Bull Vet Inst Pulawy 49, 209-213, 2005

INVESTIGATION OF Ca, Zn, Mg, Fe AND Cu CONCENTRATIONS IN BLOOD AND MILK OF COWS WITH NEGATIVE AND POSITIVE CMT RESULTS

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Received for publication January 14, 2005.

Abstract

The study was performed on 15 healthy and 21 with subclinical mastitis cows, aged 3-5 years. Forty-one udder quarters which gave negative reaction in California Mastitis Test (CMT) were considered to be healthy, and those (n=56) with +1 and +2 reactions in CMT were diagnosed to be affected by subclinical mastitis. Contents Ca, Zn, Mg, Fe, and Cu in blood serum and milk samples were determined by atomic absorption spectrophotometry. Ca levels in the milk serum of the CMT positive cows were significantly lower ($P<0.001$) than those found in healthy milk samples. It was also determined that levels of Zn and Fe ($P<0.05$ and $P<0.01$, respectively) in milk were elevated in the CMT positive cows compared to the controls. Milk serum Mg values did not significantly change. Ca levels of blood serum obtained from healthy cows were higher ($P<0.01$) and Cu values lower ($P<0.001$) than those found in the CMT positive cows. Serum levels of Mg, Zn and Fe were not significantly altered. Ca levels in milk samples with a CMT result of +1 were significantly higher than those with +2 ($P<0.05$). However, it was observed that serum values of Mg, Zn and Fe did not well correlate with CMT scores. In conclusion, the presented results have shown that Ca levels in milk from quarters with subclinical mastitis were lower and Zn and Fe levels higher than those in the controls.

Key words: cows, subclinical mastitis, Ca, Zn, Mg, Fe, Cu.

A dominant role in the aetiology of mastitis plays pathogenic bacteria, but also fungi, yeasts, and viruses are causative factors. In addition, thermic and chemical factors can cause inflammation in the udder as well (23). In subclinical mastitis, no noticeable disturbances are observed in the mammary glands and in milk samples. However, there is an increasing reduction of milk yield (23). Subclinical mastitis are of importance since their incidence is about 40-50% more than that of clinical mastitis and cause 3-26% losses in milk yield (20). Increased number of somatic cells resulting from chemotaxis of leukocytes to the inflamed tissue, passage of plasma proteins due to increased vascular permeability, raised Na, Cl, pH and electrical conductivity, decreased K and reduced synthesis of fat, casein, and lactose because of cell degeneration in the mammary tissue are seen in the milk (22). Subclinical mastitis can be detected by monitoring some biochemical parameters such as Na, Cl, K, Ca, Mg, albumin and lactose in milk and identification of pathogenic factors and somatic cell count (12, 22, 23).

Determination of Ca and Mg in mastitic milk may give important clues about the status of mastitis. Ca and Mg contents of milk originate from the blood. These elements are found in insoluble form (75%) or colloidal Ca phosphates and as colloidal complexes with casein in milk (23). It has been suggested that levels of Ca and Mg are reduced in subclinical mastitis milk (1). On the contrary, other researchers (7, 10) reported an increase in the content of these elements. There is the report that administration of Zn and Cu during mastitis treatment might be helpful for controlling and preventing mastitis by increasing the phagocytic activity of polymorphonuclear and mononuclear cells in the udder (25). It was also suggested that Zn given in feed contributes to the formation of creatinin which prevents the entry of bacteria via the teat canal (26).

Grigorian *et al*. (5) showed that Cu, Zn, and Fe levels in the milk obtained from cows wth subclinical mastitis were slightly decreased compared to those of normal milk. In another study, Zn levels of normal milk were shown to be higher than those of mastitic milk samples (9). Zn, Cu, and Ca levels determined in the hair of mastitic cows were significantly lower than the control group values, but Fe concentrations remained unchanged (24). The aim of this study was to investigate alterations in Ca, Cu, Mg, Zn, and Fe concentrations in the blood and milk (exclude Cu in milk) of the California Mastitis Test (CMT) negative and positive cows.

Material and Methods

The study was performed on 15 healthy and 21 CMT positive cows at the farm of the Firat University. Age of the animals varied between 3 and 5 years and mean body weight was 350-400 kg.

All the animals were under the same care and feeding conditions. The chemical composition of the concentrate ration given to the animals was as follows: dry matter - 93.75%; ash - 5.09%; crude fiber - 9.75%; crude protein - 15.18%; ether exract - 5.80%; organic matter - 88.66%; barley - 70.50%; sunflowerseed meal - 17.50%; soybean - 7.50%; limestone - 3.00%; DCP (dicalcium pohosphate) - 0.50%; salt - 0.50%; vitamins - 0.25% and trace elements - 0.25%. Composition of forage feed was as follows: dry matter - 95.20%; ash - 9.47%; crude fiber - 35.00%; crude protein - 3.30%; ether extract - 3.20% and organic matter - 85.73%. The milk samples were collected once, between the 3rd and 4th month after delivery.

Before collection of blood and milk samples, the CMT was performed. CMT results were evaluated as described by Schalm *et al*. (23). Forty-one healthy udder quarters belonging to 15 cows which were CMT negative were considered healthy. The remaining 19 quarters of 15 cows were healthy. Fifty-six udder quarters belonging to 21 cows which gave positive reaction as CMT +1 and +2 were considered affected by subclinical mastitis. The remaining 28 quarters of 21 cows were negative according to the CMT results. Blood samples (10 ml) from all the animals were collected from the jugular vein under aseptic conditions. They were kept for separation of serum at room temperature for two hours. Then they were centrifuged at 3000 rpm for 15 min. Milk samples (10 ml) were collected into tubes after the udder was thoroughly washed with clean warm water and dried with clean paper towel. The samples were centrifuged at 3000 rpm for 20 min and then transferred into fresh tubes. Both blood and milk samples were stored at -20ºC until analysis. Ca, Zn, Fe, Cu, and Mg concentrations in blood serum and milk (except Cu in milk) were determined by atomic absorption spectrophotometry according to the method described previously (11). The statistical analysis of the data was carried out by using Mann Whitney U test (6).

Results

Ca levels in blood serum of healthy cows were higher ($P<0.01$) than in CMT positive cows, but Cu values were lower ($P<0.001$). With regard to the concentrations of Zn, Mg and Fe in blood serum, no significant differences were observed between groups (Table 1). Levels of Ca in milk from the quarters with subclinical mastitis were significantly lower ($P<0.001$) whereas Zn and Fe values were found to be higher ($P<0.05$ and $P<0.01$, respectively) than those from the healthy animals. With regard to the values of Mg in milk, no significant change was observed between groups (Table 2).

When the findings obtained from CMT positive quarters were evaluated in view of the CMT results, milk Ca concentrations were significantly higher in CMT +1 positive samples than those with +2 reaction ($P<0.05$). Levels of Zn, Mg and Fe were not significantly different between the CMT results (Table 3).

Disccusion

In the present study, mineral concentrations determined in the blood and milk of healthy and CMT positive cows appear to be similar to those reported by others previously (3, 21, 29). Plasma Ca levels were shown to be decreased in the acute coliform mastitis while Mg concentrations remained unaltered (13). Ca levels of blood serum in the mastitic cows were also demonstrated to be lower than in healthy ones (2). The presented findings also indicated that Ca levels in cows with subclinical mastitis were lower compared to healthy cows. No significant changes in Mg levels were found.

Table 1

Mean levels of the elements in blood serum of cows with negative (N) or positive (P) CMT results

Element	N (n=15)	P (n=21)	
Ca (mg/dl)	12.53±0.42	11.31±0.42	*
Cu (mg/l)	0.44±0.02	0.97±0.04	**
Zn (mg/l)	1.05±0.04	0.96±0.04	NS
Mg (mg/dl)	2.76±0.09	2.78±0.12	NS
Fe (mg/l)	3.12±0.20	3.06±0.21	NS

NS not significant; * $P<0.01$; ** $P<0.001$; ± SE

Table 2
Mean levels of the elements in milk from quarters with negative (N) and positive (P) CMT results

Element	N (n=41)	P (n=56)	
Ca (mg/dl)	102.34±3.00	71.89±2.70	***
Zn (mg/l)	3.47±0.20	4.10±0.21	*
Mg (mg/dl)	13.71±0.57	12.47±0.54	NS
Fe (mg/l)	0.41±0.01	0.47±0.01	**

NS not significant; * P<0.05; ** P<0.01; *** P<0.001; ± SE

Table 3
Mean levels of the elements in milk serum according to CMT result

Element	CMT +1 (n=31)	CMT +2 (n=25)	
Ca (mg/dl)	77.09±3.59	63.19±4.23	*
Zn (mg/l)	4.44±0.28	3.73±0.30	NS
Mg (mg/dl)	12.54±0.72	12.28±0.82	NS
Fe (mg/l)	0.45±0.02	0.49±0.02	NS

NS not significant; * P<0.05; ± SE

It was reported that plasma Zn and Fe concentrations (but not that of Cu) were decreased in *Escherichia coli*-induced mastitis in experimental animals (3, 17). However, Lamand and Levieux (14) had shown that plasma Cu and ceruloplasmin ratios in chronic mastitis were increased while Zn levels were decreased. In another study, elevated ceruloplasmin levels in blood serum of mastitic cows were reported (2). In this study, no significant change in Zn and Fe levels in blood serum was seen whereas Cu levels in cows with subclinical mastitis were found to be higher than in healthy cows. The discrepancies between the presented Zn and Fe contents and those reported in the literature may be attributed to the use of clinically different mastitis cases in separate studies. Indeed, increased passage of some elements to milk that was reported during acute mastitis cases resulted from inflammation-induced increase in vascular permeability (3, 17). Our data with regard to the Cu levels in blood serum appear to be consistent with the findings of others (2, 14).

There is controversy on Ca levels determined in milk. Some researchers (1, 16, 19) found decreased and others (7, 10) increased Ca concentration in milk samples from animals with subclinical mastitis. Wegner and Stull (28) demonstrated Ca and Mg levels as 94±20 and 11.5 ±1.6 mg/dl in mastitic and 108±13 and 11±1.8 mg/dl in normal milk, respectively. They indicated that Ca values (but not Mg) were reduced in the mastitic cows compared to the healthy controls. Janota and Bassalik (8) reported that Ca and Mg levels in milk in experimentally induced acute clinical mastitis initially decrased and then increased during the course of the disease. In contrast to these findings, Ca and Mg levels in milk of the cows with clinical and subclinical mastitis were found to be higher than in normal milk (7, 10). We demonstrated that Ca levels were reduced in CMT positive milk and such fluctuations were not observed in terms of Mg values. Thus, our findings appear to be in parallel with the results reported by other researchers (1, 16, 28).

Varying observations were reported regarding Zn levels in the milk of clinical and subclinical mastitis cows (5, 10, 18). Some found a slight decrease (5, 10), while Banga *et al.* (1) showed that Zn values did not significantly change in the milk but increased in the inflammatory tissue. In experimentally induced mastitis, Zn levels in milk were found to be increased (18). However, reduced Zn levels obtained in milk samples of mastitic cows seem to be contradictory (9). In this study, Zn levels in CMT positive milk were higher than that in milk from healthy cows. Thus, the results are also in consistent with the reports in the literature (1, 18).

Studies performed on milk from subclinical mastitis affected quarters showed contradictory results. Milk Fe levels were slightly decreased compared to the healthy control values (5), whereas Banga *et al.* (1) reported increased Fe values. Milk concentrations of this element was also increased in experimentally induced mastitis probably owing to an inflammatory-related increase in the vascular passage of Fe to milk (15). In the present study, Fe levels in the milk with positive CMT were higher than those measured in milk from healthy quarters. Our findings appear to confirm the results reported by Banga *et al.* (1) and Lappalainen *et al*. (15).

Trejo-Guzman and Guzman (27) suggested no correlation of milk Ca and Mg levels with CMT results, however, Lee *et al.* (16) reported that Ca levels decrease in subclinical mastitis. Wegner and Stull (28) shown that Ca and Mg levels in low CMT score milk samples were 85 mg/dl and 11.2 mg/dl, respectively, and 94 mg/dl and 11 mg/dl in high CMT score milk samples indicating a difference in terms of Ca concentrations. However, Kalorey *et al.* (12) reported that Zn, Mg and Fe levels in milk did not well correlate with CMT scores in subclinical mastitis cows. In this study, Ca values in milk with CMT +1 result were higher than those with CMT +2, but the other parameters determined (Zn, Mg and Fe) showed no differences between the two CMT results. It is known that increased somatic cell count in milk correlates with an increase in CMT scores, and there is a negative correlation with Ca levels (4). Therefore, Ca findings in our study are consistent with those reported by Lee *et al.* (16), and the other parameters with the results of Kalorey *et al*. (12).

In conclusion, our results indicated that Ca concentrations in blood and milk of the cows with CMT positive are lower compared to the healthy control values. Zn and Fe levels in CMT positive milk samples were recorded to be high and with no change in the concentrations of Mg levels. We suggest that determination of Ca, Zn, and Fe levels in the blood and milk may be useful in the diagnosis of subclinical mastitis in addition to such methods as somatic cell counts, electrical conductometry and CMT.

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