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The effect of season on the microbiological status of raw milk

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SUMMARY

Many factors can influence the microbiological quality of raw cow's milk. In this study, our aim was to determine whether there was any difference between the microbiological statuses of milk produced in different seasons. Samples were collected and analysed from five dairy farms in Hajdú-Bihar County, from February to November in 2019. During our studies, total plate count (TPC), coliform count and Staphylococcus aureus count of raw cow's milk samples were determined.

There was no significant difference (P>0.05) between the mean TPC values detected in the milk collected in winter and autumn, but that values were significantly (P<0.05) lower than in the milk samples collected in spring and summer. Similarly to the TPC, in the case of coliform bacteria the lowest mean colony count was detected in the samples collected in winter. The difference was significant (P<0.05), compared to the values observed in the samples collected in summer. S. aureus *was detected in bulk milk of only two farms in excess of 1.0* log_{10} *cfu/ml. Also in case of* S. aureus*, there was a significant difference (P<0.05) between the values observed in the samples collected in winter and in summer. Samples from spring and summer contained the highest amount of* S. aureus.

Based on the results of our studies, in the case of almost all farms the mean TPC, coliform and S. aureus *counts were lower in the samples collected in winter, than in the samples collected in summer. The fact that the samples collected in winter contained the lowest amount of colonies could be attributed to the inhibition of growth of mesophilic microorganisms below 8 °C. Furthermore, the fact that we observed the highest colony counts in samples collected in summer, can be related to the heat stress of cows during the summer due to unfavorable weather conditions (high temperature and humidity).*

Keywords: *coliform bacteria, microbiological quality, raw milk, season, Staphylococcus aureus*

INTRODUCTION

There are several nutrients (for example proteins, fats, minerals, vitamins, water) in milk which necessary for the human body (Haug et al., 2007). However, many microorganism may be occurred in milk, because these components are also needed for them (Pedras et al., 2012). Pathogenic microorganisms capable of infecting and causing disease in humans may also be present in milk, e.g. *Staphylococcus aureus* (Normanno et al., 2007). There can be few microorganisms in the milk of a healthy cow. Nevertheless, contamination may occur after milking, during milk handling (Biró, 2014). The skin of the animals, the milking environment or the surface of milking equipment can also contaminate milk with microorganisms (Cilliers et al., 2014).

Coliform bacteria and *S. aureus* are also known as mastitis pathogens (Alemu and Abraha, 2017). In the case of coliform mastitis milk production can be reduced, which can cause economic problems to farms (Mbuk et al., 2016). Coliform bacteria can be considered as faecal indicator microorganisms. These bacteria are generally occur in the environment, so their presence in food can indicate contamination (Mhone et al., 2011; Wanjala et al., 2018). These bacteria can also occur in the environment of the dairy farms (e.g. milking equipment, dirt, fecal sources, water) (Kagkli et al., 2007).

The conditions in milk are appropriate for the growth of staphylococci. Some *S. aureus* strains can produce different types of enterotoxins which can cause food poisoning in humans. The symptoms of the food poisoning are abdominal cramps, diarrhea and vomiting, which begin one to eight hours after the contaminated food is consumed by humans (Korpysa-Dzirba and Osek, 2011). Raw cow's milk can be contaminated with this bacteria from the environment of the dairy farms, from the human handlers, from the equipment used during milking and from the skin of the animals (André et al., 2008). *S. aureus* can cause clinical or subclinical mastitis in cows in farms (Peton and Loir, 2014; Alemu and Abraha, 2017). In the case of *S. aureus* mastitis the milk yield decreases, the quality of the produced milk also decreases, so the milk price income decreases too, which highlights the economical importance of the disease (Ózsvári et al., 2003).

The microbiological quality of raw milk can be influenced by several factors. Few Hungarian, but several foreign authors have already discussed how the different physical, chemical and microbiological properties of raw milk change during the seasons. Cziszter et al. (2012) investigated the effect of seasons for coliform count in Romania, and observed that the highest concentration of coliform bacteria (0.9 cfu/ml) was in milk samples collected in spring. Winter samples contained the least amount of coliform bacteria (0.75 cfu/ml), which was related to the fact that the growth of mesophiles was inhibited below 8 °C. Zeinhom et al. (2016) found in Egypt that somatic cell count (SCC) and pathogen microorganisms increased in samples during the summer period, and the milk content was decreased. According to the authors, this is related to the heat stress. Heat stress causes increase in

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water consumption, the feed consumption is decreasing, so the nutritional parameters of milk change, and the cows produce less milk. Udder problems can also occur (Béri, 2011). Bouraoui et al. (2002) in Tunisia observed a significant decrease in milk fat and protein yield and a significant increase in the somatic cell count (SCC) of lactating Holstein cows during the summer compared with spring.

Therefore, in our researches, we aimed to determine how the microbiological quality of raw milk changes in different seasons. We determined in bulk milk samples the amount of some indicator microbes, i.e. total plate count (TPC) and coliform bacteria. Furthermore, we also determined the *S. aureus* count.

MATERIALS AND METHODS

Place and date of sampling

Five dairy farms (Farm „A” to Farm „E”) in Hajdú-Bihar County (Hungary) were involved in this research. Deep litter was used as keeping method in all farms, except for Farm „E”, where cubicle loose was used. In all farms TMR (Total Mixed Ration) feeding had been applied and milking was done in milking parlour. Pre-milking disinfection was used, but post-milking disinfection was not used during milking in Farm „D”. Apart from this farm, the others used pre-and post-milking disinfection of the udder.

The microbiological examinations were conducted between February and November 2019. During this period, three to seven bulk tank milk samples were collected per farm per season (n_{winter}=22; n_{spring}=27; n_{summer}=29; n_{autumn}=26). A total of 104 milk samples (n=104) were tested. The samples originated from the milk storage tanks were collected into sterile plastic tubes. The samples were tested in the Microbiological Laboratory of the Institute of Food Science at the University of Debrecen.

Microbiological analysis

Preparation of raw milk samples and the microbiological examinations were performed in accordance with the study of Petróczki et al. (2019). Raw milk samples were stored and diluted according to the MSZ EN ISO 6887-1 (2017) standard.

Microbiological examinations were performed according to the currently valid standards for the microorganisms. The TPC determination was performed according to the MSZ EN ISO 4833-1 (2014) standard and the test was done by pour plate technique.

The determination of the amount of coliform bacteria was carried out in accordance with the ISO 4832 (2006) standard. The test was also performed by pour plate technique.

The *S. aureus* count was determined according to the MSZ EN ISO 6888-1 (2008) standard, by spread plate method. *S. aureus* was separated from other *Staphylococcus* species with latex agglutination test (Prolex Staph Xtra Kit, Ferol Ltd., Hungary).

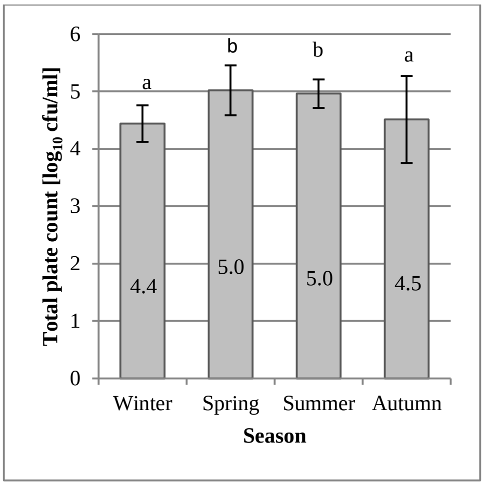
Statistical analysis

SPSS v.22.0 (SPSS, 2013) software was used to calculate averages and standard deviations (SD) and to perform logarithmic transformation of the values, the t-tests and variance analysis.

RESULTS AND DISCUSSION

The mean TPC was 3.7×10^4 (4.4 ± 0.3 log_{10}) cfu/ml in bulk milk samples collected in winter, and it was 1.9×10^5 (4.5 ± 0.8 log_{10}) cfu/ml (*Figure 1*) in the samples collected in autumn and there was no significant difference (P>0.05). But, the mean TPC were significantly (P<0.05) lower in the samples collected in winter and autumn than in the milk samples collected in spring and summer. In samples collected in spring and summer, the mean TPC were 1.6×10^5 (5.0 ± 0.4 log_{10}) cfu/ml and 1.1×10^5 (5.0 ± 0.2 log_{10}) cfu/ml, respectively. In a total of 39 (38%) samples the mean TPC was higher than the limit set in the regulation 853/2004/EC [M=1.0×10^5 (5.0 log_{10}) cfu/ml]. The mean TPC exceeded the limit in 59% of the spring samples, 52% of the summer samples, 19% of the autumn samples and 14% of the winter samples (*Table 1*).

Figure 1: **Total plate count in raw milk collected from dairy farms in different seasons**



a, b: Means with different signs differ significantly (P<0.05)

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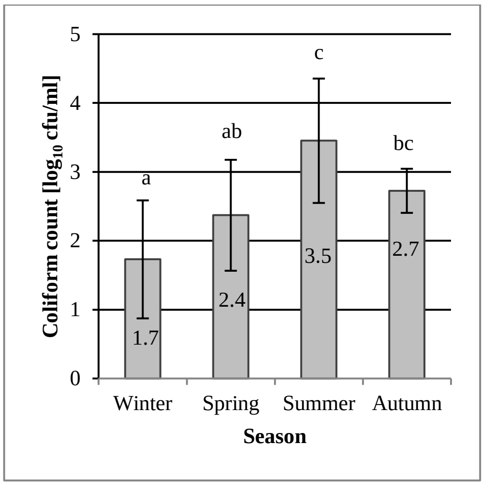
Table 1

Number and percentage of bulk milk samples exceeded the limit set in the regulations

Microbiological parameter	Number and percentage (%) of samples exceeded the „M" value				„M" value (log_{10} cfu/ml)	Regulation
	Winter	Spring	Summer	Autumn		
Total plate count	3 (14)	16 (59)	15 (52)	5 (19)	5.0	853/2004/EC
Coliform count	8 (36)	22 (81)	29 (100)	26 (100)	2.0	Regulation of Hungarian Ministry of Health 4/1998. (XI.11.)
S. aureus count	22 (100)	27 (100)	29 (100)	26 (100)	2.7	Regulation of Hungarian Ministry of Health 4/1998. (XI.11.)

Similarly to the TPC, in the case of coliform bacteria the lowest mean colony count [2.6×10^2 (1.7 ± 0.9 log_{10}) cfu/ml] was detected in the samples collected in winter. The highest mean colony count [1.4×10^4 (3.5 ± 0.9 log_{10}) cfu/ml] occurred in summer samples. There was significant difference ($P<0.05$) between the values observed in the samples collected in winter and summer. The samples collected in autumn also had a significantly higher ($P<0.05$) mean coliform count [6.8×10^2 (2.7 ± 0.3 log_{10}) cfu/ml] compared to the winter milk samples. In the samples collected in spring, the mean coliform count was 5.6×10^2 (2.4 ± 0.8 log_{10}) cfu/ml (*Figure 2*). In more than 50% (64%) of the samples collected in winter had a colony count below the „M" value (rejection limit) [$M=1.0\times10^2$ (2.0 log_{10}) cfu/ml] set in regulation of the Hungarian Ministry of Health 4/1998. (XI.11.). In 81% of the spring samples and in all samples collected in summer and autumn the mean coliform count exceeded the „M" value. Thus, in 82% of the samples, coliform bacteria could be detected in an amount above the limit (*Table 1*).

Figure 2: **Coliform count in raw milk collected from dairy farms in different seasons**

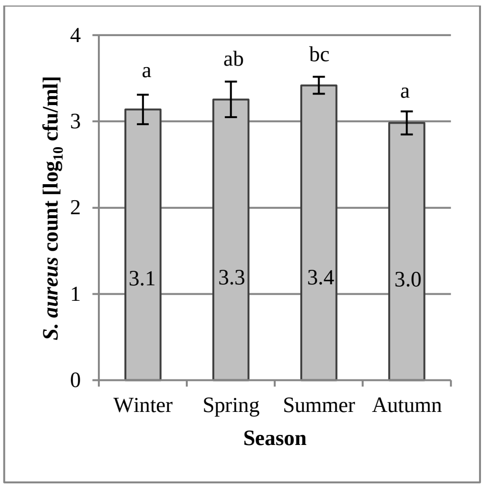


a, b, c: Means with different signs differ significantly (P<0.05)

The primary sources of coliforms are the faeces, the litter, or the surfaces of the improperly cleaned milk collection and storage devices (Murphy et al., 2001; cit. Mohamed and El Zubeir, 2007) Thus, the high amount of coliform bacteria obtained in our research indicated that improper milk collection and treatment practices at the farms may had caused the contamination of the milk with coliform bacteria. Cziszter et al. (2012) also studied the effect of seasons on coliform counts and found that the highest concentration (0.9 cfu/ml) of coliform bacteria was detected in samples collected in spring and the lowest concentration (0.75 cfu/ml) in winter samples.

S. aureus was not detected in excess of 1.0 log_{10} cfu/ml in milk of three dairy farms. The highest mean *S. aureus* count [2.7×10^3 (3.4 ± 0.1 log_{10}) cfu/ml] was obtained in summer samples and the lowest in winter [1.5×10^3 (3.1 ± 0.2 log_{10}) cfu/ml] and autumn [1.0×10^3 (3.0 ± 0.1 log_{10}) cfu/ml] samples of the two farms (*Figure 3*). There was a significant difference ($P<0.05$) between the values obtained in the samples collected during these months. The mean *S. aureus* count exceeded the „M" value [$M=5.0\times10^2$ (2.7 log_{10}) cfu/ml] specified in regulation of the Hungarian Ministry of Health 4/1998. (XI.11.) in all samples collected from the two farms.

Figure 3: ***S. aureus* count in raw milk collected from dairy farms in different seasons**



a, b, c: Means with different signs differ significantly (P<0.05)

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Zeinhom et al. (2016) found that SCC and pathogen microorganisms increased in samples during the summer, and the milk content decreased. According to the authors, this was related to the heat stress. One of the negative effects of heat stress is the increased incidence of udder infections, such as *S. aureus* infections. The highest amount of *S. aureus* was detected in our study during summer, which can be related to the heat stress in cows during summer due to unfavorable weather conditions (high temperature and humidity).

CONCLUSIONS

The average TPC, coliform and *S. aureus* count was found to be lower in the samples collected in winter than in the summer. Cziszter et al. (2012) also found that winter samples contain lower amount of coliform bacteria than in samples collected in spring.

Coliform counts exceeded the limit in 81% of the samples collected in spring and in all the milk samples collected in winter and autumn. As coliform bacteria occur in the faeces, the litter, or the surfaces of the improperly cleaned milk collection and storage devices, it is recommended that more stringent hygiene practices should be followed in these farms during these months.

S. aureus was detected above the rejection limit throughout the year in the bulk milk of two farms. The reason of it in both farms can be that milk from animals with subclinical or possibly clinical mastitis, may have been introduced into the bulk milk. It is recommended to withdrawn these animals from milk production.

Finally, since not all microbiological parameters had the same tendency, for example in the case of autumn and spring results, it was confirmed that the microbiological quality of the milk can be influence by many further factors besides the season.

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