Bulletin UASVM, Veterinary Medicine 68(1)/2011
pISSN 1843-5270; eISSN 1843-5378

Amino Acid Concentration in Normal and Subclinical Mastitis Milk

Sanda ANDREI[1], Monica CULEA[2], Sorana MATEI[1], Adela PINTEA[1], GROZA I.S. [1]

[1)]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Str. Mănăştur no. 3-5, Cluj-Napoca, Romania
[2)] ”Babes-Bolyai” University, Str. M. Kogalniceanu no.1, Cluj-Napoca, Romania
Email: sanda_m_andrei@yahoo.com

Abstract. The aim of this study was the evaluation of amino acid concentration in milk collected from cows with sub clinical mastitis compared with healthy cows. A first step was the diagnosis of mastitis, using the method based on electrical conductivity and also the determination of milk somatic cells number. Next, in all milk samples, we determined the concentration of total amino acids and the variations that occur between different types (essential and non-essential amino acids). Total amino acid content increased significantly in mastitis milk, compared to normal (mean values were 619,82±76,02 μg/ml for normal milk and 12073,06±5.564,14 μg/ml for the subclinical mastitic milk). In mastitis milk samples is an increase in the percentage of lysine, tyrosine, leucine, isoleucine, valine, glycine and alanine. Simultaneously, there were decreases in percentages of glutamic acid, ornithine, aspartic acid, methionine, proline, serine and threonine. For phenylalanine, no significant changes were observed.

Key words: amino acids**,** subclinical mastitis, milk

INTRODUCTION

Milk proteins are considered as having a high biological value, due to essential amino acid content. This occurs not only in sufficient quantities, but more, in an optimum ratio for vital activity of the body. Milk and dairy products are rich sources of protein. From technological point of view is very important to describe the amount of protein in milk as well as characterization of these proteins in terms of amino acid content, particularly essential amino acids [Andrei and Groza, 2010].

Mastitis in dairy cows are the most important diseases of the mammary gland, with economic implications, due to losses in milk production and the risk posed by consumption of infected milk to public health. All milk variety contains a certain level of somatic cells represented by polymorphonuclear cells (PMN), lymphocytes and macrophages. In bacterial infection and other inflammation processes affecting the mammary tissue, the number of somatic cell (SCC) in milk increases, especially the PMN level [Hamed et al., 2008]. Numerous studies indicate that changes in the number of somatic cells are related to changes in the composition of milk, due to injurious processes mammary cells, which reduces the synthesis of milk constituents in the gland. Another explanation is the change in membrane and interstitial spaces permeability, which increases the passage of the components of blood in milk [Groza, 2006; Petrovsky, 2006]. Milk from clinically and subclinically affected mastitis cows had very high increase in the activity of proteolytic enzymes. The main caseinolytic enzyme in milk, plasmin, is able to rapidly cleave caseins in polypeptide fragments (proteose peptones) and amino acid [Bruckmaier et al., 2004; Moussaoui et al., 2004].

The aim of this study was to determine the amino acid profile in normal milk compared with milk collected from cows diagnosed with subclinical mastitis. A first step was the diagnosis of mastitis, using the method based on electrical conductivity and also the determination of milk somatic cells number. Next, in all milk samples, we determined the concentration of total amino acids and the variations that occur between different types (essential and non-essential amino acids).

MATERIAL AND METHODS

The research was conducted in a dairy farm from Cluj County. Of the total of 120 cows (mixed race with Austrian Bălţat 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 conductibility. The somatic cells' counting was performed using the MT-04 device.

An isotopic dilution gas chromatography-mass spectrometric (ID-GC/MS) techniques was used to evaluate season effects in quantitative amino acids in fish plasma. The stable isotope internal standard used was 15N-Methionine. A Trace DSQ ThermoFinnigan quadrupole mass spectrometer coupled with a Trace GC was used. Amino acids were separated on a Rtx-5MS capillary column, 30 m x 0.25 mm, 0.25μm film thickness, using a temperature program from 50^{o}C, 1 min, 6oC/min at 100^{o}C, 4oC/min at 200^{o}C, 20^{o}C/min at 300^{o}C, (3min). The transfer line temperature was 250^{o}C, the injector temperature 200^{o}C and ion source temperature 250^{o}C; splitter: 10:1. Electron energy was 70eV and emission current, 100μA. The amino acids were purified on a Dowex 50W-W8 exchanges resin and were derivate in a procedure following two steps to obtain trifluoroacetyl butyl esters. The identification of amino acids was obtained by using NIST library but also by using amino acid standards.

RESULTS AND DISCUSSION

Following tests carried out on the 84 lactating cows, 10 of them had a positive diagnosis, representing 12% of the total so lactating cows. For the 10 cows, Waikato 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.

The content and type of amino acids employed by the mammary tissue in the biosynthesis of milk-specific proteins may be influenced by different factors, such as: blood amino acid concentration; the take-over mechanisms for amino acids by mammary cells, as well as the intracellular metabolism of these amino acids. Each of these control levels are, in their turn, influenced by different factors. Also, certain amino acids may be employed by the mammary gland in protein biosynthesis, but also as a source of energy, namely sources for carbon and nitrogen atoms necessary in the synthesis of nonessential amino acids [Schilling, et al., 1996].

The results obtained in the analysis of amino acids, in normal and mastitis milk, are detailed in Table 1 (average and standard deviation). As can be seen from the table, total amino acid content increased significantly in mastitis milk, compared to normal (measured values of over 20 times higher in subclinical mastitis). The data obtained are consistent with those presented in the literature. According to data presented by various researchers, in clinical and subclinical mastitis infections, there are significant increases in free amino acid content. These increases are directly proportional to the severity of infection (determined by the number of somatic cells and the total number of germs) [Csapo et al. 1995; Gandolfi et al., 1992].

Tabelul 1

Total amino-acids concentration in milk samples from healty and subclinical mastitis cattle

Total Aminoacids (μg/ml) Average and standard deviation	
Cattle whit sublinical mastitis	Healthy cattle
12.073,06±5.564,14	619,82±76,02

Tests carried out showed that all categories of amino acids increased in subclinical mastitis, but there are changes in the relationship between various types. Thus, as shown in Table 2 are different variations depending on the type of aminocid.

Tabelul 2

Amino-acids concentration in milk samples from healty and subclinical mastitis cattle (average and % of total amino-acids)

Amino acid	**Milk amino-acids concentration (μg/ml)**			
	Cattle whith sublinical mastitis		**Healthy cattle**	
	Average	**%**	**Average**	**%**
Alanine	716.4575	10.07	53.42	8.45
Glycine	692.6475	9.73	57.99	9.17
Threonine	92.805	1.3	11.14	1.76
Serine	260.23	3.65	28.374	4.48
Valine	773.64	10.87	62.752	9.92
Leucine	731.5738	10.28	41.688	6.59
Isoleucine	321.8625	4.52	22.846	3.61
Proline	817.64	11.49	89.192	14.1
Methionine	114.0113	1.60	56.362	8.91
Aspartic acid	306.5038	4.30	33.51	5.3
Ornithine	30.5325	0.42	7.834	1.23
Phenilalanine	254.175	3.57	23.472	3.71
Tirosine	374.3925	5.26	22.514	3.56
Lisina	771.715	10.84	17.176	2.71
Glutamic acid	855.5288	12.02	103.888	16.43

As can be seen from Figure 1, in mastitis milk samples is an increase in the percentage of lysine, tyrosine, leucine, isoleucine, valine, glycine and alanine. Simultaneously, there were decreases in percentages of glutamic acid, ornithine, aspartic acid, methionine, proline, serine and threonine. For phenylalanine, no significant changes were observed.

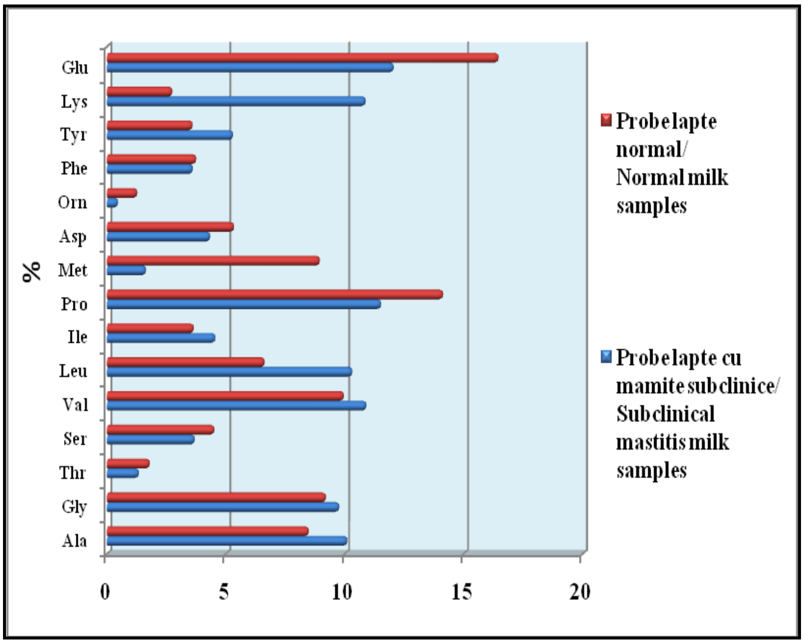

Chart 1 - Variation of amino-acids percentage in normal and mastitis milk

According to data presented by Friedman (2009), in bacterial infections there is an increase in free amino acid content. Simultaneously with the accumulation of amino acids, there are processes of isomerization, the conversion of biologically active isomers of amino acids (L form) in D isomers. Type D isomers do not show biological activity, so their accumulation in milk cause a decrease in nutritional quality of milk. This isomerisation process can be determined and physico-chemical factors such as pH and temperature. On the other hand, literature data showed that amino acids in the form D can be synthesized, *in vitro*, by microorganisms.

CONCLUSIONS

The results showed that all categories of amino acids occur in higher concentrations in milk colected from subclinical mastitis cows and also, there are changes in the relationship between various types of amino acids.

ACKNOWLEDGMENTS

This work was supported by CNCSIS –UEFISCSU, project number PNII – IDEI 1482/2009.

REFERENCES

1. ANDREI SANDA, GROZA IOAN STEFAN, *Fiziologia si patologia glandei mamare la vaca*, Editura AcademicPres, Cluj-Napoca, 2010;
2. BRUCKMAIER R., ONTSOUKA C., BLUM J., 2004, Fractionized milk composition in dairy cows with subclinical mastitis, *Vet.Med.-Czech*, 49(8): 283-290;

3. CSAPO J., CSAPO-KISS Z., STEFLER J., MARTIN TRUMAN G., NEMETHY S., 1995, Influence of mastitis on D-Amino Acid content of milk. *Journal of Dairy Science,* volume 78, Issue 11, pag. 2375-2381
4. FRIEDMAN M., 2009, Chemistry, Nutrition and Microbiology of d-Amino Acids. *J.Agric. Food Chem*., America Chemical Society, 47 (9), 3457-3479
5. GANDOLFI, I., PALLA G., DELPRATO L., DE NISCO F., MARCHELLI R., SALVADORI C., 1992, D-Amino Acids in Milk as Related to Heat Treatments and Bacterial Activity. *Journal of Food Science*, Volume 57, Issue 2, pages 377-379;
6. GROZA I.S., *Ginecologie, andrologie si obstretica veterinara – Compendiu*, Editura Academiei Romane, Bucuresti , 2006;
7. HAMED, H., A. EL FEKI, A. GARGOURI, 2008, Total and differential bulk cow milk somatic cell counts and their relation with antioxidant factors, *C.R.Biologies*, 331:144-151;
8. MOUSSAOUI F., VANGROENWEGHE F., HADDADI K., Y. LE ROUX, LAURENT F., DUCHATEAU L., BURVENICH C.**,** Proteolysis in Milk During Experimental *Escherichia coli* Mastitis, *J. Dairy Sci.* **87**:2923–2931 (2004)
9. PETROVSKI, K., E. STEFANOV, 2006, Milk composition changes during mastitis, www.milkproduction.com/library/articles;
10. SCHILLING, M., H. KHANJIAN, L. SOUZA, 1996, Gaz chromatography analysis of aminoa acids as ethyl chloroformate derivates, *JAIC*, Volume 35, Number 1, Article 4: 45-59;