Effect of coagulase-negative staphylococci on colostrum composition, properties and fatty acid profile

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Abstract

Background: Colostrum is the first food for mammals that should give a head start to the newborn organism. By providing the body with essential nutrients, colostrum plays an immune and immunostimulating function. Colostrum quality depends on multiple factors, including microbial effect. Widespread coagulase-negative staphylococci have a great impact on colostrum qualitative indicators. The aim of this study was to explore the effect of staphylococci on colostrum quality.

Results: Physical, chemical properties and fatty acid profile of cow's colostrum were determined. In our study we identified three pathogenic coagulase-negative *Staphylococcus* species in the cows' colostrum: *S. sciuri* (64.29 %), *S. xylosus* (28.57 %) *and S. warneri* (7.14 %). We noted that the somatic cell count (SCC) in the affected colostrum samples wasn't noticeably higher than that in the uninfected colostrum samples. Percentage of dry matter in infected and uninfected colostrum samples was nearly the same. Contents of fat, protein, and lactose in the studied colostrum were not significantly different. The content of butyric (C4:0) and capric (C10:0) acids was significantly higher ($P<0.05$) in the colostrum fat of cows infected with CNS compared to that of the uninfected animals.

Conclusions: According to our research infection with coagulase-negative staphylococci negatively affects colostrum patameters primarily due to an increase in SCC, acidity and decrease in β-casein level. Moreover, significant decrease in total bacterial count in infected by CNS samples may be caused by a decrease in the level of beneficial microflora. Minor changes in fatty acid profile of fat in colostrum with CNS do not have a significant negative effect on colostrum as a whole. But in view of the fact that colostrum is intended for feeding a newborn with a "sterile" gastrointestinal tract, the presence of CNS will undoubtedly leads to a great threat to an immature organism.

Background

Colostrum is the first secretion of the mammary gland after parturition [1]. It is well known that the survival rate of newborns directly depends on its nutritional value and quality [2, 3]. Colostrum not only provides newborn calves with nutrients, but also accompanies the colonization of the gastrointestinal tract with important microorganisms [4].

Yeoman and White [5] postulate that early gut colonization is crucial for the morphological and immunological development of the gastrointestinal tract, development of a functional fermentative environment, and neonatal resistance to pathogens in calves. Beneficial rumen microflora subsequently enables the fermentation of complex carbohydrates producing approximately 70% of the calf's daily energy requirement. Composition of cow's colostrum depends on many factors, including the condition of the mammary gland [6]. Microbiological quality of colostrum is very important parameter, which can be significant for calf mortality and antibodies absorption rate [7].

The quality and quantity of cow's mammary gland secretions are closely related to the udder health. Mastitis is the most frequent disease of dairy cows and has well-recognized detrimental effects on

animal wellbeing and dairy farm profitability [8]. A huge number 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.

The most common microorganisms isolated from cows' milk are coagulase-negative staphylococci (CNS) – a group of commensal microorganisms present on the skin and mucous membranes of animals and humans. Previously they have been regarded as non-pathogenic to livestock but now their connection with spontaneous abortions and mastitis is established [9, 10]. CNS are associated with subclinical mastitis and persistent increases in the bulk milk somatic cell count (SCC), resistance to antimicrobials, as well as biofilm production [11, 12]. It is clear that many CNS species can cause persistent intramammary infections [13].

Subclinical intramammary infection caused by CNS reduces milk yield and changes its composition that directly influence the product's quality [14]. High proportion of milk from infected glands in the milk tank is associated with higher losses of fat and protein in the whey during the coagulation process [15]. Moreover, CNS infections can damage udder tissue and lead to decreased milk production. Currently it is difficult to determine whether CNS species behave as contagious or environmental pathogens [16]. Shkromada et al. [17] claim that in the case of subclinical mastitis the number of somatic cells increases a thousand times.

Addis et al. [11] demonstrated that subclinical mastitis caused by CNS can induce significant changes in the milk peptidome, which may be used for mastitis detection. Bochniarz et al. [18] indicated that the level of IL-6 and amyloid A in milk of cows suffering from subclinical mastitis caused by CNS tended to be high comparably with healthy cows, while IL-4 and IL-10 concentrations were lower.

Goats with subclinical mastitis caused by coagulase-negative staphylococci had lower daily milk-fat yield, milk-lactose content and daily milk-lactose yield and higher daily milk-protein yield compared with the healthy ones [19, 20]. Moreover, changes in fatty acid profile of ewe's milk infected with Staphylococcus spp. that decrease the value of ewes' milk as a health-promoting product [21].

Tomazi et al. [22] stated that intramammary infection (IMI) caused by CNS had no effect on milk yield or on contents of fat, crude protein, casein, lactose, total solids, and solids-not-fat. But his studies were related to IMI caused by *S. chromogenes* that only increased somatic cell count but had no effect on milk yield and composition when analysed in the milk from separate quarters.

Cossignani and Blasi [23] showed that strains of *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Propionibacterium* and *Streptococcus* can be considered potential producers of conjugated linoleic acids. Changes in concentration and composition of those milk constituents synthesized and secreted by the mammary epithelium occur after secretion in the alveolar lumen and milk ducts [24]. Mammary epithelial cells fatty acid synthesis de novo accounts for all 4:0 to 12:0 więc ich poziom zależy od zyweina zwierząt [25]. Level short-chain SFA increased at 48 h, 96 h, and 5 mo, compared with samples at 24 h [26].

A lot of studies have been carried out to determine the effect of staphylococci on milk production and milk quality of cows, sheep and goats, but the effect of these pathogenic microorganisms on colostrum quality remains uncertain which was the purpose of our research.

Results And Discussion

Coagulase-negative staphylococci include various species and are traditionally regarded as minor pathogens since they seem to lack the ability to cause severe mastitis [27]. Cervinkova et al. [28] showed that CNS clearly predominated followed by streptococci and enterococci in the potential to cause mastitis.

In our study we identified three pathogenic coagulase-negative *Staphylococcus* species in the cows' colostrum. *Staphylococcus sciuri* and *Staphylococcus xylosus* were predominant among the isolated microorganisms of the *Staphylococcus spp.* group (Fig. 1). Hamel et al. [29] showed that *S. warneri*, *S. xylosus*, *S. microti*, *S. haemolyticus*, and *S. succinus* were frequent causes of intramammary infections. Park et al. [30] isolated 263 CNS from milk samples from cows with intramammary infections, identifying 11 species, including *Staphylococcus chromogenes* (72.2%), *Staphylococcus xylosus* (9.1%), and *Staphylococcus haemolyticus* (6.1%), which were the most frequent species isolated. In other study the CNS causing IMI were mainly *S. xylosus* (24/40) and *S. chromogenes* (18/26) and were predominantly associated with a subclinical presentation [31].

Physical and chemical properties of colostrum infected with CNS change insignificantly (Table 1). We noted that the somatic cell count (SCC) in the affected colostrum samples wasn't noticeably higher than that in the uninfected colostrum samples. Our results are consistent with Taponen and Pyörälä [27] who showed that CNS can persist in the mammary gland and only moderately increase milk somatic cell count. Idriss et al. [32] claimed that contamination of milk samples could be connected with low SCC in milk from which bacteria were isolated. On the other hand the samples with high SCC were found out without presence of microorganisms. In our study an average SCC in the colostrum collected directly after calving was $2 \cdot 10^6$ cells $\cdot$ mL $^{-1}$.

In the available literature, the SCC level results are divergent. Madsen et al. [33] showed a lower SCC in colostrum of cows. In colostrum, the level of SCCs in the bacteriologically negative samples was from 0.3 to $9.4 \cdot 10^6$ and in infected CNS samples 0.2 to $19.9 \cdot 10^6$ cells $\cdot$ mL $^{-1}$ [34].

The composition of cow colostrum varies considerably, depending on many factors [1, 3]. Our studies made it possible to establish that percentage of dry matter in infected and uninfected colostrum samples was nearly the same. Contents of fat, protein, and lactose in the studied colostrum were not significantly different. Concentration of total protein was about 1.41% higher in colostrum from quarters infected with CNS. The contents of fat and lactose in colostrum from non-infected mammary glands were 0.55 % and 0.28 % higher, respectively. Kayano et al. [35] approved that CNS decreased lactose content and were associated with significant changes in fat, protein and milk urea nitrogen.

The average total bacterial count (TBC) was 4.16 log CFU/mL^{-1} for uninfected colostrums samples and 3.80 log CFU/mL^{-1} for those infected with CNS. These parameters are within hygiene requirements for bovine colostrum quality, since TBC does not exceed 100000 CFU/mL^{-1} (> 5 log CFU/mL^{-1}) [36]. The difference in indicators was significant ($P < 0.05$), which we associate with the presence of another possibly useful microflora in colostrum. Gelsinger and Heinrichs [37] studies have shown a wide range of microorganisms count in colostrum 1.4 to 7.0 log CFU/mL^{-1} which is consistent with our results.

Table 1
Physical and chemical properties of cow's colostrum

Parameter	**Uninfected (n = 13)**	**CNS (n = 14)**	**SEM**	**P-value**
SCC •1000 • ml^{-1}	2537.15	2864.07	426.388	0.709
log CFU / ml^{-1}	**4.16****	**3.80****	**3.31**	**0.041**
Fat [%]	6.24	5.69	0.491	0.592
Protein [%]	12.46	13.87	0.763	0.365
α-casein [%]	17.60	15.86	0.592	0.147
β-casein [%]	**8.65****	**7.27****	**0.352**	**0.049**
κ-casein [%]	11.55	10.70	0.642	0.522
Serum albumin [%]	19.91	21.85	0.888	0.285
α-lactoalbumin [%]	5.43	5.24	0.493	0.852
Dry matter [%]	22.28	22.90	0.952	0.752
Lactose [%]	2.52	2.24	0.124	0.265
pH	**6.38****	**6.53****	**0.032**	**0.015**
Density[g/cm^3]	1.056	1.058	0.002	0.697
Resistance [Ω]	382.13	378.00	9.526	0.826

*Trend; ** significant difference, $p < 0.05$. SEM – standard error of the mean. CNS – coagulase-negative staphylococci

Some differences were identified in percentage of different casein fractions, α- lactalbumin and serum albumin in infected and uninfected colostrum samples,but only difference in the content of β-casein was significant ($P < 0.05$). Concentration of serum albumin was on 1.94 % higher in infected colostrum samples. Similar results were reported by Ogola et al. [38] who approve that in high SCC milk and infected

quarters, the concentrations of non-casein fractions, sodium, chloride, and free fatty acid were higher, while the casein content, lactose, casein-to-total protein, potassium, and calcium were lower compared to normal quarters. The results of other studies have shown that with the increasing number of somatic cells in milk and colostrum, the total number of microorganisms increases, the content of caseins, lactose and minerals decreases [39, 40].

According to our study results density and resistance infected and uninfected with CNS colostrums were almost the same, while pH of colostrum infected by coagulase-negative staphylococci samples was significantly higher ($P < 0.05$), and their value was at the level given by other authors [41]. Stewartet et al. [42] showed that pH of colostrum is lower and amounts to 5.59. pH has a relatively wide range of values, even in uninfected quarters colostrum pH does not provide a clinically useful cow-side.

The fatty acid profile of colostrum from cows uninfected and infected with coagulase-negative staphylococci is presented in Table 2. Fatty acid profile of cow's colostrum uninfected and infected with CNS did not differ significantly. The ratio of saturated fatty acids (SFA) and unsaturated fatty acid (UFA) in the uninfected and infected cow's colostrum was 1:2.23 and 1:2.32 respectively.

The content of butyric (C4:0) and capric (C10:0) acids was significantly higher ($P < 0.05$) in the colostrum fat of cows infected with CNS compared to that of the uninfected animals. According to other studies the content of lauric acid, linoleic acid, arachidonic acid were increase, and vaccenic acid was decrease in the milk fat collected from ewes infected with *Staphylococcus spp*. [21]. Gudding [43] states that milk from cows with infectious mastitis had greater concentrations of free fatty acids than milk from cows with nonspecific mastitis. Lipolysis was greatest in milk from quarters with *Staphylococcus aureus* mastitis.

Wojtas et al. [44] demonstrated a higher level of saturated fatty acids in the colostrum of Charolaise cows (approx. 75 g / 100 g of fat), with a lower proportion of unsaturated fatty acids (approx. 25 g / 100 g).

The total amount of SFA in the infected cow's colostrum tended to be higher then uninfected on 1.23 g/100 g. It happened mainly due to a slight increase in concentration of all saturated fatty acids from the list.

Table 2
Fatty acid profile of cow's colostrum

Parameters	Uninfected (n = 13)	CNS (n = 14)	SEM	P-value
C4:0[1]	**0.29****	**0.32****	**0.024**	**0.071**
C6:0[1]	0.40	0.46	0.027	0.310
C8:0[1]	0.36	0.39	0.024	0.509
C10:0[1]	**1.22****	**1.26****	**0.056**	**0.015**
C12:0[1]	2.38	2.46	0.082	0.664
C14:0[1]	12.24	12.70	0.071	0.359
C15:0[1]	0.89	0.92	0.028	0.493
C16:0[1]	40.52	40.58	0.884	0.380
C17:0[1]	0.64	0.66	0.026	0.566
C18:0[1]	4.85	5.22	0.333	0.760
C20:0[1]	0.10	0.11	0.010	0.796
∑SFA	63.86	65.09	0.646	0.953
C14:1[1]	1.07	1.20	0.058	0.253
C16:1[1]	10.42	9.45	0.342	0.499
C17:1[1]	0.32	0.31	0.013	0.289
C18:1n9c[1]	12.03	12.02	0.381	0.759
C18:1n9t [1]	0.92	0.89	0.071	0.667
C18:1n7t[1]	0.63	0.65	0.056	0.506
C18:2n6c[1]	1.55	1.63	0.040	0.156
CLA[1]	0.37	0.44	0.034	0.811
C18:3n3[1]	0.60	0.63	0.024	0.654
C20:1[1]	0.05	0.09	0.025	0.496

Parameters	Uninfected (n = 13)	CNS (n = 14)	SEM	P-value
C20:4n6[1]	0.23	0.24	0.012	0.607
∑UFA	28.74	28.15	0.508	0.739

*Trend; ** significant difference, $p < 0.05$. SEM – standard error of the mean. [1] g/100 g of the total fat concentration. SFA – saturated fatty acids. UFA – unsaturated fatty acids. CNS – coagulase-negative staphylococci

Insignificant increase in the share of myristoleic acid (C14:1), vaccenic acid (C18:1n7t), alpha-linolenic (C18:3n3) and eicosenic acid (C20:1) were observed in the colostrum obtained from the cows effected by CNS as compared to those levels observed in the colostrum from the healthy animals. Vasil et al. [45] state that *S. xylosus* and *S. warneri* infections increase C8:0, C10:0, C18:3n3, and C17:1 content, and decrease the C20:1 and EPA concentrations in milk.

Palmitoleic acid (C16:1) content in fat was higher on 0.97 g/100 g of the total fat concentration in uninfected colostrums samples comparatively to infected ones. On the contrary, infected colostrum had a higher content of linoleic acid (C18:2n6c) compared to samples free of coagulase-negative staphylococci. In general, the total amount of UFA was slightly higher in uninfected cow's colostrum then in infected. Hunt et al. [46] approve mastitis is associated with increased lipolysis in the human breast but not alterations in milk fat synthesis, as evidenced by a lack of alteration in total milk lipids.

Conclusion

According to our research infection with coagulase-negative staphylococci negatively affects colostrum patameters primarily due to an increase in SCC, acidity and decrease in β-casein level. Moreover, significant decrease in total bacterial count in infected by CNS samples may be caused by a decrease in the level of beneficial microflora. Minor changes in fatty acid profile of fat in colostrum with CNS do not have a significant negative effect on colostrum as a whole. But in view of the fact that colostrum is intended for feeding a newborn with a "sterile" gastrointestinal tract, the presence of CNS will undoubtedly leads to a great threat to an immature organism.

Materials And Methods

Animals

Experiments were carried out on 27 cows in 2nd and 3rd lactation belonging to the herd of Slovak Pied cattle breed (with share of HF blood) housed in free-stall system. Animals were clinically healthy. Cows were kept in typical buildings that meet the requirements of the European Union Directive [47]. Cow's diet was formulated according to international standards [48].

Colostrum samples for the study were collected during the first milking after calving. Udder was disinfected with 70% ethanol before sample collection. Approximately 100 ml of colostrum was milked into the sterile container from each quarter separately. Samples were immediately frozen at − 20°C and transported to the laboratory. Our study was carried out in compliance with the ARRIVE guidelines [49].

The experimental procedures were done under veterinary care of University of Veterinary Medicine and Pharmacy in Košice, according to permit number IČO 00397474 2015, licensed by Ministry of Education, Sciences, Research and Sport of the Slovak Republic.
The whole study was carried out in accordance with the relevant guidelines and regulations.

Microbiological Studies

Bacterial culture and enzymatic species determination were performed to identify udder quarters infected by S*taphylococcus spp.* Briefly all samples were cultured on 5 % blood agar and then on specific cultivation media. *Staphylococcus spp.* were selected based on colony morphology and transferred into test tubes for the coagulase test (Staphylo PK, ImunaPharm, Slovakia).

Two methods were used to identify the bacteria species from coagulase negative colonies. Biochemical enzymatic properties of bacteria were identified with the STAPHY test 24 with the identification program TNW 7.0 (Erba-Lachema, Brno, Czech Republic). The method is considered to have a detection accuracy of more than 90.0 %. Spectrum of bacterial proteins was 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.

Colostrum Composition And 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 with Somacount 150 (Bentley Instruments Inc., Minnesota, USA). Bactocount 70 analyzer (Bentley Instruments Inc., Minnesota, USA) was used to determine the total bacterial contamination (TBC CFU). Active acidity was measured with Level 2 pH-meter (Hauptstz, Germany). Colostrum density (DMA 35N Portable Density Meter) and electrical resistivity (Draminski mastitis detector) were also determined.

Protein Fraction

Fraction of colostrums protein, i.e. serum albumin, α-, β- and κ-casein, and α-lactalbumin, were determined by gel electrophoresis according to the Laemmli's method [50] on polyacrylamide gel in sodium dodecyl sulphate (SDS-PAGE), according to the method modified by Pecka et al. [40].

Declarations

Ethics approval and consent to participate

The study protocol was approved by the University of Veterinary Medicine and Pharmacy in Košice, according to permit number IČO 00397474 2015.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EPK and FZ carried out the experiment, analyzed samples and statistical data. FZ isolated samples. EPK mainly designed the study and supervised the whole program. AZ and JB participated in experiment. AP wrote the manuscript. All authors contributed to revise the manuscript. AP was corresponding author. All authors have read and approved the final manuscript.

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Emails

Not applicable.

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Figures

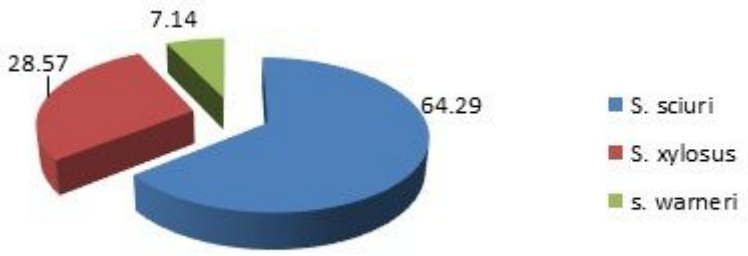

Figure 1

Percentage of the coagulase-negative staphylococci (CNS) in the cow's colostrum (%).