Evaluation of zinc in blood of cows suffering from inflammation of udder (mastitis)

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Abstract: The aim of the research was to evaluate serum zinc concentration in the experimental animals with clinical inflammation of mammary gland. Forty-four cows were divided into four groups: A, B, and C with clinical form of mastitis caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli,* respectively, and healthy control group D. Zinc concentration was determined by the atomic absorption specrtrophotometry method. The level of zinc marked low in the blood indices tested was observed in all cows with clinical mastitis. Zinc concentrations were: 108.00, 63.86, and 46,80 μg/dl in groups A, B, and C, respectively, while in the group D, the values was: 116.73 μg/dl for Zn concentration.
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1.Introduction

Inflammation of mammary gland is one of the most important diseases of dairy farms, this disease lead to decrease in quantity and quality of milk components and shorten the productive life of affected animals.(1) A number of trace minerals have important roles in immune function and may affect health in transition dairy cows. Zinc plays an important role in the oxidative stress. In the last years, many studies were performed on the importance of reactive oxygen species (ROS) on ruminant metabolism (2, 3). In many researches found that status of healthy animal results from the balance between prooxidative and antioxidative processes in the body fluids and cells (4,5). In some studies Zn has important role in immune system, glandular, reproductive and cell health. And an important in structure of some enzymes and also play role in improving diseases such as pneumonia and diarrhea. Zinc is required for more than 200 metalloenzyme including the antioxidant enzyme (6,7). Generally Zn is present in most of food which daily consume. Normally in the body, concentration of Zn is highest in muscle tissue and bone.(8). The aim of the investigations was the evaluation of serum zinc concentration in cows with clinical mastitis caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*.

2. Material and Methods

The study was conducted on 44 cows of Black and White breed, 5-8 years of age, yielding annually on average 4982 kg of milk, which contained 3.42% of fat and 3.11% of protein. On the basis of microbiological examination, the cows were divided into four groups. Groups A, B, and C were affected with mastitis (*Staphylococcus aureus, Streptococcus agalactiae*, and *Escherichia coli*) were isolated from their milk respectively, whereas the non-affected group D served as a control. During the winter, all cows were housed in high binding stands and fed hay concentrated feeding stuff. During summer, the cows stayed on pasture, and were additionally given green forage of grass and concentrated feeding stuff. Depending on the season of the year, the feeding stuff originated from a local area. Daily feeding dose was balanced with regard to energy and protein, after common acceptable standards (9). To estimate zinc concentrations, the mineral analysis of the fodder was conducted by ASA method (Atomic Sphere Approximation). The samples were mineralised by the wet method using the mixture of condensated HNO_3, $HClO_3$, and H_2SO_4, prepared in proportion 20:4:1. All the cows were subjected to an initial clinical examination, including the mammary gland palpation, evaluation of macroscopic changes in milk, and evaluation of somatic cell count in milk using the California Mastitis Test (11). The milk samples (groups A, B, C, and D) were taken simultaneously for the identifying of pathogenic microorganisms (10, 11). The somatic cell count was done using the microscopic method (12). Blood samples from all cows were collected four times from the external jugular vein immediately after clinical examination and before treatment. The statistical analysis was performed using the Student *t*- test.

3. Results

The concentrations of zinc in fodder, results of clinical examination of the mammary gland, serum

concentration of zinc, and number of milk somatic cells in cows from all the groups are presented in Tables 1, 2, and 3. The highest number of somatic cells was in the milk of cows from group C (1 596 000) and next in groups A (1 412 000) and B (938 000); the lowest one was in the group D (76 000). As it was mentioned above, *Staph. aureus*, *Str. agalactiae*, and *E. coli* were isolated from milk of cows from groups A, B, and C, respectively. No pathogenic microorganisms were isolated from milk of cows from the control group D. The results of clinical examinations of the mammary gland, organoleptic evaluation of milk, somatic cell counting, and bacteriological examination of milk indicated that cows from groups A, B, and C were affected with clinical form of mastitis.

Table 1: Concentrations of zinc in fodder

Cows	Groups	Feeding period	Zn (mg/kg d.m.)
Affected with mastitis	A	Winter	105.4±14.3
		Summer	117.2±19.5
	B	Winter	102.6±19.6
		Summer	102.6±19.6
	C	Winter	119.8±16.7
		Summer	123.4±10.5
Healthy	D	Winter	112.1±23.6
		Summer	119.2±15.3

Table 2: Clinical signs in cows

Clinical signs	Group							
	A		B		C		D	
	Number and percentage of cows with clinical signs							
	Number	%	Number	%	Number	%	Number	%
Udder swelling	11	100	11	100	11	100	0	0
Rubber of udder skin	11	100	11	100	11	100	0	0
Light painfulness of udder	9	82	4	36	2	18	0	0
Hard painfulness of udder	2	27	0	0	9	82	0	0
Induration of udder	2	18	2	18	8	73	0	0
Constipation	0	0	0	0	10	91	0	0
Indigestion	0	0	0	0	3	27	0	0
Diarrhoea	0	0	0	0	1	9	0	0
Increase in body temperature	0	0	0	0	9	73	0	0

Table 3: Serum concentration of zinc

Examined indicator	Group			
	A	B	C	D
	Infection			
	Staph. aureus	*Str. agalactiae*	*E. coli*	Negative
Zn (μg/dl)	*108.00±7.56	63.86±6.4	*46.80±3.1	116.73±4.3

*** *P* ≤0.001 when compared affected cows (groups A, B, C) to healthy ones (group D).**

The lowest serum concentration of zinc (Table 3) were noted in cows from group C (46.80 μg/dl); increased values were found in cows from groups B (63.86 μg/dl) and A (108.00 μg/dl). The highest Zn concentration were found in the cows from control group D (116.73 μg/dl).

4. Discussions

Important diseases in dairy farms, such as mastitis, are associated with oxidative stress (12, 13). However, the inflammation of the mammary gland may be preceded by the acute phase response (APR). APR occurs immediately and helps to maintain physiological homeostasis after injury. The role of APR is to prevent further injury to an organ, to destroy the microorganisms, to isolate local injury, and to remove harmful molecules and debris. The inflammatory process can be characterised by the exudation of fluids and proteins and migration of leukocytes, especially neutrophils (14, 15). The mechanism responsible for inflammatory responses is still not completely known. In other side, Zinc is part

of between 100 and 300 biological enzymes in the body, the cellular processes regulated by zinc. In our research, cows with a clinical form of mastitis, there was noted low serum concentration of zinc. And that was agree with recorded by **Klasing** K.C.(16). Some results found, zinc deficiency has been associated with reduced formation of both T and B lymphocytes and phagocytes.(17). Some result suggested that hydroxyl radicals released by activated bovine neutrophils caused the damage to mammary epithelial cells and that antioxidants may protect the mammary tissue during bovine mastitis.(18). The conducted investigations confirmed the influence of *Staph. aureus, Str. agalactiae,* and *E. coli* in clinical mastitis on zinc concentration in cows. The aim of this research is to find correlation between bacterial mastitis and level of serum zinc. And it is obvious that decrease in serum Zn concentration in dairy cows with mastitis is the result of mammary gland inflammation.

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