# THE NUMBER OF SOMATIC CELLS AND MICROORGANISMS – INDICATORS OF THE QUALITY OF RAW COW MILK IN THE REPUBLIC OF MACEDONIA

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**Aim:** The number of somatic cells and the number of microorganisms are the criteria for assessing whether raw milk is in accordance with the Regulation on specific requirements for safety and hygiene and manner for performing official controls on milk and dairy products - Official Gazette of the Republic of Macedonia 157/2007.

**Material and methods:** In order to determine the NMO, 2065 samples were used, and for determining the NSC 1625 samples were taken from farms all over the territory of the Republic of Macedonia. The samples were delivered in sterile plastic sealed cups with a capacity of 50 ml in which previously there was preservative added Azidiol (Sigma – Aldrich) in the dose of 0,25 ml, and then they were transported at a temperature of +4.0 degrees Celsius. For counting of the total number of microorganisms Bactoscan 8000 was used. The procedure for counting the NSC was carried out in accordance with the accredited method ISO 17025-FVM-SOP-398 with references from ISO 13366-2:2006/.

**Results:** Only 704 samples, i.e. 42,79% of the samples meet the criteria of the European Union (Directive 92/46 EEC) in relation to the maximum number of somatic cells in the milk. From the results of counting the somatic cells we can conclude that 682, i.e. 41,45% of the samples have over 600,001 somatic cells per ml, meaning that they do not meet the criteria of the Rulebook from 2008. 936 samples, i.e. 58,54% meet the criteria for raw milk for 2008.

**Conclusions: NMO** directly depends on the initial contamination of milk with microorganisms from the milk gland and microorganisms (contaminants) from the surroundings (afterbirth, urine, feces) that can populate the surfaces of the milking equipment and surfaces that milk comes into contactwithin a considerable number. Minimizing the milk contamination level from these sources would significantly prevent the level of psychrophilic microorganisms.

Key words: somatic cells, microorganisms, milk, contamination.

## Introduction

The Rulebook on safety and hygiene and manner of performance of official controls on milk and dairy products Official Gazette of the Republic of Macedonia Nr. 157/2007 inter alia prescribes the criteria for raw cow's milk in the period from 2008 to 2012.

According to attachment 3 which is an integral part of the said Rulebook, the NMO should be gradually reduced: 800,000 cfu/ml in 2008 to 600,000 in 2009, 400,000 in 2010, 200,000 in 2011, to 100,000 cfu/ml in 2012. Regarding the number of somatic cells, it should be gradually reduced: 600,000 scc/ml in 2008, 500,000 in 2009, to 400,000 scc/ml in 2010.

By achieving these criteria in 2010 for NSC, or in 2012 for NMO, Macedonian raw milk reaches the standards governing the quality of raw milk in EU countries. The total number of microorganisms and somatic cells in raw milk depends on a variety of factors. To reach the required criteria of the new rules, it is necessary to understand the factors that have the greatest impact on NMO and NSC. Somatic cells are a normal part of milk as its natural ingredient and in a healthy udderthey do not affect its composition and physical-chemical properties. They mainly consist of udder epithelial cells and cells originating from the blood (leukocytes). Of the total number 70% are udder epithelial cells (skin, alveoli, milk ducts and milk cistern), and the remaining 30% is composed of blood cells and other cells. The normal number of somatic cells depends on several factors, but in a healthy udder the average is 20,000 cfu/ml.

The high number of somatic cells (SCC) present in milk is the main indicator of mammary glands infection, caused by specific and non-specific types of microorganisms that cause mastitis.

Milk is a product of animal origin with high nutritional value, synthesized by special cells of the mammary glands, which are almost sterile when secreted by the alveoli of the udder. Normally, in milk from healthy mammary gland it is less than 100,000 cells/ml, while the bacterial infection can cause it to grow to over 1.000.000/ml.The high number of somatic cells in milk has a negative impact on the quality of raw milk. Subclinical mastitis is always associated with low milk production, changes in the consistency of milk (density), reduction of the possibility for proper processing of milk, low protein and high risk hygiene of milk, which may even contain pathogenic organisms. The biggest negative impact from the presence of somatic cells is associated with shorter time and less sensory content or undesirable organoleptic characteristics of the final enzyme activity because of somatic cells (Fernandes, A. etall.,2004.)

Any increase in the number of somatic cells above 250,000 or 300,000 in milk/ml is considered as an indicator of mastitis.

In studying the pathology of the milk gland and diagnosis of infected udder quarters determining the number of somatic cells milk is used. This parameter is considered the best indicator of the health of the milk gland. Indealing with mastitis the identification and classification of pathogenic microorganisms and further determination of antibiotic minimum inhibitory concentrations (MIC) for each isolate individually are applied. Apart from somatic cells, in milk from healthy cows a certain small number of microorganisms is normally found. Milk is synthesized in specialized cells in the milk gland and it is almost sterile when excreted from the alveoli into the milk cistern of the udder. That number is normally less than 1000

cfu/ml. Healthy udder has little or no influence on the total number of bacteria in the aggregate milk, whereas with cows with mastitis there is a risk of spreading a great number of microorganisms in the milk supply. In healthy cows, mammary duct, milk cistern and the top of boskata occeara can be colonized by a variety of microorganisms, but with good hygienic practice before milking they have little impact on both the total number of microorganisms in aggregate milk, and on the potential for growth in the number of microorganisms during the cooling phase. The natural micro flora generally has little impact on the number of microorganisms in milk. But the case with cows having mastitis is quite different. The impact of mastitis on the total number of microorganisms depends on the type of organism that caused the infection, the stage of infection and the percentage of infected cows. With infected cows the BMO in milk can amount up to 107 bacteria per ml.

Mastitisischaracterizedbyanincreasednumberofinflammatorycells, and SCC inmilkisusedasanindirectmeasureofthedegreeoftheudderhealth. Thesinglemostimportantfactoraffecting SCC inmilkismammaryglandinfectionandallotherfactorssuchasage, stageoflactation, weatherareofminorimportance(Eberhart*etal.*, 1979; Reneau, 1986). Kitchen (1981) commentedthatuseof SCC todiagnoseudderdiseasewasthefirstwidelyusedscreeningprocedureandeventodayithasretaineditspo sitionasthemostreliableandspecifictestformastitisdiagnosis.

The number of microorganisms is also influenced by cleaning and sanitation of the equipment. The cleannessof the milking system affects the total number of bacteria in milk. The remains of milk on the equipment milk comes into contact with support the growth of various microorganisms. The organisms that are considered natural micro flora on and in the udder do not grow significantly in raw milk during the cooling phase of milk. They mainly consist of instigators of contagious mastitis (*e.g.Staphilococcusagalactiae*) and some strains (*e.g. Coliform*) associated with environmental mastitis. The water used on the farm can also be a source of microorganisms, especially psychotropic, contaminating the surfaces of equipment and milk.

Theinflammationofthemammaryglandcanbecharacterizedbyanincreasein SCC. Thistraitisusedasanindicatorofudderhealthformanagementandselectionpurposes (Rodriguez*etal.*, 2000). SCC valueshigherthan 283,000 cell/mlindicatethepresenceofmastitis (Guidry, 1985; Reneau, 1986). Ithasalsobeenpointedoutthat SCC isalwayscomparedwithbacteriology, andthesetestscanneveragreecompletely. Recently, a SCC limitof 100,000 cells/mlissuggestedfor a healthyquarter (Hillerton, 1999) andSCC forthecompositemilkfrom a cowshouldnotexceed 100,000 cells/ml (Kromker*etal.*, 2001).

The temperature and time of storage of milk have a major impact on the number of microorganisms. Cooling prevents the proliferation of mesophilic bacteria while at the same time increases the number of psychrophilicmicroorganisms. Psychrophilic microorganisms enter the milk from the unclean udder, impure environment and contaminated equipment. Storing milk at a temperature of  $7.2^{\circ}$ C allows much faster growth of microorganisms compared with milk stored at  $4.0^{\circ}$ C.

#### MATERIALS AND METHODS

To determine the BMO 2065 samples were used, and for determining NSC1625 raw cow's milk taken from farms on territory of the Republic of Macedonia. Samples were delivered in sealed sterile plastic cups with a capacity of 50 ml to which the preservative Azidiol (Sigma-Aldriks) in the amount of 0.25 ml was previously added, and they were transported at a temperature of  $+4.0^{\circ}$ C to the laboratory for the quality of raw milk at the Faculty of Veterinary Medicine in Skopje.From reception to the beginning of the analysis the samples were stored at  $+4.0^{\circ}$ C. A common practice is to test samples at most 72 hours after taking them from subcontractors. Before starting the counting of somatic cells, the samples were heated to a temperature of  $40.0^{\circ}$ C for 15 minutes and they were analyzed twice on camera Fossomatik 6000. For counting the total number of microorganisms Baktoskan 8000 was used. The procedure for counting the NSC was performed in accordance with the accredited method ISO 17025 -FVM-SOP-398 by references ISO 13366-2:2006/.

# **RESULTS AND DISCUSSION**

## Number of somatic cells

Only 704 samples or 42.79 % of the samples met the criteria of the European Union (Directive 92/46 EEC) regarding the maximum number of somatic cells in milk. From the results of counting the somatic cells it can be found that 682, or 41.45 % of samples have over 600.001 somatic cells per ml which means that they do not meet the criteria of the Rulebook for 2008.936 samples or 58,54 % meet criteria for raw milk for 2008. The results for number of somatic cells are displayed in table 1 and graph 1. The reasons for such high number of somatic cells can be found in poor control of udder health of milking cows and irregular management of the herd.

Table 1. Results obtained fornumber of somatic cells, and the number of samples by categories according to the Rulebook

Year	NSC,ml	Number of samples	%
Outside the Rulebook	Above 600,001	682	41,45
2008	500,000 up to	131	7,96
	600,000		
2009	400,000 up to	128	7,78
	500,000		
2010	Up to 400,000	704	42,79
	Total	1645	99,98



Graph 1. The share of milk samples according to the number of somatic cells given in theRulebook expressed in percentage

Table 2 and Graph 2 represent the percentage of NSCof milk that meets the criteria under the Directive 92/46 EEC and the Rulebook of Veterinary Administration. The high percentage of samples that do not meet the criteria to 400,000scc/ml is a datum that speaks of inadequate control of milk gland. Also the failure to diagnose subclinical mastitis may be an additional reason.

Bacterial inflammation of the milk gland can be a source of pathogens and their toxins in milk that may pose a health hazard to consumers.

Table	2.PercentageofmilksamplescorrespondingtoDirective	92/46	EEC
intermsofnumber of somatic cells			

	Number of somatic cells	%
EU milk does not meet the criteria	0-400.000	42.79
Council Directive 92/46 EEC	400.001	57.21





## Number of microorganisms

From the table it can be concluded that only 221 of the total 2065 tested samples or 10.7 % met the criteria of the European Union (Directive 92/46 EEC) relative to NMO.

Over 800.001 cfu/ml were found in 1207 samples or 58.45%, which means that they do not meet the criteria for raw milk in determined by the Rulebook for 2008. The data processing for BMO showed that there was a very small number of samples that would meet the criteria set for the next four years.

Table 3. Results obtained for NMOand the number of samples by categories according to the Rulebook

Year	Number of microorganisms	Number of samples	%
Вон правилникот	Above 800,001	1207	58,45
2008	Up to 800,000	125	6,19
2009	Up to 600,000	156	7,55
2010	Up to 400,000	190	9,2
2011	Up to200,000	166	8,03
2012	Up to 100,000	221	10,7
	Total	2065	100,12



Graph 3. The share of milk samples according to the number of microorganisms given in the Rulebook expressed as a percentage

Table 4 andGraph 4 represent the percentage terms for NMOof milk that meets the criteria under Directive 92/46 EEC.

Table 4. Percentage of milk samples corresponding to Directive 92/46 EEC in relation to NMO

	NMO	%
EU milk	0-100.000	10.7
Does not fulfill the criteria of	100.001 and more	89,3
the Council Directive 92/46		
EEC		

It is notable that only about 10% of the samples meet the European criteria for the content of microorganisms in 1 ml which allows up to 100,000 microorganisms in a milliliter. The number of microorganisms presents not only makes the milk unfit for processing into dairy products, but it can also be a danger to the safety of milk and dairy products.



Graph 4. Percentage of milk samples corresponding to Directive 92/46 EEC in relation to NMO

BMO is directly dependent on the initial contamination of milk with microorganisms from the milk gland and microorganisms (contaminants) from the environment (e.g.placenta, urine, faeces) that in a significant number can populate the surfaces of milking equipment and surfaces with which milk comes in contact. Minimizing the level of contamination of the milk from these sources would significantly prevent the level of psychrotropic microorganisms. Thus from the point of keeping milk in the farm milk freezer, transporting and storing it in the farm tank, until the beginning of the processing, the number of microorganisms present in milk is kept as low as possible. Accordingly, the number of somatic cells and microorganismsmostly depend on the herd management, elimination of mastitis, milking hygiene and cooling of the milk from the milk freezer to the dairy.

## CONCLUSIONS

**1**. Only 704 (42.79 %) of the tested samples of cow milk meet the criteria of Directive 92/46 EEC and the Rulebook for specific security requirements and hygiene and the manner and performance of official controls of milk and dairy products Official Gazette of RM 157/2007.

#### The number of microorganisms%

EU milk 0 - 100,000, or 10,7do not meet the criteria of the Directive 100,001 and above 89,392/46 EEC and the Rulebook for specific security requirements and hygiene, and the manner

and performance of official controls of milk and dairy products Official Gazette of RM 157/2007.

2. Only 682 samples (41.45%) have over 600.001 somatic cells per ml, which that they means do not match the criteria of the Rulebook for specific security requirements and hygiene and the manner and performance of official controls of milk and dairy products Official Gazette of RM 157/2007 for 2008.

3. Only 221 (10,7%) samples of the examined cow milk met the criteria of the Directive 92/46 EEC in terms of the number of microorganisms in milk.

4. 1207 samples (58.45%) had more than 800.001 cfu/ml, which means that they do not meet the criteria for raw milk in determined in the Rulebook for specific security requirements and hygiene and the manner and performance of official controls of milk and dairy products Official Gazette of RM 157/2007 for 2008.

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