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Effect of Exogenous Oxytocin Administration on the Performance of Lactating Nili Ravi Buffalo

Research Article

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ABSTRACT

The objective of the study was to assess the effect of exogenous administration of oxytocin on production, composition and minerals content in milk of Nili Ravi Buffalo. At three lactation phases, milk samples were collected from two groups of eight animals each under controlled atmosphere and feeding conditions. One group was subjected to intramuscular injection of oxytocin (20 IU) and other was kept as control. Significant variations were obtained in milk composition along with lactations phases. Decrease in fat, protein, lactose, solids not fat and total solids contents and increase in toal milk was recorded in oxytocin administreted milk. Minerals' analysis of the milk samples were conducted and it was concluded that lactation stages have significant effect on minerals composition i.e. macro minerals (Na, Cl, K, Ca, Mg and P) and micro mineral (Zn and Cu) in milk. Oxytocin administration showed significant effect on milk minerals during various lactation stages as sodium, chloride and copper contents increased while potassium decreased. It was concluded that indiscriminate use of oxytocin for milk let down considerably influences minerals profile and results in detrimental variations of gross composition of milk.

KEY WORDS lactation, milk, minerals, Nili Ravi buffalo, oxytocin.

INTRODUCTION

Buffalo is playing a leading role in the national economy by producing milk, meat and draught power. Out of total milk produced in the country, buffalo contributes about 68%, followed by cattle (27%) and sheep/goat/camel (5%). Due to high fat contents of buffalo milk, it is the most preferred species in Pakistan. Milk composition and functional attributes are of considerable importance to the dairy farmer, manufacturer, and consumers. Milk is a complex colloidal dispersion of fat globules and protein (casein, whey) in an aqueous solution of lactose, minerals, and other minor constituents (Walker *et al.* 2004). Milk contains 8-12% fat, 8.5-10.5% solids not fat (SNF) and 74% of total solids. These constituents are of quite importance as these are standardized in the production of various products (Oftedal, 2004). The mineral concentrations in milk vary as a function of many kind of factors related to its secretion from the mammary gland, such as the animal species, the time of year, the breed of the animal, and human handling (Zurera-Cosano *et al.* 1994). Buffalo milk is a rich source of calcium. Buffalo milk is also loaded with other minerals such as copper, manganese, phosphorous and zinc which further helps strengthen bones. Buffalo milk has more calcium than cow milk. It may help prevent onset of bone related ailments like osteoporosis and arthritis. All 22 minerals considered to be essential to the human diet are present in smaller amount i.e. less than 1% of all constituents in milk. Mineral salts occur as solution in milk serum or in casein compounds, and the most important salts are those of calcium, sodium, potassium and magnesium. Sodium chloride content may increase at the end of lactation, while the amount of other salts decreases correspondingly (Flynn, 1992).

Buffalo milk contained (g/100 g): 4.02 protein; 7.52 fat; 0.80 ash; 82.33 water; 17.65 total solids; 5.33 carbohydrates, of which 5.02 are lactose (94.32%). Buffalo milk contained less water, more total solids, more fat more lactose, and more protein than cow's milk. Milk composition is influenced by the milking intervals, milking frequency, breed, nutritional status, health, and stage of lactation (Blum and Hammon, 2000; Blum and Baumrucker, 2002).

In dairy practice, exogenous oxytocin is frequently administered before milking, to cure disturbed milk ejection (caused by lacking or reduced oxytocin release) and for mastitis therapy. The long-term practiced exogeneous oxytocin administration reduces the release of endogenous oxytocin and sensitivity to oxytocin in the udder (Werner-Misof *et al.* 2007), possibly due to oxytocin receptor down-regulation resulting in reduced spontaneous milk ejection after withdrawal of oxytocin (Bruckmaier, 2003). Administration of exogenous oxytocin is unfortunately becoming a common practice in Pakistan, regardless of physiological effects and dose as per circumstances, only with intentions to increase milk yield. Therefore, the farmers (due to unawareness and lack of education) administer oxytocin injections before each milking. It gives temporary benefits, but in the long term perspectives, it is very harmful to animals and may alter milk composition ultimately affecting the quality of milk products.

The effect of oxytocin on milk composition is still a debatable issue. Some scientists believe that oxytocin disturbs or changes milk composition in various ways

(Hameed *et al.* 2014; Hameed *et al.* 2016) and some are of the view that it does not have any concern with milk composition (Bansode *et al.* 1996). The objective of the present study was to investigate the effect of exogenous administration of oxytocin on gross milk composition and mineral contents of Nili Ravi Buffalo milk at three lactation stages.

MATERIALS AND METHODS

Milking sampling

Forty eight Nili Ravi buffaloes were selected from Buffalo Research Institute, Pattoki, Pakistan from May to August with 8-11 litres per day milk production. Buffaloes were divided into three groups with respect to lactation stages i.e. Early lactation (post calving; 60-80 days), peak lactation (post calving; 180-240 days) and late lactation (post calving; 240-270 days).

In each lactation stage, eight out of sixteen buffaleos were normal/control (group I) and the other eight were given oxytocin injection intramuscularly (20IU; Lawrence Pharma, Pvt. Ltd) for milk let down (group II).

These animals were kept under similar atmospheric conditions and fed the similar inputs. Milking was carried out under hygienic conditions in stainless steel buckets with covers at 5 a.m. and 5 p.m. Milk samples at three different stages were collected with sterile syringe and transferred in 100 mL plastic bottles and stored at 4 ˚C throughout experiment.

Compositional analysis

The pH of milk was measured through electronic digital pH meter (Inolab WTW Series 720). Acidity in milk samples was determined by the method (No. 947.05) given in AOAC (1995). The Gerber method was used to determine fat content in milk. The nitrogen content in milk sample was estimated by using Kjeltec System-II, Tecator AB, Hoganas, Sweden based on Kjeldahl's method (991.20) of (AOAC, 2000). Lactose determination was done by the enzymatic method (AOAC, 2000). Ash concentration in milk was estimated by the method No 945.46 as given in Firestone (1990). Milk solids not fat were calculated according to procedure by Pearson (1976) using lactometer. Total solids of milk were determined according to the method described in Firestone (1990).

Mineral profiling

Milk sample (1g) was digested by the wet digestion method. It was first digested in glass flask with 10mL concentrated HNO_3 at moderate temperature (60-70 ˚C) for 20 minutes and then with 5mL $HClO_4$ at high temperature (190 ˚C) till the solution became clear. The digested sample was transferred to 100mL volumetric flask and volume was made with double distilled water and then filtered (Firestone, 1990).

Sodium, potassium and calcium were determined by flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) according to procedure given by (Firestone, 1990). Chloride in milk was determined by silver nitrate method of Mour's titration (Sawyer *et al.* 1994). Phosphorus content in the milk was determined by colorimetric estimation method as described by (Kitson and Mellon, 1944). Micro minerals (Zinc and Copper) were analyzed using atomic absorption spectrophotometer (Varian AA 240, Victoria, Australia).

Table 1 Effect of oxytocin on milk composition of Nili Ravi buffaloes at various lactation stages

Parameters	Groups (n=3)	Lactation stages (mean±standard deviation)		
		Early lactation	Peak lactation	Late lactation
Milk	Control	8.57±1.19	9.92±0.72	9.98±1.26
	Oxytocin	9.47±0.67*	10.45±2.01**	10.05±4.22*
Fat%	Control	9.47±0.46	9.65±0.05	9.22±0.28
	Oxytocin	8.01±0.04^{ns}	8.71±0.23**	8.81±0.19*
4% fat corrected milk	Control	8.96±1.29	10.09±0.44	9.73±1.48
	Oxytocin	9.52±0.27^{ns}	10.93±2.24**	10.21±2.76*
Solids not fat (%)	Control	10.32±0.19	10.35±0.11	10.14±0.16
	Oxytocin	8.92±0.29	8.79±0.34	8.77±0.23
Total solids (%)	Control	16.85±0.19	17.84±0.20	17.10±0.20
	Oxytocin	15.49±0.41	15.58±0.46	15.29±0.32
pH	Control	6.59±0.02	6.63±0.02	6.67±0.03
	Oxytocin	6.56±0.02	6.61±0.05	6.68±0.02
Acidity (%)	Control	0.1±0.001	0.097±0.002	0.092±0.001
	Oxytocin	0.098±0.003	0.095±0.002	0.091±0.001
Protein (%)	Control	3.89±0.11	3.95±0.08	4.7±0.16
	Oxytocin	3.57±0.16	3.69±0.15	3.87±0.08
Lactose (%)	Control	5.17±0.16	4.94±0.04	4.83±0.13
	Oxytocin	4.82±0.16	4.57±0.22	3.89±0.18
Ash (%)	Control	0.89±0.02	0.79±0.02	0.86±0.01
	Oxytocin	0.74±0.02	0.74±0.01	0.78±0.02

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** (P<0.01) and * (P<0.05).
NS: non significant.

The standard curves for each mineral was prepared by running samples of known strength and compared with the values of milk samples as detailed in (Firestone, 1990).

Statistical analysis

The results obtained were statistically analyzed using analysis of variance technique. Means and standard error of means were calculated and Duncan's Multiple Range (DMR) test was applied to find the difference between means (Steel and Dickey, 1997).

RESULTS AND DISCUSSION

Milk Production

Significantly higher (P<0.05) milk and 4% fat corrected milk (FCM) production while lower milk fat % were recorded in buffalo injected oxytocin before milking (Table 1). Oxytocin treatment (OT) to buffaloes intramuscularly for complete lactation triggered 11.6% higher milk yield over control (Nostrand *et al.* 1991). Likewise, milk yield was signifcantly improved (P<0.05) in animals administered oxytocin before milking (Bencini *et al.* 1992). However, Ballou *et al.* (1993) observed increased (P<0.05) milk production by 3% before and after milking thorugh OT that augmented glandular milk yield rather retained milk. Total evacuation of the udder during milking is very important in dairy business it could go a long way in minimizing production losses taht may occur when practicing once a day milking.

Bruckmaier *et al.* (1994) observed in dairy goats prestimulation of the mammary glands triggered the release of adequate amount OT to let-down substantial amount of cisternal milk without instigating the characteristic bimodal pattern of milk flow. Further more elevated OT level initiated milk let-down in ewes that needs to be sustained to maintain release of mik through the complete milking (Bruckmaier *et al.* 1997a; Bruckmaier *et al.* 1997b; Bruckmaier *et al.* 1998). However, exogenous OT inoculation at non-milking period increased milk production and improved glactopoiesis in ewes (Zamiri *et al.* 2001).

Thus OT influences cell maintenance and mammary metabolism in addition to its usual characteristic of helping milk let-down (Bruckmaier, 2003). Usage of OT to boost milk let-down, predominantly when the glands are distended with milk, can prevent udder damage and promote udder health.

Separtion of calves are being done from their dams in routineat dairy farms where machine milking is practiced. In these circumstancses the natural oxytocin release is compromised that led to the abrupt drop of milk production and flowrate (Tancin *et al.* 1995). With machine milking the pattern of milk let-down is changed, however milk yield is not significantly affected (Bruckmaier *et al.* 1996). For all the milking frequencies, the initiation of milking is followed by significantly higher circulating OT levels. Increase in milking frequency each day increases milk production and vice vers, however milk composition was also altered (Negrao *et al.* 2001; Lollivier *et al.* 2002).

Increase in milk yield was recorded by Weiss *et al.* (2003) while working on prestimulation of miking cows by hand/suckling than machine-milking as more oxytocin is synthesized and released in the blood stream. Dzidic *et al.* (2004) studied single stall automatic milking systems while monitoring milk ejection, OT release and milking characteristics and identified that OT levels didn't differ between treatments when milking process is started untill the whole milking process. In contrast, the sensitivity of mammary glands to the OT activities was declined in response to chronic OT application, subsequently there was a marked reduction in the contractibility of myoepithelial cells under normal physiological oxytocin level. Thus OT effector pathway turn out to be insensitive led to the prolonged myoepithelial/alveolar contractions (Macuhova *et al.* 2004).

Thomas *et al.* (2005) found an increase in milk yield with OT administration and reported a positive correlation between the total time OT concentrations exceeded threshold levels and milk yield harvested by machine. Lollivier *et al.* (2005) confirmed the galactopoietic effect of OT and described that additional milking amplified milk yield by 8% and milk ingredients by 6%. In addition to this, Passille *et al.* (2008) recorded improved residual milk yield in nursing buffalos than control (8.7±0.8 *vs.* 3.2±0.8 kg) subsequent to OT administration. Akhtar with his co-workers (2012) explored the properties of OT inoculation prior to milking on milk production in Nili Ravi buffalo and significant higher milk yield was recorded as compared to control treatment. While working on a project about poverty alleviation and rural development by livestock extension education in southern Punjab, author observed that mostly people use exogenous OT injection for the letdown of milk in animals especially in buffaloes and they reported that by the use of OT injection the amount of milk produced has been increased (Faraz *et al.* 2019). Administration of exogenous OT gave a similar response ($P<0.05$) in polish red cows Dymnicki *et al.* (2013).

Fat contat was decreased in oxytocin injected buffalos as compared to control. In almost all the groups milk fat was reduced when milk production was increased as milk fat and milk quantity were inversaly propotional. Differences in fat content were noticed higher at mid lactation which is the peak lactation period (Table 5). Current trend of milk fat content at successive lactation stages have supported the findings of Pavić *et al.* (2002) and Sitkowska (2008). Significant decrease in fat content in the present study at all stages as compared to control buffalos have aready been described by Bidarimath and Aggarwal, (2007) who proved that fat% in total milk decreased significantly by 11.8% and 21.3% in experimental groups of buffaloas injected with oxytocin 2.5 IU and 5.0 IU intramuscular (hip region) at 15 days interval as compared to control buffalos, respectively.

These results reflected that by removing the milk from the mammary gland at frequent intervals by forced milking may possibly retard the passage of blood fat into the gland sufficiently to decrease the amount of fat secreted by the gland. Such large variations in fat content obviously affect the economics of milk production and the composition of milk products.

In contrast Nostrand *et al.* (1991) used animals received an injection of 1 ml (20 IU) of oxytocin at each milking and Bencini *et al.* (1992) used eight lactating ewes who were milked after an intra-venous injection of 2.0 International Units (IU) of oxytocin, then milked again after a further intra-venous injection of 0, 0.5, 1.0 and 5.0 IU of oxytocin and described that OT administration did not affect the percentages of milk fat, lactose, protein, somatic-cell counts (SCC) or milk plasmin activity. Ballou *et al.* (1993) reported that the effects of OT were not demonstrated by the variations in cell remodeling. Lollivier *et al.* (2005) affirmed that milk fat and protein were positively correlated, with the increase of one component the quantity of others' will also increased with-, SNF and total solids contents following the same pattern. However, there was also significant decrease in copper and iron concentrations. While Akhtar *et al.* (2012) explored the stimulus of OT injection before milking on SCC and fat content in milk of Nili Ravi buffaloes. In their study buffaloes were treated intramuscularly with 30 IU of oxytocin daily before the start of milking for the period of 7 days and found that SCC was increased by OT injection as compared to control whereas there was no effect on milk-fat percentage. There was significant decrease in percent acidity, protein, fat, solids-not-fat (SNF) and total-solids in OT administered buffaloes (Kiran, 2001). This response may be due to variations in diet, season, the timing and dose of exogenous OT injections.

Statistical results showed non-significant difference in pH and acidity percentages and significant difference in fat, protein, lactose, ash, solids not fat and total solids percentages between oxytocin treated and normal milk samples (Table 1). The present study results showed decrease in fat content in oxytocin injected buffalo milk samples as compared to control milk samples. Differences in fat contents were noticed higher at late lactation (Table 1). Protein % in milk samples decreased in cows injected oxytocin as compared to those of control and this decrease continueduntil late lactation. While along with lactation stages proteins contents have escalation trend. Lactose content followed a declining trend with the progression of lactation stages in milk of oxyticin treated and control buffaloes. The percentage decrease in lactose of buffaloes milk administered with oxytocin respect tocontrol buffalo milk was 0.35% at early lactation, 0.47% at peak lactation and 0.41% at late lactation. Significantly, lower ash content of oxytocin injected buffalo milk was observed at all stages of lactation as compared to control buffaloes milk which shows decrease in ash content due to oxytocin injections. In case of oxytocin injected buffalo's milk, the initial content of ash in early lactation was 0.74%, which was 0.05% higher than control. Solids not fat decreased significantly in milk of buffalos administered with oxytocin as compared to buffalo's milk not treated with oxytocin injections. Percent decrease was observed as 0.4%, 0.62% and 0.87% in solids not fat along with lactation stages. Milk from oxytocin injected buffalos contained low contents of total solids while the milk from control buffalos yielded higher contents of total solids. The difference between early lactation of both oxytocin treated and control groups was 1.86% which increased to 2.26% in peak lactation milk stage and 2.81% in the end lactation milk stage. It is evident from these variations that oxytocin significantly affected the total solids of milk and this difference progressed linearly as the lactation stages increased.

In the present study, the trend of protein contents in both groups of animals along lactation stages have supported the findings of Sitkowska (2008) who found that the yield of milk increased while fat and protein contents decreased with the administration of oxytocin. Significant decline ($P<0.05$) in protein content in milk of buffalos administered with oxytocin as compared to protein content in milk of control buffalos along with lactation stages is also comparable with the findingds of workers

(Lane *et al.* 1970; Allen, 1990). The decrease in protein content of milk is explained by Ledbetter and Lubin (1977) who reported the adverse effects of elevated Na on cell function. Increases in intracellular Na:K ratios in cultured fibroblasts decreased the rate of synthesis of protein and DNA. Therefore, it seems possible that increasing the ratio of Na:K as in the present investigation in lactating mammary cells could inhibit synthesis of proteins (Rayson, 1989) which might be the reason for variation in protein content between normal and oxytocin treated milk. Stelwagen *et al.* (1999) further showed that the adverse effects on secretion were not due to a high intracellular concentration per se but were related to a change in the Na to K ratio because a reduction in the ratio also lowered milk secretion. These data support the evidence for activity of Na+-K+-ATPase in the basolateral secretory cell membranes and passive movement of these ions across the apical cell membranes.

The present results of lactose contents are in agreement with the study conducted by Werner-Misof *et al.* (2007) who reported that, chronic oxytocin administration induced increasing levels of lactose in blood and decreasing concentrations of lactose in milk. Allen (1990) concluded that the drop in yield of lactose in milk may be due to decreased synthesis or to leakage into plasma and clearance into the urine. Any variation in α-lactalbumin content may cause hinderness in the synthesis of lactose (Fox and McSweeney, 1998). Electrophoretic patterns reported by Hameed *et al.* (2016) showed that the concentration of α-lactalbumin decreased in the oxytocin injected buffalo's milk at all the stages. In a very recent study, it was concluded that lactation stages have significant variations on milk composition of Sahiwal cow. The pH, fat, protein, total solids and ash increase while lactose and acidity decrease with lactation stages. Whereas solid not fat are not affected by the lactation stages. Forceful milking by administration of oxytocin also reduces fat, lactose, protein, total solids, solids not fat and increase the ash content in milk. Oxytocin treatment to Sahiwal cows along with lactation stages significantly influenced the sodium, potassium, chloride and copper contents of milk while non-significant variations in calcium, magnesium, phosphorus and zinc were recorded (Hameed *et al.* 2021). There was an increase in ash content of treated buffalos from peak to late lactation milk, but only 0.04% increase was observed apparently in late lactation. However, there was no treatmnent effect statistically. Fox and McSweeney (1998) reported that the ash content of milk remains relatively constant at 0.7-0.8%. They further reported that the concentration of lactose is inversely related to the concentration of soluble salts. Lactose along with sodium, potassium and chloride ions play a major role in maintaining the osmotic pressure in the mammary system. Thus any increase or decrease in lactose content is compensated by an increase or decrease in soluble salt. This osmotic relationship partially explains why certain milk with low lactose content have a high ash content and *vice versa*.

The results obtained in the present trial have supported the work of Lane *et al.*(1970) who reported that solids-not-fat was significantly lower in milk removed after the injection of oxytocin than in the samples taken during normal milking. A slight difference in total solids content than previously reported values wasobserved, this may be due to breed difference as milk for chemical analysis was obtained from Nili Ravi breed of buffalo (Enb *et al.* 2009). The results of the present study are in close agreement with studies of Johansson *et al.* (1952) who reported changes in the composition of milk after three oxytocin injections, 25 IU each. The present results showed decrease in fat content and also solids not fat in milk of buffalos administered with oxytocin. Consequently this effect is also responsible for decrease in total solids of buffalo milk.

The statistical analysis showed that the administration of oxytocin significantly influenced the sodium, potassium and chloride contents in the milk of buffalo; however, variations in calcium, magnesium and phosphorus were non-significant (Table 2).

Table 2 Effect of oxytocin on micro and macro-minerals in milk of Nili Ravi buffaloes at various lactation stages

Parameters	Groups (n=3)	Lactation stages (mean±standard deviation)		
		Early lactation	Peak lactation	Late lactation
Sodium (mg/L)	Control	423±11	399±14	518±17
	Oxytocin	502±16	487±16.5	614±20
Potassium (mg/L)	Control	1491±35	1394±38	1289±31
	Oxytocin	1398±53	1267±45	1091±36
Chloride (mg/L)	Control	850±30	775±25	808±21
	Oxytocin	925±24	849±20	888±22
Calcium (mg/L)	Control	1711±29	1689±31	1619±25
	Oxytocin	1601±28	1595±30	1509±31
Magnesium (mg/L)	Control	164±32	170±34	177±37
	Oxytocin	155±34	156±35	165±3
Phosphorus (mg/L)	Control	1025±21	1085±32	1819±25
	Oxytocin	996±16	1055±25	1161±29
Zinc (µg/L)	Control	3569±150	3809±150	3098±170
	Oxytocin	3633±90	3730±120	3138±160
Copper (µg/L)	Control	161±7[a]	129±8[b]	47±7[c]
	Oxytocin	160±11[a]	134±8[b]	120±9[b]

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Significantly higher sodium contents at all lactation stages were observed in oxytocin treated milk as compared to control. Sodium contents were found higher at both stages early lactation and late lactation in both groups. Potassium contents were found significantly lower in oxytocin injected milk as compared to without oxytocin injected milk along with lactation stages. The chloride contents decreased during peak lactation but again increased slightly at the end of production. Similar to sodium, the chloride contents found in milk of oxytocin administrations were sigificantly higher as compared to milk of the control animals with lactation stages.

The results showed that oxytocin treatment had significant effect on copper but non-significantly influenced the zinc contents in milk of buffalo (Table 2). However, lactation stages showed highly significant effect on micro-minerals during the whole period. The increase in copper contents were noticed at peak lactation and late lactation in oxytocin injected buffalos milk as compared to control.

The correlation between milk composition and mineral contents for control buffalos are presented in the Table 3. It is evident that sodium correlated negatively (r=-0.998) with solids not fat. Similarly negative correlations of potassium potassium (r=-0.999, r=-0.999, r=-0.992 and r=-0.994) were established with pH, fat, protein and ash, respectively. Potassium concentration on milk had a decreasing trend from early lactation to late lactation stage while milk protein content has inverse trend as that of potassinum concentration. Milk pH did not show any treatment effect but milk fat and ash contents related negarively (Table 1). Copper was significantly and positively correlated with acidity and lactose. Both acidity (P>0.05) and copper content (P<0.05) of milk decreased towards the end of lactation.

The correlation coefficients between milk composition and mineral contents of oxytocin treated buffalos is shown in Table 4. Potassium was correlated positively with acidity and lactose (r=0.993, r=0.989) but negatively correlated with pH (P<0.01) which is in contrast to control milk samples because oxytocin might be the factor contributing to alter the natural trend of these parameters. There was a significant positive correlation (r=0.994) of sodium with ash content of milk. Copper and phosphorus were negatively correlated to protein and solids not fat (P<0.01), respectively.

The results regarding sodium content in the present study are in agreement with Hameed *et al.* (2010), who observed a significant increase of sodium content at all stages of lactation in milk of oxytocin treated buffalos. In the present study, concentration of sodium content fell within the ranges (350-900 mg/L) previously reported by Fox and McSweeney (1998). Results showed a gradual declining trend in potassium throughout the lactation, which is in agreement with the findings of Fox and McSweeney (1998) and they also reported that the concentration of potassium fell in the range of 1100 to 1700 mg/L. Allen (1990) and Hameed *et al.* (2010) also reported that doses of oxytocin of 1 IU or greater altered the milk sodium and potassium concentrations, presumably by increasing the permeability of the mammary epithelial tight junctions to small molecules, allowing electrolytes to move between the milk and interstitial space down their electrochemical gradient.

The chloride results of the present investigation are within the range (800-1400 mg/L) reported by Fox and McSweeney (1998) who further reported a significant increase in chloride content in bovine milk during lactation stages.

Table 3 Correlation coefficients between physico-chemical composition and mineral contents of milk from control Nili Ravi buffaloes

Minerals	pH	Acidity	Fat	Protein	Lactose	Ash	Solids not fat	Total solids
Na	0.755^{ns}	-0.841^{ns}	0.789^{ns}	0.683^{ns}	-0.855^{ns}	0.698^{ns}	**- 0.998****	0.629^{ns}
K	**-0.999****	**0.993***	**-0.999****	**-0.992***	0.989^{ns}	**-0.994***	0.806^{ns}	-0.980^{ns}
Cl	-0.559^{ns}	0.434^{ns}	-0.513^{ns}	-0.642^{ns}	0.410^{ns}	-0.625^{ns}	- 0.063^{ns}	-0.695^{ns}
Ca	0.189^{ns}	-0.327^{ns}	0.241^{ns}	0.086^{ns}	-0.353^{ns}	0.108^{ns}	-0.749^{ns}	0.015^{ns}
Mg	0.115^{ns}	-0.255^{ns}	0.168^{ns}	0.011^{ns}	-0.281^{ns}	0.033^{ns}	- 0.697^{ns}	-0.060^{ns}
P	0.955^{ns}	-0.987^{ns}	0.969^{ns}	0.919^{ns}	**-0.991***	0.927^{ns}	-0.938^{ns}	0.888^{ns}
Zn	-0.651^{ns}	0.753^{ns}	-0.691^{ns}	-0.569^{ns}	0.770^{ns}	-0.586^{ns}	0.979^{ns}	-0.509^{ns}
Cu	-0.969^{ns}	**0.995****	-0.981^{ns}	-0.939^{ns}	**0.997****	-0.946^{ns}	0.918^{ns}	-0.912^{ns}

** (P<0.01) and * (P<0.05).
NS: non significant.

Table 4 Correlation coefficients between physico-chemical composition and minerals content of milk from oxytocin treated Nili Ravi buffaloes

Mineral	pH	Acidity	Fat	Protein	Lactose	Ash	Solids not fat	Total solids
Na	0.860^{ns}	-0.853^{ns}	-0.977^{ns}	0.681^{ns}	-0.840^{ns}	**0.994***	- 0.922^{ns}	- 0.980^{ns}
K	**- 0.999****	**0.999****	0.722^{ns}	-0.963^{ns}	**0.999****	-0.905^{ns}	0.989^{ns}	0.734^{ns}
Cl	- 0.401^{ns}	0.413^{ns}	0.333^{ns}	-0.643^{ns}	0.436^{ns}	0.015^{ns}	0.270^{ns}	-0.318^{ns}
Ca	0.307^{ns}	- 0.294^{ns}	- 0.874^{ns}	0.026^{ns}	-0.270^{ns}	0.674^{ns}	- 0.436^{ns}	- 0.866^{ns}
Mg	- 0.164^{ns}	0.177^{ns}	- 0.554^{ns}	- 0.435^{ns}	0.201^{ns}	0.260^{ns}	0.025^{ns}	- 0.541^{ns}
P	0.988^{ns}	-0.985^{ns}	- 0.828^{ns}	0.903^{ns}	-0.981^{ns}	0.964^{ns}	**-0.999****	-0.837^{ns}
Zn	-0.836^{ns}	0.828^{ns}	0.985^{ns}	-0.647^{ns}	0.814^{ns}	-0.988^{ns}	0.904^{ns}	0.988^{ns}
Cu	-0.964^{ns}	0.968^{ns}	0.524^{ns}	**-0.999****	0.974^{ns}	-0.768^{ns}	0.918^{ns}	0.538^{ns}

** (P<0.01) and * (P<0.05).
NS: non significant.

Chronic oxytocin administration increased sodium and chloride levels in milk as well as potassium and lactose in blood as reported by Werner-Misof *et al.* (2007) who studied the milk by injecting buffalos with 50 IU intramuscular oxytocin and similar results are reported by Hameed *et al.* (2010) with 20 IU intramascular irrespective of lactation stages.

These effects are in agreement with the findings of Fox and McSweeney (1998) and Kamal *et al.* (1961) who reported the highest contents of calcium at late lactation stage. Magnesium and phosporus results are in agreement with Kamal *et al.* (1961) who reported continuous rise per week in minerals during last three month of lactation in buffalos at comfort temperature. Zinc in buffalo's milk primarily binds to casein and to a small extent with citrate. Almost 90% zinc binds to casein in Early lactation, in contrast to just 60% in the colostrums (Kincaid and Cronrath, 1992).

In casein, zinc binds primarily to colloid calcium phosphate in casein micelles (Silva *et al.* 2001). The results obtained in the present study were in agreement with these findings. Similar variations in zinc contents of milk with lactation stage have also been reported by Bedö *et al.* (1994) and Knowles *et al.* (2006). The results of the copper concentration in buffalo milk obtained during present study fell within the reported values by Murthy *et al.* (1972).

CONCLUSION

The statistically considerable differences were found in gross composition and in all the minerals during lactation periods. It was concluded that lactation stages have significant variations on milk composition of buffalo. The pH, fat, protein, total solids and ash increase while lactose and acidity decrease with lactation stages. Where as solid not fat are not affected by the lactation stages. Forceful milking by administration of oxytocin also reduces fat, lactose, protein, total solids, solids not fat and increase the ash content in milk. Oxytocin treatment to buffalos along with lactation-stages significantly influenced the sodium, potassium, chloride and copper contents of milk while non-significant variations in calcium, magnesium, phosphorus and zinc were recorded. In brief, arbitrary application of oxytocin considerably influences the minerals profile of the milk resulting in unusual variations. From this study it is concluded that regular oxytocin injections should be stopped because it not only affect the milk composition but also has a promising effect on the products manufactured from this milk and health of the consumers. Infants could be more affected than adults.

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