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Variation of Some Minerals Values in Subclinical Mastitic Milk of Buffaloduring Different Ages and Lactation Stages

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Abstract: Mastitis is one the most common inflammatory disease of mammary gland in ruminant which lead to physical, chemical and microbiological changes in milk of dairy animals. The aim of this study was to evaluate the change of some minerals concentration such as sodium (Na), potassium (K) and chloride (Cl) during different ages and lactation stagesin subclinical mastitic milk of buffaloes in Tabriz region. A total of 1151 milk sample was directly collected into sterilized tubes from each quarter of 300 buffaloes in Tabriz, Iran. All animals were clinically healthy and non-pregnant. Samples were divided according to the bacterial culture (Positive or Negative groups), age groups (< 3, 3-6 and > 6 years) and lactation stage (< 3, 3-6 and > 6 months). Milk samples were analyzed for Na, K and Cl concentrations and also submitted for bacterial culture. The concentrations of Na and K were determined by flamephotometric method and of the Clwas determined using the ISE method. Results of this study indicated that there was a significant difference between positive and negative bacterial culture concerning the Na ($p<0.001$), K ($p< 0.01$) and Cl ($p<0.001$) concentrations. In contrast to K, the concentrations of Na and Cl were increased in positive bacterial culture compared to the other group. But, there was no significant difference between culture - positive and - negative groups concerning all the studied parameters during different ages and lactation stages.It was concluded that, subclinical mastitis has an increasing effect on milk Na and Cl concentrations in contrast to the K in buffalo, but age and lactation stage had not effect on all the above mentioned parameters in the same condition.

Key words: Subclinical Mastitis · Chloride · Potassium · Sodium · Milk · Buffalo

INTRODUCTION

Milk contains all the food constituents required in human diet and its composition is affected by the breed, species, feeding regimes, stage of lactation and udder health and diseases [1, 2].

Mastitis is a serious disease of dairy animals which is found in clinical and subclinical forms and causing great economic losses in animal welfare [2-4]. Subclinical mastitis has 15-40 times more prevalence than clinical mastitis [5]. Economic losses are mainly related to reduction in milk yield and lowering its nutritive value [2, 6]. Subclinical mastitis induced no visible change in milk and udder, but milk production decreases and its composition is altered, therefore, the disease remains undetected [7, 8]. The compositional changes of milk due to subclinical mastitis reflect the degree of physical damage to the udder parenchyma [9]. Early diagnosis of udder health abnormalities is essential for dairy farmers and different diagnostic methods have been used for detection of clinical or subclinical mastitis in ruminants. As, the economic aspects interfere with the routine application of bacteriologic culture of milk samples for diagnosis of suffering dairy animals from mastitis, some alternative parameters such as determination of milk minerals are used to clear the development of udder health in dairy herds. These parameters should be indicative to inflammation. The milk Na, K and Cl are routinely measured in most studies on mastitis of dairy ruminants. When mastitis occurs in mammary gland, the concentrations of above mentioned minerals change during the disease [8-11]. The mineral contents of buffalo milk are impacted by the severity of mastitis [1]. Inger *et al.* [12] stated that the change of milk Na, K and Cl content can be used to monitor milk samples for cow mastitis. Minerals profile in milk samples can be used as an efficient method for monitoring abnormality of bovine mammary gland [13, 14]. Potassium is normally the predominant mineral in milk [15] and its value can be used as a sensitive indicator of clinical or subclinical mastitis in

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ruminant [16]. Many previous studies stated an increase in Na and Cl contents and decreases in K content in mastitic milk of most species of ruminant [1, 7-10, 17]. According to the previous reports, the Na content in milk of normal buffalo was 38.92±0.20 (mg/dl) [1] and in normal cow was 57.19±0.36 [8] and 64.11±2.6 (mg/dl) [18]. But, its value was reported 40.30±0.24 - 46.60±0.26 (mg/dl) in subclinical mastitic milk samples from buffalo [1] and 57.70±2.05 (mg/dl) in mastitic cow milk [18]. As well as, the content of K was reported 23.08±0.56 (mg/dl) [1] in milk of normal buffalo and 138.88±3.21 [8] in cow milk; and also of the Cl was reported as 69.9±3.3 (mg/dl) [10] in normal buffalo milk and 105.30±2.47 (mg/dl) in normal cow milk [8]. However, the values of K and Cl were 18.74±0.73 - 13.62±1.33 (mg/dl) [1] and 72.2±6.3- 99.1±11.4 (mg/dl) [10] in milk samples from mastitic buffalo, respectively. There is paucity of data regarding the effects of subclinical mastitis on the minerals contents of buffalo milk during different ages and lactation stage. Therefore, this study was planned to determine the variation of Na, Cl and K concentrations during different ages and lactation stages in subclinical mastitic milk of buffalo.

MATERIALS AND METHODS

Approximately 300 buffaloes were randomly selected from Tabriz district, East Azarbaijan, Iran. As, in contrast to the other ruminants, all the buffaloes were reared as a traditional method in this region; therefore, we had to select all the experiment animals from this condition. Clinical examination was performed on each buffalo before obtaining the samples. All buffaloes chosen for this study were clinically healthy and non-pregnant at the time of sampling.Before collecting the milk samples, quarters were thoroughly washed by tap water and first two streaks were discarded. A total of 1151 milk samples (20 ml) were directly collected into the sterilized tubes from individual quarter by hand stripping. The samples were immediately analyzed for bacterial culture and then frozen at -20°C until used for minerals. Samples were divided according to the results of bacterial culture (Positive or Negative groups), animals'age groups (< 3, 3-6 and > 6 years) and lactation stages including: I(< 3 months), II (3-6 months) and III (> 6 months).The submitted milk samples analyzed by routine bacteriologic examination using McConkey agar and blood agar media [19] at the Department of Microbiology, Faculty of Veterinary Medicine, Tabriz branch, Islamic Azad University, Tabriz Iran. The results of bacterial culture were reported as culture - positive or culture - negative for bacterial mastitis. Theconcentration of Na and K of milk samples were determined by flame photometer (P.F.P- Jenway, UK) as the method previously suggested by Ahmed *et al.* [1]. The concentration of Cl was determined by the Ion-Selective electrode (ISE) method using Elit ion analyzer (Nico 2000,UK). As the milk casein interfere with the Cl determination and decreases the method sensivity, milk samples mixed with diluted nitric acid (60 ml of concentrate nitric acid added to 1 liter deionized distilled water) in 1 to 2 ratio, shook for 1 min and the supernatant used for the experiment. The data was analyzed by the independent sample *t*- testfordeterminationof significant difference at the $p < 0.05$ level betweenbacterial cultures positive or negative groups concerning all the studied minerals using the SPSS v. 16 software. As well as, the mean±standard deviation (SD), median and Minimum - Maximum values of the Na, K and Cl are shown in bacterial culture positive and negative groups.

RESULTS

Descriptive statistics for the Na, K and Cl concentration in buffalo milk that were culture - positive and culture - negative for bacterial mastitis are shown in Table 1.

There were significant differences between culture - positive and culture - negative groups for bacterial mastitis concerning the Na ($p< 0.001$), K ($p< 0.01$) and Cl ($p< 0.001$)concentration and the values of Na and Cl were increased in bacterial culture - positive group, in contrast to the K value. Age and stage of lactation had not significant effect on all the studied minerals in mastitic milk of buffaloes.

DISCUSSION

Inflammatory diseases of mammary glands lead to the change of various milk compositions either because of local effects and entering serum component into the milk or the movement of some normal milk component from extra alveolar lumen into the perivascular space [20]. Results of present study indicated that there was a significant difference between culture - positive and culture - negative groups for bacterial mastitisconcerning the Na value ($p <0.001$) and its value was increased in the culture - positive group. Our result was consistent with the previous reports that regarded to the increasing effects of mastitis on milk Na values in ruminants [1, 7-9, 21, 22]. The probable reason is breakdown of the secretory cells in mammary gland during mastitis, causing

Table 1: The mean ± standard deviation (SD), median and Minimum - Maximum values of the Na, K and Cl in buffalo milk with culture - positive (n = 301) and culture - negative (n= 850) for bacterial mastitis

Analyte	Bacterial Culture	Mean ± SD	Median	Minimum - Maximum	*P*- Value
Na (mg/dl)	Negative	63.1±3.4[b]	54	51-74	< 0.001
	Positive	146.1±14.5[a]	144	125-182	
K (mg/dl)	Negative	28.1±3.5[a]	21	12-48	< 0.01
	Positive	19.1±1.1[b]	18	9-34	
Cl (mg/dl)	Negative	99.9±8.9[b]	98	67-127	< 0.001
	Positive	171.1±8.6[a]	167	144-201	

Table 2: The Na, K and Cl concentration in the milk samples with culture - positive and culture - negative for bacterial mastitis of buffalo during different ages

		Age (year)		
Analyte	Bacterial Culture	< 3	3-6	>6
Na (mg/dl)	Negative	65.1±4.40	62.1±2.40	63.9±1.40
	Positive	144.1±12.5	147.1±11.5	146.1±10.5
K (mg/dl)	Negative	24.1±5.50	27.1±6.50	26.4±4.50
	Positive	20.3±6.10	18.7±5.10	19.3±4.40
Cl (mg/dl)	Negative	100.6±7.60	97.7±8.40	100.6±6.70
	Positive	170.1±5.50	171.6±3.60	172.1±6.60

All values are shown as mean±standard deviation (SD)

Table 3: The Na, K and Cl concentration in the milk samples with culture - positive and culture - negative for bacterial mastitis of buffalo during different stages of lactation

		Lactation stage		
Analyte	Bacterial Culture	I	II	III
Na (mg/dl)	Negative	67.1±2.40	64.3±2.30	65.7±2.40
	Positive	146.1±11.3	147.9±13.5	145.1±13.5
K (mg/dl)	Negative	24.3±3.50	28.1±8.50	28.1±6.50
	Positive	22.5±6.10	19.5±2.20	19.5±2.40
Cl (mg/dl)	Negative	98.2±6.60	99.8±7.40	99.8±8.70
	Positive	169.3±6.50	172.6±6.60	172.6±9.60

All values are shown as mean ± standard deviation (SD). Stage (I): < 3 months; Stage (II): 4-6 months; Stage (III): > 6 months

blood constituents to appear into the milk [1, 3]. Similar to the Na, we found a significant difference between the culture - positive and - negative groups concerning the Cl contents of milk samples from mastitic buffalos ($p < 0.001$). Its value was higher in culture - positive group for bacterial mastitis compared to the other group. The higher value of Cl in mastitic milk was agreed with the previous reports in the same condition [7-10]. During mastitis, the increase of Cl content of milk is as the altered permeability and increased somatic pressure which can lead to entry of Cl from blood to the milk [9, 21, 22]. However, the results of this study revealed that there was a significant difference between the groups (culture - negative and - positive) concerning the K value ($p < 0.01$) and its value was significantly lower in culture - positive group compare to the other, which may be due to suppression of the normal activity of secretory cells in mammary gland [9]. Our result about the decreases of the K content in mastitic milk was justified by the other previous reports [1, 7-9]. Overall, there are some mechanisms through those, Na, K and Cl are exchanged between the secretory cells and milk, which result to markedly change in the ionic environment of mammary gland during the mastitis. The secretory cells of the mammary gland have the Na pumps which are located on the baso-lateral membrane of the cells and pumping Nainto the extracellular fluid and Kinto the cells, while Na and Kare passively transported across the apical membrane between the secretory cells and the milk. As well as, there is a paracellular pathway (tight junction) in the epithelium through that Na and Cl are moving in the milk but K is moving in to the extracellular fluid [3]. When the udder health is complicated by an intramammary infection or inflammation such as mastitis, it leads to an increase in the Na and Cl contents of milk in contrast to the K. These changes are caused by the destruction of the ion-pumping system and tight junctions. As, the mastitis damage to the secretory cells, Na and Cl leak into the lumen of the udder alveolus and K move out of the milk [1, 3]. We observed little difference with the other published reports [1, 7, 21, 22] concerning the values of all the studied minerals, that may be caused by factors which have complex and synchronous effect on above mentioned minerals values in mastitic milks and including: severity of mastitis, breed, species, feeding regime and lactation stage [1, 2]. The present study demonstrated that there was not the age- and lactation stage related variations of the milk minerals in mastitic buffalos. Although, we found no data regarding to the change of milk Na, K and Cl contents in mastitic milk samples from buffalo during different ages and lactation stage.In this study, the exact explanation of these finding is not currently possible, but it seems that severity of mastitis, nutrition status, breed and species may have the highest effect on our results.

CONCLUSION

Subclinical mastitis had a significant effect on the milk Na, K and Cl contents of buffalo and caused to increase the Na and Cl values and decrease the K value. But, age and stage of lactation had not significant effect on the above mentioned parametersin milk samples from subclinical mastitic buffalo in Tabriz region.

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