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RESEARCH ARTICLE

Effect of Recombinant Bovine Somatotropin (rbST) and Oxytocin on Health Biomarkers, Reproductive Performance and Milk Composition of Nili-Ravi Buffaloes *(Bubalus bubalis)*

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ABSTRACT

In Pakistan recombinant bovine somatotropin (rbST) is commonly used to increase milk production in dairy animals. That is why this study was conducted to check its effect on health and production in buffaloes. Selected buffaloes were divided into groups (n=10) on the basis of body condition scores (BCS) i.e. (Low) BCS 1-3, (Medium) BCS 4-6 and (High) BCS 7-9, while ten animals served as control. All the groups received rbST (Boostin-250) 35 mg on alternate days and oxytocin 1 ml (10 I.U), starting on day 70 postpartum till the end of lactation. Blood samples were collected from each animal and serum was separated. Milk samples were used for milk composition parameters. The data on reproductive performance parameters was obtained from the farm record. The results indicated that mean serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and total oxidant status (TOS) values were higher ($P \leq 0.01$) during summer, while total antioxidant status showed opposite trend. Mean serum AST and TOS values were significantly ($P \leq 0.01$) higher in medium and high BCS buffaloes. Percentage of pregnant buffaloes was high in the high BCS group during summer. Mean milk fat contents were higher ($P \leq 0.01$) in buffaloes of high BCS while mean milk protein and lactose contents were significantly ($P \leq 0.01$) higher in medium BCS buffaloes. Fat, protein, lactose and solid not-fat (SNF) contents were higher during spring than summer. Mean milk lactose and SNF contents were higher ($P \leq 0.01$) in high than low and medium milk production groups. In conclusion, milk production was increased during summer and with rbST and decreased with oxytocin during spring, rbST and oxytocin treatments showed adverse effects on general health of Nili-Ravi buffaloes.

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INTRODUCTION

There is approximately 38.8 million head of buffaloes in Pakistan, producing thousand tons of milk annually for human consumption (Anonymous, 2018). In spite of this large number, per animal productivity is low in Pakistan. The average milk yield per animal is 7-8 liter/day at farm level, which needs to be increased to make this animal more profitable, as well as to meet the protein requirements of the ever-increasing human population in the country. Low milk production, with lack of persistency, is the major problem for small dairy farmers in Pakistan. Breeding and selection have failed to increase the amount of milk produced in buffaloes as it did in cows. But, milk yield increases up to 10-15% through the use of recombinant bovine somatotropin (rbST) (NRC, 1994). Flores *et al*. (2019) reported that rbST-treated Holstein cows produced 20% greater milk yield than control cows in 305-d lactations. Similarly, 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 (bST) is a polypeptide hormone produced by the somatotrophs of the anterior pituitary gland and consists of a chain of 190 to 191 amino acids. This hormone induces marked changes in nutrient partitioning in target tissues and stimulates cell proliferation mediated by insulin-like growth factor (IGF-I). In 1982, rbST was produced by recombinant DNA technology that permitted scientists around the world to observe various aspects of its biology. In this technique, the genes that produce somatotropin were identified in bovine tissues and inserted as a part of the plasmid into specific bacteria. With bacterial replication, the new genes were also replicated and conceded along to all new bacteria. Purified rbST was extracted from these freshly grown bacteria (Bauman, 1992). The commercial formula of rbST (Posilac) is leucine variant-127, having an extra methionine at the NH2 terminus. Similarly, oxytocin is a polypeptide hormone that stimulates myometrium contractions during parturition and mammary gland muscles during milk ejection.

In Pakistan rbST is commonly used to increase milk production in dairy animals, while oxytocin is also used for milk letdown in cattle and buffaloes. However, there is relatively little information available in the literature regarding the effects of rbST and oxytocin on the animal's body and production. Therefore, the present study was designed to investigate the effect of rbST on the health of animals with different body condition score (BCS) and their milk production potential.

MATERIALS AND METHODS

In this study, 70 lactating Nili-Ravi buffaloes were divided into three groups, on the basis of their body condition scores i.e. Low (BCS 1-3), Medium (BCS 4-6) and High (BCS 7-9) with 10 animals in each group during spring and summer while 10 animals served as the untreated control. Buffaloes were multiparous in their 2nd or 3rd lactation and were maintained under same manage mental and housing conditions. Animals of all groups were treated with rbST (Boostin -250™ manufactured by LG Life Sciences, Ltd, South Korea and distributed by M/S Ghazi Brothers, Pakistan). The animals in all the groups received 35 mg of rbST (Boostin-250) on alternate days by s/c route and 1 ml (10 I.U) oxytocin I/m before each milking, starting on day 70 postpartum till the end of the lactation. About 10 mL blood was collected once in each season from each animal in a sterilized test tube without anticoagulant to harvest the serum. Blood samples were allowed to clot at room temperature and centrifuged at 448 g for 20 minutes. After separation, the serum was divided into small aliquots and preserved in Eppendorf tubes at -20°C for further analysis for various health biomarkers and enzyme activities.

Milk samples were collected to ascertain the composition parameters. About 100 mL of milk was collected in a clean dry bottle from each animal in each group. Milk sample was collected once per animal per season and bottles were labelled for its season and group. These samples were stored in a freezer at -20°C till analysis. 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 (Egan *et al.*, 1981) and SNF was determined by Fleischman formula (Khan *et al.*, 1983).

Serum samples collected from animals were analyzed for Total Oxidant Status (TOS) and Total Antioxidant Capacity (TAC) by using the protocol of Erel (2004; 2005). Activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) enzymes were determined using commercially available kits (Randox Lab. Ltd. UK).

Natural mating was practiced at farm and pregnancy was tested by rectal palpation 45 days after mating. The postpartum reproductive performance parameters like calving to estrus interval, estrus to conception, number of inseminations per conception, conception rate and calving intervals were calculated from the data available in the farm record for buffaloes under different treatment protocols. The percentages of reproductive disorders including abortion retained placenta, postpartum endometritis and dystocia during the study period were noted from the farm record. An ovarian activity like cystic ovaries, cyclic and non-cyclic ovaries was recorded by rectal palpation at the time of sampling during different seasons.

Two-way Analysis of variance was applied to extract the difference between and within groups. Duncan multiple range tests were applied for multiple means comparison, where necessary. However, Pearson's Chi-square test was used for the statistical analysis of data in percentage.

RESULTS

Difference in serum Alanine aminotransferase (ALT) activity in buffaloes of three BCS groups was non-significant. However, variations due to seasons and season×BCS interaction were significant (Table 1). During summer, buffaloes with medium BCS showed highest mean serum ALT activity (14.15±1.72 U/L), followed by high BCS (12.98±0.95 U/L) and lowest (11.31±0.81 U/L) was found in buffaloes with low BCS. In the spring season, the low and high BCS groups were having almost similar ALT values and medium BCS group had lowest (4.49±0.24; U/L) mean value of ALT. Overall mean serum ALT activity was significantly higher during summer as compared to spring (Table 1).

Variation in Aspartate aminotransferase (AST) activity was significant due to BCS ($P\leq0.01$) and seasons ($P\leq0.05$), while BCS × season interaction was non-significant. In low BCS group, overall mean AST was significantly lower ($P\leq0.01$) than that for medium and high BCS groups. However, the difference in AST activity between animals of medium and high BCS groups was non-significant. Overall mean AST was significantly higher during summer as compared to spring (Table 1).

Variations in serum Total oxidant status (TOS) activity in buffaloes due to seasons, body condition scores and their interactions were significant ($P\leq0.01$). Overall mean serum TOS was significantly ($P\leq0.01$) higher in medium and high body condition score compared to low BCS group irrespective of their seasons. Overall mean serum, TOS was significantly higher ($P\leq0.01$) in buffaloes exposed to summer season than spring (Table 1). Variations in serum Total antioxidant status (TAC) in buffaloes due to seasons, body condition scores and their interactions were significantly ($P\leq0.01$) different.

Numbers of buffaloes in the present study that were in estrus cycle was higher (66.6%) in low BCS, followed by in medium BCS (46.6%) and (26.6%) in high BCS group. Buffaloes having low BCS showed (46.6%), from medium BCS (26.6%) and from high BCS (40.0 %) cases of endometritis. None of the buffaloes with low BCS was pregnant, while seven animals with medium BCS were pregnant (46.6%) and in high BCS, only one animal (6.6%) was pregnant during spring. However, these differences among three BCS groups for these three parameters were non-significant (Table 2). Out of pregnant animals, with low BCS delivered two females (13.3%) and three males (20.0%), buffaloes with medium BCS delivered six females (40.0%) and nine males (60.0%) and animals designated high BCS delivered only one male (6.6%) and these values were statistically non-significant (Table 2). Reproductive abnormalities observed were prolapse (6.6%) and vaginal abscess (6.6%) only in animals having medium BCS (Table 2).

Analysis of variance for the effect of seasons and BCS on milk fat contents of buffaloes treated with rbST and oxytocin during spring and summer showed that fat content was higher (6.17±0.08%) in high BCS buffaloes as compared to low and medium BCS animals that the difference was significant (P≤0.05). The difference in milk fat contents between the later two groups was non-significant. Overall mean milk fat content was significantly (P≤0.01) higher during spring as compared to summer (Table 3). The interaction between season × BCS was non- significant.

Analysis of variance for the effect of seasons and BCS on milk protein contents of buffaloes revealed that difference due to seasons was non-significant, while variation due to BCS and season × BCS interaction was significant (P≤0.01). Overall mean milk protein content was (P≤0.01) higher in medium BCS, than for low BCS and high BCS buffaloes, the low and high BCS groups were having almost same values and the difference between their values was non-significant (Table 3).

Variations due to the season, body condition scores and season × body condition score interactions were significant (P≤0.01). Mean milk lactose percentage was significantly (P≤0.01) higher during spring than the summer season. Overall mean milk lactose percentage of medium BCS was significantly higher from low and high BCS buffaloes, the low and high BCS groups were having almost similar overall mean milk lactose contents.

The difference in milk SNF of buffaloes due to seasons was significant (P≤0.01). However, variation due to BCS and season× BCS interactions were non-significant. Milk SNF contents were significantly higher during spring than summer season (Table 3).

DISCUSSION

Liver enzymes and health biomarkers: When the effect of rbST treatment in relation to BCS was monitored, irrespective of milk production potential, mean serum AST and TOS values were significantly (P≤0.01) higher in medium and high BCS buffaloes than low BCS group. Mean serum TAC values differed significantly (P≤0.01) among low, medium and high BCS groups, being highest in low BCS and lowest in medium BCS group. No information in the existing literature regarding the effect of rbST treatment on liver enzymes in animals of different BCS groups could be traced. However, Khaliq *et al.* (2013) have reported a significant increase in serum ALT

Table 1: Mean values (± SE) for the effect of seasons and body condition scores on serum ALT, AST, TOS and TAC of buffaloes treated with rbST and oxytocin

Body condition scores	ALT (U/L)			AST (U/L)			TOS (µmol H2O2 eqiiv. L-1)			TAC (mmol Trolox equivalent/L)		
	Spring	Summer	Overall Mean	Spring	Summer	Overall Mean	Spring	Summer	Overall Mean	Spring	Summer	Overall Mean
Untreated control	8.58±1.48	18.00±3.01	12.35±1.74	8.98±0.99	23.80±2.97	14.91±1.96	0.238±0.00	0.431±0.035	0.315±0.024	0.39±0.04	0.53±0.11	0.45±0.07
Low	5.55±0.33^{d}	11.31±0.81^{c}	7.85±0.68	8.05±1.09	15.20±2.03	10.91±1.24^{b}	0.13±0.01^{d}	0.36±0.03^{a}	0.22±0.03^{b}	0.82±0.04^{a}	0.76±0.09^{b}	0.78±0.06^{a}
Medium	4.49±0.24^{e}	14.15±1.72^{a}	8.35±1.18	14.85±1.16	16.87±2.94	15.66±1.35^{a}	0.35±0.04^{a}	0.33±0.01^{b}	0.34±0.02^{a}	0.83±0.03^{a}	0.33±0.02^{d}	0.53±0.05^{c}
High	5.58±0.36^{d}	12.98±0.95^{b}	8.54±0.85	16.91±0.58	16.70±1.27	16.83±0.60^{a}	0.31±0.01^{c}	0.33±0.01^{b}	0.32±0.01^{a}	0.85±0.03^{a}	0.45±0.03^{c}	0.61±0.04^{b}
Overall Means	5.20±0.19^{b}	12.81±0.72		13.27±0.80^{b}	16.26±1.23		0.26±0.02	0.34±0.02		0.83±0.02	0.51±0.04	

$^{a-e}$Values with different alphabets in a row or column for each parameter differ significantly from one another (P≤0.05).

Table 2: Prevalence of cyclic ovaries, Pregnancy and Endometritis (%) in buffaloes of different body condition score groups during spring

Body condition score group	Spring			Summer		
	Animals with cyclic ovary	Pregnant animals	Animals with endometritis	Animals with cyclic ovary	Pregnant animals	Animals with endometritis
Untreated control	7(46.6)	1(6.6)	3(20.0)	0(0.00)	0(0.00)	0(0.00)
Low	10 (66.6)	0 (0.0)	7 (46.6)	4 (26.6)	4 (26.6)	1 (10.0)
Medium	7 (46.6)	7 (46.6)	4 (26.6)	4 (26.6)	4 (26.6)	2 (20.0)
High	4 (26.6)	1 (6.6)	6 (40.0)	3 (30.0)	4 (26.6)	2 (20.0)
Chi-square value	4.712 NS	3.943 NS	1.389 NS	0.220 NS	2.032*	2.212 NS

Values with parentheses are percentages; NS=Non-significant. *=significant at (P≤0.05).

Table 3: Mean values (± SE) of milk fat, protein, lactose and solids-not-fat contents (%) of buffaloes of three body condition scores treated with rbST and oxytocin

Body condition score groups	Fat (%)			Protein (%)			Lactose (%)			Solids-not-fat (%)		
	Spring	Summer	Overall means	Spring	Summer	Overall means	Spring	Summer	Overall means	Spring	Summer	Overall means
Untreated control	5.34±0.67	5.75±0.21	5.55±0.35^{b}	5.76±0.20^{b}	4.56±0.45^{d}	5.16±0.28^{b}	12.16±0.60^{a}	6.49±0.33^{c}	9.33±0.73	8.18±0.22	7.59±0.19	7.89±0.27
Low	5.96±0.40	4.86±0.24	5.41±0.26^{b}	6.71±0.30^{a}	6.55±0.65^{a}	6.63±0.35^{b}	8.48±1.24^{b}	6.80±0.31^{c}	7.64±0.65^{b}	8.54±0.28	7.29±0.35	7.92±0.26
Medium	5.91±0.40	4.97±0.37	5.44±0.29^{b}	6.09±0.46^{b}	8.33±0.97^{c}	7.21±0.58^{a}	13.86±1.02^{a}	6.28±0.18^{c}	10.07±1.01^{a}	8.94±0.15	6.94±0.49	7.94±0.34
High	6.21±0.10	6.13±0.14	6.17±0.08^{a}	6.31±0.39^{b}	4.49±0.33^{d}	5.40±0.32^{b}	8.78±0.42b	6.59±0.20^{c}	7.69±0.34^{b}	9.00±0.14	7.08±0.48	8.04±0.33
Overall Means	6.03±0.19	5.32±0.18		6.37±0.22	6.46±0.49		10.37±0.70	6.56±0.14		8.82±0.12	7.10±0.25	

a-e: Values with different alphabets in a row or column for each parameter differ significantly from one another (P≤0.05).

with 500 mg of rbST treatment after every 15 days than control animals. Increased levels of AST and ALT are most often found in acute and chronic liver disorders, starvation and the appearance of ketosis during early lactation (Steen, 2001). However, buffaloes included in the present study were clinically healthy and did not show signs of any disease during the entire study period. Garcia *et al.* (2015) reported increased AST activity in heat-stressed Holstein cows than winter months. The exact reason for increased serum AST levels and TOS values following rbST treatment in buffaloes of medium and high BCS remains unclear. Moreover, the physiological significance of these results needs further exploration.

Reproductive performance/ problems: In the present study, the incidence of endometritis was not affected significantly in buffaloes of three body condition score groups treated with rbST and oxytocin although the value was higher in high BCS and lower in low BCS group in both seasons. Same was true for buffaloes with low, medium and high milk yield. Similarly, treatment of buffaloes with rbST, oxytocin or both, irrespective of their BCS or milk yield, had no effect on the incidence of endometritis. Gohary *et al*. (2014) demonstrated that the incidence of endometritis was not different between groups when monitored after 42 days of first treatment of rbST. In the present study, the incidence of endometritis in various groups ranged from 3.44 to 37.5%. Previous studies based on clinical data have shown the prevalence of endometritis in buffaloes from 16.73 (Hanafi *et al*., 2008) to 42%.

The frequency of cyclic animals of three BCS groups treated with rbST and oxytocin was not affected significantly. Similarly, there was no difference in cyclic buffaloes of three milk yield groups given rbST and oxytocin. Same was true when animals were given rbST, oxytocin or both irrespective of their BCS or milk production. It was expected that rbST treatment would stimulate follicular development and increase in the number of cycle animals. Herrier *et al*. (1994) reported that rbST treatment to lactating cows increased the blood concentration of ST and IGF-1. Chen *et al.* (2015) reported that omitting the dry period (0 days) increased the incidence of normal resumption of ovarian cyclicity in dairy cows within 100 DIM compared with a conventional dry period (60 days). Short (<18 days) or long (>24 days) ovarian cycles during the first ovarian cycle postpartum were associated with severe negative energy balance and poor metabolic status in early lactation.

In this context, Flores *et al*. (2007) reported that a greater percentage of moderate BCS cows (82%) were detected in estrus during the first 30 days of the breeding season compared with low BCS cows (64%) treated with rbST. According to Santos *et al*. (2004), the percentage of anovulation decreased four times as the BCS increased at 70 days in milk. Flores *et al*. (2007) reported that low BCS, as well as rbST treatment, reduced the intensity of estrus in Brahman-influenced cows.

Buffaloes showing signs of true heat were served by natural mating using bulls of proven fertility. The results indicated that following rbST and oxytocin treatment pregnancy rates were significantly higher in buffaloes of high BCS group compared to low and medium BCS groups. However, Flores *et al*. (2007) reported that low (43%) and moderate (69%) BCS cows treated with rbST had greater first-service conception rates than low (40%) and moderate (15%) BCS control cows.

Milk composition: Milk analysis showed that fat content was highest in high BCS group buffaloes, while in low and medium BCS group, overall mean values were almost similar, the difference was significant (P≤0.05). Protein and lactose contents were highest in medium BCS group; its difference from low and high BCS groups was significant (P≤0.01). However, milk SNF content did not differ significantly. Between two different seasons milk fat, lactose and SNF contents vary significantly, being high during spring as compared to summer. Vandenberg (1991) reported that cow milk fat, lactose and proteins contents are slightly affected by rbST in animals having a positive energy balance. Naveed-ul-Haque *et al*. (2018) reported that milk yield decreased linearly in high-protein diet and no effect was observed on milk protein yield and contents. Garcia *et al.* (2015) reported that cows in severe heat had a 21% milk yield decrease, while lactose and protein % decreased with fat not being affected. Ling *et al*. reported that the average milk protein, fat, lactose, and total solids in Murrah, Nili-Ravi and crossbreed buffaloes (*n*=23) were 4.76, 7.31, 5.19 and 18.40 g/100 g of milk, respectively.

Naheed *et al*. (2014) reported that in Nili-Ravi buffaloes the overall milk protein, lactose and solids-not-fat (SNF) of oxytocin treated buffaloes were significantly higher as compared to control animals. During the winter season, milk fat concentration in oxytocin injected buffaloes was significantly lower, while during summer season it was higher (P≤0.05) than controls. Lammoglia *et al*. (2015) reported that intramuscular injection of oxytocin to induce milk ejection in crossbred cows under tropical conditions of Veracruz reduced the cumulative percentage of pregnant cows. Oxytocin increased serum cortisol concentrations, abortion rate and the number of open cows, but did not improve milk production. St-Pierre *et al*. (2014) reported that for cows treated with rbST-Zn, mean milk, 3.5% fat-corrected milk, fat, and protein yields were increased by 4.00, 4.04 L/day and 0.144 and 0.137% respectively; however, the concentrations of milk components did not change.

Based on various long and short term studies, it has been concluded that as a dose of rbST is increased, protein contents declines, while fat percentage increases significantly. This decline in proteins content of milk could have been due to a decline in the synthesis of proteins by mammary tissue. In the present study, milk lactose content was significantly low in low BCS buffaloes during spring and summer. It probably shows the nutritional status of low BCS buffaloes. High fat contents in the milk of high BCS buffaloes and also during spring indicate that these animals reduce the utilization of glucose and spare it for the denovo synthesis of lactose. Prathap *et al*. (2017) reported that heat stress adversely affected both quantity as well as quality of milk produced. These impacts were due to reduced feed intake, altered hormone concentration and pathological changes in udder during mastitis. Ramendra *et al*. (2016) reported that heat stress directly affect feed intake thereby, reduces growth rate, milk yield and reproductive performance, dairy breeds are susceptible since they generate more metabolic heat. In conclusion, the use of rbST was beneficial for increasing milk production, while oxytocin treatment and heat stress decreased milk production. Recombinant bovine somatotropin and oxytocin treatments showed adverse effects on the general health of Nili-Ravi buffaloes.

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REFERENCES

Anonymous, 2018. Economic survey of Pakistan, finance division, economic advisor's wing, Government of Pakistan, Islamabad.

Bauman DE, 1992. Bovine somatotropin: review of an emerging animal technology. J Dairy Sci 75:3432-51.

Chen J, Soede NM, Dorland HA, *et al.*, 2015. Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths. Theriogenology 84:1387-96.

David CL, 1977. Laboratory guide in dairy chemistry practical. FAO regional dairy development and training Centre for Asia and the Pacific, Dairy Training and Research Institute, University of Philippine, Los Banos College, Languna.

Egan H, Kirk R and Sawyer R, 1981. Person's chemical analysis of food. Churchill Livingstone, New York, USA.

Erel O, 2004. A novel automated direct measurement method for total antioxidant capacity using new generation, more stable ABTS radical cation. Clin Biochem 37:577-85.

Erel O, 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103-11.

Flores J, García JE and Mellado J, *et al.*, 2019. Effect of growth hormone on milk yield and reproductive performance of subfertile Holstein cows during extended lactations. Spanish J Agric Res 17:1-9.

Flores R, Looper ML and Rorie RW, *et al.*, 2007. Influence of body condition and bovine somatotropin on estrous behavior, reproductive performance, and concentrations of serum somatotropin and plasma fatty acids in postpartum Brahman-influenced cows. J Anim Sci 85:1318-29.

Garcia AB, Angeli N, Machado L, *et al.,* 2015. Relationships between heat stress and metabolic and milk parameters in dairy cows in Southern Brazil. Trop Anim Health Prod 47:889-94.

Gohary K, LeBlanc SJ, Lissemore KD, *et al.*, 2014. Effect of prepartum administration of recombinant bovine somatotropin on health and performance of lactating dairy cows. J Dairy Sci 97:6231-41.

Hanafi EM, Ahmad WM and Abd El Moez SI, *et al.*, 2008. Effect of clinical endometritis on ovarian activity and oxidative stress status in Egyptian buffalo-cows. American-Eurasian J Agric Environ Sci 4:530-6.

Herrier A, Einspanier R, Schams D, *et al.,* 1994. Effect of recombinant bovine somatotropin (rbST) on follicular IGF-1 contents and the ovarian response following superovulatory treatment in dairy cows: a preliminary study. Theriogenology 41:601-11.

Khaliq T, Rehman ZU and Javed I, 2013. High dose of recombinant bovine somatotropin do alter serum biochemical and hormonal profile of Nili-Ravi buffaloes. Pak Vet J 33:476-80.

Khan BB, Assad MA, Tufail M, *et al.*, 1983. Practical manual for introductory livestock management courses. Department of Livestock Management, Faculty of Animal Husbandry, University of Agriculture, Faisalabad, Pakistan.

Lammoglia MA, Domínguez MB, Alarcón MA, *et al.,* 2015. Effects of routinely oxytocin injection to induce milk ejection on some reproductive parameters of crossbred cows in the tropics of Veracruz. Ann Res Rev Biol 6:297-303.

Ling Z, Qingfeng T, Muhammad WI, *et al.*, 2018. A comparison of milk protein, fat, lactose, total solids and amino acid profiles of three different buffalo breeds in Guangxi, China. Italian J Anim Sci 17:873-8.

Ludri RS, Upadhyay RC, Singh M, *et al.,* 1989. Milk production in lactating buffalo receiving recombinantly produced bovine somatotropin. J Dairy Sci 72:2283-7.

Naheed A, Zahoor T and Rahman Z, 2014. Physicochemical profile of milk from oxytocin injected buffaloes. Pak Vet J 34:351-5.

NRC (National Research Council), 1994. Metabolic modifier-effect on the nutrient requirement of food producing animals. National Academy Press, Washington, USA.

Naveed-ul-Haque M, Muhammad UA, Rizwan M, *et al.,* 2018. Effects of increasing dietary protein supplies on milk yield, milk composition and nitrogen use efficiency in lactating buffalo. Trop Anim Health Prod 50:1125-30.

Prathap P, Archana PR, Aleena J, *et al.*, 2017. Heat stress and dairy cow: impact on both milk yield and composition. Int J Dairy Sci 12:1-11.

Ramendra D, Lalrengpuii S, Nishant V, *et al.*, 2016. Impact of heat stress on health and performance of dairy animals: A review. Vet World 9:260-8.

Steen A, 2001. Field study of dairy cows with reduced appetite in early lactation: clinical examinations, blood and rumen fluid analyses. Acta Vet Scand 42:219-28.

St-Pierre NR, Milliken GA, Bauman DE, *et al.*, 2014. Meta-analysis of the effects of sometribove zinc suspension on the production and health of lactating dairy cows. J Am Vet Med Assoc 245:550-64.

Vandenberg G, 1991. A review of quality and processing suitability of milk from cows treated with bovine somatotropin. J Dairy Sci 74:2-11.