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EFFECT OF RECOMBINANT BOVINE SOMATOTROPIN (rbST) AND OXYTOCIN ON HEALTH BIOMARKERS, REPRODUCTIVE PERFORMANCE AND MILK COMPOSITION OF NILI-RAVI BUFFALOES *(Bubalus bubalis)* DURING SPRING AND SUMMER SEASONS

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Bovine somatotropin (rbST) and oxytocin are used to increase milk production and for milk letdown respectively in dairy animals. The study was conducted to determine the effects of rbST and oxytocin on various health biomarkers, reproductive performance and milk composition of buffaloes during spring and hot summer. A total of 100 buffaloes were divided into four groups, A (control), B (oxytocin), C (rbST) and D (rbST+ oxytocin), with (n=15) animals in each group during spring and (n=10) during summer. Animals of respective groups received rbST (Boostin-250) 35mg on alternate days and oxytocin 10 IU before each milking, starting on day 70 postpartum till the end of lactation. Blood sample was collected to determine TOS, TAC, ALT and AST. Milk sample were analyzed for fat, protein, lactose and SNF contents. Reproductive performance parameters were assessed by rectal palpation and from farm record. Results revealed that oxytocin decreased the activities of ALT, AST, TOS and milk yield, while TAC was increased than controls. Buffaloes administered with rbST showed lower values of ALT, TOS and higher TAC than control. Milk fat, protein and lactose contents decreased due to rbST, oxytocin or rbST+ oxytocin treatment. Milk yield increased after rbST treatment and decreased with oxytocin during spring.
Keywords: rbST and oxytocin, buffaloes, milk composition, health indicators, reproductive performance.

INTRODUCTION

Buffalo is the main dairy animal in Pakistan. Breeding and selection have failed to improve milk production in buffaloes as it did in cows. The application of newly emerging biotechnologies**,** supported with appropriate nutrition and management**,** can be an alternate option for achieving the goal of high milk production in the buffaloes. Somatotropin is one of the biotechnological products that increase the milk production per unit of feed intake. It is a polypeptide hormone produced by the somatotrophs of the anterior pituitary gland that induces marked changes in nutrient partitioning in target tissues and stimulates cell proliferation mediated by insulin-like growth factor (IGF-I). Ludri *et al*. (1989) reported that administration of rbST resulted in improvement of 12-25% in milk yield without any substantial alteration in the composition of milk.

Bovine somatotropin is commonly used for weight gain in beef, and to increase milk production in dairy animals (Ludri *et al.*, 1989), while, oxytocin is being used for milk letdown in dairy animals. However, there is relatively little information available in the literature regarding the effects of rbST and oxytocin on health biomarkers, reproductive performance and milk composition in buffaloes. Therefore, the present study was designed to investigate the effect of rbST and oxytocin, alone or in combination, on health biomarkers, postpartum reproductive performance and milk composition during spring and summer seasons in Nili-Ravi buffaloes kept under farm conditions.

MATERIALS AND METHODS

***Experimental animals and treatments*:** In this study, a total of 100 multiparous buffaloes in their 2nd or 3rd lactation and maintained under same management and housing conditions were divided randomly into four groups, A, B, C and D, with each group having 15 buffaloes during spring (February, March) and 10 during summer (May, June). Buffaloes of group A served as control, while those of groups B, C and D were administered with oxytocin, rbST and rbST + oxytocin respectively. The rbST (Boostin-250™) was given at 35 mg on alternate days by s/c route at croup region, while oxytocin was used at the dose rate of 1 ml (10 I.U), I/m before each milking. The treatments were started on day 70 postpartum and continued till the end of lactation.

***Post-treatment monitoring*:** Experimental buffaloes were kept under close observation throughout the study period. They were milked twice daily, morning and evening, and milk

yield per milking was recorded. Any local reaction at the site of injection was recorded. About 10 mL blood samples were collected once in the middle of each season from each animal in a sterilized test tube, serum was separated and stored at -20°C for further analysis. Similarly, About 100 ml milk was collected from each animal of all groups during each season. These milk samples were stored at -20°C.

An intact bull of proven fertility was let loose in the herd daily in the morning and evening to detect the females in heat and for natural service. The dates of first postpartum and subsequent estrus were noted. The served females were examined for pregnancy through rectal palpation at least 45 days after service. Reproductive performance parameters including, calving to estrus interval, service period and calving interval were recorded. Reproductive disorders including endometritis and vaginal prolapse were recorded. Ovarian activity like, cyclic and non cyclic ovaries was observed by rectal palpation.

***Biochemical analysis*:** Serum samples collected from animals were analyzed for total Oxidant Status (TOS) and Total Antioxidant capacity (TAC) were determined using the protocol of Erel (2005) and Erel (2004), respectively. Activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) enzymes were determined using commercially available kits (Randox Lab. Ltd. UK).

Milk samples were analyzed for fat, protein, lactose and solid-not-fat (SNF) contents. Milk fat and protein contents were determined by the procedure described by David (1977). Milk lactose contents were estimated by using Fehling's solution titration method, as described by Egan *et al*. (1981) and SNF was determined by Fleischman formula, as described by Khan *et al*. (1983).

***Statistical analysis*:** Data collected on various parameters were tabulated to show means and standard error of mean for group. Two way Analysis of variance was applied to extract the difference between and within groups. Duncan multiple range test was applied for multiple means comparison, where necessary. However, Pearson's Chi square test was used for the statistical analysis of data in percentage.

RESULTS

***Health biomarkers*:** Overall mean of serum activities of ALT and AST were higher (P≤0.01) during summer compared to spring, while reverse was true for TOS and TAC (Table 1). Irrespective of seasons, overall mean serum ALT activity was highest in control group, while the lowest was in oxytocin injected buffaloes (Table 1). Serum AST activity was the highest in rbST+ oxytocin group, while lowest activity was in oxytocin treated group. Difference in AST activity between buffaloes of control and rbST treated groups was non significant. Highest mean serum TOS value was observed in rbST + oxytocin treated animals, while lowest value was observed in rbST group. Mean serum TAC was highest in oxytocin group, while lowest was estimated in rbST + oxytocin treated buffaloes (Table 1).

***Reproductive performance*:** During spring, ovarian status revealed that percentage of cyclic buffaloes was highest (66.6%) in rbST group and lowest (33.3%) in oxytocin group. The difference was not significant (Table 2). Reproductive status of buffaloes showed highest number of pregnant animals in rbST+ oxytocin group (33.3%), while lowest pregnancy rate (6.6%) was in control and rbST group. However, the effect of treatment on pregnancy rate was non-significant.

In rbST treated buffaloes highest percentage of buffaloes showed endometritis (46.6%; n=7) while lowest percentage (20.0%; n=3) was detected in control group. Statistically, these differences between groups were non significant during spring season (Table 2). Cases of vaginal prolapse and vaginal abscess were one each in control buffaloes during spring, while no such problems were seen in buffalo of three treatment groups.

Table 1. Mean values (± SE) for the effect of seasons and treatment groups on serum alanine aminotransferase, aspartate aminotransferase, total oxidant status and total antioxidant capacity of buffaloes treated with rbST and oxytocin.

Treatment groups	ALT (U/L)			AST (U/L)			TOS (μmoI H_2O_2 eqiiv. L^{-1})			TAC (mmol Trolox equivalent/L)		
	Spring	Summer	Overall means	Spring	Summer	Overall means	Spring	Summer	Overall means	Spring	Summer	Overall means
Control (A)	8.58± 1.48	18.00± 3.01	12.35± 1.74 A	8.98± 0.99 h	23.80± 2.97 b	14.91± 1.96 B	0.238± 0.007 e	0.431± 0.035 b	0.315± 0.024 B	0.39± 0.04 e	0.53± 0.11 c	0.45± 0.07 C
Oxytocin (B)	5.52± 0.28	11.37± 0.82	7.86± 0.69 D	11.20± 2.13 f	12.24± 1.28 e	11.62± 1.36 C	0.199± 0.009 f	0.327± 0.009 d	0.250± 0.014 C	0.35± 0.01 f	0.88± 0.03 a	0.56± 0.05 A
rbST (C)	5.89± 0.28	13.53± 1.78	8.95± 1.04 C	10.43± 1.55 g	18.64± 3.39 c	13.71± 1.80 B	0.179± 0.009 h	0.331± 0.010 c	0.239± 0.017 D	0.34± 0.03 f	0.82± 0.05 b	0.53± 0.06 B
rbST+ Oxytocin (D)	9.74± 0.96	12.39± 1.53	10.80± 0.86 B	14.65± 1.54 d	27.26± 2.12 a	19.69± 1.76 A	0.193± 0.013 g	0.554± 0.023 a	0.337± 0.038 A	0.38± 0.02 e	0.41± 0.07 d	0.39± 0.03 D
Overall Means	7.43± 0.50 B	13.83± 1.02A		0.202± 0.006 B	0.411± 0.018 A		11.31± 0.83 B	20.48± 1.53 A		0.36± 0.01 B	0.66± 0.05 A	

AB; Values having different alphabets in a row differs significantly from one another (P≤0.01). Values having different alphabets differ significantly from one another (P≤0.01).

Table 2. Data on reproductive performance in buffaloes of different treatment groups during spring (n=15 in each group) and summer (n=10 in each group).

Treatments	Spring			Summer		
	Animals with cyclic ovary	Pregnant animals	Animals with endometritis	Animals with cyclic ovary	Pregnant animals	Animals with endometritis
Control (A)	7 (46.6%)	1 (6.6%)	3 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Oxytocin (B)	5 (33.3%)	2 (13.3%)	5 (33.3%)	5 (50.0%)	4 (40.0%)	2 (20.0%)
rbST (C)	10 (60.6%)	1 (6.6%)	7 (46.6%)	4 (40.0%)	2 (20.0%)	1 (10.0%)
rbST+Oxytocin (D)	6 (40.0%)	5 (33.3%)	4 (26.6%)	3 (30.0%)	0 (0.0%)	1 (10.0%)
Chi-squarevalue	(4.784)NS	2.097NS	1.443NS	4.260NS	11.839**	4.640NS

Values with parentheses are percentages; NS = Non-significant; **Significant at P<0.01.

During summer, buffaloes given oxytocin showed highest cyclic ovaries (50%), while no animal of control group showed cyclic ovaries. Highest rate of pregnancy (40%) was observed in oxytocin treated group, control and rbST + oxytocin groups did not show any pregnant animal during this season (P≤0.01) (Table 2). One animal (10%) each in rbST and in rbST + oxytocin injected group was found to be suffering from endometritis, while only two buffaloes (20%) were suffering with endometritis in oxytocin injected group. However, differences in cyclic and endometritis animals among four groups were non significant.

***Milk yield and composition*:** During spring, the highest milk yield was recorded in animals treated with rbST, followed by control and rbST+ oxytocin treated buffaloes and lowest was in oxytocin treated animals (P≤0.01). Difference in milk yield between control and rbST + oxytocin group was non significant. However, in summer, oxytocin treatment resulted in lowest milk yield (P≤0.01), with the highest yield was in oxytocin+ rbST group (Fig. 1).

Differences in milk fat content of buffaloes due to seasons and season × treatment interactions were non significant. Overall mean milk fat contents in control group were significantly higher than those of three treatment groups (P≤0.01). The difference among three treatment groups were however, non significant (Table 3). Difference in milk protein contents showed significant (P≤0.01) differences due to seasons and treatment groups. However, season× treatment interaction was non significant. Overall mean milk proteins content was significantly (P≤0.01) higher during spring as compared to summer. Likewise, overall mean milk protein contents were highest in control group, the lowest in rbST group (P≤0.01). However, difference in between oxytocin and rbST+ oxytocin groups was non significant.

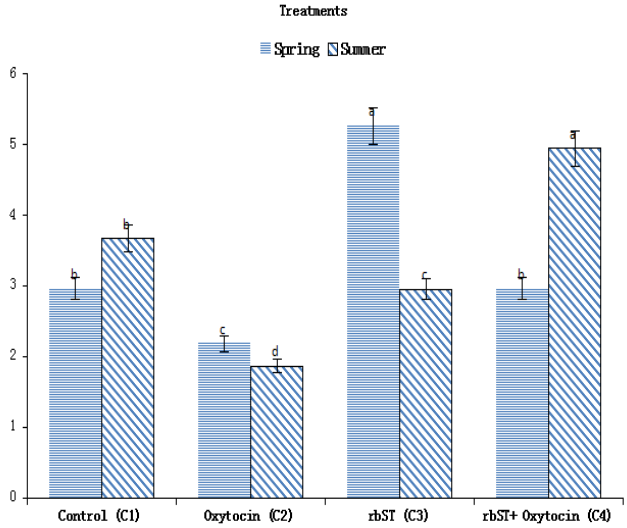

Figure 1. Mean values (± SE) for milk production of buffaloes of different treatment groups during spring and summer.

In the present study, effect of seasons, treatments and their interaction on milk lactose contents was significant (P≤0.01). Overall mean milk lactose was significantly higher during spring than summer irrespective of treatment groups. Likewise, milk lactose concentration of buffaloes was almost equal in rbST, oxytocin and rbST+ oxytocin treated groups,

Table 3. Mean values (± SE) of milk fat, protein, lactose and solids-not-fat contents (%) of buffaloes of four groups during spring and summer.

Treatments	Fat (%)			Protein (%)			Lactose (%)			Solids-not-fat (%)		
	Spring	Summer	Overall means	Spring	Summer	Overall means	Spring	Summer	Overall means	Spring	Summer	Overall means
Control (A)	5.34±0.67	5.75±0.21	5.55±0.35a	5.76±0.20	4.56±0.45	5.16±0.28a	12.16±0.60a	6.49±0.33b	9.33±0.73a	8.18±0.22	7.59±0.19	7.89±0.27b
Oxytocin (B)	4.51±0.38	5.00±0.47	4.76±0.30b	5.42±0.38	3.84±0.19	4.63±0.28b	6.95±0.85b	5.74±0.26b	6.34±0.45b	8.93±0.45	7.80±0.32	8.36±0.30b
rbST (C)	4.26±0.37	4.36±0.21	4.31±0.21b	3.96±0.18	3.14±0.34	3.55±0.21c	5.79±0.42b	5.49±0.22b	5.64±0.23b	7.80±0.23	8.34±0.42	8.10±0.26b
rbST+Oxy.(D)	4.06±0.35	5.08±0.39	4.57±0.28b	4.39±0.31	3.94±0.24	4.17±0.20b	6.58±0.74b	6.28±0.35b	6.43±0.40b	10.11±0.24	9.36±0.37	9.73±0.23a
Means	4.54±0.24	5.05±0.18		4.88±0.18a	3.87±0.17b		7.87±0.52a	6.00±0.16b		8.80±0.21	8.27±0.22	

Values with different alphabets differ significantly from one another (P≤0.01).

However, these values were significantly (P≤0.01) lower compared to control group (Table 3). Effects of treatments on milk SNF was significant (P≤0.05), while season and season × treatment interaction was non significant. Overall mean values of milk SNF were almost similar in control, oxytocin and rbST groups, however, these values were significantly (P≤0.01) lower than rbST+ oxytocin group.

DISCUSSION

The present study was conducted to determine the effects of rbST and oxytocin on various health biomarkers, reproductive performance and milk composition of buffaloes during spring and summer. In the present study, the use of rbST increased milk production whereas, oxytocin decreased milk production. Overall liver enzymes and health biomarkers were increased with the use of rbST, indicating stress and liver damage.

***Health biomarkers*:** In general the health of experimental buffaloes of all groups remained good throughout the study. However, edema like swelling at the injection site was seen in some animals. This swelling was painless and seems to be due to the local tissue reaction.

Overall the values of ALT, AST and TOS were higher during summer compared to spring season, which could have been due to environmental stress during summer. Serum ALT activity did not increase following rbST, oxytocin or rbST+ oxytocin treatment; rather it was decreased in these groups compared to control. Moreover, the magnitude of decrease was higher due to oxytocin than rbST or rbST+ oxytocin treatment. These results do not agree with those of Zafar *et al*. (2015), who recorded significant increase in serum ALT levels when buffaloes were injected 30 IU oxytocin twice daily for 6 months. This discrepancy in the results may be due to dose of oxytocin used in the two studies. The dose used in the present study was10 IU oxytocin twice daily, while Zafar *et al*. (2015) used 30 IU twice daily. Long term use of higher dose of oxytocin might have adverse effects on liver function than small dose. However, when serum AST activity was considered, it was increased in rbST+ oxytocin group, and decreased in oxytocin group compared to control, while rbST had no effect on serum AST activity. Zafar *et al*. (2015) observed increased AST activity in buffaloes given 30 IU of oxytocin twice daily. However, according to Graf *et al*. (1991), rbST treatment had no effect on serum ALT and AST activities in cows.

In the present study, TOS was increased in rbST+ oxytocin group, while decreased in animals injected with oxytocin or rbST (P≤0.01) alone compared to control. However, TAC was higher in oxytocin and rbST groups, but lower in rbST+ oxytocin group, compared to control. Zafar *et al.* (2013) injected buffaloes with oxytocin 30 IU twice daily before each milking and observed higher levels (P≤0.05) of TOS, while serum TAC level was significantly lower in these animals as compared to control. However, these workers neither tried rbST, nor rbST+ oxytocin in their study. In ruminants, liver is known to secrete IGF-I, which is dependent on the availability of both plasma growth hormone and some nutritional factors (Clemmons and Underwood, 1991). Animals with a lower nutritional status (Hodgkinson *et al*., 1991) or with negative energy balance have reduced hepatic production of IGF-I (Steen, 2001) which may lead to high ALT and AST activities.

***Reproductive performance*:** In the present study, the incidence of endometritis in various groups ranged from 0.00 to 46.6%. Based on clinical data, Hanafi *et al*. (2008) have shown the prevalence of endometritis in buffaloes from 16.73% to 42%, being lower in first calvers. This problem has been shown to have very low heritability (0.08) (Zwald *et al*., 2004) and seems to be mainly influenced by managemental practices. Treatment of buffaloes with rbST, oxytocin or rbST+ oxytocin had no effect on the occurrence of this problem. However, it does not seem to be possible to correlate this problem with hormone treatments under field condition, as a wide range of management practices such as hygienic condition at calving, service by infected bull or practice of blowing air into vagina for milk letdown can influence the results.

Buffaloes treated with oxytocin alone showed the highest pregnancy rates (40%), followed by (20.0%) in rbST treated animals (P≤0.01). while no pregnancy was recorded in control animals or in those given rbST+ oxytocin during summer. However, during spring, effect of treatments on pregnancy rate was non significant. Esteban *et al.* (1994) treated cows with rbST @ 17.2, 51.6 and 86 mg/day, starting from 70 days postpartum till the end of lactation, for 2 consecutive lactations. During the first lactation, multiparous cows treated with rbST showed decreased pregnancy rates and increased behavioral anestrus. According to St-Pierre *et al*. (2014), pregnancy rate for the first 2 breeding cycles was increased by 5.4% and for the duration of the trial it was reduced by 5.5% for rbST-Zn-treated cows, compared with controls. Besides dose rates of hormones, species differences can be blamed for these discrepancies between results of the previous and the present study. Moreover, we used only rbST, while St-Pierre *et al*. (2014) treated cows with rbST+ Zinc combination.

In spring season, 12.12% of the control buffaloes (n=2) had vaginal prolapse and vaginal abscess (n=1 each), while hormones treated animals did not show any such problem. However, this problem does not seem to have any relation with treatments used in this study, as it was not seen in any treatment group. The case of vaginal prolapse in this study was recorded prepartum, which is supported by Noakes *et al*. (2001a), who also recorded maximum number of such cases during the last 2 months of gestation. This problem is said to have genetic basis (Miesner and Anderson, 2008) and is seen mostly in animals given high estrogen feed (Shyam and

Nakao, 2003). According to Noakes *et al.* (2001b), prolapse of vagina in cattle is associated with persistent irritation and straining caused by vaginal trauma and infection.

The overall reproductive performance of the experimental buffaloes, irrespective of any treatment (measured in terms of service period, services per conception, conception rate and calving interval) was better during the spring than the summer season. Although efforts were made to protect animals from harsh summer through showering and provision of water pool, yet the hot summer season adversely affected their reproductive performance. Similar observations have been recorded previously by Jousan *et al.* (2007).

***Milk yield and composition*:** Previous studies have shown increase in milk yield in dairy cows following treatment with rbST (Ludri *et al*., 1989). However, in this study rbST treatment increased milk yield in buffaloes given rbST during spring, while in summer it resulted in decrease milk yield compared to control. Combined treatment with rbST+ oxytocin also failed to increase milk yield in spring, although it increased milk yield during summer compared to control. This shows that the effect of rbST on milk yield in buffaloes is season dependent, which might be due to seasonal breeding pattern in the buffalo. Anyway oxytocin treatment decreased the milk yield in buffaloes compared to control during both seasons, which might be due to compressive effect of oxytocin on myoepithelial cells of alveoli of mammary glands resulting in decreased ability of alveoli to secrete milk (Hafez, 1987).

Treatment of buffaloes with oxytocin, rbST or rbST+ oxytocin resulted in significant decrease in milk fat, protein and lactose contents compared to control. However, milk SNF content increased ($P \leq 0.01$) in buffaloes injected with rbST + oxytocin. Helal and Lasheen (2008) reported that in rbST treated buffaloes, milk composition (fat, total proteins, lactose and SNF) was not affected at 500mg rbST/head/14days, but milk lactose was increased ($P \leq 0.01$) when dose of rbST was increased. Milk protein and lactose contents increased significantly during spring, indicating a reduction in oxidation of amino acids as well as mobilization of labile protein reserves. Chalupa *et al.* (1996) demonstrated that when high producing animals are fed diet which is not deficient in protein or energy, they may respond to rbST treatment in increased milk proteins and fat contents. In conclusion the use of rbST was beneficial for increasing milk production, while oxytocin treatment and heat stress decreased milk production.

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