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Effect of long-term, exogenous administration of oxytocin on milk production, composition, somatic cell count, and progesterone in postpartum Nili-Ravi buffalo

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Abstract

This study sought to explore the effect of the administration of long-term, exogenous oxytocin on buffalo milk. In this study, newly-calved postpartum buffaloes (Nili-Ravi) (n = 24) were divided equally into three groups: (control without oxytocin – CON, 10 i.u. oxytocin – LOW, 30 i.u. oxytocin – HIGH). Oxytocin was injected twice a day before each 154-day milking. Milk production, milk composition, somatic cell counts, and progesterone (P4) were evaluated daily, weekly, and fortnightly, respectively. Results revealed that the HIGH group produced high milk production and pH values, but lower milk density than the CON and LOW groups. The HIGH group and LOW group had higher milk fat and solid-not-fat (SNF) compared to the CON group. SNF and protein were higher in the LOW group than the CON group. Freezing point, lactose, solids, somatic cell count, and P4 were similar in all three groups. Overall, it can be concluded that long-term, exogenous oxytocin has a positive effect on milk production, fat, SNF, and protein, with a negative effect on milk pH. None of the oxytocin treatments affected somatic cell counts and P4 concentration in postpartum buffalo milk.

Keywords: Exogenous**,** oxytocin, milk, quantity, quality, postpartum, buffalo
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Introduction

Livestock are vital to Pakistan's peri-urban and rural economy. Livestock account for 62.68% of agricultural and 14.36% of national gross domestic product of the country. The role of livestock in the rural economy can be realized by the fact that approximately eight million families in the rural population are involved in livestock raising and production activities and derive over 35% of their income from this source. Small dairy farming systems prevail in the country where dairy farmers raise an average of 1–4 cattle/buffalo and 5–6 sheep/goats each. Among the dairy animals, buffalo have a significant share of the milk production of approximately 60% (Pakistan Economic Survey, 2022–2023).

Milk is the main and only source of income for small dairy farmers. Hence, they sacrifice every male calf for more milk. Buffalo milking practices differ from cattle, and milk let-down in buffaloes is induced by the suckling of calves. Buffalo maternal bonding is strong enough to let her stand and provide milk when she has the calf (Millogo *et al*., 2009). Obtaining buffalo milk is hard for dairymen

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when there are no calves, especially when males have been sold or died. It is also challenging to get milk without a calf even if concentrate feed is offered for milking. For these complicated conditions with regular milking, a long-term exogenous oxytocin administration is considered to be an effective solution throughout lactation. Similarly, in large dairy farms where minimum labour is employed, oxytocin injections are considered time- and money-saving. Farmers usually sell male calves early and use oxytocin for milk let-down during lactation (Qureshi and Ahmad, 2008). Various reports indicate that approximately 25% of all dairy animals (Bilal *et al*., 2008) — in some reports, 10–12% or up to 15.5% of dairy farms — use exogenous oxytocin in the country (Tariq and Younas, 2013; Faraz *et al*., 2020, Murtaza *et al*., 2021).

Earlier studies have revealed the effect of exogenous oxytocin on milk production in buffalo to be an increase in milk production after short-term administration (Hanjra *et al*., 1977; Mustafa *et al*., 2008; Qureshi and Ahmed, 2008; Shahid *et al.*, 2016). Mustafa *et al.* (2008) and Shahid *et al*. (2016) reported a decrease in milk production following oxytocin injection in buffalo. Similar studies were also reported in cows with an increase in milk production due to exogenous oxytocin (Nostrand *et al*., 1991; Ballou *et al*., 1993; Knight, 1994). In another study, exogenous oxytocin did not affect milk yield in cows (Stewart and Stevenson, 1987). Some studies have also reported the effect of exogenous oxytocin on milk composition and physicochemical properties of major milk constituents, i.e. fat, protein, minerals, enzymes, and vitamins in buffalo milk (Akhtar *et al*., 2012; Abbas *et al*., 2014; Shahid *et al*., 2016). Similar studies in cows have also described the effect of oxytocin injection on milk composition (Hameed *et al*., 2010; Dymnicki *et al*., 2013; Abbas *et al*., 2014; Penry *et al*., 2017). Many studies have emphasized that oxytocin injection does not affect milk composition in dairy cows (Stewart and Stevenson, 1987; Nostrand *et al*., 1991; Ballou *et al*., 1993).

Globally, somatic cell count (SCC) is used in dairy animals as an efficient udder health biomarker for fresh milk quality (Rysanek and Babak, 2005; Dang and Anand, 2007). Microbial chemicals are mostly responsible for increasing SCC in milk. Non-microbial agents, such as exogenous oxytocin, are comparatively less targeted regarding the increase or decrease in the SCC of buffalo milk. Some studies have mentioned the effect of exogenous oxytocin on SCC in dairy buffalo milk, but for a short period, of up to one month (Prasad and Singh, 2001; Bidarimath and Aggarwal, 2007; Akhtar *et al*., 2012). However, the effect of exogenous oxytocin on SCC in buffalo milk after long-term administration (as is generally practised by the buffalo owners) has not been reported.

The concentration of progesterone (P4) is consistent in milk and plasma, either increasing or decreasing, in lactating buffalo (Batra *et al*., 1979). Dairy scientists had revealed that continuous exogenous oxytocin administration in lactating cows does not affect the P4 concentration (Stewart and Stevenson, 1987; Kotwica *et al*., 1988; Gilbert *et al*., 1989) and remained constant in normal, cyclic buffalo (Kausar *et al*., 2013), whereas Harms and Malven (1969) reported that oxytocin treatment substantially decreased the P4 concentration in cattle. However, literature is scarce on the impact of exogenous oxytocin injection on the P4 concentration in postpartum buffalo. Equally, the impact of long-term exogenous oxytocin administration on milk production, composition, and possible changes in milk quantities, qualities, and P4 concentrations in newly-calved Nili-Ravi buffalo is not available. Therefore, it is necessary to find a solution to such problems that are often highlighted in electronic and print media. This study was thus designed to evaluate the long-term effect of exogenous oxytocin management in newly-calved postpartum Nili-Ravi buffalo on milk production, composition, SCC, and P4 levels.

Material & Methods

The current study was conducted between late September and early March after approval by the Committee on Animal Care and Ethics, University of Veterinary and Animal Sciences Lahore, Pakistan. A total of 24 newly-calved Nili-Ravi buffalo (without any peripartum disorders) were randomly selected at the Buffalo Research Institute, Pattoki (B.R.I.), Kasur, Pakistan. Selected buffalo were equally divided into three groups (Control without oxytocin - normal CON; 10 i.u. oxytocin - LOW; 30 i.u. oxytocin - HIGH). Animals in the CON group were injected with normal saline whereas buffalo in the LOW and HIGH groups were administered with Oxytocin intramuscularly (Oxytocin; Star Laboratories (Pvt) Ltd, Lahore, Pakistan; 10 i.u./mL), twice daily before each milking for 154 days postpartum. These animals were raised under the same management conditions and were offered fresh and clean water *ad libitum*. Usually, each buffalo was offered 50 kg green fodder or 35 kg maize silage alternatively with 5 kg wheat straw and 4.5 kg concentrates. Crude protein (CP, %) contents in green fodder, maize silage, wheat straw, and concentrates were 19.2, 8.1, 2.5, and 17.0%, respectively. Total dry matter intake of feed and fodder was 21.47 kg, whereas the requirement was 15.9 kg. At the start of the experiment, average postpartum period (days), body condition score

(BCS), body weight (kg), age (days), and parity of buffalo in different treatment groups did not differ significantly (*P* >0.05; Table 1). BCS was recorded through visual assessment of subcutaneous fat cover on the back and pelvic regions with a score of 1 to 5 (Singh *et al*., 2017), whereas other parameters were obtained from the farm records.

Hand milking was routinely practised at 3.00 am and 3.00 pm daily during the study period. Milk production was monitored with a weighing scale daily at the milking time in each treatment group. For milk composition parameters, 50 mL milk was taken in sterile Falcon tubes directly from all the teats of every buffalo once a week after removing a few streaks of milk from each teat at the time of milking. Milk composition parameters (fat, protein, density, solids not fat (SNF), lactose, solids, and freezing point (FP) were analysed using an Ultrasonic Milk Analyzer (Master Classic LM2). Simultaneously, pH was measured on a pH meter at the Buffalo Research Institute, Pattoki, Kasur, Pakistan. Somatic cell count (SCC) was assessed using the California Mastitis Test (CMT) (Schalm and Noorlander, 1957; Sandorf *et al*., 2006), with slight modifications, on a fortnightly basis. The Porta SCC Milk Test was performed to confirm CMT results (Salvador *et al*., 2014; Havugineza *et al*., 2017). For the Porta SCC Milk Test, the Porta SCC® kits were used (UVAS and Ghazi Brothers (Pvt) Pakistan, imported from the USA). This test was performed according to standard manufacturer protocols within 2–6 h. One full drop of fresh milk was placed on Porta SSC strip well with a dropper, three drops of activator solution were added on the strip well and colour changes were noted after 45 min. The results were read with an SCC digital reader (1M001, Lot No. 00015293, Germany, for Porta Science Inc., Moorestown, USA). Blood serum was collected at fortnightly intervals from all the animals and frozen at -20 °C until P4 analysis. P4 was measured using the standard RIA technique at the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad.

The data were analysed using repeated measures ANOVA utilizing a General Linear Model (GLM) for milk production, composition, and P4. IBM SPSS version 20 (IBM Corp., Armonk, NY, USA) software was used for statistical analysis. Before statistical analysis of data, normality was checked using the Shapiro–Wilk test, and in non-normal data, normal rank transformations were applied for normal distributions. Differences were considered significant at *P* <0.05. Treatment differences were analysed using the Duncan Multiple Range (DMR) test, and SCC was analysed using the Chi-Square Test (Steel *et al*., 1997).

Results

To determine the effect of the long-term, exogenous administration of oxytocin on milk production, milk composition, and SCC in newly-calved, lactating Nili-Ravi buffalo, treatments in the CON, LOW, and HIGH groups were started at 6.37 ± 1.33, 6.87 ± 1.87, and 5.00 ± 1.51 days postpartum, respectively. The difference between all the groups at the start of treatment was statistically non-significant (*P* >0.05). Similarly, BCS, body weight, age, and parity at the beginning of the experiment was similar (*P* >0.05) between treatment groups (Table 1).

Table 1. Postpartum period, body condition score (BCS), body weight (kg), age (days), and parity of Nili-Ravi buffalo at the start of experiment (mean ± standard error, SE)

Parameters	**Treatment groups**			***P*- value**
	CON (n = 8)	**LOW (n = 8)**	**HIGH (n = 8)**	
Postpartum period (days)	6.4 ± 1.33	6.9 ± 1.87	5.0 ± 1.51	0.720
BCS	3.1 ± 0.22	3.0 ± 0.19	2.9 ± 0.22	0.717
Body weight (kg)*	528 ± 23.1	535 ± 19.4	527 ± 34.0	0.973
Age (days)	2104 ± 200.5	2310 ± 164.8	2265 ± 195.4	0.721
Parity	2.1 ± 0.48	2.4 ± 0.42	2.5 ± 0.42	0.831

*kilogram, n: number of observations, BCS: body condition score
CON = control group, without oxytocin administration
LOW = 10 i.u. oxytocin, intramuscularly, morning and evening for 154 days postpartum
HIGH = 30 i.u. oxytocin, intramuscularly, morning and evening for 154 days postpartum

Average milk production (kg; mean ± SE) in CON, LOW, and HIGH groups of buffalo was recorded as 7.81 ± 0.04, 7.57 ± 0.05 and 7.98 ± 0.05, respectively (Table 2), and the differences between and within all the treatment groups were significant (P <0.05). The increase in milk production was more pronounced in HIGH in the last 52 d than in the other two groups over the 154 d study period. The pH values of milk (mean ± SE) were higher (P <0.05) in the HIGH group (6.72 ± 0.02) than in CON (6.60 ± 0.02) and LOW (6.64 ± 0.02) groups but density (kg/m^3; mean ± SE) was lower (P<0.05) in the HIGH group (32.21 ± 0.27) than in the CON (33.08 ± 0.26) and LOW (33.82 ± 0.24) groups. Fat (%; mean ± SE) was higher (P <0.05) in HIGH (5.60 ± 0.15) than in CON (5.01 ± 0.15) but was similar (P >0.5) to the LOW group (5.24 ± 0.13). SNF (%; mean ± SE) and protein (%; mean ± SE) were greater (P <0.05) in the LOW group (9.96 ± 0.07 and 4.06 ± 0.02, respectively) than in the CON group (9.64 ± 0.06 and 3.90 ± 0.02, respectively) but were similar to the HIGH group (9.77 ± 0.07 and 3.95 ± 0.02, respectively). Freezing point, lactose, and solids did not differ significantly (P >0.05) between the three treatment groups (Table 2).

Table 2. Daily milk production, weekly milk composition, and P4 concentration after long term exogenous administration of oxytocin in Nili-Ravi buffalo (mean ± SE)

Parameters	**Treatment groups**			***P*-value**
	CON (n = 1232; 154 × 8)	**LOW (n = 1232; 154 × 8)**	**HIGH (n = 1232; 154 × 8)**	
Milk production (kg*)	7.8 ± 0.04 ^{b}	7.6 ± 0.05 ^{a}	8.0 ± 0.05 ^{c}	0.000
Milk composition	**(n = 176; 22 × 8)**	**(n = 176; 22 × 8)**	**(n = 176; 22 × 8)**	
Fat (%)	5.0 ± 0.15 ^{a}	5.2 ± 0.13 ^{ab}	5.6 ± 0.15 ^{b}	0.017
SNF (%)	9.6 ± 0.06 ^{a}	10.0 ± 0.07 ^{b}	9.8 ± 0.07 ^{ab}	0.007
Density (kg/m^3) **	33.1 ± 0.26 ^{b}	33.8 ± 0.24 ^{b}	32.2 ± 0.27 ^{a}	0.000
FP (°C) ***	-0.6 ± 0.02 ^{a}	-0.6 ± 0.02 ^{a}	-0.6 ± 0.02 ^{a}	0.151
Protein (%)	3.9 ± 0.02 ^{a}	4.1 ± 0.02 ^{b}	4.0 ± 0.02 ^{ab}	0.000
Lactose (%)	4.9 ± 0.05 ^{a}	4.9 ± 0.05 ^{a}	5.0 ± 0.05 ^{a}	0.225
Solids (%)	0.8 ± 0.01 ^{a}	0.8 ± 0.01 ^{a}	0.8 ± 0.01 ^{a}	0.246
pH	6.60 ± 0.02 ^{a}	6.64 ± 0.02 ^{a}	6.72 ± 0.02 ^{b}	0.002

^{abc} lower case values with different superscripts are significantly different from one another (P <0.05)
SNF: solids-not-fat, FP: freezing point, n: number of observations
*kilogram, **kilogram per cubic meter, *** degree Celsius
ng/ml = nanogram/millilitre
CON = control group, without oxytocin administration
LOW = 10 i.u. oxytocin, intramuscularly, morning and evening for 154 days postpartum
HIGH = 30 i.u. oxytocin, intramuscularly, morning and evening for 154 days postpartum
N = 176 (total number of samples in each group in 22 weeks; each group carrying 8 animals)

The long term effect of exogenous oxytocin administration on SCC in milk was studied fortnightly and divided into two categories; low SCC (< 0.1 million cells/mL) and high SCC (> 0.1 million cells/mL). Results in both categories were similar (P >0.05) between all treatment groups, as were progesterone concentrations (Table 3).

Table 3. Fortnightly, long-term effect of exogenous oxytocin administration on somatic cell count (SCC) in milk and progesterone (P4) concentration in serum of Nili-Ravi buffalo

Variables	**Treatments**			***P*-value**
	CON N = 80	**LOW N = 80**	**HIGH N = 80**	
Low somatic cell count < 0.1 million	71	74	73	0.704
High somatic cell count > 0.1 million	9	6	7	
Progesterone (P4 = ng/mL)	2.22 ± 0.37	2.70 ± 0.37	1.83 ± 0.37	0.290

CON = control group, without oxytocin administration
LOW = 10 i.u. oxytocin, intramuscularly, morning and evening for 154 days postpartum
HIGH = 30 i.u. oxytocin, intramuscularly, morning and evening for 154 days postpartum

Discussion

This study was carried out to determine the effect of the long-term, exogenous administration of oxytocin on milk production, milk composition, and SCC in newly-calved, lactating Nili-Ravi buffalo. In the present study, milk production was markedly increased in the HIGH group compared to the CON and LOW groups. These results are in accordance with the results of other studies in buffalo (Hanjra *et al*., 1977; Qureshi and Ahmed, 2008; Akhtar *et al*., 2012) and cows (Donker 1954; Gorewit and Sagi, 1984; Nostrand *et al*., 1991; Ballou *et al*., 1993; Knight, 1994; Belo and Bruckmaier, 2010; Dymnicki *et al*., 2013). However, they contradict the findings of some dairy researchers who reported that milk production decreased after exogenous oxytocin administration in buffalo (Mustafa *et al*., 2008; Shahid *et al*., 2016) and cows (Allen, 1990; Penry *et al*., 2017). In current study, the result of the milk production is also contrary to certain other studies that revealed no change in milk production after oxytocin injection in buffalo (Prasad and Singh, 2001; Bidarimath and Aggarwal, 2007) and cows (Stewart and Stevenson, 1987).

According to the results of the present study, it is suggested that the increase in milk production may be due to rapid mammary metabolic stimulatory mechanism and mobilization of small milk molecules within the mammary tissues and gland or due to a persistent effect on myoepithelial alveolar tissues (Lollivier and Marnet, 2005). It is conjectured that exogenous oxytocin exhibits mechanophysical pressure on mammary epithelial cells, increasing milk yield (Penry *et al*., 2017). Overall milk production increased due to the complete removal of milk from udder, leaving no or minimal residual milk at higher doses of oxytocin treatment in the HIGH group. High dose oxytocin forcefully contracts myoepithelial cells in the mammary gland and ultimately results in more effective milk removal (Gorewit and Sagi, 1984; Knight, 1994). However, it was also seen that milk production was decreased in the LOW group compared to the CON group. This was perhaps due to oxytocin receptor down-regulation. This downward regulation of the receptors influences the incomplete binding of active exogenous oxytocin with their receptors while using the low supraphysiological dose (10 i.u.) for a prolonged time in buffalo. This might be due to drug addiction, as reported in some earlier studies (Bruckmaier, 2003; Macuhova *et al*., 2004; Belo and Bruckmaier, 2010). Additionally, long term milk ejection reflex is responsible for higher milk yield and better total milk constituents in the dairy animals injected with oxytocin.

In the current study, the measured average fat percentages were 5.01 ± 0.15, 5.24 ± 0.13, and 5.60 ± 0.15& in the CON, LOW, and HIGH treatment groups, respectively. Overall, these values were closely related to the values reported by Akhtar *et al*. (2012) but unlike those of Shah *et al*. (1983), who reported values of 5.36% and 6.55% in Nili-Ravi buffalo. These differences may be due to intra breed variation, management, feeding, and environment (Ahmad *et al*., 2013). In the current study, fat% was higher (P <0.05) in the HIGH group than in the CON and LOW groups, which were similar. These findings are in line with other studies, where an increase in fat% was reported in buffalo (Abbas *et al*., 2014; Shahid *et al*., 2016; Murtaza *et al*., 2017; Murtaza *et al*., 2020) and cows (Donker *et al*., 1954; Gorewit and Sagi, 1984; Stewart and Stevenson, 1987; Dymnicki *et al*., 2013) at certain doses, season (summer) of milking, and involution interval but contrary to the findings of other studies where a decrease in milk fat value was reported at a particular time, dosage, and season (winter) during lactation in buffalo (Bidarimath and Aggarwal, 2007; Mustafa *et al*., 2008; Akhtar *et al*., 2012; Shahid *et al*., 2016). Conversely, these results also had some differences with other studies that reported that fat percentage values did not change after exogenous oxytocin administration in milch cows at certain intervals during lactation and dosages (Morag and Griffin, 1968; Allen, 1990; Nostrand *et al*., 1991; Ballou *et al*., 1993; Penry *et al*., 2017) or decreased after second milking onward (Dymnicki *et al*., 2013).

The high dose of oxytocin is probably responsible for the increase in fat value in the HIGH group, compared to the LOW and CON groups. This increase is due to more active leakage of small molecules across the membranes of myoepithelial cells in the mammary glands. It occurs via the para-cellular pathway mechanism due to active transport where more fat globules are mobilized through exocytosis, which ultimately increases the milk fat globules during milk secretion (Linzell and Peaker, 1971a&b; McManaman and Neville, 2003). Oxytocin injection exerts regular pressure at the epithelial cells' basal membrane to release maximum fat globules and other milk constituents due to higher supraphysiological and long-term treatment duration. Hence, there was an increase in certain overall milk contents. However, the mechanism of oxytocin action at the cellular and molecular level concerning milk constituent synthesis during long-term, exogenous oxytocin administration should be investigated in buffalo. One aspect of the present study is that a high dose of oxytocin increases the milk fat %.

Further results in this study indicate that pH was higher in the HIGH group than in the CON and LOW groups. The effects of pH in the present study are partially in line with findings of some dairy researchers in buffalo (Akhtar *et al*., 2012; Abbas *et al*., 2014), who reported that pH of the milk increased in the winter season and decreased in summer season. This is contrary to one study where no change in pH was reported after oxytocin injection in buffalo during the involution period (Prasad and Singh, 2001; Murtaza *et al*., 2020). The higher pH value in the HIGH group is suggested to be due to variation in mineral ion concentrations due to exogenous oxytocin administration as studied in cows (Hameed *et al*., 2010). Hence, the present results of pH in this study indicate that a high supraphysiological dose (30 i.u.) of oxytocin may have increased the pH. As a result, the taste of milk may change, which is normally unacceptable for human consumption.

Protein and SNF were higher in the LOW group than the CON group, but similar to the maximum-dose HIGH group. These results are in line with some studies in buffalo (Bidarimath and Aggarwal, 2007; Abbas *et al*., 2014) and also in cows (Morag and Griffin, 1968) but different to other studies conducted in buffalo (Shahid *et al*., 2016) and cows (Gorewit and Sagi, 1984; Allen, 1990; Werner-Misof *et al*., 2007; Hameed *et al*., 2016) that revealed a decrease in protein components. The results of protein in existing study are also different to some other studies that reported no change in protein at a certain duration and dosage in cows (Stewart and Stevenson, 1987; Nostrand *et al*., 1991; Ballou *et al*., 1993; Dymnicki *et al*., 2013; Penry *et al*., 2017).

The increase in SNF percentages are to some extent in agreement with conclusions of Abbas *et al*. (2014) at a particular interval of lactation but contrary to Hanjra *et al*. (1979) in buffalo and Lane *et al*. (1970) in cows, where no changes were observed in SNF after continuous, exogenous oxytocin usage. The fluctuations in SNF and protein values are the same and similar to the research conducted by dairy researchers in cows (Morag and Griffin, 1968). Increase in protein and SNF percentages may be suggested due to more diffusion and mobilization of these molecules in the alveolar duct in the LOW group than HIGH and CON groups. It is also recommended that milk composition be evaluated for the whole lactation at indiscriminate dosages of exogenous oxytocin in newly-calved, lactating buffalo.

In the current study, other milk components: FP°, lactose%, and solids% were unchanged in all groups, similar to a study in sheep (Zamiri *et al*., 2001) but contradictory to one study in buffalo during the involution period (Murtaza *et al*., 2020). The current study is partially comparable with the studies in cows where no change in lactose is documented (Dymnicki *et al*., 2013; Penry *et al*., 2017). Milk density (kg/m^3) decreased (P <0.05) in the HIGH group, similar to the other two groups; CON and LOW. This decrease contradicts the studies in sheep and buffalo, respectively, where no changes in density were observed (Zamiri *et al*., 2001; Murtaza *et al*., 2020). This decrease in the density may be due to an overall increase in fat value in the HIGH group. Previously, no single study explained FP° and density following any short or long term exogenous oxytocin administration in buffalo or cows due to less significance of density and FP° related milk compositional changes.

The results of lactose percentage in all treatments in the present study are contradictory to the findings of Abbas *et al*. (2014) and Shahid *et al*. (2016), where the former indicates an increase and the latter, a decrease in lactose% at certain prescriptions of exogenous oxytocin administration in buffalo. The results are analogous to some studies in cows (Ballou *et al*., 1993), where no change in lactose% was identified, but contrary to other studies in cows (Johansson 1952; Allen 1990; Werner-Misof *et al*., 2007; Hameed *et al*., 2010) that exhibited a decrease in lactose%. The percentage of solids (solids%) was in accordance with the results of one study in sheep (Zamiri *et al*., 2001) which reported that solids% remained the same in the oxytocin-injected and control groups. Though some milk solid reserves are leaked from the basal membrane of alveolar ducts during exogenous oxytocin treatment, there was no marked increase in the solids% in oxytocin-treated to control Nili-Ravi buffalo milk. Previously, solids% was not reported in oxytocin-injected dairy animals because of the small concentration of solids in terms of the component value of milk. Hence, little importance was given to this component of milk. TO the best of our knowledge, this is the first report of the solids% in Nili-Ravi buffalo milk following long-term, exogenous oxytocin administration.

The SCC in oxytocin treated-milk is crucial from all public health points of view. The current study revealed no marked difference between oxytocin-treated and control groups. Moreover, overall SCC was less than 0.1 million per mL throughout the treatment period in CON, LOW, and HIGH groups. This level of SCC in all groups indicates that the milk was not affected by a heavy somatic cell count and is considered within a normal range for raw milk and is internationally accepted for human consumption (Dhakal, 2006). These results differ from studies at particular times of lactation and dosages which induce an increase in SCC in buffalo (Prasad and Singh, 2001; Bidarimath and Aggarwal, 2007; Akhtar *et al*., 2012) and cows (Penry *et al*., 2017). However, the current SCC results are similar to the findings of other scientists (Stewart and Stevenson, 1987; Ballou *et al*., 1993; Dang and Anand, 2007). They reported no changes in milk SCC of oxytocin-treated cows. Generally, it was estimated that treatments did not influence the SCC in milk in all three groups after long term oxytocin administration. As SCC count is considered a biomarker of udder health and milk quality, it may be suggested that oxytocin has no effect on SCC in the milk of long-term, exogenously-administered lactating animals. It may also be advantageous to explore the daily effect of exogenous oxytocin on SCC and polymorphonuclear cells for the whole lactation in buffalo milk in future studies.

Progesterone concentration was similar to earlier studies conducted in lactating cows (Stewart and Stevenson, 1987; Gilbert *et al*., 1989). In these studies, there was no change in progesterone concentration with oxytocin injections. Moreover, a decrease in P4 concentration was observed in the current study with higher supraphysiological dose of exogenous oxytocin. This is perhaps due to luteolytic effect of oxytocin on the corpus luteum in cyclic animals during the postpartum period, which concurs with other studies in cows and buffalo (Chase *et al*., 1990; Murtaza *et al*., 2021). This needs to be investigated further, simultaneously, in the plasma and milk for confirmation.

Conclusion

The long-term use of exogenous oxytocin in Nili-Ravi buffalo has beneficial and harmful effects regarding animal and human health. Therefore, a high dose of exogenous oxytocin between 10 i.u. to 30 i.u. (supraphysiological) should be avoided in lactating dairy animals even though this has some beneficial effects. Furthermore, regular long-term oxytocin injection should be contraindicated to prevent any change in milk composition and public health complications. However, it can be prescribed under inevitable circumstances below the supraphysiological dose (<10 i.u.) for udder stimulation at disturbed milking.

Contributions: S. Murtaza[1]*, A. Sattar[2], N. Ahmad[2], I. R. Khan[2], M. Ijaz[2] were involved in study design and conduct and trial, T. Ahmad[1], A. A. Farooq[1], A. Basit[3], M. A. Javid[3], and M. U. Saleem[3] helped in manuscript writing and reviewing. J. Ahmad[4] helped in data analysis. A. Zia[5] & B. Azam[5] contributed to data collection and provided logistics for the trial.

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Conflict of interest

Authors have no potential conflict of interest.

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