Theses of Doctoral (PhD) Dissertation

INVESTIGATION OF FACTORS AFFECTING THE MICROBIOLOGICAL QUALITY OF MILK AND ANALYSIS OF CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM MILK

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1. Prelude and Objectives of Ph.D. dissertation

Milk and dairy products play a significant role in our diet, as these products contain nutrients valuable to the human body, e.g. proteins, fats, vitamins, minerals (HAUG et al., 2007). These nutrients are also needed for the microorganisms, so milk and dairy products can contain many of them, including pathogenic organisms, like *Staphylococcus aureus* (NORMANNO et al., 2007; HUONG et al., 2010).

Milk can be contaminated with microorganisms within the udder of the dairy animals. Improper usage of milking equipment can have a detrimental effect on the teats, teat canal, and mucosal tissue. Damage to the primary line of defense of the udder will result in loss of protection against microorganisms (TÓTH, 2015). Most microorganisms that cause mastitis can enter the udder through the teat canal (JAIN, 1979). Mastitis can be caused by, for example, coliform bacteria and *S. aureus* (PYÖRÄLÄ and TAPONEN, 2009).

Several external and internal factors can affect the microbiological quality of raw milk (ANDERSON et al., 2003). These factors need to be given special attention in order to produce safe and good quality milk. Different opinions on the microbiological quality of milk from different cow breeds have been summarized in the literature (NÓBREGA and LANGONI, 2011; BYTYQI et al., 2013). It is disputed among the authors whether the microbiological quality of milk changes at different stages of lactation and with the number of lactations (TENHAGEN et al., 2006; TESSEMA, 2016). In addition, a minimal amount of Hungarian literature deals with the changes in the microbiological properties of raw milk during the seasons. Several foreign studies (BOURAOUI et al., 2002; CZISZTER et al., 2012; ZEINHOM et al., 2016) have been made in connection with the effects of the seasons on the microbiological quality of raw milk, but these are limited to local specificities and do not reflect Hungarian relations.

S. aureus can cause mastitis in dairy farms (PETON and LOIR, 2014) and is an enterotoxin-producing pathogenic microorganism in a food safety point of view (HILL et al., 2012). *S. aureus* can enter milk directly, in the case of cows with subclinical or clinical mastitis, but it can also enter milk from the environment (SCHERRER et al., 2004). The economic significance of mastitis caused by *S. aureus* lies in the fact that because of the disease the milk yield decreases, its quality deteriorates (somatic cell count increases), so the revenue decreases (ÓZSVÁRI, 2012).

As there is few current, relevant information available in Hungary on the effect of breed, lactation stage, lactation number and season on microbiological quality and on the monitoring of the properties of *S. aureus* strains in dairy farms, we aimed to determine the following:

- Determination of contamination of raw milk from different dairy farms with *S. aureus* and indicator microbes.
- Determining whether there is a difference between the microbiological quality of milk from different cattle breeds (Holstein Friesian, Jersey) kept and milked with the same housing technology.
- To assess whether the microbiological quality of raw cow milk changes with the number of lactations (primiparous, multiparous) and at different stages of lactation (early, mid, late).
- Determining the changes in the microbiological quality of raw milk with the seasons.
- A better understanding of *S. aureus* strains that can be isolated from milk. The aim of these studies was to determine several virulence factors of the bacterium.
- Monitoring the amount of *S. aureus* in bulk milk during a *S. aureus* control program in a dairy farm.

2. Materials and methods

2.1. Main data of the dairy farms involved in the study

Seven dairy farms (T1 – T7) in Hajdú-Bihar county were included in the research. Information to help identify farms (e.g., name, code, etc.) has not been published as requested by the farms. During the selection, we managed to select farms that faithfully reflected the conditions in Hungary. Milking parlors were used in all farms. Pre-disinfection of the udder was performed in all farms except in T6 farm, post-disinfection was performed in all farms except in T4 farm. We did not have any more opportunities to collect samples from the T6 and T7 farms after the initial microbiological investigations.

2.2. The examined samples, sampling, sample preparation, data collection

Method of collecting bulk tank and individual milk samples:

The bulk milk samples were collected under sterile conditions in sterile, resealable, 50 cm^3 sampling containers, from the milk storage tanks after milking, after thorough mixing the milk. After delivering the milk samples to the microbiological laboratory, the samples were stored in refrigerator (4 °C) until the beginning of the tests and then homogenized by shaking before preparing the tenfold serial dilutions. A total of 126 bulk milk samples were collected from the seven dairy farms (n_{T1}=22, n_{T2}=23, n_{T3}=38, n_{T4}=27, n_{T5}=11, n_{T6}=4, n_{T7}=1).

Individual milk samples were collected in T4 and T5 farms. Prior to sampling, the teats were dipped in disinfectant solution and then were wiped with clean, dry paper. During sampling, the milk of individual cows showing no symptoms of clinical mastitis was collected in a 50 cm^3 sterile, resealable sampling container after the first drops of milk with a high microbial count were milked.

Data collection and individual milk sampling in the T5 and T4 farm:

Data on the daily milk yield, milk fat and protein content, and somatic cell count of the individual cows in T5 and T4 farm were collected from the results published by the Állattenyésztési Teljesítményvizsgáló Ltd. Somatic cell count were determined by flow cytometry with Bentley FCM instrument at Állattenyésztési Teljesítményvizsgáló Ltd. Data formed between September 2017 and January 2020 by 30 primiparous Holstein Friesian and

Jersey cows were included in the calculations, in case of T5 farm. During this time interval the data of the two breeds, as well as data formed during the early stage (under 100 days; n=135), the mid stage (100-200 days; n=121) and the late stage (over 200 days; n=150) of lactation were summed.

Data from 38 individuals formed between May 2015 and January 2020 were included in the calculations in case of T4 farm. During this time interval the data formed by cows in their first lactation (n = 387) and by cows in their 2-5 lactations (n = 446); as well as data formed during the early stage (under 100 days; n=275), the mid stage (100-200 days; n=249) and the late stage (over 200 days; n=309) of lactation were summed.

A total of 63 individual milk samples were collected from 30 lactating cows (16 Holstein Friesian and 14 Jersey cows), to investigate the effect of breed and lactation stage on the microbiological quality of milk in T5 farm. Of the 63 individual milk samples, 32 samples were collected from Holstein Friesian and 31 samples were collected from Jersey cows. To investigate the effect of the lactation stage on the microbiological quality of the milk, samples from the same cows were collected during the lactation stages, but in some cases this could not be done due to the removal of the cows from production. Samples were collected from individuals in their early stage ($n_{\text{Holstein Friesian}}$=12 samples; n_{Jersey}=11 samples), mid stage ($n_{\text{Holstein Friesian}}$=12 samples; n_{Jersey}=11 samples) and late stage ($n_{\text{Holstein Friesian}}$=8 samples; n_{Jersey}=9 samples) of first lactation.

To investigate the effect of lactation number and lactation stage on the microbiological quality of the milk in T4 farm, a total of 62 individual milk samples were collected from 38 lactating cows. To investigate the effect of the lactation stage, we tried to collect samples from the same cows during the lactation stages, but in some cases this could not be done due to the removal of the cows from production. The milk samples were divided into the following groups according to the stages of lactation and the number of lactations of cows during sampling occassions: 23 samples were collected in the early stage of lactation (under 100 days), 21 samples were collectd in the mid stage of lactation (100-200 days), and 18 samples were collected from cows at the end of their lactation (over 200 days). Of the 62 individual milk samples, 26 samples were collected from primiparous cows (15 individuals), and 36 samples were collected from multiparous (2-5 calvings) cows (23 individuals).

Sampling of bulk milk to examine the effect of the seasons on the microbiological quality of milk:

To analyze the effect of seasons on raw milk, three to seven bulk milk samples were collected from the T1-T5 farms per season (n_{winter}=28; n_{spring}=27; n_{summer}=29; n_{autumn}=26). A total of 110 milk samples were tested.

Sampling of bulk milk to examine the effectiveness of the *Staphylococcus aureus* control program:

A *S. aureus* control program was conducted at the T3 farm, during which we had the opportunity to collect bulk milk samples between February 2019. and December 2019. to assess efficacy. During the control program, the barns and milking parlor were cleaned and disinfected more frequently, the infected cows were kept isolated from other animals, milked separately, and their milk was destroyed. For our microbiological studies, a total of 35 bulk milk samples were collected from the same milk storage tank containing milk of all cows milked on the farm. Samples were collected during six sampling occassions. 42 to 84 days elapsed between sampling occassions.

2.3. Methods for microbiological examination of bulk milk and individual milk samples

2.3.1. Determination of total plate count

The total plate count was determined according to the requirements of the MSZ EN ISO 4833-1:2014 standard. In the case of bulk milk, the limit on the total plate count is set in Regulation (EC) No 853/2004. The limit is $M=1,0\times10^5$ ($5.00\ \log_{10}$) cfu/ml (colony forming unit).

2.3.2. Determination of coliform count

The coliform count in raw milk samples was determined according to ISO 4832:2006. In the case of bulk milk, the limits on the coliform count are laid down in Regulation 4/1998. (XI. 11.) EüM, which prescribes the compliance (“m”) limit [$m=1.0\times10^1$ ($1.00\ \log_{10}$) cfu/ml] and the rejection (“M”) limit [$M=1.0\times10^2$ ($2.00\ \log_{10}$) cfu/ml].

2.3.3. Determination of Staphylococcus aureus *count and the confirmatory tests*

The *S. aureus* count was determined according to the MSZ EN ISO 6888-1:2008 standard. Tests for identification were then performed on five typical and atypical colonies. To determine the presence of free coagulase enzyme, freeze-dried rabbit plasma (Bio-Rad Magyarország Ltd., Hungary) was used, which was prepared according to the manufacturer's instructions. *S. aureus* was isolated from other *Staphylococcus* species by latex agglutination test (Prolex™ Staph Xtra Kit, Biolab Ltd., Hungary).

In the case of bulk milk, the limit values [$m=1.0\times10^2$ (2.00 $\log_{10}$) cfu/ml, $M=5.0\times10^2$ (2.70 $\log_{10}$) cfu/ml] for *S. aureus* are laid down in Regulation 4/1998. (XI. 11.) EüM.

S. aureus strains isolated from milk samples were stored in cryotubes at -80 °C until further analysis.

2.4. Investigation of the characteristics of *Staphylococcus aureus* strains isolated from samples

Genetic level identification was also performed for all strains. The prevalence of the species-specific thermonuclease gene (*nuc*) was examined by PCR. The primers used for the tests have been shown in the literature to be specific for the *S. aureus nuc* gene (BRAKSTAD et al., 1992).

The catalase test and oxidase test were performed according to JAVID et al. (2018).

The type of hemolysis of *S. aureus* strains collected from the samples was determined in accordance with PEREIRA et al. (2009).

2.4.2. Identification of Staphylococcus aureus *isolates by MALDI-TOF mass spectrometry*

As a further confirmation, 49 *S. aureus* strains collected from milk samples were identified by MALDI-TOF mass spectrometry. Fourty-six strains were tested in the microbiology laboratory of WESSLING Hungary Ltd. in Budapest. Three strains (SA57A, SA57B, SA57C) were tested at the Department of Bacteriology, Mycology and Parasitology of the National Public Health Center, because these strains were isolated later than the other strains. Direct sample application was used with adding formic acid and α-HCCA matrix solution. MALDI BioTyper 3.1. Bruker software was used by the WESSLING Hungary Ltd. to identify isolates, the evaluation was performed based on the method of LOVÁSZ (2014).

2.4.3. The determination of spa *type of* Staphylococcus aureus *isolates*

The determination of the *spa* type of strains isolated from milk samples was performed by the Department of Bacteriology, Mycology and Parasitology of the National Public Health Center. The method used by the laboratory is the 1.1 version of the method described by the European Network of Laboratories for Sequence Based Typing of Microbial Pathogens (SeqNet) (2004) ("DNA Sequencing of the *spa* Gene"). BURP ("based upon repeat patterns") clustering of *spa* types with default parameters was performed with Ridom Staphtype software (Ridom GmbH, Germany), according to MELLMANN et al. (2008).

2.4.5. Antibiotic susceptibility testing

Antibiotic resistance of isolated strains was examined and evaluated by agar diffusion method according to CLSI (Clinical and Laboratory Standards Institute, 2017). The experiments were performed in the microbiological laboratory of the Department of Medical Microbiology and in the microbiological laboratory of the Institute of Food Science at the University of Debrecen.

Reference strain ATCC 25923 was used as a positive control. The following antibiotic discs were used for the study: cefoxitin (30 µg/disc), chloramphenicol (30 µg/disc), clindamycin (2 µg/disc), erythromycin (15 µg/disc), gentamicin (10 µg/disc), penicillin G (10U), tetracycline (30 µg/disc), trimethoprim/sulfamethoxazole (1.25+23.75 µg/disc).

2.4.6. Investigation of genes encoding Staphylococcus entertoxin by PCR

PCR tests were performed according to BIANCHI et al. (2014) with some modifications in the protocol.

Reference strains ATCC 29213 (*sea*; *seg*; *sei*), ATCC 14458 (*seb*), ATCC 19095 (*sec*; *seg*; *seh*; *sei*), ATCC 23235 (*sed*; *seg*; *sei*; *sej*), ATCC 27664 (*see*) were used as control strains. Own strains isolated from bulk milk were used as reference strains for investigating the occurrence of the *selm*, *seln*, *selo* enterotoxin encoding genes.

Extraction of genomic DNA from *S. aureus* strains was performed using PrepMan™ Ultra Sample Preparation Reagent (Biocenter Ltd., Hungary) according to the instructions of the producer.

Seven primer sets were prepared for the PCR tests. It was necessary to run the primers as separate reactions to avoid overlap of PCR products of similar size during gel electrophoresis. PCR 1 was designed to amplify the *sea*, *seb*, *sec*, *sed*, *see* enterotoxin encoding genes. PCR 2 was designed to amplify *seg* and *sei*. PCR 4 was designed to amplify *selm* and *selo*. PCR 6 was designed to amplify *seh* and *ser*. Simplex PCR was used to amplify *sej* (PCR 3) and *seln* (PCR 5) enterotoxin encoding genes and the *nuc* gene (PCR 7).

DNA amplification was performed using a T100™ Thermal Cycler (Bio-Rad Hungary Ltd., Hungary). Amplified samples were analyzed by electrophoresis at 120 V for 40 minutes with a PowerPac Basic power supply (Bio-Rad Hungary Ltd., Hungary). The gel image was visualized with a FluorChem M system (Bio-Science Ltd., Hungary).

2.5. Statistical methods

In order to perform the statistical probes and to make the results easier to represent, the cfu values were converted to logarithmic values. Mean values and standard deviation were calculated from the results obtained during the tests. SPSS v.22.0 (2013) software was used to calculate descriptive statistics, logarithmic transformation of microorganism colony counts, and to perform t-test and variance analysis. Probes were considered significant below a P value of 5%.

3. Results

3.1. The effect of various factors on the microbiological quality of raw milk

3.1.1. Effect of breed on raw milk production and microbiological parameters

The mean and standard deviations of the daily milk yield, the fat and protein content, the somatic cell count, the total plate count, coliform and *S. aureus* count of the milk produced by two cattle breeds (Holstein Friesian and Jersey) were compared at the T5 farm (**Table 1**). The mean and standard deviation of the daily milk yield of Holstein-Friesian cows was 27.61±4.78 kg/day, which was more ($P<0.05$) than the mean daily milk yield of Jersey cows (17.71±2.68 kg/day). The finding is consistent with the literature.

According to the literature, the fat content of the milk of Holstein Friesian cows is around 3.5-4.0%, the protein content is around 2.8-3.4%, while in the case of Jersey cows the milk fat content is 5.0-7.0%, the milk protein content is 4.0-4.5% (HOLLÓ and SZABÓ, 2016). In the course of our research, we obtained results that are consistent with those summarized in the literature. The mean fat and protein content of the milk of Holstein Friesian cows (4.20±0.50% and 3.45±0.20%) was found to be lower ($P<0.05$) than the mean fat and protein content of the milk of Jersey cows (5.89±0.65% and 4.39±0.34%).

The mean somatic cell count of milk from Jersey cows was 186.9×10^3 ($5.21\pm0.24\ \log_{10}$) cell/ml, and in the milk from Holstein Friesian cows the mean somatic cell count was 185.1×10^3 ($5.17\pm0.32\ \log_{10}$) cell/ml. No significant difference ($P>0.05$) was detected in somatic cell count between the two breeds.

The mean total plate count in samples collected from Holstein Friesian cows was 3.2×10^3 ($3.26\pm0.47\ \log_{10}$) cfu/ml, and in milk samples collected from Jersey cows it was 2.1×10^3 ($3.01\pm0.55\ \log_{10}$) cfu/ml. No significant difference was found between the obtained mean total plate counts ($P>0.05$).

In samples collected from Holstein Friesian and Jersey cows, no difference was observed ($P>0.05$) between the mean coliform counts [1.5×10^1 ($0.73\pm0.60\ \log_{10}$) cfu/ml and 6.9×10^0 ($0.50\pm0.50\ \log_{10}$) cfu/ml], similarly to the total plate count.

S. aureus was not present in detectable amounts in milk samples collected from cows at the T5 farm.

Table 1: Production parameters and microbiological quality of milk from Holstein Friesian and Jersey cows (T5 farm)

		Holstein Friesian ($\bar{x}\pm\sigma$)	Jersey ($\bar{x}\pm\sigma$)
Production parameter	Daily milk yield (kg/day)	27.61 ± 4.78^{a}	17.71 ± 2.68^{b}
	Fat content (%)	4.20 ± 0.50^{a}	5.89 ± 0.65^{b}
	Protein content (%)	3.45 ± 0.20^{a}	4.39 ± 0.34^{b}
Microbiological parameter	Somatic cell count (log$_{10}$ cell/ml)	5.17 ± 0.32^{a}	5.21 ± 0.24^{a}
	Total plate count (log$_{10}$ cfu/ml)	3.26 ± 0.47^{a}	3.01 ± 0.55^{a}
	Coliform count (log$_{10}$ cfu/ml)	0.73 ± 0.60^{a}	0.50 ± 0.50^{a}
	Staphylococcus aureus count (log$_{10}$ cfu/ml)	<1.00	<1.00

$^{a,\ b}$: The mean values marked with different letters in the same rows of the table differ significantly ($P<0.05$)

3.1.2. Effect of lactation number on raw milk production and microbiological parameters

Table 2 summarizes the mean and standard deviations of the daily milk yield of primiparous and multiparous cows in T4 farm and the fat and protein content, somatic cell count, total plate count, coliform and *S. aureus* count of the milk produced. The mean milk yield of primiparous cows was 25.67 kg/day, while it was 31.04 kg/day in case of multiparous cows. The difference was significant ($P<0.05$).

During the first lactation of the examined cows, the mean fat content of the milk was 3.74 ± 0.40%, which does not differ significantly ($P>0.05$) from the mean fat content detected in the milk samples collected from multiparous cows, which was 3.75 ± 0.36%. Summing up the results of our study and other foreign literature, we concluded that besides the effect of lactation number, other factors can also influence the fat content of milk.

There was no significant difference ($P>0.05$) between the mean protein content (3.24 ± 0.19%) of the milk samples collected during the first lactation of Holstein Friesian cows milked in the T4 farm, and the mean protein content (3.31 ± 0.16%) of milk samples collected from multiparous cows.

The mean somatic cell count [242.2×10^{3} (5.12 ± 0.42 log$_{10}$) cells/ml] in the milk of primiparous cows was lower ($P<0.05$) than in the milk of multiparous cows [356.3×10^{3} (5.39 ± 0.39 log$_{10}$) cell/ml], but the mean values did not exceed the limit [$M=400.0\times10^{3}$ (5.60 log$_{10}$) cfu/ml] set in Regulation (EC) No 853/2004. The results obtained was consistent with the literature.

The mean total plate count in the milk samples of the first lactating Holstein Friesian cows was 5.1×10^3 (3.36 ± 0.58 $\log_{10}$) cfu/ml, and in the milk samples collected from multiparous cows it was 4.6×10^3 ($3,30\pm0.59$ $\log_{10}$) cfu/ml; the difference was not significant ($P>0.05$).

However, the mean coliform count [1.1×10^3 (1.35 ± 1.20 $\log_{10}$) cfu/ml] was higher ($P<0.05$) in the milk of multiparous cows than in the milk of primiparous cows [1.1×10^1 (0.65 ± 0.61) $\log_{10}$) cfu/ml].

S. aureus was not occurred in any of the 26 milk samples of the primiparous cows but the bacteria occurred in seven of the 36 samples from multiparous cows (19%). The mean *S. aureus* count in the seven milk samples was 1.6×10^2 (1.92 ± 0.60 $\log_{10}$) cfu/ml. As no *S. aureus* positive sample was present in the case of primiparous cows, it was not possible to calculate significance. The values were not exceeded the limit set in Regulation 4/1998. (XI. 11.) EüM [$M=5.0\times10^2$ (2.70 $\log_{10}$) cfu/ml].

Table 2: Production parameters and microbiological quality of milk from primiparous and multiparous cows (T4 farm)

		Primiparous ($\bar{x}\pm\sigma$)	Multiparous ($\bar{x}\pm\sigma$)
Production parameter	Daily milk yield (kg/day)	25.67 ± 4.42^a	31.04 ± 5.08^b
	Fat content (%)	3.74 ± 0.40^a	3.75 ± 0.36^a
	Protein content (%)	3.24 ± 0.19^a	3.31 ± 0.16^a
Microbiological parameter	Somatic cell count ($\log_{10}$ cell/ml)	5.12 ± 0.42^a	5.39 ± 0.39^b
	Total plate count ($\log_{10}$ cfu/ml)	3.36 ± 0.58^a	3.30 ± 0.59^a
	Coliform count ($\log_{10}$ cfu/ml)	0.65 ± 0.61^a	1.35 ± 1.20^b
	Staphylococcus aureus count ($\log_{10}$ cfu/ml)	<1.00	1.92±0.56

$^{a,\ b}$: The mean values marked with different letters in the same rows of the table differ significantly ($P<0.05$)

3.1.3. Effect of lactation stages on raw milk production and microbiological parameters

Table 3 summarizes the mean and standard deviations of the daily milk yield and the fat and protein content, somatic cell count, total plate count, coliform count and *S. aureus* count of the milk of cows in their early, mid, and late lactation stages in case of T4 farm. Investigating the change in the daily milk yield with the stage of lactation, we found that the amount of milk produced per day decreases as lactation progresses. This finding, which can also be found in the literature, was confirmed in case of T4 farm. The mean daily milk yield of cows in their first stage of lactation was 32.10±4.73 kg/day, in case of cows in their mid stage of lactation it was 29.08±5.09 kg/day and in case of cows at their end of lactation 23.36±3,63 kg/day. There was a significant difference ($P<0.05$) between the mean values.

The fat content of the milk of the cows changed towards the end of lactation. The mean fat content of the milk of cows in their first and mid stages of lactation was 3.65±0.43% and 3.59±0.41%, respectively, which were lower ($P<0.05$) than in the milk of cows in their late stage of lactation (3.99±0.47%). An increase in the concentration of fat in milk in the later stages of lactation may be related to a decrease in milk yield as lactation progresses, because a decrease in the amount of milk may have a concentrating effect on milk composition.

Similar to fat content, a change could be observed as lactation progresses in case of protein content. The mean protein content at the beginning of lactation of cows was 3.08±0.15%, in the mid stage of lactation it was 3.20±0.19% and at the end of lactation it was 3.56±0.20%. The difference was significant ($P<0.05$).

At the beginning of lactation of the selected cows, the mean somatic cell count was 195.1×10^3 (5.07±0.43 $\log_{10}$) cell/ml, in the mid stage of lactation it was 370.6×10^3 (5.28±0.50 $\log_{10}$) cells/ml and in the late stage of lactation it was 336.4×10^3 (5.33±0.41 $\log_{10}$) cell/ml. The somatic cell count in milk of cows at the end of their lactation was higher ($P<0.05$) than in milk of cows in their early stage of lactation.

We also compared the total plate count of milk collected at different stages of lactation of Holstein Friesian cows. In the early stage of lactation the mean total plate count was 6.8×10^3 (3.42±0.67 $\log_{10}$) cfu/ml, in the middle of lactation it was 4.4×10^3 (3.39±0.46 $\log_{10}$) cfu/ml, at the end of lactation it was 2.7×10^3 (3.13±0.56 $\log_{10}$) cfu/ml. There was no statistically significant difference ($P>0.05$) between the mean values obtained.

The highest mean coliform count [1.3×10^3 (1.30±1.23 $\log_{10}$) cfu/ml] was detected in the samples collected at the beginning of lactation of the cows, the lowest mean coliform count [2.2×10^1 (0.76±0.79 $\log_{10}$) cfu/ml] was observed in the samples collected during mid-lactation. The mean coliform count in the samples collected in the late stage of lactation was 1.50×10^2 (0.90±0.94 $\log_{10}$) cfu/ml. No significant difference ($P>0.05$) was found between the values.

S. aureus occurred in a total of nine of the 62 milk samples (14.52%), with a mean colony count of 1.4×10^2 (1.89±0.53 $\log_{10}$) cfu/ml. *S. aureus* occurred in six of the 23 (26.09%) individual milk samples collected at the beginning of lactation, the mean colony count was 1.2×10^2 (1.81±0.56 $\log_{10}$) cfu/ml. *S. aureus* occurred in one of the 21 (4.76%) samples collected in the mid stage of lactation, the *S. aureus* count was 8.2×10^1 (1.91 $\log_{10}$) cfu/ml. *S. aureus* occurred in two (11.11%) samples collected from the end of lactation, 2.4×10^2 (2.15±0.70 $\log_{10}$)

cfu/ml. Due to the small amount of positive samples, it was not possible to calculate significance.

Table 3: Effect of lactation stages on raw milk production and microbiological parameters (T4 farm)

		Stage of lactation		
		Early ($\bar{x}\pm\sigma$)	Mid ($\bar{x}\pm\sigma$)	Late ($\bar{x}\pm\sigma$)
Production parameter	Daily milk yield (kg/day)	32.10 ± 4.73^{a}	29.08 ± 5.09^{b}	23.36 ± 3.63^{c}
	Fat content (%)	3.65 ± 0.43^{a}	3.59 ± 0.41^{a}	3.99 ± 0.47^{b}
	Protein content (%)	3.08 ± 0.15^{a}	3.20 ± 0.19^{b}	3.56 ± 0.20^{c}
Microbiological parameter	Somatic cell count ($\log_{10}$ cell/ml)	5.07 ± 0.43^{a}	$5.28\pm0.50^{a,b}$	5.33 ± 0.41^{b}
	Total plate count ($\log_{10}$ cfu/ml)	3.42 ± 0.67^{a}	3.39 ± 0.46^{a}	3.13 ± 0.56^{a}
	Coliform count ($\log_{10}$ cfu/ml)	1.30 ± 1.23^{a}	0.76 ± 0.79^{a}	0.90 ± 0.94^{a}
	Staphylococcus aureus count ($\log_{10}$ cfu/ml)	1.81 ± 0.56	1.91	2.15 ± 0.70

$^{a,\ b,\ c}$: The mean values marked with different letters in the same rows of the table differ significantly ($P<0.05$)

The effect of lactation stage on milk yield, milk composition and microbiological parameters were investigated in the case of Holstein Friesian cows in the T5 farm (**Table 4**). Investigating the changes in the daily milk yield with the lactation stages, we found that the amount of milk produced per day decreases towards the end of lactation. This finding, which can also be found in the literature, was also confirmed in the Hungarian large-scale T5 farm. The mean daily milk yield of cows in the first stage of lactation was 31.74±5.48 kg/day, in the mid stage of lactation it was 27.46±5.09 kg/day, and at the end of lactation it was 20.41±3.84 kg/day. The mean daily milk yield was significantly lower ($P<0.05$) at the end of lactation than at the beginning and middle of lactation.

In the T5 farm, the fat content of milk of the examined cows increased towards the end of lactation, but there was no significant difference ($P<0.05$) between the mean values. At the beginning of lactation, the fat content of milk was 4.07±0.50%, in the mid stage of lactation it wa 4.18±0.61%, and it was 4.49±0.73% at the end of lactation of cows.

It can be said that a change can be observed at the end of the lactation in terms of the mean protein content. The mean protein content measured at the beginning of lactation was 3.32±0.22%, in the middle of lactation it was 3.47±0.22%, and at the end of lactation it was 3.64±0.27%, which was significantly higher ($P<0.05$) than the mean values obtained at the beginning and mid stages of lactation.

In the early stage of lactation of cows selected in the T5 farm, the mean somatic cell count was 180.7×10^3 (5.12 ± 0.39 $\log_{10}$) cell/ml, in the middle of lactation it was 142.8×10^3 (5.06 ± 0.29 $\log_{10}$) cell/ml and in the late lactation stage it was 226.7×10^3 (5.15 ± 0.45 $\log_{10}$) cells/ml. No significant difference ($P>0.05$) was observed between the mean somatic cell count values.

Similarly to the T4 farm, we found that the mean total plate count did not change significantly ($P>0.05$) during the lactation stages in the case of the T5 farm. The mean total plate count in the milk of cows at their beginning of lactation was 2.0×10^3 (3.08 ± 0.50 $\log_{10}$) cfu/ml, at the mid stage of lactation it was 3.5×10^3 (3.42 ± 0.35 $\log_{10}$) cfu/ml and at the end of lactation it was 8.2×10^3 (3.53 ± 0.65 $\log_{10}$) cfu/ml.

Similarly to the T4 farm and the total plate count, no difference in the coliform count was found between the mean colony counts in the case of T5 farm. The mean coliform count in the milk of cows at their beginning of lactation was 6.7×10^0 (0.63 ± 0.47 $\log_{10}$) cfu/ml, at the mid stage of lactation it was 2.4×10^1 (0.75 ± 0.75 $\log_{10}$) cfu/ml, and at the end of lactation it was 1.1×10^1 (0.80 ± 0.57 $\log_{10}$) cfu/ml.

S. aureus did not occur in individual milk samples collected from the T5 farm.

Table 4: Effect of lactation stages on raw milk production and microbiological parameters (T5 farm)

		Stage of lactation		
		Early ($\bar{x}\pm\sigma$)	Mid ($\bar{x}\pm\sigma$)	Late ($\bar{x}\pm\sigma$)
Production parameter	Daily milk yield (kg/day)	31.74 ± 5.48^a	27.46 ± 5.09^a	20.41 ± 3.84^b
	Fat content (%)	4.07 ± 0.50^a	4.18 ± 0.61^a	4.49 ± 0.73^a
	Protein content (%)	3.32 ± 0.22^a	3.47 ± 0.22^a	3.64 ± 0.27^b
Micro-biological parameter	Somatic cell count ($\log_{10}$ cell/ml)	5.12 ± 0.39^a	5.06 ± 0.29^a	5.15 ± 0.45^a
	Total plate count ($\log_{10}$ cfu/ml)	3.08 ± 0.50^a	3.42 ± 0.35^a	3.53 ± 0.65^a
	Coliform count ($\log_{10}$ cfu/ml)	0.63 ± 0.47^a	0.75 ± 0.75^a	0.80 ± 0.57^a
	Staphylococcus aureus count ($\log_{10}$ cfu/ml)	<1.00	<1.00	<1.00

$^{a,\ b}$: The mean values marked with different letters in the same rows of the table differ significantly ($P<0.05$)

3.1.4. The effect of season on the microbiological quality of raw milk

The mean total plate count was 3.3×10^4 (4.40 ± 0.29 $\log_{10}$) cfu/ml in the bulk milk samples collected in winter, and was 1.9×10^5 (4.51 ± 0.76 $\log_{10}$) cfu/ml in the samples collected in autumn (**Table 5**). There was no significant difference ($P>0.05$). However, the mean total plate count was less ($P<0.05$) in the samples collected in winter and autumn than in the milk samples collected in spring and summer. The mean total plate count in the spring and summer samples was 1.6×10^{5} (5.02 ± 0.43 log$_{10}$) cfu/ml and 1.1×10^{5} (4.96 ± 0.25 log$_{10}$) cfu/ml, respectively. In a total of 39 (35%) samples, the mean total plate count was higher than the rejection limit set in the Regulation. However, the mean total plate count exceeded the limit in 59% of spring samples, 52% of summer samples, 19% of autumn samples, and 11% of winter samples.

The highest mean coliform count [1.4×10^{4} (3.45 ± 0.90 log$_{10}$) cfu/ml] occurred in milk samples collected in summer. There was a significant difference ($P<0.05$) between the values obtained in the samples collected in winter [1.8×10^{3} (2.19 ± 1.17 log$_{10}$) cfu/ml] and in summer. The mean coliform count in the samples collected in autumn was 6.8×10^{2} (2.73 ± 0.32 log$_{10}$) cfu/ml, and in the samples collected in spring it was 5.6×10^{2} (2.37 ± 0.81 log$_{10}$) cfu/ml (**Table 5**). In half of the samples collected in winter, the colony count was less than the “M” rejection limit. In 81% of the spring samples and in all of the samples collected in summer and autumn, the mean coliform count exceeded the “M” value. Overall, coliform bacteria were detected in excess of the limit in 83% of the samples. In our research, the large amount of coliform bacteria in bulk milk suggests that inadequate milk collection and treatment practices in dairy farms may have caused contamination of milk with coliform bacteria.

Table 5: The microbiological quality of bulk milk in different seasons (T1-T5)

Microbiological parameter	**Season**				**„M” value (log$_{10}$ cfu/ml)**	**Regulation**
	Winter	**Spring**	**Summer**	**Autumn**		
Total plate count [log$_{10}$ cfu/ml]	4.40^{a}	5.02^{b}	4.96^{b}	4.51^{a}	5.00	853/2004/EK
Coliform count [log$_{10}$ cfu/ml]	2.19^{a}	2.37^{a}	$3.45^{a,\,b}$	2.73^{b}	2.00	4/1998. (XI.11.) EüM
Staphylococcus aureus count [log$_{10}$ cfu/ml]	2.69^{a}	$3.25^{a,b}$	$3.42^{b,c}$	2.98^{a}	2.70	4/1998. (XI.11.) EüM

a, b, c: The mean values marked with different letters significantly differ from each other ($P<0.05$)

S. aureus occurred in bulk milk samples from three dairy farms (T1, T2, T5) in an amount of less than 1.00 log$_{10}$ cfu/ml. In the case of the T3 and T4 dairy farms, the highest mean *S. aureus* count [2.7×10^{3} (3.42 ± 0.10 log$_{10}$) cfu/ml] was detected in the samples collected in summer and the lowest mean count was detected in the samples collected in winter [9.3×10^{2}

(2.69±0.59 $\log_{10}$) cfu/ml] and in autumn [1.0×10^3 (2.98±0.13 $\log_{10}$) cfu/ml] (**Table 5**). Significant difference ($P<0.05$) was detected between the values obtained in the samples collected in these months. For both farms, the mean *S. aureus* count in all bulk milk samples exceeded the “M” value set in the Regulation. The highest amount of *S. aureus* was detected during the summer in our studies, which may be related to the heat stress of cows in the summer due to unfavorable weather conditions (high temperature and humidity).

3.2. Characteristics of *Stapylococcus aureus* strains isolated from milk samples

In the course of our studies, 49 *S. aureus* strains were selected from the milk samples positive for the occurrence of *S. aureus* for further studies. Forty-two strains were isolated from bulk milk while seven strains were isolated from individual milk samples. All strains were positive with the latex agglutination test, and the presence of the *nuc* gene indicative of thermonuclease enzyme production was also detected during PCR tests. Eighteen strains were isolated from milk samples collected in T3 farm and 31 strains isolated from samples collected in T4 farm.

3.2.1. Results of identification of Staphylococcus aureus *isolates*

Based on the results of the identification obtained with the MALDI-TOF mass spectrometry [best score (>2.300) and best-matching isolate], all strains from T3 and T4 farm were identified as *S. aureus*.

3.2.2. Results of determining the spa *type of* Staphylococcus aureus *isolates*

Among the 18 isolates from bulk milk samples collected from the T3 farm, two *spa* types (t164 and t1987) were identified. The *spa* types detected in our study did not belong to the same cluster according to the BURP algorithm of the Ridom Staphtype software. The most common *spa* type was the t164; 17/18 strains (94%) belonged to this type. The *spa* type of strain SA54C was t1987.

Among the 24 *S. aureus* strains collected from bulk milk samples from the T4 farm, three *spa* types (t267, t359, t693) were identified. All the seven *S. aureus* strains isolated from individual milk samples belonged to the t267 *spa* type, which was also the most common (21/24; 88%) among the strains isolated from bulk milk. Two strains (SA4, SA41C; 8%) were t693 *spa* type and one strain (SA48A; 4%) was t359 *spa* type. In the studies, the t267 and t359 *spa* types were grouped into one cluster using the BURP algorithm.

3.2.3. Results of the determination of hemolysis type

Fifteen of the 18 strains (83%) isolated from milk collected in T3 farm showed β-hemolysis on Columbia blood agar, two strains (11%) had an incomplete hemolytic phenotype (expressed dual zone), and one strain (6%) showed weak β-hemolysis (**Table 6**).

Twenty-five of the 31 strains (81%) isolated from bulk milk and individual milk samples collected from the T4 farm had an incomplete hemolytic phenotype (expressed dual zone), four isolates (13%) showed β and weak α-hemolysis, two isolates (6%) showed β-hemolysis. Five of the seven strains isolated from individual milk samples had dual zones and two strains had β and weak α-hemolysis (**Table 7**).

Table 6: Characteristics of *Staphylococcus aureus* strains isolated from raw milk (T3 farm)

#	**Strain code**	**Type of hemolysis**	**Antibiotic resistance***	**Enterotoxin genes (multiplex PCR)**
1.	SA7	β	R (P)	*seg, sei, selm, seln, selo*
2.	SA33	β	R (P)	*sei*
3.	SA34	β	R (P)	*seg, sei, selm, seln, selo*
4.	SA35A	β	R (P)	*seg, sei, selm, seln, selo*
5.	SA35B	β	R (P)	*seg, sei, selm, seln, selo*
6.	SA39A	β	R (P)	*sei, selm, seln, selo*
7.	SA39B	β	R (P)	*sei, selm, seln, selo*
8.	SA44	β	R (P)	*seg, sei, selm, seln, selo*
9.	SA45	β	R (P)	*seg, sei, selm, seln, selo*
10.	SA53A	β	R (P)	*seg, sei, selm, seln, selo*
11.	SA53B	β	R (P)	*seg, sei, selm, seln, selo*
12.	SA53D	β	R (P)	*seg, sei, selm, seln, selo*
13.	SA54A	α + β	R (P)	*seg, sei, selm, seln, selo*
14.	SA54B	α + β	R (P)	*sei, selm, seln, selo*
15.	SA54C	weak β	R (P)	*sei*
16.	SA57A	β	R (P)	*seg, sei, selm, seln, selo*
17.	SA57B	β	R (P)	*sei, selm, seln, selo*
18.	SA57C	β	R (P)	*seg, sei, selm, seln, selo*

*R: resistant; P: penicillin G

Table 7: Characteristics of *Staphylococcus aureus* strains isolated from raw milk (T4 farm)

#	Strain code	Sample type	Type of hemolysis	Antibiotic resistance*	Enterotoxin genes (multiplex PCR)
1.	SA4	Bulk milk	β	S	*seh*
2.	SA5	Bulk milk	α + β	S	-
3.	SA26A	Bulk milk	α + β	S	-
4.	SA26B	Bulk milk	α + β	S	*seh*
5.	SA27A	Bulk milk	α + β	S	-
6.	SA27B	Bulk milk	α + β	S	-
7.	SA27C	Bulk milk	α + β	S	-
8.	SA28	Bulk milk	α + β	S	-
9.	SA29	Bulk milk	β + weak α	S	-
10.	SA30A	Bulk milk	α + β	S	-
11.	SA30B	Bulk milk	α + β	S	-
12.	SA31A	Bulk milk	α + β	S	-
13.	SA31B	Bulk milk	α + β	S	-
14.	SA32A	Bulk milk	α + β	S	-
15.	SA32B	Bulk milk	β + weak α	S	-
16.	SA38A	Bulk milk	α + β	S	-
17.	SA38B	Bulk milk	α + β	S	-
18.	SA40A	Bulk milk	α + β	S	-
19.	SA41A	Bulk milk	α + β	S	-
20.	SA41B	Bulk milk	α + β	S	-
21.	SA41C	Bulk milk	β	S	*seh*
22.	SA48A	Bulk milk	α + β	S	-
23.	SA48B	Bulk milk	α + β	S	-
24.	SA56A	Bulk milk	α + β	S	-
25.	SA9503a	Individual milk	β + weak α	S	-
26.	SA9325a	Individual milk	α + β	S	-
27.	SA8582a	Individual milk	β + weak α	S	-
28.	SA9736a	Individual milk	α + β	S	-
29.	SA9979a	Individual milk	α + β	S	-
30.	SA8834a	Individual milk	α + β	S	-
31.	SA9736.2	Individual milk	α + β	S	-

S: susceptible

3.2.4. Results of the antibiotic susceptibility tests

Based on the results of the antibiotic resistance tests, it can be stated that all 18 *S. aureus* isolates collected from the bulk milk of T3 farm were susceptible to the tested antibiotics, except for penicillin G.

Since all strains from T3 farm were susceptible to cefoxitin, it can be concluded that all isolates were also susceptible to methicillin. Furthermore, according to the requirements of CLSI (2017), the results of the cefoxitin resistance test may indicate the *mecA*-mediated oxacillin resistance of the isolates. So it can be said that *S. aureus* strains isolated from bulk milk were susceptible to oxacillin in our studies.

All 31 isolates originated from T4 farm were susceptible to the tested antibiotics.

Similar but different results have been reported by other authors. In conclusion, it can be said that the *S. aureus* resistance situation at the T4 farm was more favorable than those published in the literature.

3.2.5. Results of investigation of genes encoding Staphylococcus entertoxin

All 18 strains originated from T3 farm were positive for one or more enterotoxin-encoding genes. Of the genes encoding the new types of SEs and SEls, five different genes were identified in the isolates. Twelwe of the 18 isolates (67%) harboured *seg*, *sei*, *selm*, *seln*, *selo* genes; four strains (22%) harboured *sei*, *selm*, *seln*, *selo* genes, and two strains (11%) harboured *sei* gene (**Table 6**).

Food poisoning is most commonly caused by the classical enterotoxins SEA and SEB, and among the new types of enterotoxins, SEH (ARGUDÍN et al., 2010; HNASKO et al., 2019). In our studies, none of the 18 strains harboured these enterotoxin-encoding genes. UMEDA et al. (2017) suggested that new types of SEs and SEls (e.g., toxins produced by *seg*, *sei*, *selm*, *seln*, *selo*, and *selu* genes) may also be potential causes of food-borne outbreaks. Our research revealed that all strains isolated in bulk milk from T3 farm harboured at least one of these genes, so it is essential to reduce the occurrence of *S. aureus* in the dairy farm, so implementing the *S. aureus* control program was justified. It is worth noting, that at least 10^5 cfu/ml of *S. aureus* could produce as much enterotoxin in milk as could induce food poisoning (KADARYIA et al., 2014), but this amount of bacteria was not present in the milk samples collected from the T3 farm.

HWANG et al. (2010) found in their study that the majority of t164 *spa* type isolates (seven out of eight isolates) and three of the seven t1987 *spa* type isolates harboured *seg*, *sei*, *selm*, *seln*, and *selo* genes. Similarly, in our study, 12 of the 17 t164 *spa* type isolates harboured these genes without harbouring classical enterotoxin genes. The t1987 *spa* type isolate harboured only the *sei* gene.

Three of the 31 strains (10%) isolated from bulk milk and individual milk collected from T4 farm (SA4, SA26B, SA41C) were positive for the *seh* gene, but no gene encoding classical enterotoxins and other new types of SEs and SEls was identified (**Table 7**). Only the bulk milk samples contained *S. aureus* harbouring enterotoxin; strains isolated from individual milk samples did not harbour the enterotoxin-encoding genes we examined in our research. Although vegetative bacteria can be killed during heat treatment, heat-stable enterotoxins may remain in milk (TESSEMA, 2016), so it is particularly important to reduce the occurrence of *S. aureus* in milk in the T4 farm.

Differences in results of phenotypic and genotypic characterization of *S. aureus* isolates collected from T3 and T4 farms could be detected in case of *spa* type, type of hemolysis, antibiotic resistance, and enterotoxin-producing ability. The differences may be due to the different environmental conditions or the different implementation of the hygiene regulations at the two farms.

Penicillin resistance in strains of T3 farm could be attributed to the usage of antibiotics in the farm, but it was not possible for the farm managers to share information about this. Variety can also be observed within one farm, but in both farms most of the strains have the same characterization. At the T3 farm, 11 of the 18 strains (61%) were t164 *spa* type, showed β-hemolysis, had penicillin G resistance, and harboured five *se* and *sel* genes (*seg*, *sei*, *selm*, *seln*, *selo*). At the T4 farm, 23 of the 31 strains (74%) were t267 *spa* type, showed α and β-hemolysis, were sensitive to all the tested antibiotics, and did not harbour classical enterotoxin-encoding genes or *seg*, *seh*, *sei*, *sej*, *selm*, *seln*, *selo*, *ser* genes.

3.3. Results of the investigation of effectiveness of a *Staphylococcus aureus* control program

Based on the results of the investigation of effectiveness of the *S. aureus* control program, all bulk milk samples collected from the T3 farm contained *S. aureus*. The mean values ranged from 1.1×10^2 to 3.0×10^3 (2.03 and 3.47 $\log_{10}$) cfu/ml. In our research, the amount of *S. aureus* at the end of our experiments [December; 1.1×10^2 (2.03 $\log_{10}$) cfu/ml] was lower ($P<0.05$) than in all other samples, including the samples collected during the first sampling, which was also performed in winter (February). The mean *S. aureus* count in the samples collected during the first sampling was 1.2×10^3 (3.06 $\log_{10}$) cfu/ml.

The results show that the mean *S. aureus* count in bulk milk decreased by the end of our research. The mean colony counts in the samples collected during the last two sampling occassions no longer exceeded the rejection limit. Based on the literature, fewer pathogenic bacteria can be detected in milk in winter (HILL et al., 2012; PETRÓCZKI et al., 2020). In our study, the last sampling was in winter, similar to the first sampling, but the results of the milk samples collected at the two sampling occassions differ significantly ($P<0.05$). The decreasing mean *S aureus* count can therefore be attributed to the effectiveness of the control program.

4. New scientific results

1. Different fat and protein contents in milk due to the varietal characteristics of different cow breeds (Holstein Friesian, Jersey) do not lead to a change in the microbiological quality of the milk under the same housing conditions. There was no statistically significant difference between the milk of the studied breeds in terms of total plate count (Holstein Friesian: 3.2×10^3 cfu/ml; Jersey: 2.1×10^3 cfu/ml), coliform count (Holstein Friesian: 1.5×10^1 cfu/ml; Jersey: 6.9×10^0 cfu/ml) and somatic cell count (Holstein Friesian: 185.1×10^3 cell/ml; Jersey: 186.9×10^3 cell/ml).
2. Based on the results of the studies carried out at a Hungarian large-scale dairy farm there was no difference in the total plate count of the milk samples collected from primiparous (5.1×10^3 cfu/ml) and multiparous (4.6×10^3 cfu/ml) cows.
3. In our research, we did not detect any difference in the microbiological quality of milk samples collected from different stages of lactation of dairy cows, in terms of total plate count and coliform count in the case of the two tested dairy farms. In case of T4 and T5 farms, the mean total plate count in the early stage of lactation was 6.8×10^3 cfu/ml and 2.0×10^3 cfu/ml, it was 4.4×10^3 cfu/ml and 3.5×10^3 cfu/ml in the mid stage of lactation and it was 2.7×10^3 cfu/ml and 8.2×10^3 cfu/ml at the end stage of lactation. In case of T4 and T5 farms, the mean coliform count in the early stage of lactation was 1.3×10^3 cfu/ml and 6.7×10^0 cfu/ml, it was 2.2×10^1 cfu/ml and 2.4×10^1 cfu/ml in the mid stage of lactation and it was 1.50×10^2 cfu/ml and 1.1×10^1 cfu/ml at the end stage of lactation.
4. In the case of T3 farm, 83% of the *Staphylococcus aureus* strains showed β-hemolysis and had penicillin G resistance. In the case of T4 farm, 81% of the strains showed α and β-hemolysis and were sensitive to the studied antibiotics (cefoxitin, chloramphenicol, clindamycin, erythromycin, gentamicin, penicillin G, tetracycline, trimethoprim/sulphamethoxazole).
5. In the case of T3 farm, 67% of the *Staphylococcus aureus* strains had t164 *spa* type and harboured five *se* and *sel* genes (*seg, sei, selm, seln, selo*). In the case of T4 farm, 67% of the strains had t267 *spa* type and did not harbour classical enterotoxin-encoding genes or *seg, seh, sei, sej, selm, seln, selo, ser* genes.

5. Practical usability of the results

1. In our research, we found that there is no microbiological difference between the milk of Holstein Friesian and Jersey cows, and the amount of microorganisms did not change significantly at different stages of lactation of the cows. However, the milk of multiparous cows seemed to be of lower quality than the milk of primiparous cows from a microbiological point of view, due to the high somatic cell count (356.3×10^3 cell/ml) and coliform bacteria (1.1×10^3 cfu/ml) and the higher incidence of *S. aureus*, which was detected in about 19% of milk samples collected from multiparous cows. When examining the effect of the seasons, the microbiological quality of milk collected in winter was better than the microbiological quality of milk collected in summer. The mean total plate count in bulk milk collected in winter was 3.3×10^4 cfu/ml, and it was 1.1×10^5 cfu/ml in the milk collected in summer. The mean coliform count was 1.8×10^3 cfu/ml in bulk milk collected in winter and 1.4×10^4 cfu/ml in milk collected in summer. The mean *S. aureus* count was 9.3×10^2 cfu/ml in bulk milk collected in winter and 2.7×10^3 cfu/ml in milk collected in summer. Our findings may help dairy farms, because with this information, it is possible to develop the most efficient solutions to produce proper microbiological quality milk.
2. In the course of our research the efficiency of a *S. aureus* control program was verified in a Hungarian large-scale dairy farm by collecting bulk milk samples and determining the *S. aureus* count in the samples, because based on the results the mean *S. aureus* count decreased from 1.2×10^3 cfu/ml to 1.1×10^2 cfu/ml by the end of the sampling occassions. The decrease was due to the more frequent cleaning and disinfection of barns and milking parlors, detection and isolation of infected cows from healthy animals, separate milking and destruction of their milk as soon as possible.
3. *S. aureus* is a facultative pathogenic microorganism that is widespread in the environment. *S. aureus* can be a problem in dairy farms, as it is still a major cause of mastitis in dairy animals. From the results of our studies it can be concluded that in addition to the determination and confirmation of the *S. aureus* according to the MSZ EN ISO 6888-1:2008 standard and by the latex agglutination test, it is strongly recommended to perform phenotypic and genotypic characterisation of strains isolated from milk. Data from continuous monitoring of the antibiotic resistance profile of the strains are needed for the effective and targeted implementation of possible antibiotic therapy in infected animals. Furthermore, the assessment of enterotoxin-producing ability is necessary because, although vegetative forms of *S. aureus* can be destroyed during heat treatment of milk, heat-stable enterotoxins capable of causing food poisoning can remain in milk and cause disease in consumers. Multiplex PCR can be used to determine the prevalence of enterotoxin-encoding genes, at short deadline.

6. Bibliography

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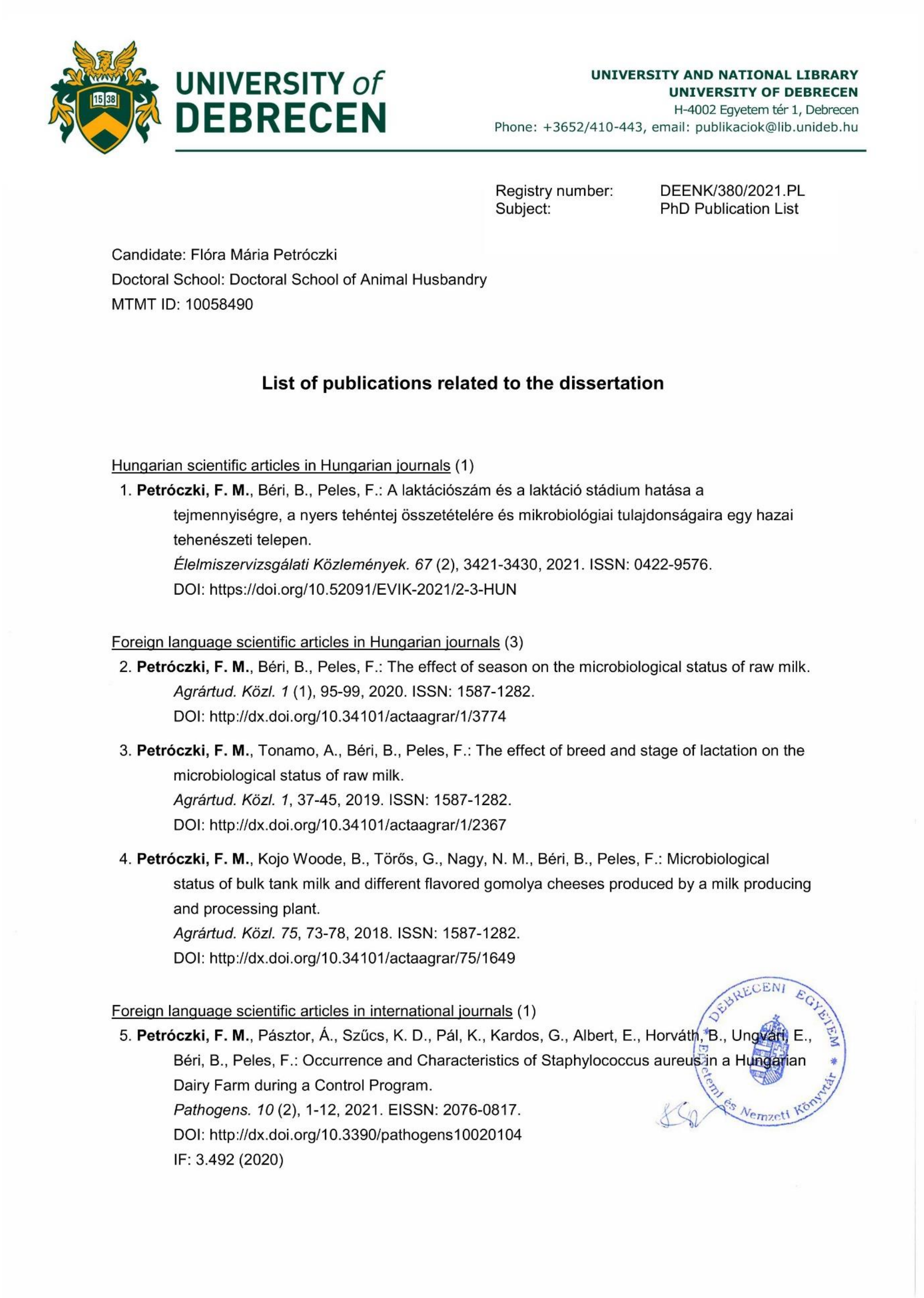
7. List of publications related to the dissertation



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Registry number: DEENK/380/2021.PL
Subject: PhD Publication List

Candidate: Flóra Mária Petróczki
Doctoral School: Doctoral School of Animal Husbandry
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List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (1)

1. **Petróczki, F. M.**, Béri, B., Peles, F.: A laktációszám és a laktáció stádium hatása a tejmennyiségre, a nyers tehéntej összetételére és mikrobiológiai tulajdonságaira egy hazai tehenészeti telepen.
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   DOI: https://doi.org/10.52091/EVIK-2021/2-3-HUN

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2. **Petróczki, F. M.**, Béri, B., Peles, F.: The effect of season on the microbiological status of raw milk.
   Agrártud. Közl. 1 (1), 95-99, 2020. ISSN: 1587-1282.
   DOI: http://dx.doi.org/10.34101/actaagrar/1/3774
3. **Petróczki, F. M.**, Tonamo, A., Béri, B., Peles, F.: The effect of breed and stage of lactation on the microbiological status of raw milk.
   Agrártud. Közl. 1, 37-45, 2019. ISSN: 1587-1282.
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4. **Petróczki, F. M.**, Kojo Woode, B., Törős, G., Nagy, N. M., Béri, B., Peles, F.: Microbiological status of bulk tank milk and different flavored gomolya cheeses produced by a milk producing and processing plant.
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6. **Petróczki, F. M.**, Veres, V., Kardos, G., Béri, B., Peles, F.: Characterisation of Staphylococcus aureus strains isolated from raw milk from vending machines in Hajdú-Bihar County, Hungary.
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8. **Petróczki, F. M.**, Béri, B., Peles, F.: The effect of breed and number of lactation on the microbiological quality of raw milk = a fajta és a laktáció számának hatása a nyerstej mikrobiológiai minőségére.
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14. Szerk. Kovács, B., Czipa, N., Peles, F., Bódi, É., Kántor, A., **Petróczki, F. M.**, Alexa, L.: Scientific researches in food production, University of Debrecen: Proceedings of abstracts. University of Debrecen, Debrecen, 30 p., 2018. ISBN: 9789634900412

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16. Horváth, B., Peles, F., Gasparik, J., Pocklán, E., Sipos, R., Erős, Á., **Petróczki, F. M.**, Szűcs, K. D., Albert, E., Micsinai, A.: Élelmiszerekből izolált Staphylococcus fajok antibiotikum rezisztencia vizsgálata.
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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