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Original article

Effects of non-*aureus* staphylococci on colostrum composition, properties and fatty acid profile in cow – a preliminary study

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Abstract

By providing the body with essential nutrients, colostrum plays an immune and immunostimulating function. Colostrum quality depends on multiple factors, including microbial presence. This study aimed to explore the effect of non-*aureus* staphylococci on colostrum quality. Physical and chemical properties, fatty acid profile of cow colostrum were determined. In our study, we identified three non-*aureus* staphylococci species in the colostrum: *S. sciuri*, *S. xylosus* and *S. warneri*. The percentage of dry matter in staphylococci positive and negative colostrum samples did not differ significantly. Contents of fat, protein, and lactose in the colostrum were similar. The content of butyric (C4:0) and capric (C10:0) acids was significantly higher in the colostrum fat from samples positive for non-*aureus* staphylococci. Total bacterial count was lower in non-*aureus* staphylococci positive samples, while pH increased. The percentage of β-casein was lower in colostrum with a positive culture for non-*aureus* staphylococci.

Key words: cow, colostrum, fatty acids, non-*aureus* staphylococci

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Introduction

Colostrum is commonly regarded as the source of immunoglobulins providing passive immunity to newborn calves. However, there is increasing evidence that components other than immunoglobulins also have a significant impact on the development of the intestinal epithelium and the formation of the gastrointestinal microbiome, as well as on the metabolic processes in calves, ensuring a stable glucose concentration and optimal growth. Non-immunoglobulin proteins serve as nutrients and growth stimulators (Santos et al. 2017). Colostrum fatty acids (FA) provide a large part of dietary energy for the newborn and it is believed that the FA composition reflects the biological needs of the neonatal calves (Wilms et al. 2022). Particular FA may also affect colostrum microbiota which play a crucial role in the development of a normal immune and barrier function of the intestinal mucosa (Walker et al. 2015). The microbiological quality of colostrum is considered an important parameter which may affect immunoglobulin absorption and passive immunity in calves. Pathogens in colostrum may also be a direct cause of morbidity and mortality of neonates (Baltrukova et al. 2019). The quality and quantity of cow mammary gland secretions are closely related to the udder health. Mastitis is the most frequent disease of dairy cows and has well-recognized damaging effects on animal wellbeing and dairy farm profitability (Ruegg 2017). A variety of factors affect the condition of the udder. Microorganisms living on the skin surface, contaminating milking equipment and present in the environment create a microbial burden and may provoke mastitis or contaminate the raw product and impact its quality (Yuan et al. 2019).

The most common microorganisms isolated from cow milk are non-*aureus* staphylococci (NAS) – a group of commensal microorganisms present on the skin and mucous membranes of animals and humans. They have been regarded as nonpathogenic or "minor pathogens" to livestock which do not impact the milk yield (Sukur and Esenda 2020, Toledo-Silva et al. 2022); however their connection with spontaneous abortions and mastitis is established as well (Hisira et al. 2020). Depending on the species NAS may be both infectious agents and contaminants in milk (Hamel et al. 2020). NAS may be associated with subclinical mastitis and persistent increases in the bulk milk somatic cell count (SCC), they often present resistance to antimicrobials, and the have the ability to produce biofilm (Addis et al. 2020). On the other hand, in other studies the presence of non-*aureus* staphylococci had no clear associacion with an increase in SCC (Chen et al. 2021).

A high proportion of milk from NAS infected udders in the milk tank may be associated with higher losses of fat and protein in whey during the coagulation process (Summer et al. 2015, Leitner et al. 2019). Moreover, according to Pyörälä and Taponen (2009) NAS infections can damage udder tissue and lead to decreased milk production. Currently, it is difficult to determine whether NAS species behave as contagious environmental pathogens or are contamina.

Bochniarz et al. (2017) indicated that the level of IL-6 and amyloid A in the milk of cows suffering from subclinical mastitis caused by NAS tended to be high compared with healthy cows, while IL-4 and IL-10 concentrations were lower.

Goats with subclinical mastitis caused by NAS had a lower daily milk fat yield,l actose content and higher daily protein yield than healthy ones (Gelasakis et al. 2018). Moreover, changes in the fatty acid profile of ewes' milk infected with *Staphylococcus spp.* that decrease the value of ewes' milk as a health-promoting product were found (Pikhtirova et al. 2020).

Cossignani and Blasi (2019) showed that strains of Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Propionibacterium and Streptococcus can be considered as potential producers of conjugated linoleic acids. Changes in the concentration and composition of these milk constituents synthesized and secreted by the mammary epithelium occur after secretion in the alveolar lumen and milk ducts (Needs and Anderson 1984).

Many studies have been conducted to determine the effect of staphylococci on milk production and milk quality of cows, sheep and goats, but the effect of NAS on colostrum quality remains uncertain, which was the purpose of our research. The aim of our study was to evaluate the influence of NAS on the features of cow colostrum predicting its biological quality.

Materials and Methods

Animals

The experiment was carried out under production conditions. The herd of cows consisted of 200 animals. Colostrum was collected from cows calving in the first quarter of the year. The animals were beginning the 2nd or the 3rd lactation. The herd consisted of cows of the Slovak Pied cattle breed (with 50% share of HF blood) housed in a free-stall system. The animals were clinically healthy and had no clinical signs of mastitis. They were kept in typical buildings that meet the requirements of the European Union Directive no. 2010/63/EU (2010). The diet was devised according to international standards (NRC, 2001). Colostrum samples for the study were collected during the first milking within 2 hours after calving.

The teats were disinfected with 70% ethanol before sample collection. Approximately 100 ml of bulk colostrum from each cow was milked into a sterile container, cooled in 4°C, and transported to the laboratory without delay. 1 ml of the sample was used for microbiological culture and the remaining material was immediately frozen to -20°C until analysis. Based on the bacterial culture results, control samples with no pathogen growth (n=13) and experimental samples positive for NAS and negative for other milk pathogens (n=14) were selected for analysis.

Our study was carried out in compliance with the ARRIVE guidelines (Percie du Sert et al. 2020). The experimental procedures were approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice, Slovakia no. EKVP 2022/05 following EU legislation 2010/63/EU, article 1:5 (practices not likely to cause pain, suffering, distress, or lasting harm equivalent to or higher than, that caused by the introduction of a need to, follow good veterinary practice).

Microbiological studies

Two methods were used to identify the species of coagulase-negative colonies. First, the biochemical enzymatic properties of the bacteria were identified using the STAPHY 24 test with the TNW 7.0 identification program (Erba-Lachema, Brno, Czech Republic). The method is considered to have a detection accuracy of more than 90.0%. The, spectrum of the bacterial proteins was the determined according to the Maldi-Biotyper (Bruker, USA). Maldi score values in the range from 2.300 to 3.000 enable highly probable species identification.

Bacterial culture and enzymatic species determination were performed to identify colostrum samples positive for non-*aureus Staphylococcus spp.* as well as negative samples. Briefly, each sample collected was cultured on plates with esculin blood agar (Oxoid, Hampshire, UK) and the plates were cultivated aerobically at 37°C and checked after a 24-hour and 48-hour incubation period. The primocultivated colony from blood agar and identification of *Staphylococcus spp.* were cultured onto different selective bacteriological media (No. 110, Baird-Parker agar, Brilliance UTI Clarity Agar, Oxoid, Hampshire, UK) and incubated at 37°C for 24 hours. Suspected *Staphylococcus spp.* were selected based on colony morphology and transferred into test tubes for the coagulase test (Staphylo PK, ImunaPharm, Slovakia).

Colostrum composition and physical properties

Fat content, total protein, dry matter and lactose were determined in the colostrum samples using Milk Analyzer 150 (Bentley Instruments Inc., Minnesota, USA). The number of somatic cells (SCCs) in the colostrum was measured using Somacount 150 (Bentley Instruments Inc., Minnesota, USA). A Bactocount 70 analyzer (Bentley Instruments Inc., Minnesota, USA) was used to determine the total bacterial count (TBC CFU). Active acidity was measured using a Level 2 pH-meter (Hauptstz, Germany). Colostrum density (DMA 35 N Portable Density Meter) and electrical resistance electrical conductivity (Draminski mastitis detector) were also determined.

Protein fraction

The fractions of colostrum protein, i.e., serum albumin, α-, β- and κ-casein, and α-lactalbumin, were determined by gel electrophoresis according to the Laemmli method (1970) on a polyacrylamide gel in sodium dodecyl sulfate (SDS-PAGE), according to the method modified by Pecka et al. (2012).

Colostrum fatty acid profile

Folch's method (Bligh and Dyer 1959) was used to extract milk fat from the colostrum. To obtain methyl esters of fatty acids, we used Christopherson and Glass's method (1969); the content of fatty acids in samples prepared using this method was determined by gas chromatography (Agilent Technologies 7890A with an FID detector, Agilent Technologies, USA). The fatty acid peak readings were compared with the standards of fatty acid methyl esters (Sigma, Aldrich, USA).

Statistical analysis

The results of the study were analyzed statistically using one-factor ANOVA in Statistica 13.0 software, $p<0.05$ was considered significant.

Results

Three species of NAS were isolated from the experimental samples: *Staphylococcus sciuri* (64.29%), *Staphylococcus xylosus* (28.57 %) and *Staphylococcus warneri* (7.14%).

We did not observe any significant changes in colostrum SCC obtained from NAS positive and negative udders (Table 1). SCC was approximately $2.5 \cdot 10^6$ cells $\cdot$ ml^{-1}. In our study the contents of fat, protein, and lactose in the NAS-positive colostrum did not change significantly compared to secretion from healthy glans.

Table 1. Physical and chemical properties of cow's colostrum.

Parameter	Uninfected	CNS	P-value
SCC ·1000 · ml^{-1}	2537.15±2180.53	2864.07±2385.15	0.709
log CFU / ml^{-1}	**4.16****±0.58	**3.80****±1.04	**0.041**
Fat [%]	6.24±2.89	5.69±2.39	0.592
Protein [%]	12.46±4.57	13.87±3.55	0.365
α-casein [%]	17.60±2.4	15.86±1.90	0.147
β-casein [%]	**8.65****±1.45	**7.27****±1.10	**0.049**
κ-casein [%]	11.55±2.50	10.70±2.20	0.522
Serum albumin [%]	19.91±5.58	21.85±6.11	0.285
α-lactoalbumin [%]	5.43±0.48	5.24±0.54	0.852
Drymatter [%]	22.28±5.87	22.90±4.39	0.752
Lactose [%]	2.52±0.27	2.24±0.59	0.265
pH	**6.38****±0.22	**6.53****±0.07	**0.015**
Density [g/cm^3]	1.056±0.01	1.058±0.01	0.697
Resistance [Ω]	382.13±66.73	378.00±32.78	0.826

$p<0.05$. ± -SD standard deviation. CNS – coagulase-negative staphylococci

The average log CFU in the negative colostrum samples was higher ($p<0.05$) than in those positive for NAS. Only minor differences were identified in the protein composition in positive and negative colostrum samples. The content of β-casein was significantly lower ($p<0.05$) in the colostrum from udders positive for NAS. According to our results, density and resistance of the NAS-positive and negative colostrum are almost the same, while the pH of the colostrum positive for NAS is significantly higher.

The fatty acid profile of colostrum from udders negative and positive for non-*aureus* staphylococci is presented in Table 2. The content of capric (C10:0) acid was significantly higher ($p<0.05$) in the colostrum fat of cows infected with NAS compared to that of NAS-negative animals. Butyric acid (C 4:0) concentration tended to increase in NAS-positive samples.

Discussion

We isolated three species of coagulase-negative staphylococci in the colostrum obtained from Slovak pied x HF cows with no signs of clinical mastitis. The results of our research on the identification of NAS coincide with the data of other researchers. Although NAS have been described as very common udder pathogens whose incidence is constantly increasing, they do not cause acute clinical mastitis (Awale et al. 2012). On the other land, NAS are considered the most prevalent cause of subclinical mastitis (Pyörälä and Taponen 2009) as well as milk contaminants (Hamel et al. 2020) belonging to phychotrophic bacteria found in milk (Yuan et al. 2019). Hamel et al. (2020) showed that *S. warneri,* and *S. xylosus,* most frequently caused intra-mammary infections (IMI) and were found as contaminants in less than 25% of cases of posivite culture. *S. sciuri*, on the other hand, was often isolated as a contaminant, but also caused IMI. Thus, all the NAS isolated in our study have infectious potential; however this potential varies significantly. We applied asepsis during sampling to minimize the risk of contamination; however, we did not use repeated cultures to confirm mastitis since this is difficult with colostrum. Nevertheless, not only infection but also the presence of bacteria may impact milk or colostrum composition. In another study, NAS causing IMI were mainly *S. xylosus* (24/40) and *S. chromogenes* (18/26) and were associated with a subclinical presentation (Wald et al. 2019). In a study by Baltrukova et al. (2019a) each colostrum sample obtained from cows in Latvia was positive for one or more non-*aureus Staphylococcus spp.*, with *S. epidermidis* and *S. haemolyticus* being the most frequent species isolated. Reports of SCC in the colostrum vary significantly. Madsen et al. (2004) showed a lower SCC in colostrum of cows compared to our results. While according to another study, SCC in the bacteriologically negative colostrum samples was from 0.3 to 9.4 · 10^6 · ml^{-1} and in the samples infected with NAS, from 0.2 to 19.9 · 10^6 cells · ml^{-1} (Puppel et al. 2020). Similarly, other researchers shower either only moderate increases in milk somatic cell count

Table 2. Fatty acid profile of cow's colostrum.

Parameters	Uninfected	CNS	P-value
C4:0[1]	0.29**±0.14	0.32**±0.12	0.071
C6:0[1]	0.40±0.17	0.46±0.12	0.310
C8:0[1]	0.36±0.11	0.39±0.09	0.509
C10:0[1]	**1.22**±0.37	**1.26**±0.24	**0.015**
C12:0[1]	2.38±0.50	2.46±0.38	0.664
C14:0[1]	12.24±1.18	12.70±1.42	0.359
C15:0[1]	0.89±0.13	0.92±0.17	0.493
C16:0[1]	40.52±4.00	40.58±5.34	0.380
C17:0[1]	0.64±0.12	0.66±0.16	0.566
C18:0[1]	4.85±1.54	5.22±1.97	0.760
C20:0[1]	0.10±0.04	0.11±0.05	0.796
∑SFA	63.86±2.74	65.09±3.92	0.953
C14:1[1]	1.07±0.33	1.20±0.29	0.253
C16:1[1]	10.42±1.92	9.45±1.64	0.499
C17:1[1]	0.32±0.07	0.31±0.07	0.289
C18:1n9c[1]	12.03±2.07	12.02±2.05	0.759
C18:1n9t [1]	0.92±0.32	0.89±0.43	0.667
C18:1n7t[1]	0.63±0.25	0.65±0.34	0.506
C18:2n6c[1]	1.55±0.24	1.63±0.18	0.156
CLA[1]	0.37±0.09	0.44±0.21	0.811
C18:3n3[1]	0.60±0.10	0.63±0.15	0.654
C20:1[1]	0.05±0.04	0.09±0.07	0.496
C20:4n6[1]	0.23±0.06	0.24±0.28	0.607
∑UFA	28.74±0.06	28.15±0.31	0.739

[1] – g/100 g of total fat concentration, SFAs – saturated fatty acids, UFAs – unsaturated fatty acids $p<0.05$. ± -SD standard deviation. CNS – coagulase-negative staphylococci

or a lack of consistent influence of NAS on SCC (Taponen and Pyörälä 2009, Chen et al. 2021). On the other hand according to Idriss et al. (2013) low SCC were found in milk in the case of contamination with potentially pathogenic bacteria. SCC is also considered a noninvasive indicator of colostrum quality (Taponen and Pyörälä 2009).

The composition of cow colostrum varies considerably, depending on many factors (Pecka-Kiełb et al. 2018). In our study the contents of fat, protein, and lactose in the NAS-positive colostrum did not change significantly compared to secretion from NAS-negative udders. The composition impacts the biological value of the colostrum in terms of newborn nutrition (Csapóa et al. 1996). A lack of alterations in the physicochemical properties suggests that colostrum from NAS positive quarters may still be a good quality feed for calves. This is a significant finding regarding the potential of NAS for main milk pathogen inhibition and considering them as "protective" bacteria (Toledo-Silva et al. 2021, Toledo-Silva et al. 2022). However, colostrum is drunk by newborns with a "sterile" gastrointestinal tract, and thus the presence of NAS may pose a threat to an immature organism without developed immunity.

The logarithm of total bacterial count (log CFU) in the negative colostrum samples was higher than in those positive for NAS (4.16 log CFU·mL^{-1} and 3.80 log CFU · mL^{-1} for negative and positive colostrum samples, respectively). Both results are within the hygiene requirements for bovine colostrum quality, since the TBC does not exceed 100000 CFU · mL^{-1} (>5 log CFU · mL^{-1}). A lower TBC together with a hig-

her pH of the infected samples might suggest that there is a lower number of beneficial flora suchsa lactic acid bacteria (LAB) crucial for maintaining gut health and contributing to the development and maturation of the systemic immunity (Chiu et al. 2014). On the other hand NAS may also inhibit other, potentially pathogenic bacteria (Toledo-Silva et al. 2022)

Protein composition analysis revealed a decrease in β-casein in the NAS-positive colostrum. However, in other studies where NAS was not associaded with an increase in colostrum SCC only a negative correlation with urea nitrogen content was found (Chen et al. 2021).The results of other studies have shown that with the increasing number of somatic cells in the milk and colostrum, the content of caseins, lactose and minerals decreases (Pecka et al. 2012).

Butyric acid concentration tended to increase in the NAS-positive colostrum in our study. On one hand, in artificial rearing systems, where calves are fed milk replacers that typically do not contain C4:0, better animal growth is achieved by supplementing them with sodium butyrate, this fatty acid induces an increased absorption capacity in the proximal part of the small intestine (Kato et al. 2011). On the other hand, butyric acid may reduce paracellular permeability during the process of gut epithelium closure. Therefore, a lower C4:0 level may extend the intestinal permeability period, thereby promoting the penetration of immunoglobulins through the intestine wall (Hiltz and Laarman 2019). No studies on the in-fluence of the C10:0 level in the colostrum on the rearing of calves have been found in the available literature. Only Wilms et al. (2022) found that the level of C10:0 acid is typically lower in normal colostrum than in milk. Capric acid has been found to have antimicrobial properties against many Gram-positive and Gram-negative bacteria. It also influences the growth and fermentation activity of rumen bacteria (Marounek et al. 2002, Zentek et al. 2012). An increase in this fatty acid in the colostrum might thus influence normal bacterial colonization of the calf gut. Vasil et al. (2016) found that *S. xylosus* and *S. warneri* infections also increase the C10:0 content in cow's milk. *Staphylococcus xylosus* was proved to produce fatty acid modifying enzyme (FAME). Lipase activity also increases in the presence of FAME. Both enzymes may together modify fat properties in the infected colostrum, thus changing the environment for the pathogen as well as for beneficial microorganisms (Lu et al. 2012). Further studies are needed to determine if changes in FA composition caused by NAS are beneficial.

Conclusion

NAS affect several colostrum parameters, primarily acidity and β-casein level. Moreover, NAS also cause a significant decrease in the total bacterial count, which may be caused by a decrease in the number of either beneficial or other potentially pathogenic microflora and this requires more detailed research. The of fatty acid profile of fat in the colostrum with NAS changes toward higher concentrations of medium-chain saturated fatty acids with a significant increase in C10:0. Since these fatty acids are characterized by bactericidal properties, NAS in the colostrum may influence the physiological bacterial colonization of the gastrointestinal tract in the calf.

References

Addis MF, Maffioli EM, Ceciliani F, Tedeschi G, Zamarian V, Tangorra F, Albertini M, Piccinini R, Bronzo V (**2020**) Influence of subclinical mastitis and intramammary infection by coagulase-negative staphylococci on the cow milk peptidome. J Proteom 226: 103885.

Awale MM, Dudhatra GB, Avinash K, Chauhan BN, Kamani DR, Modi CM, Patel HB, Mody SK (**2012**) Bovine mastitis: a threat to economy. Open Acc Sci Rep 1: 295.

Baltrukova S, Zagorska J, Eihvalde I (**2019a**) Evaluation of microbiological quality of colostrum. FOODBALT pp 45-49.

Baltrukova S, Zagorska J, Eihvalde I (**2019**) Preliminary study of bovine colostrum quality in Latvia. Food Sci 1: 234-240.

Bligh EG, Dyer WJ (**1959**) A rapid method of total lipid extraction and purification. Can. J. Biochem Physiol 37: 911-917.

Bochniarz M, Zdzisińska B, Wawron W, Szczubiał M, Dąbrowski R (**2017**) Milk and serum IL-4, IL-6, IL-10, and amyloid A concentrations in cows with subclinical mastitis caused by coagulase-negative staphylococci. J Dairy Sci 100: 9674-9680.

Chen B, Tang G, Guo W, Lei J, Yao J, Xu X (**2021**) Detection of the Core Bacteria in Colostrum and Their Association with the Rectal Microbiota and with Milk Composition in Two Dairy Cow Farms. Animals (Basel) 11: 3363.

Chiu YH, Tsai JJ, Lin SL, Chotirosvakin C, Lin MY (**2014**) Characterisation of bifidobacteria with immunomodulatory properties isolated from human breast milk. J Funct Foods 7: 700-708.

Christopherson SW, Glass RL (**1969**) Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. J Dairy Sci 52: 1289-1290. Cossignani L, Blasi F (**2019**) Fermentation as a strategy to increase conjugated linoleic acid in dairy products. Large Anim Rev 25: 101-104.

Csapóa J, Martinb TG, Csapó-Kissa ZS, Házasa Z (**1996**) Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. International Dairy J, 6: 881-902.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September **2010** on the protection of animals used for scientific purposes. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010L0063

dos Reis JF, Leite RF, de Silva CPC, Baccili CC, Melville PA, Benites NR, Gomes V **(2016)** Somatic cell counts as an indicator of mammary infection in periparturient cows. Braz J Vet ResAnim Sci 53: 161-168.

Gelasakis AI, Angelidis A, Giannakou R, Arsenos G **(2018)** Bacterial subclinical mastitis and its effect on milk quality traits in low-input dairy goat herds. Vet Rec 183: 449.

Hamel J, Zhang Y, Wente N, Krömker V **(2020)** Non-S. *aureus* staphylococci (NAS) in milk samples: Infection or contamination? Vet Microbiol 242: 108594.

Hiltz RL, Laarman AH **(2019)** Effect of butyrate on passive transfer of immunity in dairy calves. J Dairy Sci 102: 4190-4197.

Hisira V, Slovák P, Marčeková P, Kadaši M, Mudroň P **(2020)** Assessment of udder health in lame cows. Acta Vet Brno 89: 133-140.

Hunt KM, Williams JE, Shafii B, Hunt MK, Behre R, Ting R, McGuire MK, McGuire MA **(2013)** Mastitis is associated with increased free fatty acids, somatic cell count, and interleukin-8 concentrations in human milk. Breast Feed Med 8: 105-110.

Idriss SE, Tancin V, Foltys V, Kirchnerová K, Tancinová D, Vršková M **(2013)** Relationship between mastitis causative pathogens and somatic cell counts in milk of dairy cows. Potravinarstvo 7: 207-212.

Kaskous S **(2021)** Physiological Aspects of Milk Somatic Cell Count in Dairy Cattle. Internat J Livest Res 11: 1-12.

Kato SI, Sato K, Chida H, Roh SG, Ohwada S, Sato S, Guilloteau P, Katoh K **(2011)** Effects of Na-butyrate supplementation in milk formula on plasma concentrations of GH and insulin, and on rumen papilla development in calves. J Endocrinol 211: 241-248.

Laemmli UK **(1970)** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Leitner G, Rovai M, Merin U **(2019)** Clinical and subclinical intrammamay infection caused by coagulase negative staphylococci negatively affect milk yield and its quality in dairy sheep. Small Rum Res 180: 74-78.

Lu T, Park JY, Parnell K, Fox LK, McGuire MA **(2012)** Characterization of fatty acid modifying enzyme activity in staphylococcal mastitis isolates and other bacteria. BMC Res Notes 5: 323.

Madsen BD, Rasmussen MD, O'Nielsen M, Wiking L, Larsen LB **(2004)** Physical properties of mammary secretions in relation to chemical changes during transition from colostrum to milk. J Dairy Res 71: 263-272.

Marounek M, Skřivanová V, Savka O **(2002)** Effect of caprylic, capric and oleic acid on growth of rumen and rabbit caecal bacteria. J Anim Feed Sci 11: 507-516.

National Research Council. **(2001)** Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001. Washington, DC: The National Academies Press.

Needs EC, Anderson M **(1984)** Lipid composition of milks from cows with experimentally induced mastitis. J Dairy Res 51: 239-249.

Nutrient requirements of dairy cattle / Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council **2001**. 7th rev. ed., National academy Press, Washington DC, USA. p 401.

Pecka E, Dobrzanski Z, Zachwieja A, Szulc T, Czyz K **(2012)** Studies of composition and major protein level in milk and colostrum of mares. Anim Sci J 83: 162-168.

Pecka-Kiełb E, Zachwieja A, Wojtas E, Zawadzki W **(2018)** Influence of nutrition on the quality of colostrum and milk of ruminants. Mljekarstvo 68:169-181.

Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H **(2020)** Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol 18: e3000411.

Pikhtirova A, Bujok J, Pecka-Kiełb E, Zachwieja A, Vasil M, Elečko J, Zigo F **(2020)** Fatty acid profile of ewe's milk infected with *Staphylococcus spp.* Iranian J Vet Res 21: 216-220.

Puppel K, Gołębiewski M, Grodkowski G, Solarczyk P, Kostusiak P, Klopčič M, Sakowski T **(2020)** Use of somatic cell count as an indicator of colostrum quality. PLoS ONE 15: e0237615.

Pyörälä S, Taponen S **(2009)** Coagulase-negative staphylococci – Emerging mastitis pathogens. Vet Microbiol 134: 3-8.

Ruegg PL **(2017)** 100-Year Review: Mastitis detection, management, and prevention. J Dairy Sci 100: 10381-10397.

dos Santos G, da Silva JT, da Santos FH, Bittar CM **(2017)**. Nutritional and microbiological quality of bovine colostrum samples in Brazil. Rev Bras Zootec 46: 72-79.

Sukur H, Esendal OM **(2020)** Presence and antimicrobial resistance of coagulase-negative staphylococci isolated from animals in a Veterinary Teaching Hospital in Cyprus. Vet Med-Czech 65: 191-198.

Summer A, Franceschi P, Formaggioni P, Malacarne M **(2015)** Influence of milk somatic cell content on Parmigiano-Reggiano cheese yield. J Dairy Res 82: 222-227.

Taponen S, Pyörälä S **(2009)** Coagulase-negative staphylococci as cause of bovine mastitis-Not so different from *Staphylococcus aureus*? Vet Microbiol 134: 29-36.

Toledo-Silva B, de Souza FN, Piepers S, Mertens K, Haesebrouck F, De Vliegher S **(2021)** Metabolites of bovine-associated non-aureus staphylococci influence expression of Staphylococcus aureus agr-related genes in vitro. Vet Res 52: 62.

Toledo-Silva B, Beuckelaere L, De Visscher A, Geeroms C, Meyer E, Piepers S, Thiry D, Haesebrouck F, De Vliegher S **(2022)** Novel Quantitative Assay to Describe In Vitro Bovine Mastitis Bacterial Pathogen Inhibition by Non-aureus Staphylococci. Pathogens 11: 264.

Vasil M, Pecka-Kiełb E, Elečko J, Zachwieja A, Zawadzki W, Zigo F, Illek J, Farkašová Z **(2016)** Effects of udder infections with *Staphylococcus* xylosus and *Staphylococcus warneri* on the composition and physicochemical changes in cow's milk. Pol J Vet Sci 19: 841-848.

Wald R, Hess C, Urbantke V, Wittek T, Baumgartner M **(2019)** Characterization of *Staphylococcus* species isolated from bovine quarter milk samples. Animals 9: 200.

Walker WA, Iyengar RS (**2015)** Breast milk, microbiota, and intestinal immune homeostasis. Pediatr Res 77: 220-228.

Wilms JN, Hare KS, Fischer Tlustos AJ, Vahmani P, Dugan MER, Leal LN, Steele MA (**2022)** Fatty acid profile characterization in colostrum, transition milk, and mature milk of primi- and multiparous cows during the first week of lactation. J Dairy Sci 105: 2612-2630.

Yuan L, Sadiq FA, Burmølle M, Wang NI, He G (**2019**) Insights into Psychrotrophic Bacteria in Raw Milk: A Review. J Food Prot 82:, 1148-1159.

Zentek J, Buchheit-Renko S, Männer K, Pieper R, Vahjen W (**2012)** Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets. Arch Anim Nutr 66:14-26.