PRESENCE OF SOMATIC CELLS AND HETEROTROPHIC BACTERIA IN MILK, INDICATOR FOR ASSESSING ANIMAL HEALTH AND MILK QUALITY

Klementina PUTO
Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania
Ermelinda NEXHIPI
Institute of Food and Veterinary Safety, Tirana, Albania
Linda LUARASI and Anida BASHURI
Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

---

ABSTRACT

The safety/ quality of milk and dairy products, which is delineated by a series of challenges, has been one of the major societal concerns. It unavoidably requires continuous investments**.** The increase of investments in small farms, livestock complexes and new diary processing plants in 2019 led to an increase by 0.49% of milk production when compared to 2018, unavoidably affecting nutritional values and its quality. Low bacterial counts and low somatic cell counts are the key indicators of milk quality, and as their numbers increase, there is a higher risk for contamination of milk and cheese with pathogens. Their increased number indicates the presence of pathogenic microorganisms and negatively affects the quality and technological performance of milk. The paper identifies the amount of bacterial microflora and the number of somatic cells (SCC) present in milk for the period January - June 2020. A total of 50 milk samples collected from the milk provided by the farms in the Tirana region were analyzed for the presence of SCC and heterotrophic microorganisms in milk, and the results showed high levels of SCC (38%) and heterotrophic microorganisms (45%). The European Community Directives 92/46 and 92/47 (European Economic Community, 1992) define regulations that established hygienic standards for raw milk collection and transport that focus on issues, such as temperature, sanitation, and microbiological standards, enabling the production of raw milk of the highest possible quality. Cow milk shall conform to the following standards: a standard plate count at 30°C of <100,000 cfu/mL and somatic cell counts of ≤400,000 $mL^{-1}$of milk. For the heterotrophic microorganisms the permissible levels are < 10^5. Dairy producers are responsible for the safety of their products and

must guarantee food safety through the implementation of Hazard Analysis Critical Control Point (HACCP).
Keywords: heterotrophic bacteria, milk, number of somatic cells (SCC), public health

1. INTRODUCTION

Milk is a nutrient-rich liquid food produced mammals. It is the primary source of nutrition for young mammals (including breastfed human infants) before they are able to digest solid food. Early-lactation milk, which is called colostrum, contains antibodies that strengthen the immune system, and thus reduces the risk of many diseases. Milk contains many other nutrients, including protein and lactose. It must provide the necessary nutritional values and meet the requirements on food quality. From milking to its final use, milk is subjected to various physical, enzymatic and thermal treatments, which ensure its preservation.

Producers are responsible for the safety of their products and must guarantee the food safety of dairy products, for which the dairy industries are subject to the Hazard Analysis Critical Control Point (HACCP). This allows for the quality of the final products through a control of the entire product management chain. The quality and safety of raw milk is essential for the quality and safety of milk and dairy products. The quality and safety of milk is related to the contamination of milk with microorganisms, chemical residues and other contaminants.

Livestock is a very important agro-food sector in Albania. About half of the farmers are engaged in livestock, including the dairy sector. Dairy products occupy an important part in the consumer basket of Albanian households. Milk production is dominated mainly by cow's milk (more than 4/5).

Albania faces serious problems in the national food safety control system in terms of legislation, infrastructure, institutional capacity, control and law enforcement, which affect the real and perceived safety risks for consumers.

Imami *et al*. (2011) and Verçuni *et al*. (2016) stated that food safety a major concern for Albanian consumers.

Food safety and consumers' health is at the heart of Albanian government's policies (https://sane27.com/wp-content/uploads/Law-no.9863-of-28.01.2008.pdf.). The law sets out the government's requirements to be met by food chain operators to ensure food safety and quality for humans and animals, to some extent in line with EU provisions.

The International Dairy Federation Report (1980) clearly states the importance of monitoring the key indicators that ensure a high-quality product.

Dairy products are generally destined for the domestic market, so the increase in production is mainly driven by the increase in domestic demand.

But most farmers do not have enough knowledge about the microbiological content and quality of raw milk. Although most farmers state that they have a register of farm animals, they do not know which institution is responsible for their control (Zhllima *et al*. 2015).

Dairy farms with a small number of cows usually milk the cows by hand. Only farms whose main activity is milk production (normally with more than six cows) have started to buy simple milking machines. When a farm has more than 50 cows, it usually invests in a milking parlor, milk storage tanks and cooling systems (Verçuni *et al*. 2016).

The data here reported are based on the information obtained from the interviews. Hygienic conditions and the quality of raw milk remain of great concern. Milk is mostly provided by small farms; more than 59.2% of which have one cow and considerable problems with dairy standards. From all the farms that have up to 20 cows, with a total of 94,481 cows, individual farms produced 28.8% of the amount of milk (INSTAT 2017 - data published by the agricultural census).

The organization system (fragmentation) is the source of bad sanitary conditions which unavoidably affects the quality and quantity of milk production. Milking doesn't occur in milking barns, and containers used for milking, storing and transporting milk are substandard. In addition to the organization system, lack of knowledge, information and skills on dairy hygiene, breast health, milking techniques, storage and cooling, affects the quality standards.

Given the aforementioned situation, analyzing somatic cell count (SCC) as a basic indicator of cow health and milk quality is of great concern. Leukocytes are somatic mammary cells and depend on the intensity of cellular immune defense. Some of the cells derive from the mammary pathways. Mastitis, one of the most common diseases of dairy cows, might cause significant economic losses to dairy farmers. The number of somatic cells in milk has been accepted as the world standard for the diagnosis of mastitis (Dairy Federation Report 148A, 1995).

Regulation no 853/2004 of the European Parliament and of the Council lays down specific hygiene rules for food of animal origin. The increased number of SCC shows the presence of pathogenic microorganisms which unavoidably affect both quality and technology of milk production.

SCC is defined as the number of cells per ml of milk. In general terms:

- A cow of 100,000 SCC or less indicates an uninfected cow, where there is no significant production loss due to subclinical mastitis.

- A SCC threshold of 200,000 would determine if a cow is infected with mastitis. With a SCC greater than 200,000 they are more likely to become infected in at least 25%.
- Cows infected with significant pathogens have an SCC of 300,000 or higher.
- Milk with a value of 400,000 or more is estimated to be unfit for human consumption.

SCCs vary, however, due to many factors, including seasonal and management effects. Dairy farmers are financially rewarded if they have low SCCs and are penalized for high ones.

Table 1. Impact of somatic cells on the reduction of milk production, according to the standards of National Mastitis Council, US (NMC Standard)

Number of somatic cells	Coefficient	Losses in %
200,000	1	0
200,000-500,000	0.94	6
500,000-1,000,000	0.82	18
1,000,000-1,500,000	0.71	29

Good-quality raw milk is required to make good-quality dairy products. Once raw milk is defective, it cannot be improved during processing, and defects often become more pronounced. Therefore, it is important that raw milk be produced and handled from farm to plant under conditions that do not reduce its quality or, consequently, the quality of the product. Many factors can influence the quality of raw milk.

The present study overviews freshness, quality, safety and naturalness of milk by analyzing the quality and quantity of bacterial microflora based on public health safety standards. In addition, it reports about the effect of somatic cell count (SCC) which affects milk yield and composition (National Mastitis Council).

2. MATERIALS AND METHODS

Basically, somatic cell count (SCC) indicates milk quality. Lower the SCC, higher the milk quality and vice versa. The present study reports about the presence of somatic cells and heterotrophic bacteria in milk as a means to address animal health and milk quality and the impact on human health as based on the horizontal method ISO 4833: 2003 at 30^{o}C. The investigation was carried out at the Institute of Food Safety and Veterinary (IFSV).

A total of 50 milk samples were collected from milk obtained from the farms in the Tirana region and analyzed for mesophilic loads and the total number of somatic cells to investigate the safety of the milk.

The horizontal method was applied as defined by the ISO 4833-1: 2013 for the counting of microorganisms that are able to grow and form colonies in a solid environment once aerobic incubation at 30°C was applied to determine the total microbial number. The method is also applicable to the products that require a reliable count when a low detection limit is specified (below 10^2 / ml for liquid samples).

Plate Count Agar (PCA) was employed to determine the total number of live, aerobic bacteria in a sample. The number of bacteria is expressed as colony forming units per ml (CFU / ml) in liquid samples. Here, the pouring plate technique would be recommended. The samples were diluted and the appropriate dilutions added to the Petri dishes. Sterile melted agar is added to these dishes. Once the sterile melted agar is added to the dishes, the dishes were incubated at 20 or 30°C for three days. Subsequently, the number of colonies is calculated on the plate with 25-250 colonies, which is considered to give the most accurate result.

The following formula was employed for the calculation:

$$N = \frac{\Sigma C}{V \times [n_1 + (n_1 + 0.1n_2)] \times d}$$

where:

- ∑C is the sum of the colonies numbered in all dishes obtained from two successive dilutions at least where one of them contains at least 15 colonies
- V is the volume of inoculate, in milliliters, cast on each plate
- n_1 is the number of dishes taken in the first dilution
- n_2 is the number of dishes in the second dilution
- d is the dilution factor corresponding to the first dilution obtained [d = 1 in cases (liquid products) when the test sample is inoculated directly].

Microbiological samples of milk were classified based on the quality criteria applied. The criteria of <100000 col/ml for quality premium rate and > 100000 col/ml for milk unsuitable for processing at the industrial level are set in accordance with the requirements of Regulation no. 853/2004.

Microscopic technique involving the ISO 13366-1: 2008 was used for the somatic cell count. Laboratory milk samples are stored at 4°C and analyzed within 6 hours after the arrival at the laboratory.

Initially, the sample is heated in the bath for a short time at 40°C, left at room temperature and then diluted with PSB (Phosphate buffer solution)

which were recorded. 0.1 ml of the prepared samples was taken and all the spaces of the marked surface of the blade were carefully filled. The inoculated blades are completely dried and then immersed in the Newman Stain Solution dye where they are left for 15 minutes. Once rinsed, the count was performed under a microscope (Fig. 1). The following formula was used for the interpretation of the results:

$$C = fwx\left[\frac{N_t}{N_b} \times \frac{1}{d}\right]$$

where:

- C - number of somatic cells
- f - displacement factor
- wx - track width in mm
- Nt- total number of numbered cell nuclei
- Nb- total number of transits
- d- dilution factor

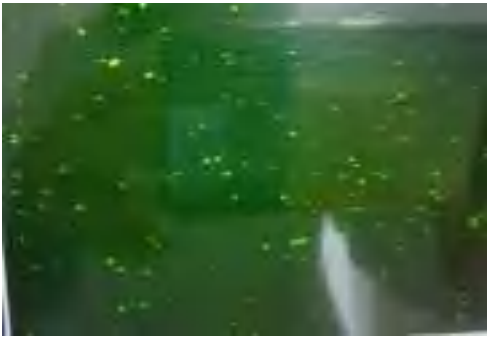

Fig. 1: View of somatic cells

3. RESULTS AND DISCUSSIONS

Monitoring the total number of bacteria at 30°C is necessary for the hygienic and sanitary quality of milk. The current EU regulation requires MAB calculations to be below 100,000 CFU/mL (Regulation EC 853/2004), while EU standards by food business operators normally set a stricter limit for the production of high-quality milk (30,000 CFU/mL).

The number of somatic cells in milk has been accepted as the world standard for the diagnosis of mastitis (International IDF Standard 148A, 1995). SCC is a key indicator of milk quality.

We note that the 50 samples analyzed for the presence of heterotrophic bacteria and the number of somatic cells were from fresh milk of individual cows (Figure 2 and 3).

The mesophilic bacteria were analyzed as based on the Regulation 853/2004 for product safety as it defines the quality criteria of bacterial colonies per ml of milk, and information about the load of mesophilic bacteria for all the samples analyzed are in the Table 2 reported. Exceeding values could be clearly noted.

Table 2. Number of mesophilic bacteria load colonies in milk and number of somatic cells in milk

Number of samples	Number of mesophilic bacteria, rate 100 000	Somatic cells, rate 400000
1	210 000	2 209 000
2	19 000	1 702 000
3	25 000	449 000
4	800 000	222 000
5	60 000	56 000
6	150 000	13 000
7	65 000	134 000
8	600 000	74 000
9	17 000	254 000
10	78 000	17 000
11	120 000	300 000
12	100 000	43 000
13	18 000	43 000
14	3 500	220 000
15	52 000	434 000
16	10 000	66 000
17	270 000	76 000
18	16 000	90 000
19	350 000	730 000
20	4 600 000	357 000
21	35 000	15 000
22	250 000	1 712 000

23	2 200 000	2 100 000
24	27 000	2 200
25	5 500	436 000
26	4 800	430 000
27	4 000	310 000
28	26 000	684 000
29	4 700	332 000
30	17 000	315 000
31	98 000	414 000
32	74 000	358 000
33	123 000	158 000
34	5 300	198 000
35	7 200	279 000
36	10 000	498 000
37	45 000	520 000
38	38 000	230 000
39	65 000	100 000
40	100 000	174 000
41	55 000	50 000
42	33 000	1 000 000
43	99 000	453 000
44	230 000	330 000
45	24 000	231 000
46	32 000	110 000
47	7 500	98 000
48	24 000	76 000
49	6 500	65 100
50	8 400	

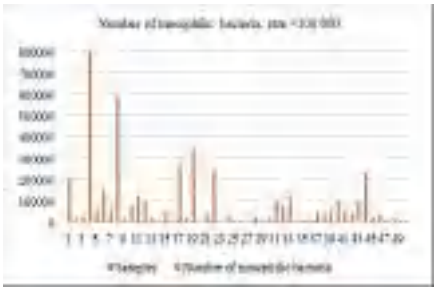

Fig. 2: Number of mesophilic bacteria

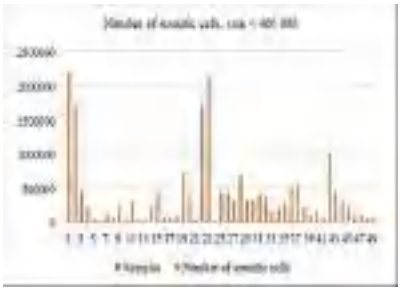

Fig. 3: Number of somatic cells

Regulation no. 853/2004 defines <100000 col/ml as a quality premium rate, and >100000 col/ml for industrially unsuitable milk (Table 3).

Table 3. Bacteriological standards of fresh milk

CFU/ml	ASSESSMENT
Not more than 100,000 col/ml	Acceptable
Over 100 000 col/ml	Not acceptable

About 72% of the analyzed samples meet the microbiological criteria and are safe for consumption. The Figure 4 depicts the test results for all the milk samples.

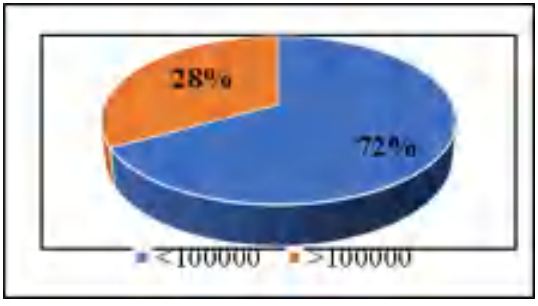

Fig. 4: % e mesophilic bacteria according the norms (rate < 100 000 col/ml)

Results on somatic cell count

A somatic cell count (SCC) is a cell count of somatic cells in a fluid specimen, usually milk. In dairying, the SCC is an indicator of the quality of milk—specifically, its low likeliness to contain harmful bacteria, and thus its high food safety.

An increase in SCC causes a decrease in milk yield and affects milk composition, which leads to reduced production of dairy products.

SCCs vary due to many factors, including seasonal effects and management. Milk with an SCC of more than 400,000 is deemed unfit for human consumption by the European Union. A particularly low SCC is sometimes considered a sign of a weak immune response, but in general terms this does not necessarily have to be true; it may happen that there is simply a low level of current infection. The immune response is best measured by how quickly the immune system responds to the challenges of the disease; not how many white blood cells are present before infection occurs.

High SCC is not only associated with udder health and milk losses, but also negatively affects the longevity (Sewalem *et al*., 2006) and fertility (Rekik *et al*.,2008) of dairy cows.

A low SCC means increased income from more milk, increased quality premiums, and decreased mastitis costs.

Table 4. Number of colonies with somatic cells according to certain norms

	Number of samples	**< 400000 col/ml**	**> 400000 col/ml**
Milk	50	35	15

As it could be noted from the Table 4, 35 samples out of 50 resulted in a number of somatic cells ranging from 13000 colonies/ml to 400000 colonies/ml—70% of the analyzed samples meeting the microbiological criteria. The reminder did not meet the criteria. The values are quite variable, showing in some cases considerable exceedance of the permissible values. The milk provided by individual farmers is of poor quality and dangerous for human consumption. Milk becomes a source of infections if overloaded with bacterial microflora and the number of somatic cells.

4. CONCLUSION

The following conclusions could be drawn: i) milk produced by the individual farmers is of poor quality; not only dangerous for human consumption, but also a source of milk-borne infections, ii) 72% of the cases resulted with heterotrophic bacteria load less than 100000 col / ml, iii) 70% of the cases resulted with somatic cell counts less than 400000 col/ml, iv) 28% of the cases resulted in a load of heterotrophic bacteria greater than 100000 col/ml and, v) 30% of the cases resulted with somatic cells count greater than 400000 col/ml.

5. RECOMMENDATIONS

The present paper highlights the importance and demand of estimating somatic cell count (SCC) and its effects on milk quality and human health. Dairy producers are responsible for the safety of their products and must guarantee food safety through the Hazard Analysis Critical Control Point (HACCP).

REFERENCES:

Committee on Infectious Diseases and Committee on Nutrition of the American Academy of Pediatrics. 2014. Consumption of raw or unpasteurized milk and milk products by pregnant women and children. *Pediatrics* **133**:175-179.

Imami D, Chan-Halbrendt C, Zhang Q, Zhllima E. 2011. Conjoint analysis of consumer preferences for lamb meat in central and southwest urban Albania. *International Food and Agribusiness Management Review*, **14(3).**

INSTAT. 2017. Baza e të dhënave www.instat.gov.al

International Dairy Federation Report (IDFR): 1980. Factors influencing the bacteriological quality of raw milk, International Dairy Federation, Document 120, Brussels.

International IDF Standard 148A: 1995. Milk enumeration of somatic cells. International Dairy Federation, Brussels, Belgium. International IDF Standard 141B: 1996. (1996). Whole milk: Determination of milk fat, protein and lactose content—guide for the operation of mid-infrared instruments.

ISO 13366-1:2008(en) Milk — Enumeration of somatic cells

ISO 4833-1:2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique.

Law, *On food*, no. 98638, Albania, dated 28.01.2008.

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

Rekik B, Ajili N, Belhani H, Ben Gara A, Rouissi H. 2008. Effect of somatic cell count on milk and protein yields and female fertility in Tunisian Holstein dairy cows. *Livestock Science*, **116**:309–17.

Sewalem A, Miglior F, Kistemaker GJ, Van Doormaal BJ. 2006. Analysis of the relationship between somatic cell score and functional longevity in Canadian dairy cattle. *Journal of Dairy Science* (JDS); **89**:3609–14.

Verçuni A, Zhllima E, Imami, D, Bijo B, Hamiti X, Bicoku, Y. 2016. Analysis of consumer awareness and perceptions about food safety in Tirana, Albania. *Albanian Journal of Agricultural Sciences*, **15(1): 19**.

Zhllima E, Imami D, Canavari M. 2015. Consumer perceptions of food safety risk: Evidence from a segmentation study in Albania. *Journal of Integrative Agriculture*, **14(6):** 1142-1152.