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EVALUATION OF MICROBIOLOGICAL QUALITY OF COLOSTRUM

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Abstract

Bovine colostrum is an important source of different biologically active compounds: immunoglobulins, lactoferrin, lysozyme, lactoperoxidase etc., therefore vital for a dairy calf's ability to survive. There is the lack of information about microbiological quality of colostrum. Still it is very important parameter, which can be significant for calf mortality and antibodies absorption rate, the aim of the study was to evaluate microbiological quality of colostrum obtained from Latvian cows. Colostrum samples (n=20, 50 mL^{-1}) were collected in conventional farm with 500 cows (Zemgale, Latvia) during December 2018 to January 2019 one hour after calving. Samples were immediately frozen (-19±1 °C, within 30 min) and delivered to the laboratory. The colony forming units (LVS EN ISO 4833-1:2013) and presence of β-glucuronidase positive *Escherichia col* (LVS EN ISO 16649-2:2007) were detected in colostrum samples. Descriptive statistics were used for data analysis. The average total plate count of analysed colostrum samples was 5.62 log CFU mL^{-1}, colony forming units ranged from 4.97 to 5.90 log CFU mL^{-1}. In the current research β-glucuronidase positive *Escherichia coli* CFU ranged from >1 to 8300 mL^{-1} in colostrum sample. Research results associated with low antibodies absorption rate by calf and high risk of diarrhoea in the farm.

Keywords: colostrum, total plate count, Ig concentration

Introduction

Bovine colostrum is an important source of different biologically active compounds: immunoglobulins (Ig), lactoferrin, lysozyme, lactoperoxidase etc., therefore vital for a dairy calf's ability to survive. Studies show that the highest concentration of biologically active components in colostrum is collected in cows at the first milking after calving (Hurley, Theil, 2011), reporting maximum concentration in the first four hours (Sacerdote et al., 2013) to six hours (Borad, Singh, 2018) after calving.

The concentration of Ig in bovine colostrum affects the passive immunity acquisition in new-born calves (Swan et al., 2007; Quigley et al., 2013; Mann et al., 2016; Arsenopoulos et al., 2017). Therefore, accurate measurement of Ig is essential to provide healthy growth of the younger generation on the dairy farms, which will be able to provide new-born calves with necessary Ig concentration (>50 mg mL^{-1}) in colostrum (Lago et al., 2018). Quigley et al. (2013), Morrill et al. (2015), Yaylak, Yavuz, Özkaya (2017) and other authors used Brix refractometer to estimate Ig concentration in colostrum. Authors concluded that Brix measurement of total solids in fresh colostrum is a sufficiently accurate method for estimating IgG concentration, which is confirmed by the use of alternative methods in studies. Quigley et al. (2013) suggested, that predicted IgG concentration in colostrum based on Brix percentage is variable. Current industry recommendations designate discarding colostrum should contain <50 mg of IgG mL^{-1} and > 5 log10 CFU mL^{-1} total bacteria count (Morrill et al., 2012; Quigley et al., 2013; Morrill et al., 2015; Yaylak et al., 2017).

Colostrum bacterial contamination is another important quality parameter. Microbiologically contaminated colostrum can reduce animal performance as well as increase morbidity and mortality rates in the farms (Elizondo-Salazar et al., 2010; Mohammed et al., 2018). Microorganisms can bind to free immunoglobulins and block absorption of these molecules by enterocytes (Morrill et al., 2012; Santos et al., 2017).

Last data reported about microbiological quality of colostrum from Latvian dairy herds is from 2008, when microorganisms count ranged from 4.3 log to 6.0 log CFU mL^{-1} (Gala ziņojums, 2008).

Information about microbiological quality of colostrum in Latvia is limited and outdated; still it is very important parameter and can be significant factor detecting calf mortality rate. Therefore, the aim of the study was to evaluate microbiological quality of colostrum obtained from Latvian cows.

Materials and Methods

Colostrum was collected from Holstein Black cows, lactation period ranged from: 1st to 4th. Colostrum samples (n=20, 50 mL) were collected in conventional farm with 500 cows (Zemgale, Latvia). Colostrum samples were classified according lactation number 1st (n=4), 2nd (n=8), 3rd (n=5) and 4th (n=3). Colostrum samples were collected from December 2018 to January 2019 one hour after calving according LVS EN ISO 707:2011 Milk and milk products – Guidance on sampling. Samples at 20 °C were immediately after collection used for immunoglobulins and total solids content detection.

Samples for microbiological analysis were immediately frozen (-19±1 °C, within 30 min) and delivered to the laboratory, stored less than 30 days before analysis. Before microbiological tests, samples were removed from freezer, thawed and homogenized in a water bath (~45±2 °C), then prepared according the following standards: LVS EN ISO 6887-1 and 5:2011 Microbiology of food and animal feeding stuffs –

Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions, Part 5: Specific rules for the preparation of milk and milk products.
The total plate count (TPC) was detected in colostrum samples according to standard LVS EN ISO 4833-1:2013 Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 °C by the pour plate technique; presence of *Escherichia coli* (*E.coli*) according to LVS EN ISO 16649-2:2007 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase positive *Escherichia coli*. Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
The colostrometer (COLOSTROMETER^{tm} Biogenics, USA) was used for evaluation of Ig concentration in mg mL^{-1}. Percentage of Ig in colostrum was measured using an optical Brix refractometer (Model BX, UK) with a range of 0 to 34% Brix.
Descriptive statistics were used for data analysis.

Results and Discussion

One of the most important factors for bovine neonatal immune function is the first feeding of colostrum. Colony forming units is very important parameter, which indicate quality of many products, colostrum isn't exception. One of the quality criteria for bovine colostrum is the total plate count based on Commission Regulation lays down the same hygiene requirements for bovine colostrum quality as for raw bovine milk, TPC does not exceed 100 000 CFU mL^{-1} (>5 log CFU mL^{-1}) (European Commission, 2006). Previous studies have shown a wide range of microorganisms count in colostrum, ranged from 1.4 to 7.0 log CFU mL^{-1} (Gelsinger, Heinrichs, 2017), from 3.0 to 6.8 log CFU mL^{-1} (Morrill et al., 2012), from 5.4 to 7.2 log CFU mL^{-1} (Dunn et al., 2017), from 1.86 to 11.02 log CFU mL^{-1} (Swan et al., 2007).
During the research, samples were divided into two groups by TPC and its correspondence to Regulation. Colony forming units and distribution of analysed samples are shown in Table 1.

Table 1

Proportion of colostrum samples by colony forming units

Group	Proportion %	min	max
		Log CFU mL^{-1}	Log CFU mL^{-1}
1	5	3.97	3.97
2	95	5.08	5.90

Among the colostrum samples in the current study, there was a wide variation of TPC, from 3.97 to 5.90 log CFU mL^{-1}. Other authors reported more significant total plate count variations from 3 to 6.80 log CFU mL^{-1} (Morrill et al., 2012) and from 2.34 to 6.94 log CFU mL^{-1} (Quigley et al., 2013).

In this study only 5% of the colostrum samples meet microbiology safety criteria that matched <5 log CFU mL^{-1}, in 95% of samples total bacterial count ranged from 5.09 to 5.90 log CFU mL^{-1}. Similar results, high percentage of microbiologically contaminated colostrum, that exceed higher permissible limit of guidelines for microbial criteria, were obtained in other studies: overall 81% samples (n=1239) (Dunn et al., 2017).
Gelsinger & Heinrichs (2017) proved, that high TPC, detected in analysed dairy farm (average 5.62 log CFU mL^{-1}), can negatively influence Ig absorption by calves. Microbiological contamination is an indicator of colostrum quality. In the current study it was not satisfactory and according to Arsenopoulos et al. (2017) can be a reason for neonatal calf diarrhoea, still storage of colostrum practised in farms for the longest period of time can enhance negative influence, promoting bacteria growth. Morrill et al. (2012) reported about TPC reduction by 0.5 log in colostrum samples after freezing, in comparison with the samples that were stored chilled.
Authors (Elmoslemany et al., 2010; Zhao et al., 2010; Sacerdote et al., 2013; Mann et al., 2016) mentioned different factors influencing bacteria count in colostrum – stage of lactation is one of them. Average total plate count and *E. coli* distribution of cow's lactation are show in Table 2.

Table 2

Distribution of colostrum samples by total and *E. coli* bacteria count

Lactation period	Proportion %	Average TPC	Average *E.coli*
		Log CFU mL^{-1}	Log CFU mL^{-1}
mean	100	5.62	2.64
1st	20	5.76	3.32
2nd	40	5.49	1.43
3rd	25	5.63	1.52
4th	15	5.66	0.88

According to our results, the highest total bacteria count was in colostrum obtained after 1st lactation (5.76 log CFU mL^{-1}), followed by samples obtained from 3rd and 4th lactation period with average bacteria count ranged from 5.62 to 5.65 log CFU mL^{-1}, respectively. Lowest TPC was determined in colostrum after 2nd lactation – 5.49 log CFU mL^{-1}. Other authors presented contradictory results, higher bacteria count after 3rd lactation, were presented in Sacerdote et al. (2013) study, in colostrum after 2nd and 3th lactation period total bacteria count increased from 7.5 log to 7.8 log CFU mL^{-1}. Different tendency was detected by Morrill et al. (2012), who reported lower TPC in colostrum obtained after 2nd lactation, comparing to 3rd lactation colostrum. According to the research results bacteria count significantly increased after 1st lactation from 4.5 log to 4.7 log in 2nd lactation samples, but decreased till 4.3 log CFU mL^{-1} after 3th lactation, respectively (Morrill et al. 2012). Our results can be

explained with tissue damage in older animals and easier microorganism migration inside.

Enterobacteria presence was detected in 100% of the samples, results can be explained with poor hygiene rules adherence (Santos et al., 2017) in the farm. In the current study, used β-glucuronidase positive *Escherichia coli* count determination method allows to identify about 90% to 100% *E. coli* strains (Public Health England, 2014). *E. coli* is commonly used as alternative marker of poor sanitary practice, direct and indirect fecal bacteria contamination. Levels of these bacteria often act as an indirect measure of the potential for dangerous fecal pathogens to be present in food (Neeliah, Arlandoo, Kureemun, 2016).

Presence of β-glucuronidase positive *E. coli* was identified in 85% of colostrum samples analysed during research (Table 3).

Table 3

Proportion of colostrum samples by *E. coli* count

Group Log CFU mL^{-1}	Proportion %	min Log CFU mL^{-1}	max Log CFU mL^{-1}
No growth	15	–	–
<1	35	0.00	0.85
1–1.99	40	1.08	1.93
2–2.99	5	2.23	2.23
>3	5	3.92	3.92

Compared to other authors results proportion of colostrum samples by *E. coli* count is significantly higher ($p<0.05$) than those reported by Mohammed et al. (2018) – 12% and Phipps et al. (2016) – 37% study results. In the current research *E. coli* count ranged from 0.00 to 3.92 log CFU mL^{-1}, and corresponds to US standards of bacterial contamination of colostrum <4 log CFU mL^{-1}. Other authors data about total coliform count 0.00–4.87 log (Quigley et al., 2013), 3.78–6.91 log (Gelsinger, Heinrichs, 2017) and 0.00–9.48 log CFU mL^{-1} (Swan et al., 2007) confirm our research results.

In the current study, significant number of samples met the industry recommendations in terms of total coliform count (100%), however; only 5% of the analysed colostrum samples were within limits in terms to TPC. Colostrum microbiological contamination can occur in different ways: milk can be contaminated with commensal bacteria from the teat skin, epithelial lining of teat canal, the lactiferous duct while it is being excreted of milk during collection, processing, handling and storage (Alegbeleye et al., 2018). Some countries practise heat treatment as for improving microbiological quality of colostrum, as for reducing bacterial infection in neonatal calves and increasing Ig absorption (Elizondo-Salazar et al., 2010; Gelsinger, Heinrichs, 2017). For ensuring calves with high quality colostrum such experience can be recommended for Latvian dairy herds.

The level of Ig in bovine colostrum after first milking can be highly variable, from 60 to 100 mg mL^{-1} (Sanchez et al., 2004), from 1.4 to 204 mg mL^{-1} (Dunn et al., 2017), from <1 to 200 mg mL^{-1} (Morrill et al., 2012), from 12.8 to 154.3 mg mL^{-1} (Morrill et al., 2015). The concentration of immunoglobulins in analysed colostrum samples ranged from 40 to 118 mg mL^{-1}, mean value – 85 mg mL^{-1} (Figure 1).

Other authors reported significant ($p<0.05$) lower Ig concentration in colostrum – 63.6 mg mL^{-1} (Lago et al., 2018). Only in one analysed colostrum sample Ig concentration was less than 50 mg mL^{-1} – 40 mg mL^{-1}, but other 95% of samples contained above 53 mg mL^{-1} of Ig. Other studies showed lower Ig concentration (<50 mg mL^{-1}) in individual colostrum samples, overall 44% samples (Dunn et al., 2017), 30% (Morrill et al., 2012), 17% (Yaylak et al., 2017) were not according recommendations, but mean concentration of Ig still exceeds industry recommendations for IgG concentration in bovine colostrum.

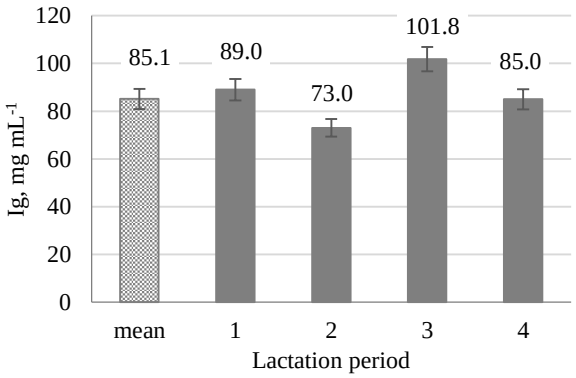

Figure 1. Mean Ig concentration in colostrum samples

Researchers found a relationship between lactation and Ig concentration in colostrum (Zhao et al., 2010; Morrill et al., 2012; Yaylak et al., 2017). The first lactation cows were found to have significantly lower IgG concentration in colostrum samples compared to cows in the second or higher lactation. This may be associated to increased tissue damage in older animals that increase the leakage of Ig from the serum in milk (as observed in somatic cells) (Sanchez et al., 2004). Colostrum production is often lower in cattle of the first lactation, which implies less development of the mammary glands and potentially reduces the transport ability of IgG to the mammary gland (Morrill et al., 2012).

Comparing Ig concentration after 1st and 2nd lactation, results were similar to Yaylak et al. (2017), higher Ig concentration was after 1st, comparing to 2nd lactation 94.1 and 88.03 mg mL^{-1}, respectively.

Contradictory results obtained by Morrill et al. (2012), Ig concentration (95.5 mg mL^{-1}) after 3rd lactations was higher compared with first two lactations – 1st (42.4 mg mL^{-1}) and 2nd (68.6 mg mL^{-1}). Latvian researchers' results detected the mean concentration of Ig tends to decrease with each subsequent lactation (from 1st to 4th) in the colostrum – 101.3 mg mL^{-1}, 89.6 mg mL^{-1}, 88.1 mg mL^{-1} and 89.7 mg mL^{-1} (Eihvalde et al., 2012).

In the current study, higher Ig concentration in colostrum after 1st lactation can be explained with high TPC and *E.coli* count in colostrum, which resulted in a mammary gland protection mechanism.

Obtained results did not reveal any tendency to reduce or increase Ig concentration depending on the lactation period as other authors reported. This may be related to a small number of samples, breed, nutrition, length of the dry period of cows, vaccination and other factors (Dunn et al., 2017).

Figure 2 shows the distribution of Brix refractometer readings for the analysed samples. The percentage of Ig in colostrum in the analysed farm ranged from 20 to 32%, mean 24% Brix.

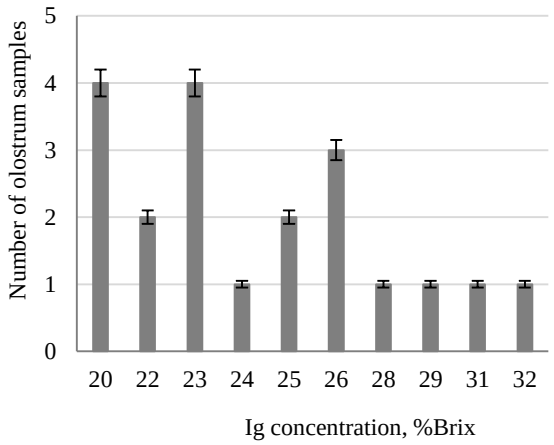

Figure 2. Distribution of Ig concentrations (% Brix) by the number of samples

The distribution of samples was the same as in a case with colostrometer measurements: higher concentration (28%) was in 3rd colostrum, then follow 1st (26%), 4th (22%), and 2nd (21%).

Recommended concentration 21% is considered the break point for high-quality (>50 mg mL^{-1}) bovine colostrum (Morrill et al., 2012; Quigley et al., 2013). Based on this recommendation, 80% of the samples should be included in a high-quality colostrum group.

Other authors reported lower mean Ig concentration – 21.24% (Morrill et al., 2015), – 23.8% Brix (Quigley et al., 2013), – 20.3% (Lago et al., 2018). Yaylak et al. (2017) report higher concentration of Ig – 24.61%.

Obtained research results showed poor microbiological quality of colostrum regarding total plate count in the colostrum from analysed dairy farm. Nowadays there is no control for microbiological quality of colostrum in Latvian dairy farms. However it can be a determinative factor in mortality rate of calves, as for passive immunity transfer, still bacteria can bind to free immunoglobulins in the intestinal lumen and block absorption of these molecules by enterocytes (Santos et al., 2017). For comprehensive assessment of Latvian colostrum quality, number of analysed samples should be increased and further research should be done.

Conclusions

Poor microbiological quality of colostrum obtained from analysed farm was proved through a high total plate count (average – 5.65 log CFU mL^{-1}) exceeding permissible level for such product and Enterobacteria presence in 100% of analysed samples.

Despite sufficient concentration of Ig in analysed samples (mean 85.1 mg mL^{-1} and 24% Brix) colostrum microbiological quality should be improved.

Research results can be associated with low Ig absorption rate by calves and high risk of diarrhoea in the farm.

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