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Effect of mastitis on macro-minerals of bovine milk and blood serum in Sudan

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ABSTRACT

Milk and blood serum from clinically mastitis infected, subclinically mastitis infected and healthy Friesian cows (15 samples from each of 3 groups) were evaluated for macro-minerals (sodium, potassium, calcium, magnesium and phosphorus). The milk from cows infected with subclinical mastitis revealed a significant decrease in potassium ($P < 0.001$) and a significant increase in sodium and phosphorus content ($P < 0.01$). Similarly, the milk from cows with the clinical form of the disease showed a significant increase in sodium ($P < 0.001$) and a significant decrease in potassium, magnesium ($P < 0.001$) and calcium ($P < 0.01$). Comparison of healthy cow's milk with that from cows with subclinical mastitis revealed a highly significant increase in sodium ($P < 0.001$). Comparison of healthy cow's milk with that of clinically mastitic milk showed a highly significant decrease in levels of calcium, magnesium ($P < 0.001$) and potassium ($P < 0.01$). However, sodium increased highly significantly ($P < 0.001$). Comparison of macro-minerals in milk from cows with subclinical and clinical mastitis revealed a significant decrease in potassium contents ($P < 0.05$) compared with that of healthy cows. Potassium levels were found to decrease significantly ($P < 0.05$) in subclinically infected cow's blood serum. However, calcium and phosphorus showed a significant decrease ($P < 0.01$) in blood serum samples from the clinically infected cows.

Key words: blood serum, dairy cows, Friesian, macro-minerals, mastitis, milk, Sudan.

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INTRODUCTION

Macro-minerals in milk provide the primary mineral requirements of the neonate calf during the critical period after birth when it cannot forage for itself[1]. Buruiana *et al.*[4] reported that milk whey from healthy cows contained more potassium and calcium, and less sodium, than that from cows with evidence of clinical mastitis. El Deeb and Hassan[7] reported significantly higher levels of ash, sodium and chloride in mastitic milk compared with healthy milk, while significantly lower levels of calcium, magnesium and inorganic phosphorus in secretions obtained from *E. coli*-infused glands were reported[3]. However, clinically and subclinically mastitic milk samples had higher sodium, magnesium and calcium, and lower potassium levels than normal milk[9].

Wagner and Stull[17] found differences in mean potassium and calcium contents in blood serum between mastitic and healthy cows.

The present study was conducted to compare the levels of the important macro-minerals (sodium, potassium, calcium, magnesium and phosphorus) in the milk and blood serum of healthy cows and cows with subclinical and clinical mastitis.

MATERIALS AND METHODS

This study was carried out on lactating Friesian cows of a herd raised under an intensive management system, on a farm belonging to the Arab Company for Agricultural Production and Processing, Dairy Unit (Dairy Land Farm).

The farm is located 30 km south of Khartoum. It was established in 1984 with a stock of Holstein-Friesian cattle originally imported from the Netherlands. The animals on the farm were housed separately as young calves, heifers, dry herds, milking herds, pregnant cows, and stud bulls. The most common breeding practice is artificial insemination. The cows are machine-milked twice a day. Screening for mastitis is carried out twice monthly. Vaccination of animals against diseases is also carried out. The farm records contain information about each individual animal, regarding production, reproduction and health.

Experimental design and procedures

According to clinical signs and California Mastitis Test (CMT) scores, the cows were grouped as clinically infected, subclinically infected and healthy cows (45 cows altogether, 15 cows in each group). In addition, the CMT result was confirmed using standard plate count agar for total bacteria[10]. Quarter milk samples were collected in sterile bottles and blood samples were collected in sterile vacutainers from the udder vein. Three weeks after the 1st collection, milk and blood were collected and analysed for all the cows in the study.

Laboratory procedures

Atomic absorption spectrophotometry. The concentration of minerals in milk and blood serum was determined with an Atomic Absorption Spectrophotometer 2380 (AAS) (Perkin Elmer, Germany).

Milk and blood serum samples were prepared according to the procedures described in the technical manual of the AAS. The procedure is based on ignition of pre-prepared ash, with concentrated nitric acid and then dilution with distilled water to 100 mℓ. Then a series of standard metal solutions (sodium, potassium, calcium and magnesium) in parts per million (ppm) were prepared by dilution according to the procedures described in the manuals.

The AAS was calibrated and the absorption of the different concentrations of the metal standards were measured at specific wavelengths (i.e. calcium 442.7, magnesium 285.2, sodium 589.0 and potassium 766.5 nm). The standards and sample solutions were run through the AAS, their values in ppm were recorded directly and calculated according to the procedures described in the technical manuals. The data obtained were then expressed as mg/100 mℓ.

Determination of phosphorus. Concentrations of blood serum phosphorus were

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Table 1: **Mean values of macro-minerals in healthy and subclinically and clinically mastitic cow's milk, initially and after 3 weeks.**

Macro-minerals	Healthy (mg/100 mℓ)		Subclinically mastitic (mg/100 mℓ)		Clinically mastitic (mg/100 mℓ)	
	R1*	R2	R1	R2	R1	R2
Sodium	038.94 ± 04.60	040.33 ± 09.9	062.97 ± 18.52	045.01 ± 08.63	090.88 ± 12.24	036.78 ± 06.190
Potassium	160.08 ± 14.35	142.91 ± 12.5	134.78 ± 12.16	159.32 ± 12.16	126.16 ± 08.38	154.81 ± 12.470
Calcium	105.49 ± 14.25	109.38 ± 12.44	075.26 ± 12.84	097.56 ± 16.48	055.61 ± 15.68	105.19 ± 16.920
Magnesium	015.34 ± 02.28	014.17 ± 01.68	009.97 ± 01.62	018.79 ± 27.32	007.81 ± 01.39	010.32 ± 03.130
Phosphorus	005.48 ± 01.19	005.89 ± 01.25	006.02 ± 00.94	004.97 ± 01.09	005.07 ± 01.04	005.28 ± 01.001

*R1: initial mean values; R2: mean values 3 weeks later.

determined according to the method of Varley[16]. This method is based on the removal of protein by trichloroacetic acid with subsequent treatment of the filtrate with molybdate and reduction by methanol, giving a coloured, measurable complex. The concentrations of inorganic phosphate were then measured using a Spectrophotometer (UV-120-02; Shimadzu, Japan) at 680 nm according to the following formula:

$$\text{Concentration of phosphorus (mg/100m}\ell\text{)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}}$$

The phosphorus content of milk samples was measured using the molybdovandate yellow colormetric method of Chapman and Pratt[6].

Statistical analysis

The data were analysed by a randomized complete block design, using co-variance analysis and *t*-tests[8].

RESULTS

Macro-mineral composition and content in milk

Table 1 shows that the level of sodium in the milk increased with mastitis (38.939 ± 4.599, 62.967 ± 18.516 and 90.878 ± 12.24 mg/100 mℓ) for healthy, subclinically and clinically mastitis-infected cows, respectively. Three weeks after the 1st milk samples were tested, sodium showed increased levels in milk from healthy cows (40.326 ± 0.99 mg/100 mℓ), while it decreased in subclinically and clinically mastitic milk to about 45.006 ± 8.63 and 36.783 ± 6.194 mg/100 mℓ, respectively.

The reduction in potassium was more obvious in the clinically mastitic milk samples followed by subclinical samples compared with those of healthy milk (126.161 ± 8.38, 134.78 ± 8.87 and 160.08 ± 14.35, respectively), both during the 1st, and 3 weeks after the milk samples were tested. Potassium levels were 154.81 ± 12.47, 159.32 ± 12.16 and 142.91 ± 12.5 mg/100 mℓ for clinically, subclinically mastitic and healthy milk, respectively.

Calcium levels in milk decreased as a result of mastitis for clinical and subclinical mastitis infection (55.61 ± 15.68 and 75.26 ± 21.84, respectively) compared with healthy milk (105.49 ± 14.25 mg/100 mℓ). Magnesium decreased in clinically and subclinically mastitic milk samples compared with those of healthy milk (7.81 ± 1.4, 9.97 ± 1.62 and 14.17 ± 1.68 mg/100 mℓ, respectively). Phosphorus showed a decrease in the clinically mastitic milk samples, while it increased in subclinically mastitic milk samples compared with that of healthy milk samples (5.07 ± 1.04, 6.02 ± 0.94 and 5.47 ± 1.19 mg/100 mℓ, respectively). Three weeks after the 1st collection of milk samples, the levels of phosphorus were 5.28 ± 1.001 and 4.97 ± 1.09 for clinically and subclinically mastitic milk samples, respectively, compared with healthy milk (5.89 ± 1.25 mg/100 mℓ).

Table 2 shows the differences in means, standard deviations and standard error of the means for healthy cow's milk and that from subclinically and clinically mastitic cows, initially and after 3 weeks. The subclinical cases showed a significant decrease in potassium ($P < 0.001$) and a significant increase in sodium content ($P < 0.01$). The clinical cases showed a significant increase in sodium ($P < 0.001$) and a significant decrease in potassium, magnesium ($P < 0.001$) and calcium ($P < 0.01$).

When comparing healthy cow's milk with subclinically mastitic milk, the level of sodium revealed a highly significant increase ($P < 0.001$) as shown in Table 3. Similarly, comparison of healthy milk with clinically mastitic milk samples revealed a significant increase in sodium ($P < 0.001$) and a significant decrease in calcium, magnesium ($P < 0.001$) and potassium ($P < 0.01$). Comparison between subclinically and clinically mastitic milk samples also showed a highly significant increase in calcium ($P < 0.001$) and sodium ($P < 0.01$), and a significant decrease in potassium ($P < 0.05$).

Blood serum macro-minerals

Blood serum mineral level (Table 4) did not change noticeably with mastitis infection, except for magnesium, which appeared higher in the subclinically mastitic cases when examined 3 weeks after the 1st collection of samples (6.14 ± 15.44 *vs* 2.26 ± 0.46 mg/100 mℓ).

Table 5 shows that, in the clinically infected blood serum samples, the levels of phosphorus and calcium decreased

Table 2: **Comparison of mean differences of macro-minerals in healthy and subclinically and clinically mastitic cow's milk, initially and after 3 weeks.**

Macro-minerals	Healthy (mg/100 mℓ)			Subclinically mastitic (mg/100 mℓ)			Clinically mastitic (mg/100 mℓ)		
	Difference mean	Standard deviation	Standard error of mean	Difference mean	Standard deviation	Standard of mean	Difference mean	Standard deviation	Standard error of mean
Sodium	-1.387	11.444	2.955	17.961	20.946	5.400**	54.095	12.699	3.279***
Potassium	17.171	18.928	4.887**	-24.545	10.740	2.773***	-22.303	14.388	3.715***
Calcium	-3.885	20.852	5.384	-0.579	18.757	4.843	-2.552	28.132	7.264***
Magnesium	1.162	3.277	0.846	18.828	27.590	7.124	-2.552	2.552	0.692***
Phosphorus	-0.415	1.509	0.390	1.056	1.343	0.347**	-0.212	1.397	0.361

**, $P < 0.01$; ***, $P < 0.001$.

Table 3: **Comparison of mean differences of macro-minerals in healthy and subclinically and clinically mastitic cow's milk.**

Macro-minerals	Healthy and subclinically mastitic (mg/100 mℓ)			Healthy and clinically mastitic (mg/100 mℓ)			Subclinically and clinically mastitic (mg/100 mℓ)		
	Mean difference	Standard deviation	Standard error of mean	Mean difference	Standard deviation	Standard error of mean	Mean difference	Standard deviation	Standard error of mean
Sodium	14.355	11.449	2.956***	24.198	10.007	2.584***	9.843	12.117	3.129**
Potassium	-4.440	14.923	3.853	-11.007	12.785	3.01**	-0.567	12.299	3.176*
Calcium	-47.043	12.686	3.276	-21.030	14.225	3.673***	26.012	20.493	5.291***
Magnesium	-0.370	13.229	3.416	-5.665	2.267	0.585***	-5.295	13.596	3.510
Phosphorus	-0.191	0.932	0.241	-0.509	1.014	0.262	–0.319	1.016	0.262

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4: **Mean values of blood serum macro-minerals in healthy and subclinically and clinically mastitic cows, initially and after 3 weeks.**

Macro-minerals	Healthy (mg/100 mℓ)		Subclinically mastitic (mg/100 mℓ)		Clinically mastitic (mg/100 mℓ)	
	R1*	R2	R1	R2	R1	R2
Sodium	2.158 ± 0.88	2.332 ± 0.94	2.743 ± 0.17	2.511 ± 0.53	2.517 ± 0.65	2.936 ± 0.39
Potassium	2.581 ± 0.56	2.528 ± 0.57	2.665 ± 0.69	3.148 ± 0.56	2.668 ± 1.23	2.668 ± 0.82
Calcium	8.697 ± 0.66	10.035 ± 0.92	8.093 ± 1.45	7.687 ± 1.23	7.649 ± 1.45	8.885 ± 1.50
Magnesium	2.476 ± 0.66	2.119 ± 0.37	2.262 ± 0.46	6.136 ± 15.44	2.188 ± 0.59	2.367 ± 0.67
Phosphorus	14.647 ± 3.72	14.909 ± 3.69	12.741 ± 4.77	11.447 ± 4.83	10.673 ± 3.71	13.80 ± 3.42

*R1: Initial mean values; R2: Mean values 3 weeks later.

Table 5: **Mean differences in blood serum macro-mineral contents in healthy and subclinically and clinically mastitic cows.**

Macro-minerals	Healthy (mg/100 mℓ)			Subclinically mastitic (mg/100 mℓ)			Clinically mastitic (mg/100 mℓ)		
	Difference mean	Standard deviation	Standard error of mean	Difference mean	Standard deviation	Standard error of mean	Difference mean	Standard deviation	Standard error of mean
Sodium	-0.0175	0.897	0.231	0.2315	0.575	0.148	-0.4183	0.848	0.219
Potassium	0.0533	0.880	0.227	-0.4837	0.828	0.214*	0.0203	1.620	0.418
Calcium	-1.3387	2.859	0.738	0.4062	2.166	0.559	-1.2283	1.548	0.400**
Magnesium	0.3565	0.736	0.190	-3.8742	15.422	3.982	-0.2480	0.779	0.201
Phosphorus	-0.2620	4.476	1.156	1.2933	4.708	4.216	-3.1600	4.702	1.214**

*, $P < 0.05$; **, $P < 0.01$.

significantly ($P < 0.01$), while in cows with subclinical mastitis, blood serum potassium levels showed a significant decrease ($P < 0.05$).

DISCUSSION

Comparison of means (Table 1) revealed increased levels of sodium in milk from cows with mastitis, and reduction of potassium with increased levels or scores of the disease. This finding is in agreement with those of Buriana *et al.*[4], Chaiyabutr *et al.*[5], Smith *et al.*[15] and Mahran *et al.*[9], and may be due to bacterial infection of the udder, resulting in damage to the ductal secretory cells and increased permeability of the blood capillaries[12]. Thus, sodium and chloride (which are high in extra-cellular fluid) flow into the lumen of the alveolus, and to maintain osmolarity and potassium levels, decrease proportionately.

The decrease in calcium content in milk samples (Table 1) was similar to that reported by Sbodio *et al.*[13] and Munro *et al.*[11], who found that mastitic milk contained less calcium than healthy cow's milk. This could be due to damage to the mammary gland by pathogens, often disrupting the junctional complex of the secretory epithelium which is essentially impermeable to calcium transport from milk to blood.[2] However, the present results are in contrast to those of Mahran *et al.*[9], who found higher concentrations of calcium in clinically and subclinically mastitic milk than in healthy cow's milk.

The reduction of magnesium levels that occurred in subclinically and clinically mastitic milk were transient, since after 3 weeks of the 1st collection, there was an increase in magnesium levels. This is similar to the findings of El Deeb and Hassan,[7] and in contrast to those of Mahran *et al.*[9]. The non-significant difference shown in the present data (Table 5) for magnesium levels in subclinically mastitic milk was, however, similar to the results of Singh and Ganguli[14] and Wagner and Stull[17]. Moreover, these authors concluded that the magnesium content of mastitic milk remained relatively constant with increased somatic cell counts.

The reduction in mean values for phosphorus in the clinically mastitic milk samples (Table 1) is in accordance with the results of Bogin and Ziv[3] and El Deeb and Hassan[7].

Blood serum macro-mineral levels in milk from mastitic cows did not show noticeable changes in the present study, with the exception of magnesium (Table 4). This might be, as posited by Wagner and Stull[17], due to homeostatic mechanisms that are sufficiently dynamic to cope with mineral losses from blood into the mastitic gland. They also considered that the magnitude of minerals passing into mastitis-diseased udder sections is so small as to be negligible compared to the total electrolyte pool in the blood, since milk yield is reduced greatly in a mastitic gland.

We conclude that the changes in macromineral levels in individual milk samples (especially increased sodium and decreased potassium) could be a good indicator of udder or quarter infection.

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