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Compositional and Mineral Profile of Sahiwal Cow Milk at Various Lactation Stages as Influenced by Oxytocin Administration

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Milk, Sahiwal cow, Minerals, Oxytocin, Lactation

ABSTRACT

The objective of the study was to assess the effect of exogenous administration of oxytocin on gross composition and mineral contents in milk of Sahiwal cow. At three lactation stages, milk samples were collected from two groups of sixteen 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 stages. Decrease in fat, protein, lactose, solids not fat and total solids contents and increase in ash contents were noted in oxytocin administreted milk. Minerals' analysis of the milk samples were conducted and it was found that lactation stages have significant effect on minerals composition i.e. macro minerals (Na, Cl, K, Ca, Mg and P) and micro minerals (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.

INTRODUCTION

Cow's milk composition and functional attributes are of considerable importance to the dairy farmer, manufacturer, and consumer. 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 8.5-9.5% solids not fat (SNF) and 14% of total solids of milk. These constituents are of quite importance as these are standardized in the production of various products (Oftedal, 2004).

Minerals are divided into two groups: macro and micro. The mineral concentrations in milk vary as a function of many kinds 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). 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 cow 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).

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). Sahiwal cow originated from the Sahiwal district of the Punjab province of Pakistan and still regarded as one of the best dairy animals in Indo-Pak region (Khan *et al*., 2005).

In dairy practice, exogenous oxytocin is frequently administered to cows 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

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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*., 2016; Hameed *et al*., 2014) and some are of the view that they do 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 Sahiwal cows' milk at three lactation stages.

MATERIALS AND METHODS

Milk sampling

Forty eight Sahiwal cows were selected from Livestock and Dairy Development Farm at Bahadur Nagar, Okara, Pakistan from May to August with average milk production 5-9 litres per day. Forty eight cows were divided into three groups with respect to lactation stages i.e. Mature milk (Post calving; 60-80 days), Peak production (Post calving; 180-240 days) and End production (Post calving; 240-270 days). In each lactation stage, eight out of sixteen cows were normal/control (group I) and the other eight were given oxytocin injection (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 5am and 5pm. Milk samples at three different stages were collected with sterile syringe and transferred in 100 ml plastic bottles and stored at 4^{o}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 by Horwitz and Horwitz (2000). 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 Horwitz and Horwitz (2000). Lactose determination was done by the enzymatic method of Horwitz and Horwitz (2000). Ash concentration in milk was estimated by the method No 945.46 as given by 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 by Firestone (1990).

Mineral profiling

Milk sample (1g) was digested by the wet digestion method. It was first digested in glass flask with 10 ml concentrated HNO_3 at moderate temperature (60-70°C) for 20 minutes and then with 5 ml $HClO_4$ at high temperature (190°C) till the solution became clear. The digested sample was transferred to 100 ml 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). The standard curves for each mineral was prepared by running samples of known strength and compared with the values of milk samples as detailed by 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 composition

Statistical results showed non-significant difference ($P>0.05$) in pH and acidity percentages and significant difference ($P<0.05$) in fat, protein, lactose, ash, solids not fat and total solids percentages of both oxytocin treated and normal milk samples (Table I). Present study results showed decrease in fat contents in oxytocin injected sahiwal cow milk samples as compared to control milk samples. Difference in fat contents were noticed higher at end production. Protein results in this study indicated decrease in oxytocin injected milk samples as compared to control one's and this decrease continue till end production. While along with lactation stages protein contents have inclined trend. Lactose content followed a declining trend with progression of lactation stages in milk of oxytocin treated and control sahiwal cows. Percent decrease in lactose of Sahiwal cow's milk administered with oxytocin and control cow's milk was observed 0.35% at mature milk, 0.47% at peak production and 0.41% at end production. Significantly highest ash content of oxytocin injected Sahiwal cow's milk was observed at all stages of lactation as compared to control cows milk which shows increase in ash content due to oxytocin injections. In case of oxytocin injected cow's milk, the initial content of ash in mature milk was 0.74%, which was 0.05% higher than control. Solids not fat decreased significantly in milk of Sahiwal cows administered with oxytocin as compared to Sahiwal cow's milk those were 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 cows contained low contents of total solids while the milk from control cows yielded higher contents of total solids. The difference between mature milk of both oxytocin treated and control groups was 1.86% which increased to 2.26% in peak production 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.

Table I. Effect of oxytocin on milk composition of Sahiwal cow at various lactation stages.

Parameters	Groups	Lactation stages		
		Mature milk	Peak production	End production
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
Fat (%)	Control	4.05±0.04	4.49±0.11	5.02±0.08
	Oxytocin	2.58±0.18	2.85±0.24	2.02±0.16
Protein (%)	Control	3.39±0.11	3.75±0.08	4.0±0.16
	Oxytocin	3.27±0.16	3.49±0.15	3.60±0.08
Lactose (%)	Control	5.17±0.16	4.84±0.04	4.23±0.13
	Oxytocin	4.82±0.16	4.37±0.22	3.82±0.18
Ash (%)	Control	0.69±0.02	0.73±0.02	0.76±0.01
	Oxytocin	0.74±0.02	0.74±0.01	0.78±0.02
Solids not fat (%)	Control	9.32±0.19	9.35±0.11	9.14±0.16
	Oxytocin	8.92±0.29	8.73±0.34	8.27±0.23
Total solids (%)	Control	13.35±0.19	13.84±0.20	14.10±0.20
	Oxytocin	11.49±0.41	11.58±0.46	11.29±0.32

n, 3; ±, SD.

The fat content in milk showed an increasing trend at successive lactation stages that is in agreement with the findings of Pavic *et al*. (2002) and Sitkowska (2008). Significant decrease in fat content in present study at all stages as compared to control cows are in line with the study of 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 2.5IU and 5.0IU respectively, intramuscular (hip region) at 15 days interval as compared to control cows. 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 present study results, inclined trend of protein contents in both groups of animals along with lactation stages are in line with those of Sitkowska (2008). Significant decline ($p<0.05$) in protein content in milk of cows administered with oxytocin as compared to protein content in milk of control Sahiwal cow along with lactation stages which is in agreement with the work of scientists (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 can inhibit synthesis of proteins (Rayson, 1989) which might be the reason for variation in protein content between normal and oxytocin treated milk.

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 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 hindrance in the synthesis of lactose (Fox and McSweeney, 1998). Electrophoretic pattern as indicated by Hameed *et al*. (2016) showed that the concentration α-Lactalbumin decreased in the oxytocin injected cow's milk at all the stages. This could be the reason for reduction in lactose biosynthesis in mammary gland.

The oxytocin treated cows were addicted of oxytocin this could be the reason of higher content of ash in treated cows at mature milk. There was an increase in ash content of treated cows from mature milk to peak production milk, but again 0.04% increase was observed in end production. 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 study are in line with 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. Slight difference in total solids contents than reported values were observed, may be due to breed difference as milk for chemical analysis was obtained from Sahiwal breed of cow (Enb *et al*., 2009). The present results showed decrease in fat contents and also solids not fat in milk of cows administered with oxytocin. Consequently this effect is also responsible for decrease in total solids of Sahiwal cow milk.

Mineral contents

The statistical analysis showed that the administration of oxytocin significantly influenced the sodium, potassium and chloride contents in the milk of Sahiwal cow; however, variations in calcium, magnesium and phosphorus were non-significant (Table II). 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 mature milk and end production 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 production but again increased slightly at the end of production. Similar to sodium, the chloride contents found in milk of oxytocin administrations were significantly 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 Sahiwal cow (Table II). However, lactation stages showed highly significant effect on micro-minerals during the whole period. The increase in copper contents were noticed at peak production and end production in oxytocin injected cows milk as compared to control.

The correlation between milk composition and mineral contents for control Sahiwal cows are presented in the Table III. It is evident that sodium was correlated negatively (r=-0.998) with solids not fat. Similarly negative correlations of potassium (r=-0.999, -0.999, -0.992 and -0.994) were established with pH, fat, protein and ash, respectively. Potassium concentration of milk had a decreasing trend from mature milk to end production stage while pH, fat, protein and ash contents were found the lowest at the end production of milking. Copper was significantly and positively correlated with acidity and lactose. Both acidity and copper content of milk decreased towards the end of lactation. The correlation coefficients between milk composition and mineral contents of oxytocin treated cows is shown in Table IV. Potassium was correlated positively with acidity and lactose(r=0.999, r=0.999) 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.

Table II. Effect of oxytocin on micro and macro-minerals in milk of Sahiwal cow at various lactation stages.

Parameters	Groups	Lactation stages		
		Mature milk	Peak production	End production
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	994±29	894±31	1019±25
	Oxytocin	988±28	881±30	1019±31
Magnesium (mg/L)	Control	94±3	80±3	96±3
	Oxytocin	97±3	82±3	93±3
Phosphorus (mg/L)	Control	984±21	1015±32	1119±25
	Oxytocin	996±16	1025±25	1101±29
Zinc (µg/L)	Control	3569±150	3809±150	3098±170
	Oxytocin	3633±90	3730±120	3138±160
Copper (µg/L)	Control	161±7	129±8	47±7
	Oxytocin	160±11	134±8	120±9

n, 3; ±, SD.

Table III. Correlation coefficients between physico-chemical composition and mineral contents of milk from control Sahiwal cows

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

**, Highly significant ($p<0.01$); *, Significant ($p<0.05$); NS, Non-significant ($p>0.05$).

Table IV. Correlation coefficients between physico-chemical composition and minerals content of milk from oxytocin treated Sahiwal cows.

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

**, Highly significant ($p<0.01$); *, Significant ($p<0.05$); NS, Non-significant ($p>0.05$).

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 cows. In 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 1IU 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. 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 cows with 50 IU intramuscular oxytocin and similar results are reported by Hameed *et al*. (2010) with 20IU intramuscular irrespective of lactation stages. These effects are in agreement with the findings of Fox and McSweeney (1998) who reported the highest contents of calcium at late lactation stage. Magnesium and phosphorus results are in agreement with Webb *et al*. (1974) who reported continuous rise per week in minerals during last three month of lactation in cows at comfort temperature.

Zinc in cow's milk primarily binds to casein and to a small extent with citrate. Almost 90% zinc binds to casein in mature milk, 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 are in agreement with these findings. Similar variations in zinc contents of milk with lactation stage have also been reported by Bedo *et al*. (1994) and Knowles *et al*. (2006). The results of the copper concentration in cow milk obtained during present study fell within the reported values by Murthy *et al*. (1972).

CONCLUSION

The statistically considerable discrepancies are found in gross composition and in all the minerals during lactation periods. It is 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. 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 manufacture from this milk and health of the consumers. Infants could be more affected than adults.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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