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Research article

IMPROVEMENT OF HYGIENE PRACTICES AND MILK HYGIENE DUE TO SYSTEMATIC IMPLEMENTATION OF PREVENTIVE AND CORRECTIVE MEASURES

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The Total Bacterial Count (TBC) and the Somatic Cells Count (SCC) in the milk are important indicators of its hygiene and quality. Hygienic conditions in barns, milking procedures, udder hygiene before, during and after milking, milking machine hygiene, as well as milk cooling procedures immediately after milking, have direct or indirect influences on milk hygiene indicators. Poor results of milk hygiene quality, when it comes to SCC and TBC, which are often seen in dairy cattle farms in our country, indicate numerous omissions before and during milking. To determine the possibility of improving milk hygiene quality, training of extension service professionals and farmers was conducted, corrective and preventive measures were determined and the achieved state was monitored on 128 dairy farms where SCC and TBC were observed at regular monthly intervals for 6 months. The results showed a continuous statistically very significant improvement in hygienic conditions in barns, milking procedures, udder hygiene before, during and after milking, milking machine hygiene, as well as milk cooling procedures. This has contributed to a statistically very significant improvement in the hygienic quality of milk, both in SCC and TBC indicators, continuously from month to month, with a visible improvement at the end related to the beginning of the study period**.** At the begining and the end of survey 19.7% and 50.0% of milk samples belong to 1st class of milk quality, respectively, indicating a significant improvement after implementation of corrective measurements.

Key words: corrective and preventive practices, hygiene quality, milking practice, the somatic cells count, the total bacterial count

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INTRODUCTION

Important indicators of cow's milk hygiene quality are the Total Bacterial Count (TBC) and the Somatic Cells Count (SCC) [1-3]. The microorganisms from the barn bedding [4], contaminated parts of the cow's body (i.e., ventral abdomen, upper and lower parts of extremities, tail, udder and teats) [5,6], farmers hands and milking unit [7,8], and diseased udder quarters can reach the cow's milk [9-11]. On the surfaces of barns, cows bodies and udders, there are also pathogenic microorganisms from the environment, which enter the mammary gland through the teat sphincter, causing intramammary infections and as a result contribute to the increase of SCC and TBC in the milk [12,13]. Pathogenic microorganisms, also, can be transmitted during milking from one cow to another, most often to the next 6-8 cows [14-16]. It is a well-known fact that there is a connection between the cleanliness of the upper and lower parts of the extremities, ventral abdomen, tail, and especially the teats and udder of cows and the TBC in the milk [6,17-23]. The presence of dirt on these parts of the cow's body, as a consequence of inadequate barn hygiene, especially bedding, can cause the TBC in the milk to increase significantly [4,19,20]. The increase of the TBC can be additionally influenced by inappropriate hygienic procedures that are carried out before, during and immediately after milking cows [18,19,23-26], as well as during inadequate milk cooling procedures [27].

Despite the improvements made in many areas of the dairy industry, the ability to keep cows clean and to reduce the bacterial load at the teat end has been improved a little [26,27]. The literature emphasizes that farmers are often unaware of these connections and in practical conditions; as a consequence, not enough attention is often paid to the assessment of the contamination of the previously mentioned areas of the barn, body and udder of cows [4-7,14,17]. Important components hereof are the daily decision regarding mastitis control program with exception of good hygiene practices, as well as SCC and TBC decrease in cow's milk [15-17].

Based on the presented, the paper aimed to analyze the extent to which the TBC and SCC in the milk can be improved by a collaborative work of farmers and extension service professionals in assessing, defining and controlling the application of corrective and preventive hygienic measures related to the barn, cow's body and udders, milking procedures, as well as milk cooling procedures immediately after milking.

MATERIAL AND METHODS

The research was performed in compliance with the Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia, No. 41/09) and Ordinance on the conditions for registration for trial animals and the keeping of such a register, training programs on welfare for trial animals, request forms for approval for conducting experiments on animals, keeping, treatment and killing trial animals and reproduction, circulation, or implementation experiments on animals (Official Gazette of the Republic of Serbia, No. 39/10).

Farms selection and visits

A repeated cross-sectional longitudinal survey was carried out for hygiene evaluation on 128 cattle farms that are characterized with 10-15 dairy cows, the use of a milking machine and a lactofreeze. The farms were different regarding the systems and technology of rearing, milking procedures, size of the herd, hygiene and health management.

Farms were visited 6 times from February until July (designated as Controls) for assessment of hygiene and milking procedures corrections. Assessment was done by the scoring system described below. At the beginning, farms were evaluated and comprehensive recommendations were prepared for every production unit and training was done by extension service professionals based on a detailed consideration of the current situation.

Analysis of hygiene aspects before, during and after milking

The hygiene scoring was based on the methodologies applied by Reneau et al. [5], De Vries et al. [12], Cook and Reinemann [19], Kurwijila [28], Atasever et al. [29], and Relić and Hristov [30] with the necessary modifications following the current state of all hygiene aspects on farms.

The applied methodology included 65 hygiene indicators (In) grouped in the following groups: Group 1: hygiene of farm professionals; Group 2: hygiene of the barn; Group 3: hygiene of cows body and udder; Group 4: udder hygiene before, during and after milking; Group 5: milking procedures; Group 6: maintaining of on-farm milk processing equipment and cooling of milk. All indicators were scored in the range from 1 (the lowest score) to 5 (the highest score).

Milk sampling and analysis

Milk samples for SCC and TBC were taken on every farm control, in the morning and evening milking, and were analysed in certified laboratories in order to determine the class of milk quality (Rulebook on the quality of raw milk, "Official Gazette of RS", No. 106/17). The criteria for SCC were graded as excellent (5) for less than 200.000 somatic cells/mL, very good (4) for 200.001 - 300.000; good (3) 300.001 - 400.000; sufficient (2) 400.001 – 500.000; and insufficient (1) for more than 500.000 somatic cells/mL.

The criteria for TBC were defined as excellent (5) for less than 100.000 bacteria /mL, very good (4) 100.001-300.000; good (3) 300.001 - 500.000; sufficient (2) 500.001-1.000.000; and insufficient (1) for more than 1.000.000 bacteria/mL. Milk quality is presented as percentage of milk samples belong to 1st class according to Regulations (106/17) after first and last farm control during the survey period that presents an indicator of the efficacy of the extension service professionals advisory activities.

Corrective and preventive measures

Based on the results obtained after the first visit and farm scoring, recommendations for a set of efficient corrective and preventive measures were prepared by extension service professionals; proposed measures application for hygienic and sanitary conditions improvement before, during and after milking was insured through instructions of extension service professionals and collaborative activities of extension service professionals and farmers.

Statistical analysis

Statistical analyses were conducted in SPSS for Windows. The correlations between variables in the model were calculated by Pearson's coefficient of correlation. Data analysis was carried out by General Linear Model (GLM) repeated measures; applied univariate approach (split-plot or mixed-model approach) considers the dependent variables as responses to the levels of farms visits (Control) as within-subjects factors. The validity of the F statistic used in the univariate approach was assured as the variance-covariance matrix was circular. To test this, Mauchly's test was used, testing the sphericity on the variance-covariance matrix of an orthonormalized transformed dependent variable; since the statistical significance of the test is large, the hypothesis of sphericity was assumed. LSD test was used as a *post-hoc* test for pairwise comparisons of differences in mean values of hygiene scoring. The significance was evaluated on levels $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

The mean values with their variations presented as mean standard error for hygiene scoring during the survey are shown in Table 1.

Table 1. Descriptive statistics for mean values with standard $(\bar{x} \pm S_{\bar{x}})$ error of hygiene scoring depending on the farm controls, group of measures, SCC and TBC

Measure: Hygiene evaluation			
Control	$(\bar{x} \pm S_{\bar{x}})$	**Group of measures**	$(\bar{x} \pm S_{\bar{x}})$
1	3.247±0.027	1	3.126±0.050
2	3.388±0.026	2	3.670±0.033
3	3.489±0.026	3	3.282±0.021
4	3.779±0.025	4	3.882±0.030
5	3.933±0.025	5	3.687±0.035
6	3.986±0.022	6	4.058±0.032
Overall	3.637±0.021	SCC	3.537±0.100
		TBC	3.854±0.100

It can be assumed that the mean value for hygiene scoring was slightly increased from the first (3.247±0.027) to the sixth farm control (3.986±0.022). The score mean

value is higher at the end of the observation period compared to the time before the introduction of corrective and preventive measures. The overall mean value of hygiene scoring for all dairy farms included in the survey was 3.637±0.021. The highest mean score was recorded for Group 6 (scoring for maintaining on-farm milk processing equipment and cooling procedures of milk), while the lowest mean score was recorded for hygiene measures of Group 1 (scoring for the hygiene of farmers). The mean values for milk hygiene scoring were 3.537±0.100 for SCC scoring and 3.854±0.100 for TBC scoring (score related to range from 10^5-5x10^5).

Estimation of interdependence between controls as dependent variables included in the GLM repeated measures were performed with Pearson's coefficient of correlation, as shown in Table 2.

Table 2. Pearson's coefficient of correlation between dependent variables as repeated measures included in the statistical analysis

Pearson's	Control 2	Control 3	Control 4	Control 5	Control 6
Control 1	0.835**	0.739**	0.569**	0.448**	0.314**
Control 2	1	0.830**	0.652**	0.500**	0.383**
Control 3		1	0.735**	0.532**	0.388**
Control 4			1	0.671**	0.494**
Control 5				1	0.448**

** Correlation is significant at the $p<0.01$ level

It can be assumed that a high statistically positive correlation exists between the levels of within-subjects factors is reflecting the improvement of repeated hygiene measures. The correlation between two subsequent controls is stronger and the positive statistical significance showed the trend of weakening on every subsequent level of control compared to the previous control level, regarding the first level control. This means that implementation of corrective and preventive measures for improvement of hygiene practices and milk hygiene is a progressive process until the final reach of the desired improved hygiene level and therefore a strong positive correlation exists between two consecutive controls.

ANOVAs with repeated measures (within-subject factors) are particularly susceptible to the violation of the assumption of sphericity. Sphericity can be linked to the homogeneity of variances in a between-subjects ANOVA. Therefore, determining whether sphericity has been violated is very important. To test the data set for sphericity, Mauchly's Test of Sphericity was used (Table 3). Mauchly's Test of Sphericity in SPSS indicated statistically high significance that the assumption of sphericity had not been violated, $\chi^2=8027.775$, $p<0.001$.

Considering the data set that has been used for repeated measures GLM, the "Sphericity Assumed" showed that sphericity has not been violated during calculations of the significant value for within-subjects effects of control on hygiene measures ($F=366.537$, $p<0.001$); and also interaction between Control and Farm ($F=4.083$,

$p<0.001$); Control and Group of measures ($F=40.656$, $p<0.001$); and interaction between Control, Farm and Group of measures ($F=2.106$, $p<0.001$).

Table 3. Mauchly's Test of Sphericity

Measure: Hygiene evaluation				
Within Subjects Effect	**Mauchly's W**	**Approx. Chi-Square**	**df**	**Sig.**
Control	0.342	8027.775	14	p<0.001

The results from GLM repeated measures clearly indicated that Farm and Group of controlled measures (Scorings, SCC, TBC) highly significantly ($p<0.001$) affect the on-farm hygiene evaluation. Their interaction significant influence hygiene evaluation at level $p<0.01$ (Table 4).

Table 4. Between-Subjects effects on hygiene scoring in GLM repeated measures, univariate analysis

Measure: Hygiene evaluation				
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**
Intercept	237135.453	1	237135.453	31081.049***
Farm	1875.054	126	14.881	1.950***
Group of hygiene measures	4851.245	7	693.035	90.835***
Farm * Group of measures	7544.711	882	8.554	1.121**
Error	57168.467	7493	7.630	

***significant at the p<0.001 level; **significant at the p<0.01 level

Following the analysis of variance in repeated measures, significance between groups for mean values of hygiene scoring was estimated by the LSD test, as a *post-hoc* test for pairwise comparisons of differences in mean values of hygiene scoring (Table 5).

Table 5. LSD-test results for pairwise comparisons of differences in mean values of hygiene measures for within-subjects effects of controls

Control	**2**	**3**	**4**	**5**	**6**
1	0.141*	0.242*	0.532*	0.686*	0.739*
2		0.101*	0.391*	0.545*	0.598*
3			0.290*	0.443*	0.497*
4				0.154*	0.207*
5					0.053

*significant at the p<0.05 level

Regarding the Controls as dependent variables, only between Control 5 and Control 6 a significant difference does not exist. The general trend is that the last significant differences in score mean values are highest as the next number of control is far one from another one. Differences in mean values from hygiene scoring are greater with

every consecutive control, which again confirms the hypothesis that on-farm hygiene improvement is a progressive process.

Significant improvement after implementation of suggested measures could be seen from Table 6. that shows a precentage (%) of milk samples that belong to 1st class of quality according to regulations, at the begining and at the end of survey period. It could be seen that at the begining just 19.7% of samples belong to the 1st class while at the begining that share increased significantly to 50.0%.

Table 6. Percentage of milk samples which belong to the 1st class according to Regulative (106/2017) after the first and last control of farms during survey

Control	Milk quality	1st Class	2nd Class	Total
1	TBC*	19.7%	80.3%	100.00%
	SCC**	76.4%	23.6%	100.00%
6	TBC*	50.0%	50.0%	100.00%
	SCC**	87.9%	12.1%	100.00%

*1st class TBC ≤100.000/ml; 2nd class 100.001/ml – 400.000/ml;
**1st class ≤400.000/ml (score 3,4,5); 2nd class > 400.00/ml (score 1 and 2)

DISCUSSION

Improving the hygiene of barns, cows bodies and udders, which was achieved with the implementation of corrective and preventive measures, as well as applied methodology for assessment, through the cooperation and collaborative activities of extension service professionals and farmers, has contributed to the continuous reduction of SCC and TBC in the milk, and therefore significant improvement of milk quality. The observed changes in hygiene scores are mostly expected since they represent the implementation of the complete system of hygiene measures, which are in line with the literature data. The rate of new intra-mammary infections is related to the number of bacteria that the teat end is exposed to [1,2,4,6,7,13,31], and several studies have made associations between clean housing, clean cows and lower bulk tank somatic cell counts [1,3,5,12,13,22]. Thus, ensuring the cleanliness of farmers hands [7,31], various aspects of barn hygiene [5-7,12,14,32], different body parts of cows [11,14,21,22], udder hygiene before, during and after milking [8,18,23] and hygiene of milking machines and cooling procedures [27] are very important hygienic activities that contribute to SCC and TBC increase in the milk. Indeed, the occurrence of a completely wet and soiled floor is a risk factor for poor hygiene in the stalls and a potential source for mastitis pathogens to enter the udder through the teat orifice. It was found [12] that poor udder hygiene was associated with poor barn hygiene, longer lying was associated with poor upper leg/hip and udder hygiene. Also, a longer-standing time before milking is associated with poor udder hygiene [7,25,26] and lower parts of legs [12]. To decrease the splashing of manure on the udder, cows should not be rushed to and from the milking parlour or feed area, and alleys should be kept as

clean as possible. This will help minimize exposure to environmental bacteria, which are the primary mastitis pathogens on most dairies. The positive impact of proper cooling and other raw milk procedures was obvious from the obtained hygienic scores over time, which is confirmed by the literature data [27,33].

Somatic cells do not multiply in milk, so they are a very good indicator of udder health [3,5,13]. On the other hand, the microbiological quality expressed through the TBC value, directly depends on numerous factors such as the health condition of the udder, as well as hygiene before, during and after milking [1,3,5,12,13,22]. These findings suggest that environmental and management factors, such as cleanliness, bedding, and barn design, may be the most important factors contributing to udder health [9,15,16].

Not only in the conducted study, but also the attitude of farmers towards the undertaken hygienic and general biosafety procedures is widely recognized. The importance of staff training level for SCC, TBC and udder health was demonstrated [1,24-26,28].

In conclusion, it should be borne in mind that the differences in mean values from hygiene scoring were greater with each consecutive control, which confirms the hypothesis that on-farm hygiene improvement is a progressive process. It seems more likely that the ability to keep the barn and cows clean and to reduce the bacterial load at the teat end could increase milk hygiene quality. Control and training systems around the world deal with the same issues: training farmers how to handle raw milk, the hygiene of the cows and the milking, the udder health of the cows, the prevention of infections of the cows and the contamination of milk, and finally, the control of the cooling chain.

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Authors' contributions

LjM carried out the hygiene practices and milk hygiene practices studies, participated in preparation of preventive and corrective measures and drafted the manuscript. MC participated in the design of the study and performed the statistical analysis. DN participated in the design of hygiene practices and milk hygiene practices and performed the statistical analysis. BS participated in preparation of preventive and corrective measures and drafted the manuscript. JM participated in preparation of preventive and corrective measures. SH conceived of the study, and participated in its design and coordination and supervised the draft of manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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UNAPREĐENJE HIGIJENSKE PRAKSE I STANJA HIGIJENE MLEKA BAZIRANO NA SISTEMATSKOM SPROVOĐENJU PREVENTIVNIH I KOREKTIVNIH MERA

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Ukupan broj bakterija (TBC) i broj somatskih ćelija (SCC) u mleku su važni pokazatelji njegovog higijenskog kvaliteta. Higijenski uslovi u stajama, postupci muže, higijena vimena pre, za vreme i posle muže, higijena aparata za mužu, kao i postupci hlađenja mleka, neposredno nakon muže krave, direktno ili indirektno utiču na ove veoma važne pokazatelje. Loši rezultati higijenskog kvaliteta mleka, kada je reč o SCC i TBC, koji se često utvrđuju na farmama mlečnih goveda u našoj zemlji, ukazuju na brojne propuste muzača, posebno prilikom muže krava. U cilju utvrđivanja mogućnosti poboljšanja kvaliteta higijene mleka, sprovedena je obuka stručnih lica savetodavnih službi i muzača, utvrđene korektivne i preventivne mere i praćeno je postignuto stanje na 128 farmi na kojima su SCC i TBC praćeni u redovnim mesečnim intervalima tokom 6 meseci. Rezultati su pokazali kontinuirano statistički veoma značajno poboljšanje higijenskih uslova u stajama, postupaka muže, higijene vimena pre, tokom i posle muže, higijene aparata za mužu, kao i postupaka hlađenja mleka. Ovo je doprinelo statistički veoma značajnom poboljšanju higijenskog kvaliteta mleka, kako u SCC, tako i u TBC indikatorima, kontinuirano iz meseca u mesec, uz vidljivo poboljšanje na kraju u odnosu na početak perioda istraživanja.