

Serum Metabolic Parameters in Healthy and Subclinical Mastitis Cows

Sorana Teodora MATEI, Ioan GROZA, Sanda ANDREI, Liviu BOGDAN,
Simona CIUPE, Anamaria PETREAN

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
Faculty of Veterinary Medicine
Department of Veterinary Obstetrics, Gynecology and Reproduction
3-5 Manastur Street, Cluj-Napoca, Romania, soranamatei@yahoo.com

Abstract. Subclinical mastitis continues to be the most frequent and expensive disease of dairy cows. It negatively affects the quality of milk and economic effectiveness of farms. The purpose of the study was to identify changes that occur in some serum metabolic parameters (total protein, serum albumin, serum globulin, ALP, ASAT, GGT, CK) in healthy and subclinical mastitis cows. First objective was to diagnose subclinical mastitis using the indicator of mastitis Waikato and determining the number of somatic cells in milk using the MT-04. Following tests carried out on the 84 lactating cows, 10 of them had a positive diagnosis, representing 12% of the total lactating cows. For the 10 cows, positive diagnosis was confirmed by the increased number of somatic cells present in milk. Thus, values obtained were between 500.000 and 1.5 million cells/ml. In healthy cows, somatic cell count has not exceeded the value of 270.000 cells/ml. Total serum protein (9.14 ± 2.74 g/dl) values and serum globulin (5.76 ± 1.82 g/dl) from subclinical mastitis cows were higher compared to healthy cows. Increased proteins and globulin in the blood of cows indicate an activation of immune response following infection of the mammary gland. We obtained low values of the two enzymes, ASAT (87.20 ± 30.16 U/l) and GGT (18.80 ± 8.90 U/l), along with a decrease activity of CK (221.80 ± 80.53 U/l). Contrarily, ALP activity (71.80 ± 50.70 U/l) increased in cows diagnosed with subclinical mastitis compared with healthy cows enzymes.

Keywords: subclinical mastitis, diagnosis, serum metabolic parameters, cows.

INTRODUCTION

Subclinical mastitis continues to be the most frequent and expensive disease of dairy cows. It negatively affects the quality of milk and economic effectiveness of farms (Groza *et al.*, 2006; Hillerton, Barry, 2005). Mastitis is the inflammatory reaction of the udder to invading pathogens. One of the most apparent reactions is the increased influx of immunoreactive cells from blood into milk inducing a dramatic increase of milk somatic cell counts (Bruckmaier *et al.*, 2004).

Subclinical mastitis causes considerable changes in milk composition and serum, which may contribute to the impaired immune defence (Megalia *et al.*, 2001). The somatic cell count in bovine milk is an indicator of udder health and milk quality and can be related to the cellular immune response after an inflammatory stimulus (Lindmark-Mansson *et al.*, 2005). Subclinical mastitis increases capillary permeability, which facilitates passage of proteins from blood to milk (Andrei *et al.*, 2009).

Subclinical mastitis milk shows evidence of direct passage of blood into the milk as indicated by the changes of some blood enzymes level (Ibtisam El Zubeir, 2005). Alkaline phosphatases are a group of enzymes found primarily the liver and bone. What is measured in

the blood is the total amount of alkaline phosphatases released from these tissues into the blood. In the liver AST and GGT show high activity and are most often determined if there is a suspicion of acute and chronic liver disease. The activity of AST and GGT enzymes shows occasional irregular changes during pregnancy and early lactation. Creatinine kinase is an enzyme found primarily in the heart and skeletal muscles, and to a lesser extent in the brain. The implications of serum enzymes levels must consider more than just bone, liver and muscles function (Stojević *et al.*, 2005).

The purpose of the study was to identify changes that occur in some serum metabolic parameters (total protein, serum albumin, serum globulin, ALP, ASAT, GGT, CK) in healthy and subclinical mastitis cows. First objective was to diagnose subclinical mastitis using the indicator of mastitis Waikato and determining the number of somatic cells in milk.

MATERIAL AND METHODS

The research was conducted during February 2010 - March 2010, in a dairy farm from Cluj County. Of the total of 120 cows (mixed race with Austrian B 1 at with Red Holstein and Red Holstein metis) were 84 lactating cows.

Mastitis diagnosis was achieved with the aid of Waikato mastitis indicator, a physical method for determining the quality of milk by measuring the conductivity. The *somatic cells' counting* was performed using the MT-04 device.

Blood and milk samples were taken from cows diagnosed with subclinical mastitis, and from healthy cows. Blood samples were collected by jugular venepuncture with the use of heparin as anticoagulant. After collection, all the sample was immediately transferred to the laboratory for performing somatic cell counting and determination of biochemical parameters of oxidative stress.

Determination of serum total protein concentration was done by biuret photometric method, based on color reaction with CuSO₄ in basic medium, using a calibration curve obtained with solutions of bovine serum albumin (BSA) (Pintea *et al.*, 2008). *Determination of serum albumin concentration* was performed by an enzymatic – colorimetric method. The bromocresol green reacts with albumin and forms a green complex in pH 3.8, whose intensity is proportional to the albumin concentration in sample (λ max = 630nm). *Serum globulin concentration* was determined by difference between total protein concentration and albumin concentration in the samples.

Determination of alkaline phosphatase activity was based on the hydrolysis reaction of p-nitrophenylphosphate when p-nitrophenol is formed, a yellow colored compound that can be dosed photometrically at λ max = 410nm (Andrei, Pintea, 2004).

Determination of AST activity was performed by a photometric method based on the condensation reaction of keto acids (resulting in the presence of enzyme) with dinitro phenylhydrazine (DNPH). The reaction, in basic medium, form a red compound that can be dosed photometrically at λ max = 546nm (Andrei and Pintea, 2004).

Determination γ -glutamyl transpeptidase activity (GGT) was based on the fact that the enzyme catalyzes the reaction between glycyl-glycine and L-glutamyl-3-carboxy-4-nitroanilide. In this reaction, p-nitroanilide result, which can be can be determined photometrically at λ max = 405nm (Pintea *et al.*, 2008).

Determination of creatine kinase activity was performed using an analysis Hospitex Diagnostic kit. Creatine kinase catalyze the conversion of creatine phosphate and ADP to creatine and ATP. Glucose and ATP are converted to glucose-6-phosphate and ADP.

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphogluconate and reducing NADP to NADPH. The rate of conversion of NADP/NADPH, monitored at 340nm, is proportional to CK activity.

RESULTS AND DISCUSSION

Following tests carried out on the 84 lactating cows, 10 of them had a positive diagnosis, representing 12% of the total lactating cows. The diagnosis was based on electrical conductivity (EC) of milk which is an indicator trait for mastitis over the last decade. The EC is determined by the concentration of anions and cations. If the cow suffers from subclinical mastitis, the concentration of Na⁺ and Cl⁻ in the milk increases, which leads to increased EC of milk from the infected quarter (Norberg *et al.*, 2004). For the 10 cows, positive diagnosis was confirmed by the increased number of somatic cells present in milk. Thus, values obtained were between 500.000 and 1.5 million cells / ml. In healthy cows, somatic cell count has not exceeded the value of 270.000 cells / ml.

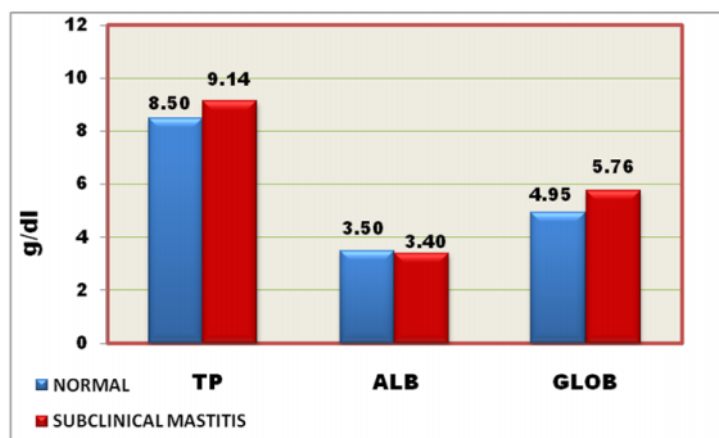
Results obtained from the analysis of total serum protein, serum albumin and serum globulin (mean value and standard deviation) are shown in Table 1.

Tab.1

Total serum protein, serum albumin and serum globulin values from healthy and subclinical mastitis cows

Serum samples	Total Proteine (g/dl)	Albumin (g/dl)	Globulin(g/dl)
Normal	8.50±0.70	3.50±0.20	4.95±0.55
Subclinical Mastitis	9.14±2.74	3.40 ±1.06	5.76 ±1.82

We obtained an increase of total serum protein and serum globulin from subclinical mastitis cows compared to healthy cows (Graphic 1). Increased proteins and globulin in the blood of cows indicate an activation of immune response following infection of the mammary gland. These proteins are mainly serum albumin and immunoglobulins that are implicated in udder defense mechanisms (Tsenkova *et al.*, 2001). Immunoglobulin plays an important role in host immunity and inflammation, and there is a correlation between total serum protein (globulins and albumin) and somatic cells count in milk (Pandey, 2005).



Graphic 1 - Total serum protein, serum albumin and serum globulin values of subclinical mastitis cows compared with healthy cows

Were observed some changes in serum enzymes from mastitis cows compared with blood samples collected from healthy cows (mean value and standard deviation) (Tab. 2).

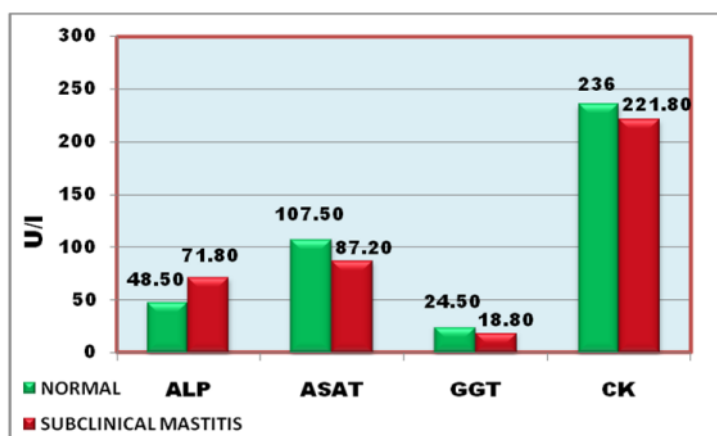
Tab. 2

Serum enzymes activity from healthy and subclinical mastitis cows

Serum samples	ALP (U/L)	ASAT (U/L)	GGT (U/L)	CK (U/L)
Normal	48.50±3.50	107.50±5.50	24.50±1.50	236.50±68.50
Subclinical Mastitis	71.80±50.70	87.20±30.16	18.80±8.90	221.80±80.53

The activity of AST and GGT enzymes shows occasional irregular, changes during pregnancy and early lactation (Stojevi *et al.*, 2005).

In our study we obtained low values of the two enzymes, ASAT and GGT, along with a decrease activity of CK. Contrarily, ALP activity increased in cows diagnosed with subclinical mastitis (Graphic 2). There are many experimental studies that indicate an increase of serum alkaline phosphatase from mastitis cows, which may suggest that this enzyme plays a role in the pathogenesis of the disease (Vangroenweghe, 2004).



Graphic 2 - Serum enzymes activity from subclinical mastitis cows compared with healthy cows

CONCLUSIONS

Research conducted on the diagnosis of sub clinical mastitis shows that the Waikato method presents a good precision. Data obtained by this method were correlated with those obtained in determining the number of somatic cells with the MT-04. Thus, cows diagnosed positive with the Waikato in an increasing number of somatic cells beyond the normal allowance.

The activity of total protein, serum globulin and serum enzymes studied vary in different ways in serum mastitis cows compared with healthy cows:

- We obtained an increase of total serum protein and serum globulin from mastitis cows compared to healthy cows.
- Increased proteins and globulin in the blood of cows indicate an activation of immune response following infection of the mammary gland.

- In our study we obtained low values of the two enzymes, ASAT and GGT, along with a decrease activity of CK.
- ALP activity increased in cows diagnosed with subclinical mastitis. There are many experimental studies that indicate an increase of serum alkaline phosphatase from mastitis cows, which may suggest that this enzyme plays a role in the pathogenesis of the disease.

This study was supported by research program PNII “IDEI”, 1482/2009.

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