71	0.02
HPE diet+rbST	0.63	0.95	0.73	0.02
P-value	P>0.05			

3.4. Chlorides Content in rbST Treated and Untreated Milk

The chlorides content in rbST treated and untreated milk of kundhi buffalo are depicted in Table 5. The noticed chlorides content average indices were as 0.11±0.05, 0.11±0.003, 0.13±0.007 and 0.15±0.02 (%) in milk of LPE diet, LPE diet+rbST treatment, HPE diet and HPE diet+rbST treatment, respectively. However, non-significance difference was observed between all the groups. An increase (P<0.001) in chlorides contents was observed in milk from buffaloes given HPE plus rbST treatment compared to HPE and LPE diets fed animals.

Table 5. Chlorides content (%) in rbST treated and untreated milk

	Maximum	**Minimum**	**Mean**	**S.E**
LPE diet	0.08	0.17	0.11	0.05
LPE diet+rbST	0.08	0.16	0.11	0.003
HPE diet	0.07	0.27	0.13	0.007
HPE diet+rbST	0.07	0.80	0.15**	0.02
P-value	P<0.001			

3.5. Sodium Level in rbST Treated and Untreated Milk

The sodium level in rbST treated and untreated milk of kundhi buffalo are depicted in Table 6. The observed mean values of sodium were as 70.16±1.65, 78.52±2.31, 75.67±2.25 and 73.74±1.94 mg/100ml in milk of LPE diet, LPE diet+rbST, HPE diet and HPE diet+rbST, respectively. The significance difference was observed between all the groups. An increase was observed (P<0.001) in sodium concentration of milk from buffaloes maintained on HPE diet and HPE diet plus rbST treatment compared to buffaloes given LPE diet. While non-significant difference was observed when each group compared each other.

Table 6. Sodium level (mg/100ml) in rbST treated and untreated milk

	Maximum	**Minimum**	**Mean**	**S.E**
LPE diet	58	93.02	70.16	1.65
LPE diet+rbST	60.00	94.00	78.52**	2.31
HPE diet	54.26	95.00	75.67**	2.25
HPE diet+rbST	54.26	98.00	73.74	1.94
P-value	P<0.001			

4. Discussion

SCC and mineral salt contents are biomarkers of mammary gland health and milk quality, and elevation above normal limits indicates the sign of mastitis. As a consequence an increase in chlorides, sodium and calcium may occur due to passage from blood into the milk (Harding, 1995). In fact, minerals and/or salts may have a direct bearing on processing consideration as formation of curd during cheese making, the storage stability of concentrated milk, evaporated milk and ultra high temperature (UHT) milk, and stability of milk fats (Packard, 1996). It was also reported that administration rbST did not

affect composition of milk including milk fat, protein, lactose, ash, calcium and phosphorus concentrations, or somatic cell count (Hartnell *et al.,* 1991 and Barbano *et al.,* 1992). The present study was predicted, whether rbST have impact on milk composition of buffalo especially in kundhi breed, due to extensive usage of milk in dairy products (cheese, yoghurt). In present study, SCC was increased with rbST treatment but within normal ranges, which coincides with Harding (1995) findings. Previous reports suggested that SCC was present during mastitis (Bartlett *et al.,* 1991; Miller *et al.,* 1991). The SCC secreted into milk in normal conditions without presence of udder infection (Emanuelson and Funke, 1991) and their count is increased after one month of lactation. Nevertheless, high SCC in milk has adverse effect on quality of milk and milk products. Harding (1995) reported that high number of SCC in milk influenced on storage stability and flavor quality of pasteurized milk, and also observed a high incidence of unclean flavor, pasty texture and increase moisture content in cheese made from high SCC milk. It could be argued that SCC observed in rbST treated milk was within normal ranges, and no significant impact of SCC could be expected on milk quality and milk products.

In current study calcium concentration was increased in rbST treated milk groups. However, calcium level reported to be unaffected when bovine growth hormone treated to cows (Packard, 1996), whereas Bauman *et al.,* (Bauman *et al.,* 1992) reported that minerals were not substantially altered when rbST was used and did not differ in product process.

The ash content is an amount of non-combustible matter in milk which remains rather constant about 0.7%, a value much higher than normal is indicative conditions in the secreting glands. Indeed, it almost always gives the level of minerals in milk, which have direct bearing on processing considerations like formation of curd during cheese making, the strong stability of concentrated/evaporated milk or UHT milk and stability of milk lipids (Packard, 1996). However, content of ash in present study was relatively similar to that of reported by Webb *et al.,* (Webb *et al.,* 1974), with non-significant effect of rbST treatment. Pathan (2002) also investigated no significant effect of rbST treatment on ash content in buffalo milk.

Chlorides of milk are considered to be salt of milk which influences the stability of milk towards heat or alcohol coagulation, the age thickening of sweetened condensed milk and/or the coagulation of milk by rennin (Webb *et al.,* 1974). Fluctuation in the level of chlorides of milk is most significant factor in dairy processing, and has been used as index of mastitis in calculation of Kostler number (Packard, 1996). However, no significance difference in chloride content was observed between the various groups in present study, while significance difference was seen in rbST treated group milk. Nevertheless, the maximum concentration of chlorides in buffalo milk observed in present investigation expected to be no adverse impact on dairy products (Harding, 1995).

In present study sodium level was decreased in milk of HPE + rbST treated animals, while increased in milk of animals maintained on LPE diet. In fact, sodium is an important element, often

associated with high blood pressure (Packard, 1996). An increase in sodium may have adverse effect on human health. Remarkable increase in sodium was reported due to passage from blood into milk during mastitis condition and may have significant effect on rennet coagulation of milk during cheese making. Consequently, rise in labor charges due to longer cheese making time could be expected (Harding, 1995). It is noteworthy, that increase or decrease in sodium concentration of milk in rbST treated buffalo was non-significant and within normal limits as reported by many researchers (Harding, 1995; Packard, 1996; Hoeben *et al.,* 1999). Thus, no adverse effect on human health and/or on processing conditions during cheese making could be expected from rbST treated milk.

5. Conclusion and Recommendations

It was concluded from the present study that the concentration of ash and sodium contents were unaffected through rbST treatment. The SCC, calcium and chloride contents were elevated in milk of buffaloes given LPE and HPE diets, but within normal ranges. Thus, it is expected to be no adverse effects on the quality of milk and milk products. Since present study was of initial nature with limited time period, a long term trial should be carried out on large population of buffaloes. Further studies should be carried out to produce different types of cheese and fermented products from rbST treated milk to observe its effect on their yield and chemical composition.

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