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Lactose, sodium, potassium and chloride levels in milk of cows with subclinical mastitis administered intramammary levamisole*

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Summary: In this study, therapeutic effect of intramammary levamisole infusion in subclinically infected cows were evaluated. Totally, 4-5 years old Holstein breed 40 cows were used. Twenty cows with CMT (-) and SCC≤300,000 as control group and 20 cows with CMT (+) and SCC≥300,000 as trial group were used. Strong immunomodulatory substance levamisole administered 20 ml as 4% solution for 6 days intramammarial to the cows with subclinical mastitis and in which manner levels of lactose, sodium, potassium and chloride levels of immunoglobulin G affected was investigated. After administration of levamisole to the cows with subclinical mastitis, there was no change in lactose, sodium and potassium levels, but the levels of chloride was raised statistically as $p \leq 0.01$. It was found out that subclinical mastitis criteria in milk did not change after a 6 day intramammary levamisole administration to the cows with subclinical mastitis. Levamisole showed no healing effect on the spoiled ion balance and lactose synthesis during mammary tissue infections.

Key words: Chloride, cow, lactose, levamisole, potassium, sodium, subclinical mastitis

Meme içi levamizol uygulanan subklinik mastitisli ineklerin sütlerinde laktoz, sodyum, potasyum ve klor seviyeleri

Özet: Bu çalışmada subklinik mastitisli memelere uygulanan levamizolün iyileştirici etkisi araştırılmıştır. Çalışmada 4-5 yaşlarında, Holstein ırkı toplam 40 inek kullanılmıştır. CMT (-) ve SCC≤300.000 olan 20 inek kontrol grubunu, CMT (+) ve SCC≥300.000 olan 20 inek ise deneme grubunu oluşturmuştur. Subklinik mastitisli ineklere, güçlü immunomodülatör etkiye sahip olan levamizolün 20 ml %4'lük solüsyonu, meme içi 6 gün boyunca uygulanmış ve sütte laktoz, sodyum, potasyum ve klor düzeylerinin ne yönde etkilendiği araştırılmıştır. Subklinik mastitisli ineklerde, levamizol uygulanmasından sonra süt laktoz, sodyum ve potasyum düzeylerinde herhangi bir değişiklik olmamıştır ($p \geq 0.05$). Klor düzeyleri istatistiki olarak $p \leq 0.01$ düzeyinde artmıştır. Subklinik mastitisli ineklerde, meme içi, 6 gün boyunca levamizol uygulanmasından sonra sütteki subklinik mastitis kriterlerinin değişmediği belirlenmiştir. Meme dokusu enfeksiyonlarında, bozulan iyon dengesinde ve laktoz sentezinde, levamizol kullanımının iyileştirici etkisi olmadığı anlaşılmıştır.

Anahtar kelimeler: İnek, klor, laktoz, levamizol, potasyum, sodyum, subklinik mastitis

Introduction

Mastitis is an inflammation of the glandular tissue and it can be defined as the reaction of the mammary gland to irritant factors. It may be seen in all animals, however, in cows, it is more likely to occur because of productivity of the animal and sensitivity of the over-working mammary gland (16).

Mastitis causes great loss in dairy farming in our country, and if it is diagnosed on time and treated at subclinical stage, it can be stopped and prevented to pass on to other animals (9,10,13).

In subclinical mastitis cases, generally changes in mammary tissue and milk cannot be observed clinically, so the disease can be diagnosed in the laboratory by determining the increase of somatic cells in milk and changes in the levels of milk components. The number of somatic cells in milk covers the total count of cells including the number of leukocytes. The count of the neutrophil leukocytes is accepted to be the best criterion in order to indicate the existence and severity of the inflammation in the mammary gland (18).

Subclinical mastitis is characterized by an increase in the number of milk cells, degeneration in mammary gland epithelial cells, a decrease in the synthesizing procedures in cells and changes in ions. Because of the damage in the synthesizing cells, the synthesis of such milk components as lactose, casein and fat is decreased. Ionic changes caused by an increased levels of sodium, bicarbonate and chloride are decrease the levels of hydrogen and potassium ions. When the intercell canals get damaged, the concentration of chloride ions increases, therefore, 'a salty taste' is formed and this increases the

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electrical conductivity of the milk. The result of the development of the inflammation in mammary is that substance permeation from blood vessels to mammary gland increases, so the composition of milk resembles blood gradually (12).

Some of the researchers have taken the advantage of the immunomodulatory effect of levamisole to stop the development of mastitis diagnosed at the subclinical stage (2,7). In this study, levamisole as a strong immunomodulatory substance administered intramammarial to the cows with subclinical mastitis to see lactose, sodium, potassium and chloride levels in milk to understand whether whole cell structure and ionic balance regained or not again.

Material and Methods

The materials of the study were 40 Holstein breed cows of 4-5 years of age, provided by three private dairy farming companies in the town of Kırıkkale. The determination of the control and trial groups was realised according to the results of California mastitis test (CMT) and somatic cell count (SCC). Twenty of the cows with CMT( ) and SCC<300.000/ml milk formed the control group while the other 20 with CMT(+) and SCC>300.000/ml milk was used as the trial group.

Before the intramammary levamisole administration, milk samples were taken from two of the mammary lobes of each cow separetely into the 20 ml vials. The levels of lactose, sodium, potassium and chloride were determined in the serum of milk samples. Then, 4% of 20 ml levamisole was administered to one of the two lobes of the cows from control and trial groups, whereas no administration was applied to the other lobe. Levamisole administration was carried out once a day following morning milking and it continued for 6 days. When the levamisole administration ended, milk samples were taken from the same cows second time and the same tests mentioned were carried out.

The California mastitis test (CMT) was carried out according to the method described by Schalm et al. (18). SCC was carried out according to the method described by Scandinavian scoring system was used to evaluate the test results (15).

In order to extract milk serums, the method described by Alais (1) was used. The measurement of sodium and potassium levels in milk serum was done through the flame photometric method used by Ersoy and Baysu (6). The amount of lactose in milk was measured through the method described by Frais (8). The measurement of the amount of chloride in milk was also performed using a method described by Egan et al. (5). In order to evaluate the results of analysis, t-test described by Sumbuloglu and Sumbuloglu was used (20).

Results

Before the intramammary administration of levamisole, the level of lactose in milk serum was measured as 4.25±0.08 g/dl in the cows with subclinical mastitis, and this level was found to be statistically lower by $p \leq 0.001$ than the value of 4.90±0.05 g/dl measured in the cows in the control group. In the cows with subclinical mastitis, the levels of milk serum lactose were measured as 4.25±0.08 g/dl before the levamisole administration, and as 4.34±0.05 g/dl after the administration ($p \leq 0.001$) (Table 1)..

In healthy cows, the sodium level was measured as 46.9±14.5 mg/dl while it was found to be 82.346.9±25.7 mg/dl in the cows with subclinical mastitis, and the difference between the two groups was found to be statistically significant at the level of $p \leq 0.001$. After the intramammary administration of levamisole, a relative increase was observed in the level of sodium in milk serum of both the healthy cows and the cows with subclinical mastitis but, there was no change statistically ($p \geq 0.05$) (Table 1).

The levels of chloride were measured as 61.01±0.04 mg/dl in the cows of control group, and 111.66±0.05 mg/dl in the cows with subclinical mastitis ($p \leq 0.001$). The milk serum chloride level, which was 111.66±0.05 mg/dl before the intramammary levamisole administration to the cows with subclinical mastitis, was measured as 113.51±0.05 mg/dl after the administration, and a difference was observed statistically between the groups at the level of $p \leq 0.001$ (Table 1).

The levels of milk serum potassium levels were 169.8±16.2 mg/dl in the healthy cows and 130.5±18.4 mg/dl in the cows with subclinical mastitis. The milk serum potassium levels in the cows with subclinical mastitis were statistically lower at the level of $p \leq 0.001$ than the healthy cows. After the levamisole administration, it was seen that the milk serum potassium levels did not change statistically in the cows with subclinical mastitis ($p \geq 0.05$) (Table 1).

Discussion and Conclusion

The mineral content of milk changes during mastitis and the changes are of great importance in terms of characteristic of milk as well as its nutritive value. The mammary tissues selective permeability of ions reduces during mastitis, but passive permeability increases (12,17).

Table 1. Levels of lactose, sodium, potassium and chloride in milk cows of control and subclinical mastitis.

Tests	Control				Trial				Signature
	A		B		A		B		
X±Sx	Preadmin X±Sx (a)	Postadmin X±Sx (a)	Preadmin X±Sx (a)	Postadmin X±Sx (a)	Preadmin X±Sx (a)	Postadmin X±Sx (a)	Preadmin X±Sx (a)	Postadmin (a)	
Lactose g/dl	4.90 ±0.05	4.97 ±0.02	4.90 ±0.03	4.91 ±0.02	4.25 ±0.08	4.34 ±0.05	4.51 ±0.09	4.54 ±0.08	a–b:p≥0.05 e–f:p≥0.05 a–e:p≤0.001 b–f:p≤0.001 c–d:p≥0.05 g–h:p≥0.05 c–g:p≤0.001 d–h:p≤0.001 a–b:p≥0.05 e–f:p≥0.05
Sodium mg/dl	46.9 ±14.5	48.0 ±12.8	50.0 ±6.8	50.0 ±5.9	82.3 ±25.7	83.1 ±28.0	82.3 ±25.7	83.1 ±28.0	a–e:p≤0.001 b–f:p≤0.001 c–d:p≥0.05 g–h:p≥0.05 c–g:p≤0.001 d–h:p≤0.001 a–b:p≥0.05 e–f:p≥0.05
Potassium mg/dl	169.8 ±16.2	169.4 ±16.2	191.8 ±26.1	187.2 ±23.7	130.5 ±18.4	129.2 ±16.9	138.2 ±22.1	133.5 ±21.4	a–e:p≤0.001 b–f:p≤0.001 c–d:p≥0.05 g–h:p≥0.05 c–g:p≤0.001 d–h:p≤0.001 a–b:p≥0.05 e–f:p≤0.001
Chloride mg/dl	61.01 ±0.04	61.40 ±0.04	66.37 ±0.01	66.58 ±0.01	111.66 ±0.05	113.51 ±0.05	108.58 ±0.06	108.68 ±0.06	a–e:p≤0.001 b–f:p≤0.001 c–d:p≥0.05 g–h:p≥0.05 c–g:p≤0.001 d–h:p≤0.001

A: Mammary lobe with levamisole administration.
B: Mammary lobe with no levamisole administration.

As a result of the development of the inflammation in the mammary gland, substance permeation from the blood vessels to the mammary gland increases, so the composition of milk resembles blood gradually. Milk is in osmotic balance with blood. Since the amount of lactose has decreased in milk with mastitis, the reduced osmotic pressure is balanced by the permeation of chloride and sodium ions and blood proteins to milk. It is pointed out that in mastitis cases, ion levels permeating milk increase by 10 times as much as the normal values (12).

After the intramammary levamisole administration, no significant improvement was recorded in the level of lactose in milk, but a relative increase was observed.

Major anions and cations related to mammary gland secretion disorders are chloride, sodium and potassium. Normal secretion of sodium and potassium is controlled by the active pump systems in the basal and lateral membranes of the secreting cells. These pumps sodium out of the secreting cells, that is, into the extracellular liquid, whereas they pump potassium in the reverse direction. These ions are passively moved towards the apical membranes of the secreting cells. This is because milk is electrically more positive than intracellular liquid. The mechanism of chloride secretion is not as clear as of sodium and potassium, but it is moved towards the basal and apical membranes both actively and passively, and its concentration is higher in blood and extracellular liquid than in milk (11).

Levamisol, which arouses the antibody synthesis by strengthening the functions of peripheral T-lymphocytes and phagocytes, has useful effects over cow mastitis (19). However theoretical arguments recommend acting prudently in finding out the pathogen of mastitis and using levamisole (14).

In other studies, levamisole, known for its immunomodulatory effect, was administered both intramammary and parenterally in order to treat subclinical mastitis. In these studies, where reportedly levamisole was used to treat mastitis, no information was given on the change of subclinical mastitis criteria in milk (3,4).

In present study, it was found out that subclinical mastitis criteria in milk did not change after a 6 day intramammary levamisole administration to the cows with subclinical mastitis. Mammary tissue infections increase permeability in blood capillaries and spoil the ion balance, and levamisole showed no healing effect over that.

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