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The Effect on Serum Mineral Levels of Acute Septic Mastitis and Clinical Mastitis in Cows

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Summary: The purpose of this study is to identify serum Na, K, Ca, Mg, Zn, Cu and Fe levels in cows with acute septic mastitis (ASM) and clinical mastitis (CM). The study consisted 80 cows between the ages of three and five in four groups (ASM, n=20; CM, n=20; Control-1 C1=20, healthy cows, together with ASM or CM cows; Control-2, C2=20, healthy cows, without ASM or CM cows). Serum Na, K, Ca, Mg, Zn, Cu and Fe levels were determined by using atomic absorption spectrophotometry. The Mg levels were lower in the ASM group compared to C1 and C2 groups ($P<0.05$, and $P<0.001$ respectively). Zn levels in the ASM and CM animals were lower than those in control groups ($P<0.01$). Fe concentrations that of the ASM group were found to be statistically lower than in the other groups ($P<0.001$). The glutaraldehyde (GLA) test was positive in the ASM and CM groups but negative in the control groups. In conclusion, lower levels of Mg, Zn and Fe were found with acute septic mastitis. In clinical mastitis, on the other hand, only the Mg and Zn levels were low. Na, K, Ca and Cu values were not affected. It may also be beneficial to apply minerals such as Mg, Zn and Fe in mastitis treatment.

Key words: Acute septic mastitis, clinical mastitis, cow, minerals

İneklerde akut septik mastitis ve klinik mastitisin kan serumu mineral düzeylerine etkisi

Özet: Bu çalışmada, akut septik mastitisli (ASM) ve klinik mastitisli (KM) ineklerde serum Na, K, Ca, Mg, Zn, Cu ve Fe düzeylerinin belirlenmesi amaçlandı. Çalışmada 3-5 yaş arası 80 adet inek kullanıldı ve 4 grup (ASM, n=20; KM, n=20; Kontrol1, K1=20, ASM ve KM'li ahırların sağlıklı hayvanları; Kontrol2, K2=20, ASM ve KM şikayeti olmayan ahırların sağlıklı hayvanları) oluşturuldu. Gruplardan alınan kan serumu örneklerinde atomik absorbsiyon spektrofotometre ile Na, K, Ca, Mg, Zn, Cu ve Fe düzeyleri belirlendi. Ayrıca tüm gruplara glutaraldehyde (GLA) testi yapıldı. Ölçüm sonuçlarına göre Na, K, Ca ve Cu düzeylerinin tüm gruplarda benzer olduğu saptandı ($P>0.05$). Mg düzeyinin ASM grubunda K1 ve K2'ye göre daha düşük olduğu (sırasıyla $P<0.05$, $P<0.001$) belirlendi. Zn düzeyinin ise ASM ve KM'li hayvanlarda kontrol gruplarına göre daha düşük düzeyde olduğu saptandı ($P<0.01$). Fe konsantrasyonunun ise ASM grubunda diğer gruplara göre istatistiksel olarak daha düşük düzeyde tespit edildi ($P<0.001$). GLA testi ASM ve KM gruplarında pozitif, kontrol gruplarında ise negatif olarak bulundu. Sonuç olarak, akut septik mastitiste Mg, Zn ve Fe düzeylerinde düşüş saptandı. Klinik mastitiste ise yalnızca Mg ve Zn düzeylerinin düştüğü belirlendi. Na, K, Ca ve Cu değerlerinin ise etkilenmediği görüldü. Ayrıca mastitis tedavisinde Mg, Zn ve Fe gibi minerallerin uygulaması faydalı olabilir.

Anahtar kelimeler: Akute septik mastitis, inek, klinik mastitis, mineraller

Introduction

Mastitis results in significant economic losses to dairy farms because it affects the quality and quantity of milk (1,26,30). Acute septic mastitis (ASM), which is characterized by various symptoms of general disease, can form in cows as bacteria producing endotoxin multiply in one or more quarters of the udder (22). Several risk factors create a predisposition for the disease, including lesions on the udder, incontinentia lactis, the shape of the udder, sagging udder, asymmetrical udder, periparturient oedema of the udder, the breed and age of the cow as well as number of lactations and vitamin and mineral deficiencies (23). If ASM does not occur early, it is not a difficult disease to diagnose (10). Clinical mastitis (CM), on the other hand, can be caused by several pathogenic microorganisms and results in changes to the color of the milk as well as clotting, flaking and watery appearance (7). Changes that may occur to biochemical parameters of the blood and milk can be used to diagnosis of mastitis (32,36). One study found that serum levels of Zn, Cu and Fe are lower in CM caused by *Escherichia coli* (9). However, a study conducted with *Staphylococ-*

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cus aureus found a statistically significant decline only in serum Zn levels but no changes in Cu and Fe levels (24).

The purpose of this study is to identify changes in serum levels of Na, K, Ca, Mg, Zn, Cu and Fe in cases of acute septic mastitis (ASM) and clinical mastitis (CM) that may be encountered during lactation in cows.

Material and Methods

The study was conducted after receiving approval from the Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK / 2014-037).

Animals

The study material consisted of animals in smallholder dairy farms from Kars and surrounding areas. The study material was obtained from 80 Simental cows from 3 to 5 years of age. The cows' body condition scores ranged from 2.75-3.25 on a 5-point scale with increments of 0.25 (7) and daily milk production was 15 ± 3 liters.

Study groups

The cows were divided into group one (ASM, n=20), group two (CM, n=20), group three (Control-1, C1, n=20, healthy cows, which together with ASM or CM cows), and group four (Control-2, C2, n=20, healthy cows, which together without ASM or CM cows). After the clinical examinations, cases with high body temperature, depression, rapid heart and respiratory rates, downer cows, symptoms of shock, dehydration, diarrhea, infective udder and abnormal milk secretion were classified as ASM (10,11,23). Cows with physical changes in the milk accompanied by local symptoms such as udder edema, redness and sensitivity were classified as CM (4,32).

Biochemical analysis

Blood was collected from selected cows' *vena coccygea* using empty 10 mL vacuum tube (BD®, Tıpkimsan, Turkey) without anticoagulant. The collected blood samples were centrifuged (Hettich Universal 320®, Hettich, Germany) for 10 min at 3500 rpm and then the serum samples were stored at -20°C until the serum samples were analyzed.

The serum Na, K, Ca, Mg, Zn, Cu and Fe concentrations were determined by using Atomic Absorption Spectrophotometry (Thermo Elemental S4®-Thermo Electron Corporation, UK) equipped with a flame system. In order to demonstrate the reliability of the measurements performed by the machine, standard solutions with previously specified concentrations were read by the machine after every fifth sample measurement. This data was used in the calculation of coefficient variation (CV). Following the CV results for each mineral, the following determinations were made – Na: 2.04%, K: 2.33%, Ca: 2.98%, Mg: 2.73%, Zn: 1.88%, Cu: 3.21%, and Fe: 6.31% (15,17,19).

The glutaraldehyde (GLA) test solution in the study was prepared using 5.6 mL glutaraldehyde (Merck S30895 027, Germany), 200 mg disodium ethylenediaminetetraacetic acid (Merck 14.3517, Germany), 900 mg sodium chloride (Merck 106404, Germany) and 94.4 mL distillated water to make 100 mL. Whole blood was mixed with the GLA test solution described above at a ratio of 1:1. The vial was inverted every 15 sec, and the coagulation status of the mixture was checked. Complete coagulation was recorded as the final test time. If coagulation did not occur in 16 min or more, the GLA test was considered negative and the test time was recorded as 16 min (13,14).

Statistical analysis

The SPSS® (SPSS 20, IL, USA) program was used for the statistical analysis of the serum Na, K, Ca, Mg, Zn, Cu and Fe levels. Statistical differences between the groups were assessed with the ANOVA and post-hoc Tukey HSD test. Comparison of the GLA test values between the ASM and CM groups was conducted using the Independent Samples T test. The results that were obtained were given as Mean ± Standart error of mean (SEM). Values of $P<0.05$ or lower were considered statistically significant in the statistical assessment.

Results

The measurements established no statistical difference in serum Na (P=0.667), K (P=0.958), Ca (P=1.000) and Cu (P=0.139) levels between the groups. The change in Mg concentrations were similar within the mastitis groups (ASM and CM groups, P=0.65) and within the control groups (C1 and C2 groups, P=0.986). The ASM group Mg levels were statistically lower than the control groups ($P<0.001$). The CM group Mg levels were also statistically different from that of the control groups (P=0.014, P=0.005 respectively) (Table 1).

It was determined that the change in serum Zn levels was not statistically significant within the mastitis groups (ASM and CM, P=0.531) and

within the control groups (C1 and C2, P=0.995). In the ASM group, Zn levels were affected by the infection and declined in comparison with the control groups (P<0.001). CM group serum Zn concentrations were statistically different from that of the control groups (P=0.02, P=0.01 respectively) (Table 1).

The Fe concentrations were similar between CM and the control groups (P>0.05), but statistically different between ASM and CM and control groups (ASM and CM P=0.01, ASM and C1 P=0.001, ASM and C2 P<0.001 respectively) (Table 1).

The results of the GLA test were negative in the control groups. However, in the ASM group, GLA was 11.8 ± 3.41 min. and in the CM group it was 15.35 ± 1.59. It was determined that the GLA test results of these two groups were statistically different (P<0.001) (Table 1).

Table 1. Change in serum Na, K, Ca, Mg, Cu, Zn and Fe concentrations by groups

Parameters	ASM (n=20) Mean ± SEM	CM (n=20) Mean ± SEM	C1 (n=20) Mean ± SEM	C2 (n=20) Mean ± SEM	P-value
Na %mg	210.68 ± 5.47	220.28 ± 6.78	211.33 ± 6.90	216.13 ± 5.97	0.667
K %mg	21.66 ± 0.40	21.65 ± 0.54	21.47 ± 0.40	21.81 ± 0.38	0.958
Ca %mg	7.59 ± 0.22	7.57 ± 0.25	7.59 ± 0.20	7.58 ± 0.19	1.000
Mg %mg	1.49 ± 0.08[a]	1.59 ± 0.05[a]	1.85 ± 0.05[b]	1.87 ± 0.05[b]	<0.001
Cu µg/dL	74.04 ± 3.26	67.13 ± 2.88	65.95 ± 1.97	73.78 ± 4.01	0.139
Zn µg/dL	58.06 ± 2.00[a]	62.84 ± 2.30[a]	73.37 ± 2.91[b]	74.21 ± 2.67[b]	<0.001
Fe µg/dL	132.31 ± 3.12[a]	149.57 ± 2.39[b]	154.26 ± 3.90[b]	155.22 ± 5.20[b]	<0.001
GLA Test (min)	11.8 ± 0.76[a]	15.35 ± 0.36[b]	Negatif	Negatif	<0.001

ASM: Acute septic mastitis (n=20), CM: Clinical mastitis (n=20), C1: Healthy cows, which together with ASM or CM cows (n=20), C2: Healthy cows, which together without ASM or CM cows (n=20)
SEM: Standart error of mean, GLA: Glutaraldehyde, min: Minute
[a, b]: The difference between values with different letters on the same line is significant at the P value

Discussion and Conclusion

Trace elements and macro minerals are vitally important to human and animal health (25). Numerous studies have been conducted on the change in the serum levels of these elements in clinical mastitis as they play a role in several biological functions (2,8,9,30). However, information on the changes in serum levels of trace elements and macro minerals is more limited (3).

In a study conducted by Rişvanlı et al. (31), the authors reported that serum Na and K levels were lower in CM cows compared to the control group. However, similar studies have found that serum Na and K levels are not affected by mastitis (2,8,32). There are also researchers who claim that serum Na levels decrease but that K levels are not affected (34). In our study, however, no statistical difference was found in serum Na and K levels in any of the groups (P=0.667 and P=0.958 respectively).

Serum Ca levels reportedly do not change (31,32,34) or decrease (8) in CM. Bleul et al. (3) found that serum Ca levels remained within the reference range in most of the cows they diagnosed with ASM. Studies have shown that Cu concentrations are not statistically affected in mastitis experimentally induced with *E. coli* or *S. aureus* in cows (9,24). However, in some studies (30,37), the serum Cu levels of animals with mastitis were statistically different from those of healthy animals. Our study found that serum Ca and Cu concentrations were similar in ASM, CM and control groups (P=1.000 and P=0.139 respectively).

Mg concentrations reportedly decrease under conditions of stress and inflammation (16,18,33). Researchers have also noted that serum Mg levels decrease in CM and ASM, but were unable to establish a statistically significant difference with control groups (3,8,32). In our study, on the other hand, the Mg concentrations of the ASM and CM groups were lower than those of the control groups and the difference was statistically significant. These values are different from the information available in the literature and might be due to the rations being fed or the severity of the infection. Plasma Mg may be related to lypolysis during stress, cold, or starvation. Diet with inadequate Mg can reduce blood Mg (33).

Zn is an essential component of DNA and RNA synthesis. Cu and Zn join the superoxide dismutase (SOD) structure and serve in the antioxidant system. They may play a role in cell replication and proliferation (35). Zn is an essential element required for the normal growth of bacteria, and serum Zn concentrations decrease in response to infection (21). Studies on cows have shown that administration of oral Zn increases teat canal keratin levels and reduces the somatic cell count and the formation of mastitis (28). The lower serum Zn levels in clinical mastitis and experimentally-induced mastitis have been shown to be statistically significant compared to control groups (9,24,30). In our study, serum Zn levels were statistically different in ASM and CM compared to the control groups ($P<0.001$). This result is consistent with other studies (9,24,30). It is thought that serum Zn levels are affected by infection and decrease, and this may be due to the fact that Zn plays a non-specific role in host immunity.

Fe is required for the growth of gram-negative bacteria, it is thought that there are immune systems that reduce levels of Fe during infections and restrict the growth of bacteria (5,9). Studies have reported that Fe levels may decrease in cases of stress and inflammation (12,27). Serum Fe concentrations in cows with mastitis are not statistically different than those in healthy animals (24,32,37). In our study, there was no statistically significant difference between the group with clinical mastitis and the control groups ($P=0.548$). Serum Fe levels in the ASM group were statistically lower than those of the CM and control groups ($P<0.001$). In cows with experimental mastitis induced with *E. coli*, which is one ASM factor, serum Fe concentrations are reported to be statistically lower than pre-administration (9). The Fe findings obtained in our study are consistent with the literature. It was determined that serum Fe levels can be affected by severe infection originating in the udder.

Identification of proteins in the blood can provide important information about inflammation in the body. The GLA test is a quick, simple and semi-quantitative way to determine blood protein and fibrinogen conditions (20). Coagulation time on the GLA test has been shown to be shorter in cattle with traumatic reticuloperitonitis (13,14). In our study, the GLA test on the control groups was negative, but it was positive for the ASM and CM groups with a mean of 11.8 ± 3.41 and 15.35 ± 1.59 min, respectively. These results indicate that there are changes in blood protein that indicate mild generalized inflammation in the cattle with mastitis in groups ASM and CM. The decline in Zn and Fe levels parallel to the results of the GLA test are remarkable (Table 1). This finding is similar to the decline in Zn levels that Karademir (16) found in cases of inflammation with the Foot and Mouth Disease vaccination in cattle. Similar studies that induce inflammation and stress with the infectious bovine rhinotracheitis in cattle have reported a decline in serum Zn and Mg levels like those in this study (6,29).

In conclusion, inflammation and stress that occurs with ASM and CM do affect serum mineral levels in cows. The presence of inflammation is supported by the GLA test results. The decline in Mg, Zn and Fe levels was expected with acute septic mastitis, but the fact that only Mg and Zn levels declined in clinical mastitis is noteworthy. No significant changes in Na, K, Ca and Cu values were observed. There are not many studies related to changes in serum trace elements and macro minerals in cows with ASM, so it was decided that these results would be a contribution to the literature. It may also be useful to measure Mg, Zn and Fe levels in order to diagnose mammary infections and this type of minerals can be applied in addition to treatment of mastitis.

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