CVJ CLUJ VETERINARY JOURNAL

Article

Presence of subclinical mastitis and economic losses in dairy farms in Serbia

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Abstract: The objective of the study was to determine the presence of subclinical mastitis in smallholder dairy farms in Serbia. We also aimed to show economic losses due to the presence of subclinical mastitis on the farms. The physical-chemical parameters of bulk tank milk samples were analysed: fat content, protein content, lactose and non-fat dry matter content. The total number of somatic cells ranged from 21000-4690000 in pooled milk samples. From 2020 to 2022, based on the number of somatic cells in the milk, there was a statistically significant ($p<0.05$) increase in the number of collective bulk tank milk samples that did not meet the requirements for Class I milk. Economic losses due to the reduction of milk quality 0.033 euro/l lead to economic losses of approximately 20 euro per cow every month due to the reduced price of milk based on the classification of milk into classes based on the number of somatic cells.

Keywords: bulk tank milk, cows, milk components, somatic cells, subclinical mastitis

Received: 02.07.2024
Accepted: 14.08.2024
Published: 12.09.2024

DOI: 10.52331/h2bspw41

1. Introduction

Control of raw milk from the bulk tank is essential to monitor the presence of subclinical and clinical mastitis on the farms. Subclinical mastitis is common in dairy cows and is mainly caused by infectious and non-infectious agents. Subclinical mastitis leads to a decrease in the amount of milk, a decrease in the production of milk components, and economic losses due to the decline in milk quality, manifested by an increase in the number of bacteria and somatic cells [1]. A higher somatic cell count is an indicator of udder health [2]. The number of somatic cell counts is an indicator of subclinical mastitis. Level of somatic cells in milk >250,000 cells/mL and indicates subclinical mastitis in herds [3]. Determining the number of somatic cells in collected milk can indicate the presence of subclinical mastitis and help timely detection of subclinical mastitis to prevent economic losses due to a reduction in the amount of milk and milk components altered [4]. National regulation of the Republic of Serbia for milk quality prescribes the following conditions for milk, based on the results of testing the quality of raw milk in an authorized laboratory. Raw cow's milk is, depending on the total number of microorganisms and somatic cells, classified into: Class I milk - contains up to 100,000 cfu/ml (colony forming unit per ml) of the total number of microorganisms and the total number of somatic cells up to 400,000/ml; cow's raw milk has at least 3.2% milk fat, has at least 3.8% protein, has at least 8.5% dry matter without fat; Class II milk - contains from 100,001 to 400,000 cfu/ml of the total number of microorganisms and the total number

of somatic cells up to 400,000/ml; Class III milk - contains more than 400,000 cfu/ml of the total number of microorganisms and the total number of somatic cells up to 400,000/ml [5] (Serbian Regulation of quality raw milk 106/2017). The appearance of subclinical mastitis is associated with changes in the contents of milk components such as milk protein, fat, lactose, fatty acid, and pH value [6]. Bulk tank milk with a high SCC (somatic cell count) affects the shelf life of milk products [7]. The presence of subclinical mastitis is associated with udder injuries, mastitis pathogens, defective milking equipment, and awareness of milkmen. The average contents of milk components in milk cows are as follows: protein 2.9-5.0%, milk fat 2.5-6.0%, lactose 3.6- 5.5%, minerals 0.6-0.9% and water 85.5%-89.5%. The main factors that affect the contents of milk components are breed, stage of lactation, diet, parity, age, health status, and occurrence of mastitis and metabolic diseases [6 ,8, 9]. The presence of inflammatory reactions in the udder might cause disorders in the contents of milk components [10, 11].

To our knowledge, there are no scientific reports on information presented on subclinical mastitis and milk quality from Serbian smallholder dairy farms in bulk tank milk analysis. The aim of this study was to determine the presence of subclinical mastitis based on the number of somatic cells which determine the milk quality of raw milk from bulk tank milk samples originating from Serbian smallholder dairy farms with a capacity of up 20 cows and from this, to determine the economic losses due to the lower price of raw milk due to a decrease in milk quality.

2. Materials and Methods

In total, 161 bulk tank milk samples were collected from 2020 to 2022 from smallholder's farms from three Republic of Serbia districts (Mačvanski, Kolubarski, and Sremski district). All farms were considered smallholder dairy farms, with an average of 16 Simmental or Holstein dairy cows in lactation and a milk yield average of 19.3kg/cow/day or approximately 5,886 kg/cow/year. Milk samples were taken from farms of up to 20 cows. Samples were collected from September to November during 2020, 2021 and 2022. The diets had similar contents: corn silage, alfalfa hay, and concentrate with about 16% protein in the meal. Samples were collected in 50 ml sterile screw-cap tubes. All analyses were performed within 24 h of collection at the farm. Physical-chemical parameters of milk were analysed: fat content, protein content, lactose and non-fat dry matter content (NFDM). Standard methods used were the acid butirometric method for fat content - SR EN ISO 1211:2010 (Funke DR.N-Gerber Labortechnik GmbH (Nova-Safety), the Kjeldahl method for protein content - SR EN ISO 8968-1-4:2016 using an automatic distillation unit Buchi K-350 (BUCHI Labrotechnik AG, Switzerland) and oven drying at 105°C for the total dry substance – SR ISO 6731:96 (ISCO, NTC 9000 (temperature range 25-300°C)). High-performance liquid chromatography (HPLC) was used to determine the lactose content in raw milk samples, using a Waters (Milford, USA) HPLC system, with a Waters 2414 refractive index detector (RID). A lactose standard was supplied by DR Ehrenstorfer (LGC, Germany) with a certified purity of 99.0%. A Fossomatic 5000, Foss-electric (Hillerød, Denmark) was used to determine the number of somatic cells in milk samples.

Milk quality parameters were analysed using a GraphPad® Prism®6 and SPSS ver. 22 software for descriptive statistic parameters and ANOVA using the Tukey post hoc test. For SCC and protein data, which were not normally distributed, Kruskal Wallis and Mann-Whitney post hoc nonparametric tests were conducted.

3. Results

A total of 161 bulk tank samples were evaluated for each the components milk fat, protein non-fat dry-matter and somatic cell counts of milk samples from Serbian smallholder farms. Contents of milk components in each year are shown in (Table 1).

Table 1. Contents of milk components in each year

Year	Parameter	Observation	Min.	Max.	Mean	Median	Std. Deviation	Coefficient of variation
2020	**Somatic cell count**	57	27000	688000	172456	132000	149890	86.9%
	Milk fat	57	3.2	7	4.08	3.9	0.683	16.7%
	Protein	57	2.65	3.86	3.34	3.34	0.281	8.42%

	NFDM	57	5.4	10	8.85	9.0	0.944	10.7%
2021	**Somatic cell**	44	31000	1429000	396614	320000	324285	81.8%
	Milk fat	44	2.4	7.8	3.93	3.62	1.15	29.2%
	Protein	44	2.74	4.04	3.25	3.14	0.3	9.23%
	NFDM	44	7.6	15	9.25	9.20	1.07	11.6%
2022	**Somatic cell**	60	21000	4690000	504350	248000	760752	151%
	Milk fat	60	2.4	7.9	4.45	4.25	1.35	30.3%
	Protein	60	2.92	3.99	3.22	3.20	0.248	7.69%
	NFDM	60	7.7	10	8.77	8.71	0.535	6.11%

The average content of milk fat, protein, NFDM and the number of somatic cells were 4.15%, 3.27%, 8.96% and 357.807 cells/ml, respectively, across the three years. The quality of milk based on the number of somatic cells in shown in (Table 2).

Table 2. Distribution of milk samples according to requirements milk quality

	2020	2020	2021	2021	2022	2022
0-400,000	54	33.5%	24	14.9%	36	22.4%
400,000–1,000,000	3	1.9%	18	11.2%	19	11.8%
>1,000,000	0	0.0%	2	1.2%	5	3.1%

During 2020 to 2022, it was found that on average 24.8% of herds had a number of somatic cells >400,000 cells/ml and so did not meet the requirements for class I milk quality. Based on the number of somatic cells in the milk, there was a significant ($p<0.05$) increase in the number of BTM (bulk tank mil) samples that did not meet the requirements for class I quality (Figure 1).

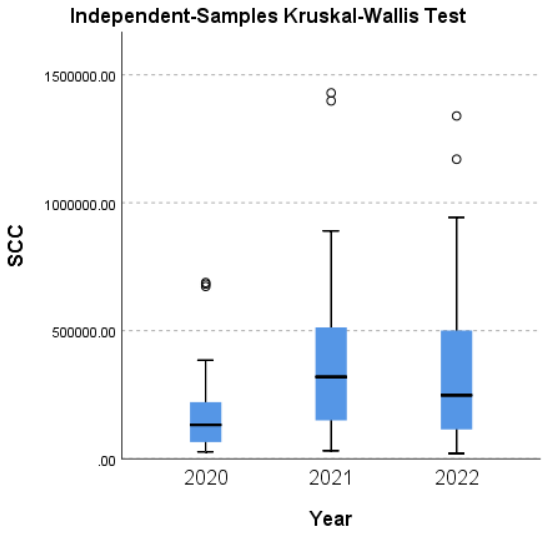

Figure 1: Somatic cell counts over the period 2020 - 2022 ($\bar{X}$±SD).

There was a drop class I quality BT samples from 33.5%, over 14.9%, to 22.4% in year 2020, 2021 and 2022 respectively. Analysis of milk components such as milk fat , NFDM and SCC showed a statistically significant differences in observed period $p<0.05$ (Table 3).

Table 3. Milk components analysis of bulk tank samples

Parameters	Period	Significant	Adjusted P Value
Somatic cell count	2020 vs. 2022	Yes	0.002
Somatic cell count	2020 vs. 2021	Yes	0.001
Milk fat	2021 vs. 2022	Yes	0.049
NFDM	2021 vs. 2022	Yes	0.015

4. Discussion

The appearance of subclinical mastitis is associated with an increase in the number of somatic cells in milk, which affects the quality of raw milk as well as the price of milk. The most common non-infectious cause of the increase in the number of somatic cells is pain, which can occur due to mechanical trauma, inadequate pressure in the milking machine, lameness, and the presence of other diseases that can affect the retention of milk in the mammary gland [12]. Many factors that affect the number of somatic cells include udder infection, stage of lactation, parity, individual response to the infection, inadequate microclimate conditions, milking equipment, metabolic disease, and lameness [13, 14]. Determining the level of SCC in bulk tank samples is a useful tool for monitoring udder health and detecting contagious mastitis agents such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis* [2, 15]. The increased somatic cell counts lead to reduced cheese production and affect the sustainability of cheese [16]. For manufacturing high-quality dairy products, raw milk must have an adequate composition (e.g., protein and fat levels), be free from unpleasant taste and smell, free from detectable drug residues, added water and have low SCC. In our study, the average bulk tank somatic cell count (BTSCC) from 2020 to 2022 was 339,536 cells/ml, and these somatic cell numbers are very similar to those of the study by [17]. The numbers of somatic cells obtained in our study are close to the threshold value of 400,000 cells/ml, indicating the presence of subclinical mastitis in these smallholder farms. However, the study by [18] reported that an average BTSCC was 521,583 cell/ml and indicated widespread udder health in Northern Cyprus. Our results are similar to [19] in Holland, which reported a BTSCC of 392,220 cells/ml. Many authors have reported an increased number of somatic cells associated with an increase in milk fat, protein and non-fat dry matter [4]. The presence of mastitis affects the yield of milk and the contents of milk components [8, 20]. However, compared with our results, [21] reported an average content of milk fat of 3.39%, protein content 3.13%, lactose 4.27%, non-fat dry matter 8.13% and 615,500 somatic cells/ml which were significantly lower values of the content of components, as well as a significantly higher number of somatic cells.

Economic losses due to reduced milk quality can be calculated on the basis of the chemical and hygienic composition of milk. In most dairies in Serbia, the price of raw milk is based on the classification of milk into classes [5], with a difference of about 4 dinars (0.033 euros) per 1L milk between classes based on the quality of raw milk. If all the milk production for small farms (up to 20 cows) was found to be in Class II instead of Class I, the average economic losses calculated on the basis of a yearly production of *ca* 6000 L would be 24,000 dinars (about 200 euros per year) or 16.67 euros per cow/month due to the increased number of somatic cells in milk, together with the economic cost of reduced milk production. Our study provides information on raw milk quality from bulk tank samples from smallholder farms in Serbia and is a measure of udder health to prevent the occurrence of subclinical mastitis and showed typical economic losses due to decreased milk quality. Determining bulk tank somatic cell numbers is the first step in detecting subclinical mastitis, after which the California mastitis

test would be important to detect simply and easily cows that have an increased numbers of somatic cells.

5. Conclusions

A reduction of milk quality worth 0.033 euro/L would lead to economic losses of nearly 20 € per cow on a monthly basis due to the reduced price of milk based on the classification of milk into classes according to the number of somatic cells and the content of milk components. This research shows the importance of surveillance of bulk tank milk to detect subclinical mastitis in farms and to prevent economic losses from lower milk prices due to reduced production of milk components, leading to reduced profitability.

Author Contributions: "Conceptualization and methodology, M.N.; software, N.Z.; formal analysis and investigation , A.T.; data curation, N.Z.; writing—original draft preparation, M.N.; writing—review and editing, N.Z and A.T.; All authors have read and agreed to the published version of the manuscript".

Funding: This research was funded by the Serbian Ministry of Science, Technological Development and Innovation, grant number. 451-03-66/2024-03/200030**.**

Institutional Review Board Statement: "Not applicable."

Conflicts of Interest: "The authors declare no conflict of interest."

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