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CONSEQUENCE OF OXYTOCIN INJECTIONS ON MINERALS CONCENTRATION IN SAHIWAL COW MILK

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The major objective of this study was to find out the consequence of exogenous administration of oxytocin to Sahiwal cow, local breed of Pakistan (to enhance milk let down) on mineral composition. Milk samples were collected from two groups of eight animals under controlled atmosphere and feeding input. The analysis of fodder and feed samples were also carried out to support this study. Means of macro and micro minerals of fodder and feed samples were determined. One group of Sahiwal cows was subjected to intramuscular injection of oxytocin (20 IU). The milk obtained from oxytocin injected animals possessed significantly higher sodium and chloride with increment in ash content. Lactose content decreased by 0.39% and ash content increased by 0.08% in milk treated with oxytocin injections. Oxytocin administration increased the level of Na (19.74%), Cl (9.39%) and Cu (146.89%) while K content decreased (10.06%).
Keywords: Oxytocin, milk composition, minerals, Sahiwal cows

INTRODUCTION

Sahiwal cow is the best breed existing in Indo-Pak region with per lactation capacity of 1500-2200 liters having 4.5 % fat content (Khan *et al.,* 2005). A good milking process in dairy cows requires optimization of management, technological and physiological processes. For fast and complete milk removal, the active role of dairy cows must be considered. The active role means that the milk ejection reflex must be to shift milk from the alveolar tissue to lower parts of the udder - cistern from which milk is available for mechanical removal. Before milk ejection occurs, only very small amounts of milk in cistern are available for removal. Complete milk ejection during each milk removal is necessary for the maintenance of high production level and udder health. Milk yield in dairy cows is regulated by numerous factors, such as genetics, environment, hormonal status, nutritional state and milking frequency (Crowley and Armstrong, 1992). The release of oxytocin into blood circulation represents the beginning of the endocrine way. After arriving via systemic circulation, oxytocin binds at specific receptors in the mammary gland to influence the myoepithelial cell activity (Soloff *et al.,* 1980; Zavizion *et al.,* 1992). Myoepithelial cells are located between the basement membrane and the epithelial cells of the mammary alveoli and along the mammary duct system. Due to the binding of oxytocin, the myoepithelial cells contract and the intraalveolar pressure is increased, leading to the expulsion of milk from the alveoli to an individual maximum (Bruckmaier and Blum, 1996; Mayer *et al.,* 1991).

In dairy practice, oxytocin injections are administered to cows before milking to cure disturbed milk ejection caused by lacking or reduced oxytocin release or for mastitis therapy. However, the effect of oxytocin injection on blood pattern and milk ejection efficiency is unclear. Moreover, long-term oxytocin treatment in cows reduces spontaneous milk ejection after withdrawal of oxytocin (Donker *et al.,* 1954; Bruckmaier 2003). Chronic administration of exogenous oxytocin inhibits the release of endogenous oxytocin and sensitivity to oxytocin in the udder, possibly due to an oxytocin receptor down-regulation.

Unfortunately administration of exogenous oxytocin is very common practice in Pakistan and number of farmers is increasing day by day who use oxytocin only for milk let down. They are uneducated about the use of oxytocin with respect to dose as circumstances. Due to unawareness and lack of education of farmers they administer oxytocin injections every time of milking which may leads to effect the composition of milk and ultimately interfere with the quality of milk and milk products.

All 22 minerals considered to be essential to the human diet are present in cow milk which are less than 1%. Mineral salts occur in solution in milk serum or in casein compounds, and the most important salts are those of calcium, sodium, potassium and magnesium. Lactose is a major carbohydrate in milk (Gopal and Gill 2004) and ranges from 4.4 to 5.2% with average content of 4.8% (Nickerson, 1974). Lactose is reducing disaccharide comprised of glucose and galactose, linked by a β1-4-O glycosidic bond (Fox, 2003). The aim of the present study is to investigate the effect of administration of exogenous oxytocin to Sahiwal cattle on potassium, sodium, chloride, calcium and phosphorus as well as ash and lactose.

MATERIALS AND METHODS

Analysis of fodder and feed samples

Five samples were drawn from the fodder supplied to control and five from oxytocin injected Sahiwal cows. For feed analysis, five samples were drawn from concentrate supplied to control and oxytocin injected cows. These fifteen samples were analyzed for mineral analysis in triplicates.

All the fodder and feed samples were oven dried at 60^{o}C upto constant weight and ground through a Wiley mill (1mm screen). One gram of dried samples (fodder and feed) was subjected to wet digestion method as described by Richards (1968). Then analysis was conducted through atomic absorption spectrophotometer (Varian AA 240, Victoria, Australia) for determination of macro and micro minerals (Mg, Zn and Cu,) using standard curve. Analysis for sodium, potassium and calcium were conducted through flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK). Chloride content in samples was determined by using method as described by Sawyer et al. (1994) and phosphorus content was determined following method described by Kitson and Mellon (1944).

Analysis of milk samples

Sahiwal cow milk was taken from Bahadur Nagar, Okara. Milking was carried out under normal and hygienic conditions in stainless steel buckets with covers. Milk samples were collected from two groups of eight animals under similar conditions and feeding input. One group of Sahiwal cows was subjected to intramuscular injection of oxytocin (20 IU). The animals were kept under routine management and feeding conditions throughout the experiment. Milk samples were collected and placed in 100 mL sterile plastic bottles. Milk samples were kept at 4^{0}C throughout the experiment.

Ash was estimated by the method as given in AOAC 945.46 (1990). A well mixed and homogenized milk sample (5 ml) was taken in a crucible and moisture was evaporated to dryness of sample on steam bath. Then crucible was placed in muffle furnace at 550^{o}C until ash is carbon free. The crucible containing ash was placed in dessicator for 30 minutes then weighed and ash% was calculated as:

% Ash = Wt. of ash/ Wt. of sample × 100

Lactose contents were estimated by using Fehling's solution titration method as described by Egan *et al.* (1981). Milk Lactose (%) was calculated as:

$$\text{Lactose (\%)} = \frac{\text{Dilution factor} \times \text{equivalent obtained from lactose}}{\text{Volume of sample used} \times 10} \times 100$$

Where Dilution factor = 100

Lactose equivalent was 0.087 = 10 ml Fehling's solution

Sample (0.5 g) was digested by the wet digestion method. It was first digested with 10 ml HNO_3 at gentle temperature (60-70°C) for 20 min. Then the sample was digested with $HClO_4$, at high temperature (190°C) till the solution become clear. The digested sample was transferred to 250mL volumetric flask and volume was made with deionized water and then filtered (Duhan *et al*., 2002). Sodium, potassium and calcium were determined by wet digestion method by flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) and magnesium, zinc and copper through atomic absorption spectrophotometer (Varian AA 240, Victoria, Australia) according to AOAC (1990).

Ammonium vandate was used to determine phosphorus by aminonaphthol sulphonic acid method (Kitson and Mellon, 1944). In the test tubes, ammonium molybdate solution (1 ml), amminonaphthol sulphonic acid solutions (0.4 ml) and 0.1 ml sample or standard solutions at various dilutions were added. Distilled water was added to make up the volume (10 ml). This solution was shaken and allowed to stand for 5 min to develop color. Blank was used to set the zero absorbance at 720 nm wavelength.

Chloride was determined by silver nitrate method of Mour's titration (Sawyer *et al.*, 1994). One mL of digested sample was taken in a 100 mL beaker and was made basic by adding calcium carbonate about 0.05-0.08g. Then one drop of potassium chromate was added as indicator. It was titrated against silver nitrate drop-wise. End point was precipitate of brick red color.

Results obtained from each parameter were subjected to statistical analysis using T- test described by Steel *et al.* (1997) to determine the level of significance and comparison of means.

RESULTS AND DISCUSSION

The analysis of fodder and feed samples for minerals were carried out along with milk samples to support the results of this study. The means for macro minerals in fodder samples used for control cows have been given in Table: 1a, while for oxytocin injected cows were given in Table 1b. The sodium content in fodder used for control cows was 843mg/Kg and for oxytocin injected cows was 806mg/Kg while potassium content of control cows and oxytocin injected cows were 16863mg/Kg and 16217mg/Kg, respectively. The fodder provided to the control cows contained 1335mg/Kg chloride, 8800mg/Kg calcium, 1310mg/Kg phosphorus and 1730mg/Kg magnesium. Fodder

Table 1a. Macro minerals (mg/Kg) of fodder samples used for control experimental Sahiwal cows (Means[1] + SD)

Fodder	Na	K	Ca	P	Mg	Cl
Fodder C1	826 ± 18.50	15570 ± 105	8500 ± 115	1300 ± 80	1767±195	1275±85
Fodder C2	861 ± 20.50	19519 ± 110	9500 ± 78	1300 ± 95	1724±85	1347±158
Fodder C3	796 ± 30.00	16557 ± 150	9500 ± 110	1400 ± 88	1735±70	1229±136
Fodder C4	903 ± 25.38	17089 ± 98	8500 ± 175	1350 ± 125	1678±120	1548±120
Fodder C5	828 ± 20.00	15578 ± 57	8000 ± 55	1200 ± 75	1744±130.5	1278±38.9
Overall Means	843 ± 41	16863 ± 1622	8800 ± 671	1310 ± 74	1730±33	1335±126

C = Control
SD = Standard deviation
Means[1] of three replicates

Table 1b. Macro minerals (mg/Kg) of fodder samples used for experimental Sahiwal cows injected with oxytocin (Means[1] + SD)

Fodder	Na	K	Ca	P	Mg	Cl
Fodder O1	767 ± 25.75	16962 ± 398	8000 ± 179	900 ± 156	1443 ± 105	1184 ± 54.5
Fodder O2	800 ± 25.98	16888 ± 340	7500 ± 258	1000 ± 110	1634 ± 125	1235 ± 110
Fodder O3	766 ± 39.90	15577 ± 350	8000 ± 270	1050 ± 95	1433 ± 79	1182 ± 98
Fodder O4	798 ± 24.50	14890 ± 409	8500 ± 385	1200 ± 75	1400 ± 98	1232 ± 84
Fodder O5	900 ± 38.00	16766 ± 510	7000 ± 305	1200 ± 98	1599 ± 125	1389 ± 94
Overall Means	806 ± 55	16217 ± 932	7800 ± 570	1070 ± 130	1502 ± 107	1244 ± 85

O = Oxytocin
SD = Standard deviation
Means[1] of three replicates

supplied to oxytocin treated cows contained 1244mg/Kg chloride, 7800mg/Kg calcium, 1070mg/Kg phosphorus and 1502mg/Kg magnesium.

Means and ranges for micro minerals in fodder of control and oxytocin injected cows have been given in Tables 2a and 2b. The micro minerals in fodder fed to control cows were Zn 19.6mg/Kg (19-21mg/Kg), Cu 17.8mg/Kg (16-20mg/Kg), while in fodder fed to Oxytocin injected cows Cu and Zn were found 19.4mg/Kg (18-21mg/Kg) and 17.6mg/Kg (16-20mg/Kg) respectively for control group.

Table 2a. Micro minerals (mg/Kg) of fodder samples used for control experimental Sahiwal cows (Means[1] + SD)

Fodder	Zn	Cu
Fodder C1	19 ± 1.51	20 ± 1.85
Fodder C2	20 ± 1.18	16 ± 1.90
Fodder C3	21 ± 1.98	19 ± 0.58
Fodder C4	19 ± 1.10	16 ± 1.10
Fodder C5	19 ± 0.95	18 ± 1.70
Overall Means	19.6 ± 0.9	17.8 ± 1.8

C = Control
SD = Standard deviation
Means[1] of three replicates

Table 2b. Micro minerals (mg/Kg) of fodder samples used for experimental Sahiwal cows injected with oxytocin (Means[1] + SD)

Fodder	Zn	Cu
Fodder O1	19 ± 1.20	19 ± 0.52
Fodder O2	19 ± 1.00	16 ± 2.15
Fodder O3	20 ± 1.16	17 ± 1.99
Fodder O4	21 ± 0.98	20 ± 0.68
Fodder O5	18 ± 1.15	16 ± 1.00
Overall Means	19.4 ± 1.1	17.6 ± 1.8

O = Oxytocin
SD = Standard deviation
Means[1] of three replicates

The similar feed fed to control and oxytocin injected cows. Feed samples were also analyzed for macro and micro minerals (Table 3 and 4). The mean (range) of sodium, potassium, chloride, calcium, magnesium and phosphorus were recorded as 4595mg/Kg (4009-5565mg/Kg), 11154mg/Kg (10000-12600mg/Kg), 6896mg/Kg (6327-7589mg/Kg), 7200mg/Kg (7000-7500mg/Kg), 1607mg/Kg (1577-1645mg/Kg), 18460mg/Kg (17000-20000mg/Kg) for control feed fed to cows and oxytocin groups, respectively. The micro minerals such as zinc and copper were found in the range of 13.0mg/Kg (12.8-13.0mg/Kg) and 15.8mg/Kg (15-18mg/Kg), respectively.

Table 3. Macro minerals (mg/Kg) of feed samples used for experimental Sahiwal cows (Means[1] + SD)

Feed	Na	K	Ca	P	Mg	Cl
Feed 1	4366 ± 25.15	10000 ± 298	7000 ± 210	18200 ± 915	1622 ± 85	6738 ± 137
Feed2	5565 ± 40.00	11568 ± 357	7000 ± 180	19200 ± 870	1645 ± 110	7589 ± 145
Feed3	4478 ± 35.50	10658 ± 365	7500 ± 110	20000 ± 978	1599 ± 95	6912 ± 129
Feed4	4099 ± 39.00	12600 ± 295	7000 ± 165	17000 ± 965	1577 ± 126	6327 ± 148
Feed5	4466 ± 35.50	10943 ± 395	7500 ± 120	17900 ± 865	1590 ± 95	6912 ± 129
Overall Means	4595 ± 563.4	11154 ± 985	7200 ± 273	18460 ± 1165	1607 ± 27	6896 ± 455

SD = Standard deviation
Means[1] of three replicates

Table 4. Micro minerals (mg/Kg) of feed samples used for experimental Sahiwal cows (Means[1] + SD)

Fodder	Zn	Cu
Feed 1	13 ± 0.95	15 ± 1.15
Feed2	13 ± 1.20	18 ± 1.10
Feed3	13 ± 1.55	16 ± 0.95
Feed4	13 ± 1.50	15 ± 1.00
Feed 5	12.8 ± 1.50	15 ± 1.00
Overall Means	13.0 ± 0.1	15.8 ± 1.3

SD = Standard deviation
Means[1] of three replicates

Milk analysis revealed significant effect of oxytocin on ash% and lactose% of milk as shown in Table 5. Ash percentage was increased with mean values in control milk samples 0.69% and in oxytocin injected samples 0.77%. Lactose percent in milk was highly significant affected by administration of oxytocin and percent decrease was observed 0.39%.

Table 5. Means of ash and lactose percentage in oxytocin treated milk samples of Sahiwal cow with significance level

Parameter	Control	Oxytocin	T-Value
Lactose (%)	4.730±0.16	4.336±0.16	1.79**
Ash (%)	0.69±0.01	0.77±0.004	–5.22**

P ≤ 0.01=**

Sahiwal cow milk showed significant effect of oxytocin injections on minerals concentration except phosphorus, calcium and magnesium as shown in Tables 6 and 7 with T values. Control milk samples showed 1391 mg/L of potassium but decrease was observed as 1251 mg/L in contrast sodium and chloride content (535 mg/L and 887 mg/L respectively) increased in oxytocin treated samples. Phosphorus concentrations in Sahiwal cow milk showed non significant effect of oxytocin with slight change in values as 1038 mg/L in control milk samples and 1041 mg/L in oxytocin treated samples. Results further revealed that copper content in treated milk was found to be higher (250.6 μg/L) than control milk samples. Results in present study are supported by several researchers that immediately following a single dose of 100 milli units has no significant effect but effect on milk composition has been evident after 1 IU milk sodium and chloride were significantly increased (Gachev, 1963, 1965, 1971; Cowie, 1969; Linzell and Peaker, 1971; Peaker and Taylor, 1975). Twenty-four hr after 1 IU oxytocin, milk sodium and chloride was decreased while potassium was increased. The passage of sucrose, sodium and chloride from blood to milk also increased. While milk yield remains relatively high, the concentrations of lactose and potassium in milk decrease while those of sodium and chloride increased. Increased milk sodium and chloride and decreased lactose concentration also results from supraphysiological doses of oxytocin, apparently due to tight junctions between mammary epithelial cells becoming leaky.

Oxytocin increases the permeability of a paracellular pathway, may be through 'tight junctions', allowing substances to pass directly between blood and milk down their respective concentration gradients. Due to this, they proposed that milk is secreted in the alveoli with plasma concentrations of sodium and potassium, that, as it lies in the ducts between milkings, there is an exchange of sodium for potassium and lactose and that by milking very frequently one can obtain a solution approaching the primary secretion. These were seen only when the milk yields was very low and when a very large dose of oxytocin was administered. It is suggested that the increase in sodium and chloride might be partly due to contamination of the milk already in the teat by tissue fluid expressed from the tissues by compression during manual milking. Allen (1990) reported that doses of oxytocin of 1 IU or greater changed milk sodium and potassium concentration, most likely 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. Second, lactose concentration decreased slightly, and lactose yield declined to a greater extent after oxytocin in these experiments.

Table 6. Means of macro minerals concentration in oxytocin treated milk samples of Sahiwal cow with significance level

Minerals	Control	Oxytocin	T-Value
Potassium (K) (mg/L)	1391 ± 2.4	1251 ± 13	10.36**
Sodium (Na) (mg/L)	446.8 ± 14	535 ± 9.2	-5.19**
Chloride (Cl) (mg/L)	810.8 ± 5.5	887 ± 2.9	-12.26**
Calcium (Ca) (mg/L)	975.4 ± 21	956.2 ± 23	0.61 NS
Phosphorus (P) (mg/L)	1038.3 ± 17	1041.0 ± 11	-0.14NS
Magnesium (Mg) (mg/L)	90.96 ± 2.9	89.64 ± 2.6	0.34 NS

$P \leq 0.01$=**

Table 7. Means of micro minerals concentration in oxytocin treated milk samples of Sahiwal cow with significance level

Minerals	Control	Oxytocin	T-Value
Zinc (Zn) (mg/L)	3.517 ± 0.11	3.449 ± 0.10	0.45 NS
Copper (Cu) (µg/L)	101.5 ± 1 2	250.6 ± 22	6.03 **

$P \leq 0.01$=**

CONCLUSION

The requirement, benefits and safety of oxytocin at different concentrations during bovine lactation is not thoroughly understood. It is concluded from the study with the help of supportive data that oxytocin have significant effect on milk composition as sodium, chloride and copper were to increase while potassium and lactose decreased. Indiscriminate use of oxytocin for milk let down should be restricted and awareness should be generated on farmer level.

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