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RESEARCH ARTICLE

Physicochemical Profile of Milk from Oxytocin Injected Buffaloes

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ABSTRACT

A study was conducted to evaluate the impact of continuous exogenous use of oxytocin in Nili Ravi breed of buffaloes on the physicochemical composition of milk. A group of animals (n=12) were injected with oxytocin (20IU) daily at morning and evening while the other group of buffaloes (n=12) were kept as control and injected with normal saline. Morning and evening milk samples were pooled for analysis. Analysis was made on fortnight basis starting from January to the end of June. Appropriate statistics was applied. Overall milk pH was lower ($P \leq 0.05$) while proteins, lactose and SNF of oxytocin injected buffaloes were significantly higher as compared to control animals. These differences were due to a significant lower pH during 8th and 9th fortnights, higher milk proteins during 9th fortnight, a higher ($P \leq 0.05$) lactose and SNF during 5th and 9th fortnights of oxytocin injected buffaloes during the experimental periods. Milk fat, proteins and sold not fat was significantly higher during the months of March and May. Milk pH was higher ($P \leq 0.05$) while milk fat was lower during January and February. During April and May, pH was lower ($P \leq 0.05$) and milk fat was higher ($P \leq 0.05$) in oxytocin treated buffaloes as compared to control animals. During winter season, milk fat concentration from oxytocin injected buffaloes was significantly lower, however during summer seasons fat concentration was higher ($P \leq 0.05$) than controls. In conclusion, long term use with a high dose of oxytocin significantly alters the composition of milk and these changes are also significant during different months and with different seasons.

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INTRODUCTION

Milk yield of Nili-Ravi breed of buffalo which is the best breed in Pakistan is 1800-2500 liters per lactation with 6.5% fat and average milk yield of 7.5 liters per day (Bilal *et al.,* 2006; Bhutto *et al*., 2012; Khan *et al*., 2013; Hussain *et al*., 2013).

Exogenous oxytocin (OT) has been used in cow and buffaloes as frequently administered medicine before milking for quick let down and enhancement of milk production without realizing its impact on the health and production indices of animals. Till date there is only one study which demonstrates that OT was quite stable to pasteurization in OT spiked milk (Prakash *et al.*, 2009; Mahmood *et al*., 2012). In dairy practice, exogenous oxytocin is commonly administered to buffaloes before milking in a dose of 10-20 IU to cure troubled milk ejection caused by lacking or reduced oxytocin release or due to mastitis therapy (Bruckmaier, 2003; Akhtar *et al*., 2012). Moreover, oxytocin treatment for a long time in buffaloes decreases spontaneous milk ejection after withdrawal of oxytocin. Qureshi and Ahmad (2008) observed dams given oxytocin injection for milk let down at a dose rate of 5IU I/M and sometimes as high as 25IU. OT correlated positively with placental expulsion time and post-partum ovulation intervals. This was also observed that one injection/day of OT shorten while no injection or two injections per day increased post-partum estrus intervals in cow. Oxytocin injection may be used as an important tool for better milking management in buffalo and effects of oxytocin on the milk composition/profiles are not well studied for long duration in buffaloes. Rizzo *et al.* (2012) investigated the impact of OT on the hemodynamic characteristic of milk vein in Holstein-Friesian cows and suggested that OT may exert a vasodilatory effect as well as functional status of the mammary gland (Piccione *et al.*, 2004). Antonic *et al.* (2013) demonstrated that OT treatment in ewes increased peak milk flow and milk yield but were unable to change in milk composition. It has also been reported that OT injection in lactating buffaloes resulted in elevated oxidative stress by increasing the total homocysteine and ceruloplasmin oxidase activity and decreasing enzymatic activities of antioxidant enzymes including paraoxonase-1 and arylesterase; that might render the animals to poor productive and reproductive potential (Zafar *et al.*, 2013).

The purchase of OT does not require any prescription and is easily available even from general stores of the villages. The effect of one dose of OT may persist for several days but the farmers inject oxytocin before each milking. This practice may be due to lack of awareness and illiteracy of the farmers which may result in temporary gains but in the long term this practice can have harmful effects on animal and human health as well as on the quality of milk. Therefore the present study was designed to study the long term use with a high dose of oxytocin on the quality of buffaloes milk.

MATERIALS AND METHODS

Sample Collection: Twenty four Nili Ravi buffaloes in their first and second lactation for milk were selected at Tarir Buffalo Dairy Farm, Dhanola, near Faisalabad during 2010. Twelve (12) buffaloes were kept as control (group I) and were injected with normal saline and the other twelve (12) were selected for oxytocin injection (group II; 20IU/IM). The treatment continued from January to June and milk samples were collected at fortnight intervals. Environmental temperature was obtained from the Department of Crop Physiology, University of Agriculture, Faisalabad – Pakistan. The minimum and maximum environmental temperature (°C) recorded during the month of January (6.0-16.2), February (9.5-22.2), March (16.5-30.4), April (21.4-38.4), May (25.4-40.7) and in June (27.7-40.1) was used for splitting the data into winter (January and February), spring (March and April) and summer (May and June) season. Data obtained was pooled to analyze the months and seasonal effect. These animals were kept under similar atmospheric conditions and were fed similar feed. Homogenous milk samples were obtained (morning, 50 ml and evening 50 ml) and transferred in plastic bottles to store at -4°C for subsequent analysis.

Sample analysis: Milk pH was measured using electronic digital pH meter (Model Inolab WTW Series 720). The Gerber method as described by Marshall (1993) was used to determine fat content in milk samples. 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 (2007). Lactose percentage was carried out by Lane and Eynon method using the procedure described in AOAC (2007). Milk solids not fat were determined using lactometer as described by David (1977). For each parameter two way analysis of variance was applied to determine the difference between fortnights and groups, months and groups and between seasons and groups. In case of significant difference, Duncan Multiple Range test was applied.

RESULTS

pH: Mean milk pH was significantly lower during 8th and 9th fortnight in oxytocin treated buffaloes as compared to control animals, therefore overall mean milk pH was significantly lower in oxytocin treated buffaloes as compared to controls buffaloes (Table 1). Mean milk pH

Table 1: Mean±SE pH, fat (%), protein (%), lactose (%) and solid-not-fat (%) of milk from oxytocin injected buffaloes vs control at different fortnight intervals

Fort-nights	pH		Fat		Protein		Lactose		Solid-Not-Fat	
	Control	Oxytocin Treated	Control	Oxytocin Treated	Control	Oxytocin Treated	Control	Oxytocin Treated	Control	Oxytocin Treated
1st	6.68 ±0.029^{b-d}	6.65 ±0.093^{b-d}	4.01 ±0.43^{a-d}	1.92 ±0.17^{e}	3.25 ±0.14^{b-e}	3.55 ±0.10^{a-d}	4.74 ±0.21^{a-d}	5.24 ±0.16^{ab}	8.73 ±0.39^{a-d}	8.96 ±0.25^{a-d}
2nd	6.68 ±0.031^{b-d}	6.79 ±0.043^{ab}	3.75 ±0.52^{a-e}	1.81 ±0.19^{e}	3.15 ±0.15^{cde}	3.24 ±0.15^{b-e}	4.60 ±0.23^{bcd}	4.76 ±0.21^{a-d}	8.47 ±0.43^{bcd}	8.70 ±0.39^{a-d}
3rd	6.69 ±0.027^{bcd}	6.75 ±0.028^{abc}	3.94 ±0.39^{a-d}	2.06 ±0.15^{de}	3.48 ±0.11^{a-d}	3.30 ±0.10^{a-e}	5.11 ±0.16^{abc}	4.82 ±0.15^{a-d}	9.32 ±0.32^{abc}	8.83 ±0.27^{a-d}
4th	6.70 ±0.025^{a-d}	6.87 ±0.025^{a}	3.97 ±0.37^{a-d}	2.25 ±0.34^{cde}	3.40 ±0.11^{a-e}	3.65 ±0.09^{a-c}	4.99 ±0.17^{a-d}	5.36 ±0.13^{ab}	9.11 ±0.32^{a-d}	9.80 ±0.24^{ab}
5th	6.61 ±0.037^{c-e}	6.67 ±0.037^{b-d}	3.28 ±0.57^{b-e}	4.18 ±0.29^{a-c}	2.92 ±0.09^{e}	3.59 ±0.12^{abc}	4.24 ±0.13^{d}	5.11 ±0.19^{abc}	7.79 ±0.24^{d}	9.43 ±0.34^{abc}
6th	6.57 ±0.029^{de}	6.69 ±0.024^{bcd}	3.50 ±0.53^{a-e}	4.35 ±0.43^{ab}	3.01 ±0.09^{de}	3.46 ±0.08^{a-e}	4.36 ±0.12^{cd}	5.01 ±0.10^{a-d}	8.01 ±0.23^{cd}	9.22 ±0.19^{a-d}
7th	6.69 ±0.027^{bcd}	6.60 ±0.044^{cde}	3.94 ±0.39^{a-d}	4.34 ±0.34^{ab}	3.48 ±0.11^{a-d}	3.44 ±0.06^{a-e}	5.11 ±0.16^{abc}	4.99 ±0.08^{a-d}	9.32 ±0.32^{abc}	9.17 ±0.16^{a-d}
8th	6.70 ±0.025^{a-d}	6.36 ±0.019^{fg}	3.97 ±0.37^{a-d}	4.15 ±0.37^{abc}	3.40 ±0.11^{a-e}	3.42 ±0.11^{a-e}	4.99 ±0.17^{a-d}	4.95 ±0.16^{a-d}	9.11 ±0.32^{a-d}	9.08 ±0.30^{a-d}
9th	6.61 ±0.037^{cde}	6.25 ±0.017^{g}	3.28 ±0.57^{a-e}	4.30 ±0.41^{ab}	2.92 ±0.09^{e}	3.81 ±0.12^{a}	4.24 ±0.13^{d}	5.54 ±0.17^{a}	7.79 ±0.24^{d}	10.17 ±0.32^{a}
10th	6.57 ±0.029^{de}	6.59 ±0.021^{cde}	3.50 ±0.53^{a-g}	4.35 ±0.43^{ab}	3.01 ±0.09^{de}	3.46 ±0.08^{a-e}	4.36 ±0.12^{cd}	5.01 ±0.10^{a-d}	8.01 ±0.23^{cd}	9.22 ±0.19^{a-d}
11th	6.59 ±0.027^{cde}	6.55 ±0.020^{de}	4.25 ±0.39^{ab}	4.69 ±0.34^{ab}	3.69 ±0.14^{abc}	3.45 ±0.07^{a-e}	5.36 ±0.21^{ab}	5.09 ±0.10^{abc}	9.84 ±0.37^{ab}	9.29 ±0.19^{a-d}
12th	6.58 ±0.024^{cde}	6.44 ±0.25^{ef}	4.30 ±0.36^{ab}	5.45 ±0.20^{a}	3.76 ±0.15^{ab}	3.62 ±0.07^{abc}	5.47 ±0.22^{a}	5.22 ±0.10^{ab}	10.04 ±0.39^{a}	9.61 ±0.17^{ab}
Overall Means	6.64 ±0.009^{A}	6.60 ±0.019^{B}	3.81 ±0.13	3.65 ±0.14	3.29 ±0.04^{B}	3.50 ±0.03^{A}	4.80 ±0.06^{B}	5.09 ±0.04^{A}	8.79 ±0.11^{B}	9.29 ±0.08^{A}

Mean sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction of means in column for each parameter and capital letters in rows for each parameter are used for overall mean.

Table 2: Mean±SE pH, fat (%), protein (%), lactose (%) and solid-not-fat (%) of milk from oxytocin injected buffaloes vs control at various months of experimental period

Months	pH		Fat		Protein		Lactose		Solid-Not-Fat	
	Control	Oxytocin Treated	Control	Oxytocin Treated	Control	Oxytocin Treated	Control	Oxytocin Treated	Control	Oxytocin Treated
1stJanuary	6.68 ±0.02^{ab}	6.72 ±0.05^{a}	3.88 ±0.33^{ab}	1.87 ±0.12^{d}	3.20 ±0.10^{bc}	3.39 ±0.0^{ab}	4.67 ±0.15^{bc}	4.99 ±0.14^{ab}	8.60 ±0.28^{cd}	8.83 ±0.23^{bcd}
2ndFebruary	6.69 ±0.02^{ab}	6.81 ±0.02^{a}	3.96 ±0.26^{ab}	2.15 ±0.18^{cd}	3.44 ±0.08^{ab}	3.47 ±0.07^{ab}	5.05 ±0.11^{ab}	5.09 ±0.11^{ab}	9.21 ±0.22^{abc}	9.32 ±0.21^{abc}
3rdMarch	6.59 ±0.02^{bc}	6.68 ±0.02^{ab}	3.39 ±0.38^{bc}	4.26 ±0.26^{ab}	2.96 ±0.06^{c}	3.52 ±0.07^{ab}	4.29 ±0.09^{c}	5.06 ±0.10^{ab}	7.90 ±0.16^{d}	9.33 ±0.19^{abc}
4thApril	6.69 ±0.02^{ab}	6.48 ±0.04^{cd}	3.96 ±0.26^{ab}	4.25 ±0.24^{ab}	3.44 ±0.08^{ab}	3.43 ±0.06^{ab}	5.05 ±0.11^{ab}	4.97 ±0.09^{ab}	9.21 ±0.22^{abc}	9.13 ±0.16^{abc}
5thMay	6.59 ±0.02^{bc}	6.42 ±0.04^{d}	3.39 ±0.38^{bc}	4.32 ±0.29^{ab}	2.96 ±0.06^{c}	3.64 ±0.08^{a}	4.30 ±0.09^{c}	5.27 ±0.12^{a}	7.90 ±0.16^{d}	9.70 ±0.21^{ab}
6thJune	6.58 ±0.02^{bc}	6.50 ±0.02^{cd}	4.27 ±0.26^{ab}	5.07 ±0.13^{a}	3.73 ±0.10^{a}	3.54 ±0.05^{ab}	5.42 ±0.15^{a}	5.15 ±0.07^{ab}	9.94 ±0.27^{a}	9.45 ±0.13^{abc}
<u>Season</u>										
Winter	6.69 ±0.14^{ab}	6.76 ±0.03^{bc}	3.92 ±0.21^{b}	2.01 ±0.11^{c}	3.32 ±0.07	3.43 ±0.06	4.86 ±0.10	5.05 ±0.09	8.91 ±0.18	9.07 ±0.16
Spring	6.64 ±0.02^{a}	6.58 ±0.03^{c}	3.67 ±0.23^{b}	4.25 ±0.17^{ab}	3.20 ±0.06	3.48 ±0.05	4.67 ±0.09	5.01 ±0.07	8.56 ±0.17	9.23 ±0.12
Summer	6.59 ±0.01^{c}	6.46 ±0.02^{d}	3.83 ±0.24^{b}	4.70 ±0.17^{a}	3.34 ±0.08	3.59 ±0.05	4.86 ±0.12	5.21 ±0.07	8.92 ±0.22	9.57 ±0.12

Mean sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction of means in column for each parameter and capital letters in rows for each parameter are used for overall mean.

was significantly lower during April and May of the experimental period in oxytocin treated buffaloes than the controls (Table 2). Seasonal data showed a decrease (P≤0.05) in milk pH of oxytocin injected buffaloes during spring and summer as compared to winter (Table 2).

Fat (%): At the beginning of the experiment (1st fortnight), milk fat from oxytocin treated buffaloes showed lower (P≤0.05) fat concentration, it further dropped from 2nd to 4th fortnights and then started increasing and this difference was non-significant (Table 1). Overall mean milk fat concentration was lower in oxytocin injected buffaloes. Mean milk fat concentration was lower (P≤0.05) during the month of January and February in oxytocin treated buffaloes and was lower (P≤0.05) during winter and high (P≤0.05) during spring and summer season (Table 2).

Proteins (%): Milk protein concentration was higher (P≤0.05) on 9th fortnight and overall mean was significantly higher in oxytocin injected milk of buffaloes (Table 1). A significant increase in milk protein concentration was observed during the month of March and May (Table 2) in oxytocin injected buffaloes as compared to control animals. Mean protein concentration was higher during all seasons; however these differences were non-significant (Table 2).

Lactose (%): Milk lactose concentration was higher (P≤0.05) during 9th fortnights in oxytocin treated as compared to control buffaloes (Table 1). Overall mean milk lactose concentration was significantly higher (P≤0.05) in oxytocin treated buffaloes as compared to controls. Significantly higher concentration of lactose was observed during the month of March and May in lactating buffaloes subjected to oxytocin injection (Table 2). There were no differences among seasons in the lactose concentration of milk between oxytocin treated and control buffaloes (Table 2), even though the values of lactose concentration were much higher than control buffaloes.

Solid Not Fat (%): Mean milk solid not fat (SNF) concentration was significantly lower (P≤0.05) during 2nd and high (P≤0.05) during 12th fortnights in the milk of oxytocin treated buffaloes (Table 1). Overall mean milk SNF was significantly higher in the oxytocin treated animals as compared to control buffaloes. Mean milk SNF concentration was significantly higher during the month of March and May in oxytocin injected buffaloes as compared to controls animals (Table 2) and it was higher than control during various seasons.

DISCUSSION

Difference in composition of buffalo's milk in different localities would be due to breed, managements feeding and environment (Medhammar *et al.,* 2011; Ahmad *et al.*, 2013). The significance of measuring pH of raw milk was to determine its shelf life. Enb *et al.* (2009) reported the milk pH in Egyptian buffalo which is similar to the one reported in the present study. Significant decrease in milk pH during spring and summer could be attributed to change and availability of fodder during different seasons.

Large variations in milk fat concentration were observed at various fortnights particularly in the early part and then at the end of the experimental period. The increase in milk fat may be due to the cumulative effect of this hormone as the buffaloes are approaching towards decline in their milk production. El-Loly (2011) reported that in milk 90% or more milk fat is present in droplet forms and been given the name as milk fat globules (MFG) in cows. Prasad and Singh (2001) reported that the milk fat percentage vary from 6.22 to 6.88% during different days in buffaloes. However in the present study the variation of fat percentage ranged from 3.28 to 4.3% in normal while in oxytocin treated buffaloes the range was 1.81 to 5.45%. Tonhati *et al.* (2011) reported milk fat in buffalo's milk to be 90.g.kg-1. Therefore, arguing that oxytocin was not a limiting factor when the daily milking frequency was increased. Enb *et al.* (2009) reported 4.9-7.52% milk fat in Egyptian buffaloes. Milk fat concentration of oxytocin treated buffaloes was significantly low during winter followed by a significant increase in the summer throughout the experimental period. Abid El-Aziz *et al.* (2012) concluded that buffaloes milk fat concentration are slightly affected by the type and level of the feeding diet supplementation. Beata (2008) reported that milk fat content showed an increasing trend at successive lactation stages. Also, Pavici *et al.* (2002) reported a significantly high fat milk contents in the middle and at the end of lactation. Buffalo's milk composition can vary widely depending upon stage of lactation, lactation month, parturition day, seasonal feeding level and breeds (George, 2006).

The total lipid in buffalo milk are in higher contents (El-Fattah *et al.*, 2012) when compared with cow milk. Milk lipid contributes to sensorial and nutritional quality improvement of various values added products to benefit human health (Mansson, 2008). In cows injected with oxytocin, Hameed (2010) reported a high percentage of fat in control as compared to oxytocin treated groups in Sahiwal cattle. When residual milk is removed by the use of oxytocin, the deficit in milk fat contents became evident. It has also been pointed out that oxytocin pre-propeptide gene can increase milk yield and oxytocin to have insulin like activity thus lipogenic in nature can affect cheese production therefore effecting negatively the economic return (Mancini *et al.*, 2013). In a comparative study, seasonal variation has been reported on buffaloes, cow, goat and sheep milk where the pasture by season has shown a higher concentration of total poly unsaturated, followed by trans and conjugated linoleic acid contents, while short fatty acid has shown an opposite trend indicating that grass during summer affects the bio-hydrogenation pathway (Talpur *et al.*, 2008).

In the present study milk proteins vary from 2.92 to 3.76% along with lactation in normal and in oxytocin injected buffaloes it ranged from 3.24 to 3.81%. Of the total proteins of buffaloes 80% are caseins and 20% are whey proteins with traces of other minor proteins (Ahmad *et al.,* 2013). In the present study, early response of oxytocin was a decrease in the protein contents which increased on the 9th fortnight of oxytocin injection. Beata (2008) concluded that protein contents in milk of cows were high between 200 to 300th day of lactation after which it decreased to a level lower than the beginning of lactation. In contrary to this, buffaloes milk proteins contains 2-3 times high concentration of alanine, glycol, glutamic acid and proline (Mihaiu *et al.,* 2013). Hameed (2010) also reported a decline in the protein contents of milk from oxytocin injected Sahiwal cows. It is not known yet if long term use with high dose of oxytocin effects on the amino acids of milk.

Change in the yield of lactose can be the result of either due to lack of synthesis or its movement through tight junction to circulation or the combination of both. In the present study, overall lactose milk concentration did increase in oxytocin treated buffaloes. Buffaloes milk is richer source of lactose than cow, sheep, goat and camel to provide a good source of energy for body and brain activities in addition to hormonal regulation (Ahmad *et al.,* 2013). Lactose is the least variable component and has close relationship between lactose synthesis and amount of water being added by the animal. Pollott (2004) reported that the secretion rate of lactose and water are stable and constant throughout the lactation. Mech *et al.* (2009) showed a close relationship between lactose synthesis and the quantity of water being drawn into the milk. The decline of lactose in winter and significantly high during spring and summer may be related to the effect of oxytocin injection on experimental buffaloes. Opposite trend was observed in the concentration of lactose in Sahiwal cow being low during beginning and then increase toward the end of lactation (Hameed, 2010). The results for lactose in present study are in line with the work of Misof *et al.* (2007) indicating that chronic oxytocin administration induced a high level of lactose in blood. It is also possible that certain ions like sodium, potassium and chloride has an important role to play in maintaining the osmotic pressure and could be responsible for an increase and/or decrease of lactose and protein concentration in the milk. Very few studies document the effects of season on buffaloes milk however negative effect of hot environments on animal production in term of quality and quantity of milk of farm animals has been reported by Bernabucci *et al.* (2013).

Buffalo milk, because of its higher fat, solid not fat (SNF) and total solid (TS) contents yield relatively more cream, butter, cheese, condensed milk as well as other dairy products (Pandya and Khan, 2009). Similarly, higher proportion of solid not fat makes buffalo butter harder and less spreadable. In the present study the overall SNF was significantly high in oxytocin treated buffaloes. Hameed (2010) on the other hand in a study with Sahiwal cow reported that SNF decreased ($P \leq 0.05$) in cows when injected with oxytocin.

Sharma *et al.* (2002) reported SNF contents to be high in winter and low in rainy season and attributed this change to the availability of green fodder during rainy season. Mech *et al.* (2009) reported lack of any significant change during various lactation stages. Oftedal (2004) reported that milk contain 74% of total solid (TS) and 8.5 to 9.5% of solid not fat, and these constituents are very important in standardization of quality and specification of milk products

Conclusion: From the present study it is evident that long term use as well as high doses of oxytocin buffalo milk altered the pH, fat, proteins, lactose and SNF as compared to normal animals. These changes are alarming and needs further investigation using in vitro studies to elucidate the mechanism and also the effect of continuous injection on the welfare of animal health.

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