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### THE IMPROVEMENT OF THE BODY AND UDDER HYGIENE INFLUENCE ON THE MILK SAFETY AND COMPOSITION ON SMALL DAIRY FARMS IN SERBIA

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Abstract: The hygienic condition of dairy cows and their udders in 128 small household farms in different regions of Serbia, producing mostly milk, having 5-15 cows, a milk cooler and at least one milking machine, was evaluated by the following indicators: 1. general assessment of body cleanliness, 2. visual inspection of teats and udder base, and 3. maintenance of udder cleanliness, rated on a scale of 0 and 1 or 1 to 5. The samples were taken at each visit to the farm after cooling and tested for milk protein and fat content, somatic cell count (SCC) and the total number of microorganisms (TNM). The milk fat content was determined by the Gerber method, the protein content by the Kjeldahl method, the TNM according to the ISO 4833-1:2013 method and the SCC using Fossomatic TM. The IBM SPSS program was used for statistical data processing. Capacity, housing system, breed, milking system, number of cows, and capacity occupancy mostly had a very significant or significant impact on hygiene parameters, protein and fat content, SCC and TNM in milk. The hygiene parameters showed a continuous trend of improvement in relation to the visits. TNM mean scores increased after each visit, with significant differences between visits (F=9.63, P<0.0001). SCC scores varied very significantly between visits (F=5.17, P<0.0001). The number of visits tended to show a significant influence on the milk fat rate (F=2.221; P<0.1), but no influence on the milk protein rate (P=0.480; F=0.901).

Key words: improvement, body, udder, hygiene, milk, safety, composition, small dairy farms.

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### Introduction

Milk production is an important financial resource for the survival and life of small farmers and their families. Today, it is increasingly important for them to produce milk and dairy products that meet food safety standards and to take advantage of the growing demand for their products.

Therefore, research often examines the relationship between the hygiene parameters of the farm, the cows and the milkers with the hygiene and milk composition. Success in milk production and processing practically depends on the adequate implementation of hygiene measures before and during milking and the procedures with the milk immediately after milking (Tamime, 2009; Oumer et al., 2017; Berge and Baars, 2020). These areas are closely related and are particularly evident in cows on small and medium-sized family farms.

The literature indicates that a clean cow body is an important factor in obtaining and maintaining milk quality (Schreiner and Ruegg, 2002; Sant'anna and da Costa, 2011; Robles et al., 2020). When cows lie on dirty bedding, feces, urine, soiled mats, and feed stick to the cow's body. During milking, this muck from the cow's body may easily get into the milking machines and milk. Regular removal of bedding and daily care of the cow's body may reduce the contamination of the cow's body and thus the milk contamination, especially on the parts of the body that are important for milking hygiene: hindquarters, udder, teats, abdomen, groin and extremities. A new mat should be added daily to keep the lying area clean and dry (Hristov, 2002; Relić and Hristov, 2016).

Maintaining animal health is extremely important for hygienic milk production (Schukken et al., 2003; Malik et al., 2018), whereby special attention should be paid to the occurrence of udder infections that lead to contamination of milk through the teats, and gastrointestinal infections increase the contamination of teat surfaces.

#### **Material and Methods**

During the study, the hygienic condition of dairy cows and their udders on farms was evaluated using a defined questionnaire, and sampled milk was tested for protein and milk fat content, SCC and the total number of microorganisms (TNM). On-farm examinations were performed three to six times, depending on whether on-farm hygiene measures needed to be corrected. The assessment and analysis of the situation were carried out on 128 small farms with free or tied rearing in different regions of Serbia. The selection of farms was made by the advisers of the Agricultural Advisory and Expert Services (AAES) of Požarevac, Jagodina, Kragujevac, Kruševac, Negotin, Niš, Prokuplje, Leskovac, Vranje and Pirot. The prerequisite was that the farmers are predominantly engaged in milk production and possess 5–15 cows, a milk cooler tank and at least one mechanical

milking machine. The structure of the questionnaire was defined based on the methods of Kurwijila (2006), Cook and Reinemann (2007), Atasever et al. (2012), De Vries et al. (2012), and Relić and Hristov (2016), with the necessary modifications for a more detailed overview for the assessment of hygiene of cows, milking units as well as the procedures during milking. The questions corresponded to indicators and were rated on a scale of 0 and 1 or 1 to 5 (1 – poor, 2 – sufficient, 3 - good, 4 - very good and 5 - excellent). Based on the analysis of the obtained results, recommendations for correcting the hygienic procedures in milking were defined and agreed with the farmers. Questions 1–3 of the questionnaire were related to: 1. general assessment of body cleanliness, 2. visual inspection of teats and udder base for soiling and 3. maintaining of teat cleanliness visually or by using wet wipes or cotton wool.

For the application of the proposed corrective hygiene measures to improve hygienic conditions before, during and after milking of cows, farmers were trained by advisors with additional printed instructional materials.

The samples were collected after cooling of the total amount of milk and delivered to the selected accredited laboratory for analysis. These samples were taken at each farm visit, which makes a total of six samples per farm during the study period. The milk fat content was determined by the Gerber method (Anon., 2018), the protein content by the Kjeldahl method (Anon., 2014), the TNM value according to the ISO 4833-1:2013 method (Anon., 2013) and the SCC value by the fluoro-opto-electronic method on the Fossomatic TM apparatus and by the method of cytological staining of milk cells and counting under a microscope.

The IBM SPSS program was used for statistical data processing with a descriptive presentation of farm characteristics, scores for all questions of the questionnaire and value and score for milk composition, considering average scores, differences and proportions of scores, using ANOVA analysis and  $\chi^2$  test. To examine the relationship between the farm characteristics and the milk quality scores and the questions from the questionnaire, the size of the effect of the farm factors on the mentioned scores was determined using a partial  $\eta^2$  test.

### **Results and Discussion**

Analyzing the general score of body cleanliness at the herd level in Table 1, the most common score determined was 4 with 45.7%, followed by score 3 with 31.9%.

When evaluating body hygiene at the herd level, the score assigned to the largest number of examined animals was taken into account, as well as the average score. The most frequently obtained score of teat and udder base cleanliness by visual inspection was 5 (65.2%), while the scores for classes 3 and 4 were very similar (13.8% and 15.9%, respectively, see Table 2).

Table 1. General evaluation of the cows' body cleanlines	s.
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	Parameter	Frequency	%	Cumulative %
	2	56	8.7	8.7
	3	206	31.9	40.6
Score	4	295	45.7	86.2
	5	89	13.8	100
	Σ	646	100	100

Table 2. The inspection of teats and udder bases for soiling.

	Parameter	Frequency	%	Cumulative %
	1	14	2.2	2.2
2 3	19	2.9	5.1	
	3	89	13.8	18.9
Score	4	103	15.9	34.8
	5	421	65.2	100
	Σ	646	100	100

Regarding the hygiene of the teats (Table 3), which was determined visually and by using wet wipes or cotton wool to wipe the top of the teat, as well as by assigning the appropriate scores taking into account the number and percentage of teats whose tips were dirty, the most common score was 3 (good) amounting to 41.6%, followed by the percentage representation of score 4 (29.1%) and score 5 (24.5%).

Table 3. Maintenance of teat cleanline
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	Parameter	Frequency	%	Cumulative %
	1	11	1.7	1.7
	2	20	3.1	4.8
G	3	269	41.6	46.4
Score	4	188	29.1	75.5
	5	158	24.5	100
	Σ	646	100	100

Table 4 shows the univariate analysis of variance for the results related to the body hygiene parameters at the herd level (as a criterion, the score assigned to the largest number of examined animals and the average score) as hygiene measures.

The capacity, rearing system and capacity occupancy had a significant impact on of cow body hygiene and daily milk production. Other parameters did not have a statistically significant influence on cow body hygiene. The partial  $\eta^2$  test

revealed that the greatest impacts on daily milk production, capacity occupancy and capacity were 19.2%, 13.2% and 10.4%, respectively.

Table 4.	Univariate	analysis	of v	ariance	for 1	the	assessment	of th	e parame	ters of
body hyg	giene of cow	vs.								

Tests of effects between factors and parameters								
Dependent variable	Question 1							
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$		
Corrected model	225.435a	200	1.127	2.362	0.000	0.515		
Section	24.689	1	24.689	51.730	0.000	0.104		
Capacity	24.755	14	1.768	3.705	0.000	0.104		
Rearing system	7.526	1	7.526	15.768	0.000	0.034		
Breed	0.248	1	0.248	0.519	0.471	0,001		
Milking system	0.834	1	0.834	1.747	0.187	0.004		
No. of cows in a facility	7.300	10	0.730	1.530	0.126	0.033		
Capacity utilization	32.207	32	1.006	2.109	0.001	0.132		
No. of cows milked	11.308	16	0.707	1.481	0.102	0.051		
Daily milk production	50.385	75	0,672	1.408	0.020	0.192		
Daily milk production per cow	15.614	25	0.625	1.309	0.147	0.068		
Error	212.387	445	0.477					
Total	9023.000	646						
Total corrected	437.822	645						

a.  $R^2 = 0.515$  (Adjusted  $R^2 = 0.297$ ).

Table 5 shows the univariate analysis of variance for the results related to the parameter considering whether the teats and the base of the udder are visually inspected.

A highly significant influence for the visual inspection of teats and udder base and their soiling was found for capacity, milking system, capacity occupancy, daily milk production, daily milk production per cow, and number of dairy cows. Other parameters did not show significance for the visual examination of the teats and the base of the udder and their soiling. Taking into account the partial  $\eta^2$  value, the greatest impact on the visual inspection of the teat and the base of the udder and their soiling was found for daily milk production (27.0%) and capacity occupancy (15.0%).

Table 6 shows the univariate analysis of variance for the results related to the parameter of maintaining teat cleanliness (determining visually or using wet wipes or cotton wool, which are used to wipe the top of the teat and evaluate accordingly; for the teat cleanliness rating, it was important to determine the number and percentage of teats whose tips were soiled), as hygiene measures in the conducted tests.

Table 5.	Univariate	analysis	of	variance	for th	ne	assessment	of teat	and	udder	base
cleanline	ess.										

	Tests of effects bet	tween fac	ctors and pai	ameters		
Dependent variable	Question 2					
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$
Corrected model	384.176 <sup>a</sup>	200	1.921	3.724	0.000	0.626
Section	62.548	1	62.548	121.269	0.000	0.214
Capacity	21.432	14	1.531	2.968	0.000	0.085
Rearing system	1.921	1	1.921	3.724	0.054	0.008
Breed	1.465	1	1.465	2.840	0.093	0.006
Milking system	5.938	1	5.938	11.514	0.001	0.025
No. of cows in the facility	8.359	10	0.836	1.621	0.098	0.035
Capacity utilization	40.467	32	1.265	2.452	0.000	0.150
No. of cows milked	17.460	16	1.091	2.116	0.007	0.071
Daily milk production	84.896	75	1.132	2.195	0.000	0.270
Daily production per cow	25.182	25	1.007	1.953	0.004	0.099
Error	229.521	445	0.516			
Total	13064.000	646				
Total corrected	613.697	645				

a.  $R^2 = 0.626$  (Adjusted  $R^2 = 0.458$ ).

Tests of effects between factors and parameters									
Dependent variable	Question 3								
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$			
Corrected model	334.680 <sup>a</sup>	200	1.673	3.402	0.000	0.605			
Section	34.006	1	34.006	69.127	0.000	0.134			
Capacity	23.282	14	1.663	3.381	0.000	0.096			
Rearing system	0.459	1	0.459	0.934	0.334	0.002			
Breed	1.477	1	1.477	3.003	0.084	0.007			
Milking system	2.018	1	2.018	4.103	0.043	0.009			
No. of cows in the facility	6.219	10	0.622	1.264	0.248	0.028			
Capacity utilization	43.730	32	1.367	2.778	0.000	0.167			
No. of cows milked	21.230	16	1.327	2.697	0.000	0.088			
Daily milk production	99.206	75	1.323	2.689	0.000	0.312			
Daily milk production per cow	25.534	25	1.021	2.076	0.002	0.104			
Error	218.911	445	0.492						
Total	9470.000	646							
Total corrected	553.591	645							

Table 6. Univariate analysis of variance for the evaluation of the teat cleanliness.

a.  $R^2 = 0.605 (R^2 = 0.427)$ .

In the category of parameters related to the hygiene of the cow's body, the general evaluation of the cleanliness of the body, the visual inspection of the teats and the base of the udders for soiling and the maintenance of the cleanliness of the teats were considered. The results of the test showed that during the implementation of the hygiene measures there was an increase in the scores for the general evaluation of the cleanliness of the cows' bodies (Figure 1) from the first to the sixth farm visit (F=21.47, P<0.0001). The increase resulted from a decrease in scores in the range 1-3 (presence of dried and fresh faeces) and an increase in the frequency of scores 4 and 5, indicating slight soiling or a clean body ( $\chi^2 = 100.38$ , P<0.0001). In addition, the results showed that the value of scores for the visual inspection of the teats and the base of the udder for soiling during the implementation of hygiene measures (Figure 2) increased from the first to the sixth visit to the farm (F=8.83, P<0.0001). This increase resulted from a decrease in the frequency of scores indicating that the teats and the base of the udder were not visually inspected for soiling and an increase in the frequency of scores indicating that this inspection was performed daily ( $\chi^2 = 66.83$ , P<0.0001). When examining the parameter of maintaining the cleanliness of the teats during the implementation of hygiene measures, an increase in the frequency value and rating for maintaining the cleanliness of the teats (Figure 3) was observed from the first to the sixth visit to the farm (F=13.12, P<0.0001). It was observed that the increase was due to the decrease in the frequency of scores 1 (the presence of a greater amount of older, neglected, dried dirt), 2 (a greater amount of dirt on the teats) and 3 (a smaller amount of dirt on the teats), and an increase in the frequency of score 4 (traces of disinfectant, without traces of impurities) and score 5, indicating the absence of traces of disinfectant colour or impurities ( $\chi^2 = 76.30$ , P<0.0001).



Figure 1. General assessment of body cleanliness during visits.



Figure 2. Visual inspection of teat and udder base soiling during visits.



Figure 3. Maintenance of the cleanliness of the udder during visits.

Table 7 presents the results of univariate analysis of variance for TNM in milk in the conducted tests.

Table 7 shows that capacity, rearing system of cows, breed, number of cows in the facility and number of cows milked had a very significant effect on TNM. The factors of capacity occupancy, daily milk production and daily milk production per cow did not have a statistically significant impact.

The partial  $\eta^2$  test shows that the percentage of milk protein was most influenced by daily milk production (14.8%), capacity occupancy (12.5%), daily milk production per cow (6.6%), number of cows milked (5.0%), capacity (4.0%), and number of cows in a facility (3.8%).

Tests of effects between factors and parameters									
Dependent variable	TNM								
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$			
Corrected model	539.283 <sup>a</sup>	200	2.696	1.620	0.000	0.421			
Section	31.387	1	31.387	18.863	0.000	0.041			
Capacity	30.925	14	2.209	1.328	0.187	0.040			
Rearing system	3.352	1	3.352	2.015	0.156	0.005			
Breed	1.577	1	1.577	0.948	0.331	0.002			
Milking system	1.167	1	1.167	0.701	0.403	0.002			
No. of cows in the facility	29.029	10	2.903	1.745	0.069	0.038			
Capacity utilization	105.594	32	3.300	1.983	0.001	0.125			
No. of cows milked	39.288	16	2.456	1.476	0.104	0.050			
Daily milk production	128.588	75	1.715	1.030	0.416	0.148			
Daily milk production per cow	52.576	25	2.103	1.264	0.179	0.066			
Error	740.457	445	1.664						
Total	8772.000	646							
Total corrected	1279.740	645							

Table 7. Univariate analysis of variance for TNM.

a.  $R^2 = 0.421$  (Adjusted  $R^2 = 0.161$ ).

It should be taken into account that the most pronounced increase in scores related to TNM in milk was found between the  $3^{rd}$  and  $4^{th}$  visits and that the ANOVA test revealed significant differences between visits (F=9.63, P<0.0001), and by using the LSD test, very significant differences were found between the  $1^{st}$  and  $4^{th}$ ,  $1^{st}$  and  $5^{th}$  and  $1^{st}$  and  $6^{th}$  visits. Using the  $\chi^2$  test, very significant differences in TNM were found between visits. In essence, the number of scores 4 and 5 was found to increase in relation to the number of visits, especially for the  $4^{th}$ ,  $5^{th}$  and  $6^{th}$  visits, which should be taken into account when defining good hygiene practices.

Table 8 shows a univariate analysis of variance for the results related to SCC in milk in the conducted tests. A gradual increase was observed in the mean values of SCC from the first to the third visit, then a more pronounced increase between the third and fourth visits, and a slightly less pronounced increase at the fifth visit compared to the fourth. Some decrease in this parameter was observed between the fifth and sixth visits. Furthermore, significant differences between visits were found in the analysis of variance for SCC (F=5.17, P<0.0001). In addition, SCC values varied depending on the visit and the application of control measures. Using the LSD test, a very significant difference was found between the 1<sup>st</sup> and 5<sup>th</sup> visits, as well as a very significant difference between the 2<sup>nd</sup> and 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup>, and between the 3<sup>rd</sup> and 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> visits, respectively. Finally, using the  $\chi^2$  test,

very significant differences were found in SCC values between visits. It was found that the rate of scores 4 and 5 increased in relation to the ordinal number of visits, especially at the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> visits.

Tests of effects between factors and parameters									
Dependent variable	SCC								
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$			
Corrected model	403.831 <sup>a</sup>	200	2.019	1.460	0.001	0.396			
Section	34.137	1	34.137	24.691	0.000	0.053			
Capacity	32.294	14	2.307	1.668	0.059	0.050			
Rearing system	4.639	1	4.639	3.355	0.068	0.007			
Breed	2.772	1	2.772	2.005	0.157	0.004			
Milking system	1.425	1	1.425	1.030	0.311	0.002			
No. of cows in the facility	40.129	10	4.013	2.903	0.002	0.061			
Capacity utilization	101.318	32	3.166	2.290	0.000	0.141			
No. of cows milked	21.476	16	1.342	0.971	0.488	0.034			
Daily milk production	138.103	75	1.841	1.332	0.043	0.183			
Daily milk production per cow	28.332	25	1.133	0.820	0.718	0.044			
Error	615.227	445	1.383						
Total	10756.000	646							
Total corrected	1019.059	645							

Table 8. Univariate analysis of variance for SCC.

a.  $R^2 = 0.396 (R^2 = 0.125).$ 

All of the above results should be taken into account when determining farm and hygiene parameters that influence the increase in the SCC mean values. These parameters should be included in the consideration of corrective and preventive measures within the framework of good hygiene practices. It should also be taken into account that the analysis of the determined values shows that the number of visits had an influence on the percentage of milk fat (p<0.1, F = 2.221), while there was no influence on the protein percentage.

According to the research results, it is clear that certain hygiene procedures were of great importance before milking cows, such as visual examination of the udder for the presence of signs of inflammation or damage, visual examination of the teats and the base of the udder for their soiling, application of the pre-milking test, washing and disinfecting of teats and udders, application of the mastitis test, teat wiping procedures, udder massages, hygiene procedures before and during the installation of milking units, control of the milking machine, automatic vacuum interruption, total duration of milking from the beginning to the end of contact with the milker, as well as interruption of milking (poor actions of the milking personnel, noise, other cows, other animals, etc.), as determined by other authors (Hristov et al., 1997; Tamime, 2009; Lemma et al., 2018). Furthermore, the research found that the cleanliness of the cow's body, a visual inspection of the teats and the base of the udders for their soiling by the milker, the overall cleanliness of the teats and trimming the hair from the udders had an impact on milk quality. Hygienic procedures after milking include the application of various disinfectants as soon as possible after removing the teat cups by dipping or spraying the teats (Hristov et al., 1997). The importance of maintaining the hygiene of the materials for wiping the udder and the accessories for the application of disinfectants was also highlighted. It has been stated that numerous studies show that the occurrence of new udder infections is directly related to greater soiling of the udder (Stewart et al., 2002; Berge and Baars, 2020). It is necessary to disinfect the teats of the udder immediately after milking with an agent that has the ability to close the teat opening. In addition, post-milking udder disinfection should be done regularly after each milking on farms, which is not the case on small farms where it is mostly poorly performed (Hristov et al., 1997; Hristov, 2002; Tamime, 2009).

Presently, there are several programs addressing the best management practices in milk production, including good hygiene practices (Costa et al., 2005), emphasizing that the key factor for milk quality is avoiding contamination of raw milk and dairy products. The failure to maintain adequate sanitary practices contributes to the bacterial contamination of milk, chemical substances or physical deterioration from various sources. Common predisposing factors for milk contamination include the milking environment, cow body, milking personnel, milking equipment, milk transport, utensils and liquids used during milking, which has been unequivocally confirmed in research. Finally, it is crucial to apply good hygienic practices in milk production, regardless of the size of the farm, to effectively protect public health in accordance with the claims made by Bekuma and Galmessa (2018).

European Union regulations require raw milk to come from animals without any symptoms of infectious diseases transmitted to humans through milk. The cows should be in good health and free of udder wounds, both of which could affect the quality of the milk. It is envisaged that milk from cows treated with approved medicinal products should be separated from milk originating from healthy cows (European Commission, 2004a). Therefore, animal health management aims to achieve and maintain a disease-free herd (Hillerton, 2004). This can be achieved when infected animals are cured or removed from the herd, and new infections are prevented. A closed herd production, when there is no introduction of animals from other farms, is an important measure for maintaining a disease-free herd. Treatment and separation of infected animals from the rest of the herd prevent the transmission of pathogenic microorganisms from cow to cow (Hillerton, 2004), as well as high-quality feed, facility hygiene and hygienic milking conditions. Mastitis control is an important issue for the dairy sector, so mastitis control programs have been developed and implemented in many countries (Ekman et al., 2005; Olde Riekerink et al., 2005; Van der Zvaag, 2005). They are usually based on five crucial principles: disinfection after milking, antibiotic therapy for dry cows, appropriate treatment of clinical cases, removal of chronically infected cows and regular maintenance of milking machines (Akam et al., 1989). In Norway, in 1982, a successful udder health program was implemented, with the main focus of this program on milking and the repair of milking machines, although treatment of cows' udders by drying and dipping teats in disinfectant was not required to the same extent. Nevertheless, this, combined with a change in farmer attitudes and cow breeding programs, led to a 50% reduction in clinical mastitis treatment, a reduction in SCC (somatic cell count – an indicator of subclinical mastitis) from 250,000 to 114,000 in 1 mL of milk, and a significant reduction in treatment costs during ten years since 1994 (Østeras and Sølverød, 2005). Contemporary mastitis control programs anticipate limiting the frequency of mastitis, and thus reducing SCC in milk to an economically tolerable level is mainly achieved by applying strict hygienic and sanitary measures in milking parlors and barns. Limiting the frequency of infections in the udder quarters of cows involves teat disinfection after each milking, service and maintenance of the milking machine, back-washing of the milking cups and washing of the udders with running water before milking, as well as the application of other hygienic and sanitary measures (Hristov, 2002).

### Conclusion

Based on the results of the conducted study, it can be concluded that farm characteristics (capacity, housing system, breed, milking system, number of cows, capacity occupancy mostly had a very significant or significant impact on all hygiene parameters classified in the aforementioned categories, as well as on protein and fat content, SCC and TNM in milk. Very significant variations were found in the listed hygiene parameters of the farm depending on the farm visits, as well as a continuous trend of improving the scores of hygiene parameters of the farm in relation to the regular number of farm visits. Based on the obtained results, an increase in the mean values of the TNM scores was observed after each visit. The TNM ANOVA test revealed significant differences between visits (F = 9.63, P < 0.0001). For SCC mean values, an increase in scores was found from the first to the third visit, then a more pronounced increase between the third and fourth visits, and a slightly less pronounced increase in the fifth visit. Some decrease was observed for this parameter between the fifth and sixth visits. Analysis of variance for SCC revealed highly significant differences between visits (F = 5.17, P<0.0001). The SCC significantly varied across all visits and control measures applied. Highly significant or significant differences were found between all visits by the LSD test and  $\chi^2$  test. It was observed that the number of visits tended to have a significant influence on the milk fat rate (F=2.221; P<0.1), but no influence on the milk protein rate (P = 0.480; F = 0.901).

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#### References

- Akam, F.D., Dodd, F.H., & Quick, A.J. (1989). Milking, Milk Production Hygiene and Udder Health, Report No. 78, Food and Agriculture Organization of the United Nation, Rome.
- Anon (2013). 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.
- Anon (2014). ISO 8968-1:2014 [IDF 20-1:2014] Milk and milk products Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation, International Dairy Federation.
- Anon (2018). ISO 19662 I IDF 238: 2018 Milk Determination of fat content Acido-butyrometric (Gerber method), International Dairy Federation.
- Atasever, S., Erdem, H., & Demiryurek, K. (2012). Association of some milking parameters with milk quality of smallholder dairy farms in Samsun region, Turkey. *Journal of Environmental Biology*, 33 (1), 123-126.
- Bekuma, A., & Galmessa, U. (2018). Review on hygienic milk products practice and occurrence of mastitis in c ow's milk. Agricultural Research & Technology, 18 (2), 1-11.
- Berge, A.C., & Baars, T. (2020). Raw milk producers with high levels of hygiene and safety. *Epidemiology & Infection*, 148, e14, 1-7.
- Cook, N.B., & Reinemann, D.J. (2007). A tool box for assessing cow, udder and teat hygiene. Proceedings of 46th annual meeting of the NMC. (pp. 21-24). San Antonio, Texas, USA.
- Costa, H.B.A., Dantas, R.M., Alvarenga, M.B., Peripolli, V., Tanure, C.B., Dargatz, D.A., Strohmeyer, R.A., Morley, P.S., Hyatt, D.R., & Salman, M.D. (2005). Characterization of *Escherichia coli* and *Salmonella enterica* from cattle feed ingredients. *Foodborne Pathogens* and Disease, 2, 341-347.
- De Vries, T.J., Aarnoudse, M.G., Barkema, H.W., Leslie, K.E., & von Keyserlingk, M.A.G. (2012). Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. *Journal of Dairy Science*, 95 (10), 5730-5739.
- European Commission (2004a) Regulation (EC) No 852/2004 on the hygiene of foodstuffs. Official Journal of the European Union, L139, 1-54.
- Ekman, T., Landin, H.L., Waldner, J., Gyllensward, M., & Hallen Sandgren, C. (2005). 'Friskko– Juver' ('Healthyc ow–Udder') A systematic veterinary approach to udder disease in dairy herds. (Ed. H. Hogeveen), *Proceedings 4th IDF International Mastitis Conference*, (pp. 605-610). Wageningen Academic Publishers, Wageningen, The Netherlands.
- Hillerton, J.E. (2004). Control of MAP in milk. In (Ed. Robert C.) New Applications of Mid-Infra-Red Spectrometry for the Analysis of Milk and Milk Products, *Proceedings of IDF Symposium* of Advancement in Analytical Techniques (pp. 17–19), Document No. 383, International Dairy Federation, Brussels.

- Hristov, S., Lazarević, N., Radovanović, M., & Pavlović, M. (1997). Streptokokni mastitis krava. Zbornik naučnih radova XI Savetovanja agronoma i tehnologa sa međunarodnim učešćem, Aranđelovac, 3 (1), 415-424.
- Hristov, S. (2002). Najznačajniji aspekti utvrđivanja ukupnog broja mikroorganizama u svežem mleku krava. *Mlekarstvo*, 1 (7), 208-216.
- Kurwijila, L.R. (2006). Hygienic milk handling, processing and marketing: reference guide for training and certification of small-scale milk traders in Eastern Africa. International Livestock Research Institute.
- Lemma, D.H. Mengistu, A., Kuma, T., & Kuma, B. (2018). Improving milk safety at farm-level in an intensive dairy production system: relevance to smallholder dairy producers. *Food Quality and Safety*, *2* (3), 135-143.
- Malik, T.A., Mohini, M., Mir, S.H., Ganaie, B.A., Singh, D., Varun, T.K., Howal, S., & Thakur, S. (2018). Somatic cells in relation to udder health and milk quality-a review. *Journal of Animal Health and Production*, 6 (1), 18-26.
- Olde Riekerink, R.G.M., Barkema, H.W., Scholl, D.T., Kelton, D.F., Keefe, G.P. & Sandgren, C.H. (2005). Implementation of recommended mastitis prevention management practices and the herd level prevalence of contagious mastitis pathogens on Canadian dairy farms. In (Ed. H. Hogeveen) *Proceedings of 4th IDF International Mastitis Conference* (pp. 587-592). Wageningen Academic Publishers, Wageningen, The Netherlands.
- Oumer, E., Tsegaye, S., Damtew, A., & Feleke, A. (2017). Hygienic practices and bacteriological quality of cow raw milk from selected smallholder dairy farms of Mersa Town, North Wollo, Ethiopia. *European Journal of Experimental Biology*, 7 (4), 22.
- Østeras, O., & Sølverød, L. (2005). Mastitis control system: the Norwegian experience. In (Ed. H. Hogeveen), *Proceedings of 4th IDF International Mastitis Conference* (pp. 91-101). Wageningen Academic Publishers, Wageningen, The Netherlands.
- Relić, R., & Hristov, S. (2016). *Praktikum iz zoohigijene*. Poljoprivredni fakultet Univerzitet u Beogradu, Beograd.
- Robles, I., Kelton, D.F., Barkema, H.W., Keefe, G.P., Roy, J.P., Von Keyserlingk, M.A.G., & DeVries, T.J. (2020). Bacterial concentrations in bedding and their association with dairy cow hygiene and milk quality. *Animal*, 14 (5), 1052-1066.
- Sant'anna, A.C., & da Costa, M.P. (2011). The relationship between dairy cow hygiene and somatic cell count in milk. *Journal of Dairy Science*, 94 (8), 3835-3844.
- Schreiner, D.A., & Ruegg, P.L. (2002). Effects of tail docking on milk quality and cow cleanliness. Journal of Dairy Science, 85 (10), 2503-2511.
- Schukken, Y.H., Wilson, D.J., Welcome, F., Garrison-Tikofsky, L., & Gonzalez, R.N. (2003). Monitoring udder health and milk quality using somatic cell counts. *Veterinary research*, 34 (5), 579-596.
- Stewart, S., Godden, S., Rapnicki, P., Reid, D., Johnson, A., & Eicker, S. (2002). Effects of automatic cluster remover settings on average milking duration, milk flow and milk yield. *Journal of Dairy Science*, 85, 808-823.
- Tamime, A.Y. (2009). Milk processing and quality management. John Wiley & Sons.
- Van Der Zwaag, H.G. (2005). Mastitis control program in the Netherlands: goals, tools and conditions. In (Ed. H. Hogeveen), *Proceedings of 4th IDF International Mastitis Conference* (pp. 599-604). Wageningen Academic Publishers, Wageningen, The Netherlands.

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## UTICAJ POBOLJŠANJA HIGIJENE TELA I VIMENA NA BEZBEDNOST I SASTAV MLEKA NA MALIM FARMAMA U SRBIJI

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### Rezime

Higijensko stanje muznih krava i njihovih vimena na 128 malih gazdinstava lociranih u različitim regionima Srbije, pretežno angažovanih na proizvodnji mleka, 5–15 krava, laktofriza i najmanje jedne mašine za mužu, procenjeno je korišćenjem sledećih indikatora: 1. opšta procena čistoće tela, 2. vizuelni pregled sisa i osnove vimena, i 3. održavanje čistoće vimena, ocenjeno na skali od 0 do 1 ili od 1 do 5. Uzorci su uzeti nakon hlađenja pri svakoj poseti farmi i testirani na sadržaj mlečnih proteina i masti, broj somatskih ćelija (engl. somatic cell count -SCC) i ukupan broj mikroorganizama (engl. total number of microorganisms -TNM). Sadržaj mlečne masti određen je Gerberovom metodom, sadržaj proteina Kjeldahlovom metodom, ukupan broj mikroorganizama metodom ISO 4833-1:2013, a broj somatskih ćelija upotrebom aparata Fossomatic TM. Program IBM SPSS je korišćen za statističku obradu podataka. Kapacitet, uslovi smeštaja, rasa, sistem muže, broj krava i popunjenost kapaciteta su uglavnom imali veoma značajan ili značajan uticaj na higijenske parametre, sadržaj proteina i masti, broj somatskih ćelija i ukupan broj mikroorganizama u mleku. Higijenski parametri su imali kontinuirani trend poboljšanja u odnosu na posete. Srednje vrednosti ocena za ukupan broj mikroorganizama su se povećavale posle svake posete, uz značajne razlike između poseta (F=9,63, P<0,0001). Ocene broja somatskih ćelija su veoma značajno varirale između poseta (F=5,17, P<0,0001). Broj poseta je pokazao tendenciju značajnog uticaja na masnoću mleka (F=2,221; P<0,1), ali ne i na udeo proteina (P=0,480; F=0,901).

Ključne reči: unapređenje, telo, vime, higijena, mleko, bezbednost, sastav, mala farma.

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