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# Bovine colostrum casein: Post-partum dynamics of micelle size, content, and associated traits

Andres Sats [a,*], Tanel Kaart [b], Ivi Jõudu [a,c]

[a] Chair of Food Science and Technology, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51006, Estonia
[b] Chair of Animal Breeding and Biotechnology, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51006, Estonia
[c] ERA Chair for Food (By-) Products Valorisation Technologies of the Estonian University of Life Sciences (VALORTECH), Kreutzwaldi 1, Tartu 51006, Estonia

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ABSTRACT

In milk, casein exists in the form of colloidal casein micelles (CM), which are crucial for various biological functions such as neonatal nutrition, calcium phosphate control, and amyloid fibril suppression. However, bovine colostrum casein has received less attention despite its importance. The objective of this study was to investigate the size of colostrum CM and their relationship with compositional traits during four post-partum milkings. A total of 184 samples (collected at 12 ± 1 h intervals) were obtained from 46 cows over four sequential milkings. The results demonstrated a significant decrease in CM size of colostrum from 227.2 nm to 201.9 nm over the four post-partum milkings. Fat, protein, casein content, and Brix values also declined across milkings and showed a positive correlation with CM size, although the relationships were nonlinear. Understanding CM size can be valuable for decision-making regarding colostrum and transition milk processing due to its significant role.

## 1. Introduction

Rapidly increasing global energy and food demand, in parallel with increasing pressure to lower carbon footprints brings “zero waste” and “full valorisation” production concepts into the spotlight. However, food production under these concepts requires new and more in-depth understanding of the materials and technologies used. One of the important foods is milk, which can be divided into colostrum, transition milk, and mature milk during the lactation phase. Over the centuries improved farming practices have multiplied the individual milk production of cows while the intake by newborn calves has not changed. Therefore, milk production is accompanied by a considerable surplus (about 1% of milk production) of colostrum and transition milk as a by-product. Colostrum, which is a source of bioactive materials and vital fluid for calves, has been studied for decades. Some of the components in colostrum, such as bioactive components (McGrath, Fox, McSweeney, & Kelly, 2016) and fats (O'Callaghan et al., 2020; Sats et al., 2022) are well studied; at the same time, much less is known about the main protein of mature milk, casein.

In milk, casein is in the form of colloidal casein micelles (CM). The structure of CM is associated with various biological functions, including neonatal nutrition, regulation of calcium phosphate precipitation, and inhibition of amyloid fibril formation. (Holt, Carver, Ecroyd, & Thorn, 2013). These functions relate to the cow's ability to lactate and to the calf's health and future reproductive success. Holt and Carver (2012) suggested that the CM is a means by which protolacteal fluid could became complex, nutrient-rich milk without jeopardising the mother's reproductive prospects. It is well established that the release of casein from the micelle is related to milk salts, pH and heat (Holt et al., 2013). However, there is still no clear consensus about the mechanism of the assembly of the CM. Studying colostrum CM size during post-partum milkings can further knowledge about the functions and assembly of the CM, as CM biological functions relate primarily to the calf's life.

CM plays an essential role in milk processing, specifically in the production of numerous dairy products such as curd, cheese, and yoghurt (Holt et al., 2013). The colloidal micelles are necessary for the transport of calcium, phosphate, and casein proteins in high, insoluble concentrations (Holt & Carver, 2012). Individual milk samples' average CM size is between 150 and 230 nm (Bijl, de Vries, van Valenberg, Huppertz, & van Hooijdonk, 2014; De Kruif & Huppertz, 2012; Mootse et al., 2014). This wide range does not

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[*] Corresponding author.
*E-mail address:* Andres.Sats@emu.ee (A. Sats).

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seem to be related to the fat or protein contents of the milk, parity, or lactation stage (De Kruif & Huppertz, 2012). However, it is not known how variable CM size is in colostrum samples. From the dairy processing aspect, milk containing smaller CM coagulates faster and forms stronger gels than larger micelles (Glantz et al., 2010; Zhang et al., 2020). Zhang et al. (2020) related this difference in CM size to the observation that smaller micelles contain higher concentrations of micellar calcium, phosphate, and κ-casein. In contrast, Huppertz, Heck, Bijl, Poulsen, and Larsen (2021) observed a negative correlation between milk CM size and non-sedimentable casein. Therefore, CM size can be a valuable criterion for milk (and colostrum) processing and production decisions. Furthermore, the knowledge of colostrum's particle size can be utilised in developing new membrane processing technologies and combining them with unconventional methods in the dairy industry, such as chromatography or precipitation-assisted recovery. This is particularly important because the utilisation of existing conventional dairy technologies is challenging due to the poor heat stability of colostrum (McGrath, Grandison, & Lewis, 2016; Tsioulpas et al., 2007).

Colostrum (and transition milk) differs from mature milk produced later, reflecting the neonate's needs during the first postpartum days. The composition and quality of colostrum are influenced by various factors, including farming conditions and individual factors such as season, parity, pre-partum diet, and the health status of the cow (Puppel et al., 2019), as well as the sex of the new-born (Nazir et al., 2018). These differences in colostrum composition, both in terms of composition and functionality, are also associated with variations in particle size distribution (PSD). There are reports about the size of colostrum whey (Sats et al., 2020) and fat globules (Sats et al., 2022), while description of colostrum CM size is lacking. Only one study (based on eight Friesian dams) has reported colostrum CM size (Tsioulpas et al., 2007), which demonstrated that CM is larger on the first day post-partum, and that the size does not change during the subsequent 90 days.

The aim of this study was to measure colostrum CM size during four post-partum milkings. It was hypothesised that CM size decreases over the four post-partum milkings. An additional purpose was to investigate CM size relationships with animal specific (parity, successive milkings) and colostrum compositional traits (contents of main components, Brix value, fat globule sizes).

## 2. Materials and methods

### 2.1. Sampling

Colostrum samples were collected from 46 Holstein cows from the experimental farm of the Estonian University of Life Sciences over a two-year period. The average milk yield in the previous lactation of the multiparous cows included in this study was 95,00 kg. The average lactation and dry period durations were 300 and 60 days, respectively. All cows included in this study were in good health. To prevent suckling, calves were separated from dams. Cows were milked using a portable milking machine. Four consecutive post-partum samples were collected from each cow, resulting in a total of 184 samples ($n = 4 \times 46 = 184$). The first milking samples were collected 30–120 min after calving, and the subsequent three milkings were taken at 12 ± 1 h intervals. Specifically, the first and second milking samples were collected on day one post-partum, while the third and fourth milking samples were obtained on day two post-partum. After milking, the colostrum of each animal was stirred, and the sample (50 mL) was collected. Then the samples were frozen at −20 °C and stored. During the dry period, and after calving, cows were fed ad libitum. Daily diets were based on hay and grass silage with the addition of a concentrate feed (barley flour, rapeseed cake) and Rindavital VK mineral feed (Schaumann Agri International GmbH, Germany). The composition and nutritional value of the feed during the dry period and after calving are described in a previous study (Sats et al., 2022). Sampled cows were selected according to their parity (9 in their first parity, 9 in the second, 10 in the third, 9 in the fourth, 3 in the fifth, 4 in the sixth, and 2 in the seventh).

### 2.2. Size distribution of casein micelles

Colostrum samples were diluted 100-fold in RPMI 1640 (Mediatech Inc., Manassas, USA). The diluted sample was then filtered through a 0.45 μm pore diameter syringe filter (Whatman UNIFLO 25/0.45 RC, Maidstone, UK) into a disposable sizing cuvette (Sarstedt 10 × 10 × 45 mm; Sarstedt AG&Co, Nümbrecht, Germany). The casein micelles size distribution analyses were performed at 20 °C using a Zetasizer Nano ZS analyser equipped with a 633 nm He-Ne laser and software version 7.13 (Malvern Instruments Ltd., Worcestershire, UK). The size distribution was determined at a fixed 173° backscattered angle using the default ‘protein analysis mode’ with automatic duration and attenuation selection, similar to the procedure described and used previously (Mootse et al., 2014; Sats et al., 2014). A cumulative analysis correlation function was used to obtain each sample's intensity mean diameter (z-average diameter) using a dispersant viscosity of 0.733 mPa s [RPMI 1640 viscosity according to Poon (2022)] and a refractive index of 1.335. At least two cumulative z-average measurements were obtained for each sample. The preliminary experiment in our laboratory, where 1L of raw tank milk was divided into 15 × 5 mL and 15 × 50 mL samples and subjected to freezing for 5 weeks at −20 °C, has confirmed that freezing time and sample volume (freezing speed) have no significant effect on CM size (Table 1).

### 2.3. Size distribution of fat globules

The size distribution of fat globules was determined with a laser diffraction analyser (Malvern Mastersizer 3000, Malvern Instruments Ltd., Worcestershire, UK) as described earlier by Sats et al. (2022). The colostrum fat globules (CFG) size was expressed by diameter median (Dv50) and CFG diameter volume moment mean (D[4; 3]).

### 2.4. Compositional traits and colostrum quality

Casein contents were analysed by the Kjeldahl method. Casein was extracted by adding acetic acid and sodium acetate solution at pH 4.6 in a Kjeldahl tube and filtered according to ISO/DIS 17997−2 | IDF 29-2 standard (ISO, 2004). The nitrogen content of the

**Table 1**
Casein micelle size (nm) depending on sample volume and freezing week.[a]

| Week | Sample volume | |
|---|---|---|
| | 5 mL | 50 mL |
| 0 | 208.6 (2.73) | |
| 1 | 207.9 (6.27) | 204.6 (0.64) |
| 2 | 203.8 (1.15) | 206.6 (1.46) |
| 3 | 207.3 (1.90) | 207.9 (0.72) |
| 4 | 204.1 (1.95) | 204.2 (1.40) |
| 5 | 208.1 (0.42) | 208.7 (0.56) |

[a] Values are the mean with standard error in parentheses. At each week and sample volume three samples were analysed; according to two-way analysis of variance there was no effect of sample volume ($p = 0.930$), freezing week ($p = 0.363$) and their interaction ($p = 0.886$).

precipitate was determined according to ISO 8968 | IDF 20 standard (ISO, 2014).

Lactose, protein, fat, and total solids contents (%) were determined in samples with a near infra-red multipurpose analyser, equipped with an LSM module (Bruker Optik GmbH, Ettlingen, Germany). Prior to measurement of the spectra, the sample was pumped through an LSM module homogenisation unit. The spectra were analysed and converted to compositional results using the instrument OPUS software with licensed milk composition calibration (OPUS v. 7.5, Bruker Optik GmbH). Each sample was analysed in triplicate (three sequential pumping and spectra measurements).

The quality of colostrum, particularly with respect to immunoglobulins, was evaluated at 20 °C using a digital Brix (%) refractometer (Atago Co., Ltd, Tokyo, Japan).

### *2.5. Statistical analyses*

To study the effect of post-partum milking and parity number on colostrum traits, general linear mixed models were fitted. In these models, fixed effects of the post-partum milking, parity and their interaction, and the random effect of cow were considered. The model-based means (alias least square means with standard errors and 95% confidence intervals) were estimated and compared by milkings and parities with the Tukey HSD test. The magnitude of the cow effect was estimated using the intraclass correlation coefficient. Three parity groups were used in the analyses: 1st, 2nd and ≥3rd parities (n = 9, n = 9 and n = 28, respectively).

Linear relationships between colostrum traits were studied with Pearson correlation analysis and potential nonlinear relationships with Lowess smoothing and Spearman rank correlation analysis. To summarise the results of univariate analyses and discover more general patterns, principal component analysis (PCA) was used. To analyse to what extent these general patterns reflected the differences between milkings and parities, general linear mixed models with principal components as dependent variables, milkings and parity groups and their interactions as fixed effects and cow identity as a random effect were fitted.

All statistical analyses (significance was declared at $p \leq 0.05$) were performed, and all figures were constructed with R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria). For modelling, the packages' car', 'lme4', 'emmeans', and 'multcompView' were used and for PCA, the package 'ade4' was used.

## 3. Results and discussion

### *3.1. Protein, fat, and lactose contents*

The mean total solids (TS) content in the first milking samples (28.5 ± 1.0%) was nearly twice as high as in the fourth milking samples (16.3 ± 1.0%), which were collected approximately 36 h later. The most rapid decrease in TS content occurred between the second and third milkings, with TS dropping from 23.9% to 16.5% (SE ± 1.0%; Fig. 1, Table 2). Similarly, the protein and casein contents and refractive index (Brix) followed similar trends to the TS content. In contrast, the proportion of casein to total protein exhibited an opposite pattern to the TS content, increasing two to three times over the four sequential post-partum milkings. These findings are consistent with previous reports (El-Fattah, Rabo, El-Dieb, & El-Kashef, 2012; Elfstrand, Lindmark-Mansson, Paulsson, Nyberg, & Akesson, 2002; Madsen, Rasmussen, Nielsen, Wiking, & Larsen, 2004) and can be attributed to compositional changes as colostrum transitions into transition milk. The increase in the proportion of casein relative to total protein can be explained by a decrease in whey proteins, particularly immunoglobulins (Elfstrand et al., 2002; Sats et al., 2020). The effect of parity was statistically significant ($p < 0.05$) on fat globule diameter volume moment mean and lactose content. This effect of parity is likely explained by the less developed udder of primiparous cows. The interaction between milking and parity did not show a statistically significant effect on any of the variables studied ($p > 0.05$).

The fat contents in the first and second milkings were similar (6.6% and 6.4%, SE ± 0.47, respectively, $p = 0.998$) but decreased significantly in the third milking (4.5 ± 0.47, $p < 0.05$ in comparison with first and second milking). In the fourth milking, the fat content increased again, reaching 5.8 ± 0.47% (Fig. 1, Table 2). As with these findings, Elfstrand et al. (2002) also reported a fat content decrease

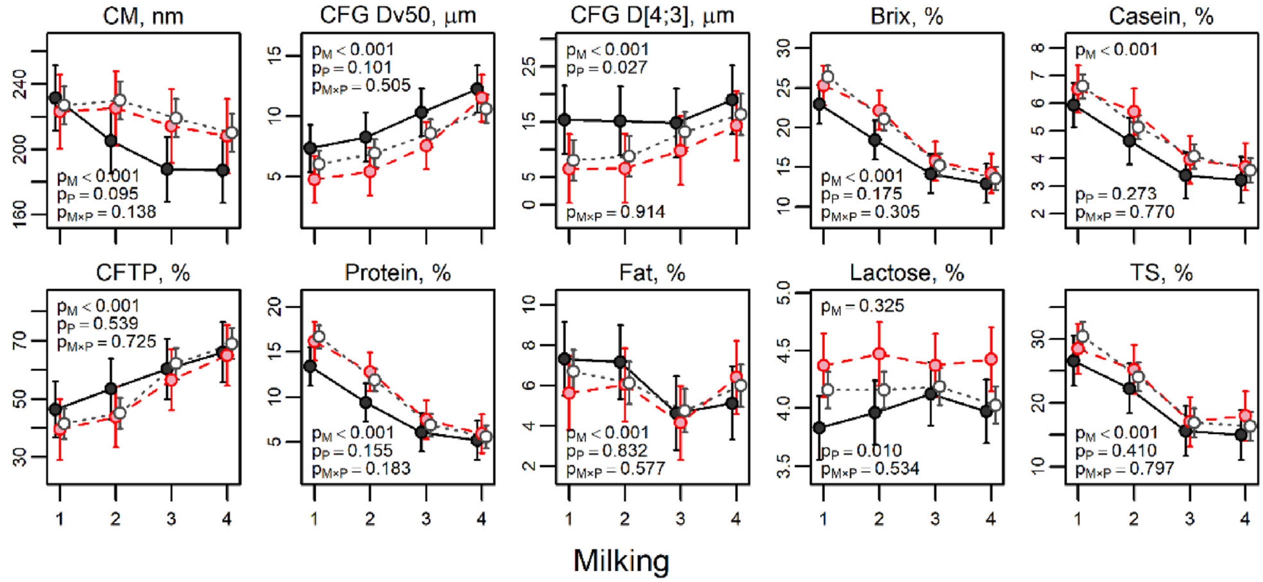

**Fig. 1.** Model-based means (with 95% confidence interval) of studied traits depending on milking and parity: —●—, parity 1; –○–, parity 2; ··○··, parity 3–7. Statistical significance of milking ($p_M$), parity ($p_P$) and milking by parity interaction ($p_{M \times P}$) effects are presented (AbbCM: casein micelles, z-average; CFG Dv50: colostrum fat globules diameter median; CFG D[4; 3]: colostrum fat globules diameter volume moment mean; CFTP: casein from total protein; TS: total solids).

**Table 2**
Model-based means (with standard errors) of studied traits depending on milking and parity.[a]

| Variable | Milking | | | | Parity | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3–7 | M | P | M × P |
| CM, nm | 227.2 (5.43)$^{a}$ | 220.1 (5.43)$^{ab}$ | 207.1 (5.43)$^{bc}$ | 201.9 (5.43)$^{c}$ | 202.9 (7.44) | 217.7 (8.43) | 221.6 (4.36) | <0.001 | 0.095 | 0.138 |
| CFG Dv50, μm | 6.05 (0.50)$^{a}$ | 6.88 (0.51)$^{a}$ | 8.83 (0.50)$^{b}$ | 11.47 (0.50)$^{c}$ | 9.55 (0.76) | 7.32 (0.76) | 8.05 (0.45) | <0.001 | 0.101 | 0.505 |
| CFG D[4; 3], μm | 9.99 (1.61)$^{a}$ | 10.19 (1.61)$^{a}$ | 12.60 (1.60)$^{ab}$ | 16.53 (1.61)$^{b}$ | 16.09 (1.84)$^{a}$ | 9.30 (1.84)$^{b}$ | 11.59 (1.09)$^{ab}$ | <0.001 | 0.027 | 0.914 |
| Brix, % | 24.9 (0.63)$^{a}$ | 20.6 (0.63)$^{b}$ | 15.1 (0.63)$^{c}$ | 13.6 (0.63)$^{c}$ | 17.1 (0.99) | 19.4 (0.99) | 19.1 (0.58) | <0.001 | 0.175 | 0.305 |
| Casein, % | 6.35 (0.21)$^{a}$ | 5.15 (0.21)$^{b}$ | 3.80 (0.21)$^{c}$ | 3.49 (0.21)$^{c}$ | 4.29 (0.33) | 4.96 (0.35) | 4.84 (0.18) | <0.001 | 0.273 | 0.770 |
| CFTP, % | 42.5 (2.57)$^{a}$ | 47.4 (2.64)$^{a}$ | 59.6 (2.64)$^{b}$ | 66.7 (2.64)$^{b}$ | 56.5 (3.46) | 51.2 (3.56) | 54.4 (1.84) | <0.001 | 0.539 | 0.725 |
| Protein, % | 15.42 (0.55)$^{a}$ | 11.36 (0.55)$^{b}$ | 6.79 (0.55)$^{c}$ | 5.55 (0.55)$^{c}$ | 8.51 (0.86) | 10.60 (0.86) | 10.24 (0.50) | <0.001 | 0.155 | 0.183 |
| Fat, % | 6.55 (0.47)$^{a}$ | 6.44 (0.47)$^{a}$ | 4.51 (0.47)$^{b}$ | 5.84 (0.47)$^{ab}$ | 6.06 (0.61) | 5.56 (0.61) | 5.90 (0.36) | <0.001 | 0.832 | 0.577 |
| Lactose, % | 4.12 (0.07) | 4.20 (0.07) | 4.23 (0.07) | 4.14 (0.07) | 3.97 (0.10)$^{a}$ | 4.41 (0.10)$^{b}$ | 4.13 (0.06)$^{ab}$ | 0.325 | 0.010 | 0.534 |
| TS, % | 28.5 (1.00)$^{a}$ | 23.9 (1.00)$^{b}$ | 16.5 (1.00)$^{c}$ | 16.4 (1.00)$^{c}$ | 19.9 (1.43) | 22.2 (1.43) | 22.0 (0.84) | <0.001 | 0.410 | 0.797 |

[a] Abbreviations are: CM, casein micelles, z-average; CFG Dv50, colostrum fat globules diameter median; CFG D[4; 3], colostrum fat globules diameter volume moment mean; CFTP, casein from total protein; TS, total solids. *P*-values indicate milking (M), parity (P) and milking-by-parity interaction (M × P) effects. Means without common superscript letters in the same row among four milkings/three parity groups are significantly different ($p \leq 0.05$, Tukey post-hoc test).

in the second milking and an increase in the fourth milking. In contrast, Tsioulpas et al. (2007) demonstrated low fat contents on the first post-partum day which increased on the third day and decreased again on the fifth day. El-Fattah et al. (2012) reported a decrease in the fat content of Holstein cow milk samples from 8.0% at parturition to 3.9% on the fifth day; in contrast to buffalo milk samples, where the fat content increased slightly from parturition to the third milking and decreased thereafter. In a review, McGrath et al. (2016) concluded that the fat content of colostrum varies in a very wide range, being generally higher than that of milk. The results of the current study support the pattern that the fat content decreases from parturition, but more research is needed to advance current understanding of the dynamics of colostrum fat contents.

Overall, our results indicated that the lactose content of colostrum was stable over four sequential post-partum milkings ($p = 0.33$) (Fig. 1, Table 2). Earlier studies (El-Fattah et al., 2012; Madsen et al., 2004; Tsioulpas et al., 2007) have demonstrated lower lactose contents in the first and second post-partum milkings and this increases in the subsequent milkings, with a similar range as found in our results. Lactose and inorganic salts are responsible for the osmotic pressure of milk; a low level of these two results in the production of viscous milk that contains little water (Bleck, Wheeler, Hansen, Chester-Jones, & Miller, 2009; Holt & Jenness, 1984). The stable level of lactose contents while TS decrease (Fig. 1, Table 2), means that the water content and inorganic salts in milk increase. Generally, the lactose content reaches its normal and stable level in milk (around 4.8%) within seven days post-partum (El-Fattah et al., 2012; McGrath et al., 2016).

### 3.2. *Fat globules sizes*

Our results demonstrated that the CFG size Dv50 (diameter median) and D[4; 3] (diameter volume moment mean) almost doubled during four sequential post-partum milkings (both $p < 0.001$, Fig. 1, Table 2) which were from 5.8 to 11.2 μm and 9.5–16.1 μm, respectively (Sats et al., 2022). Higher D[4; 3] values, compared with Dv50, means that the CFG size distribution is positively skewed. The parity effect was statistically significant for D[4; 3] ($p = 0.03$) but not significant for Dv50 ($p = 0.1$). Milking by parity interaction effects were not significant for either of the CFG size characteristics (D[4; 3] and Dv50 $p > 0.05$). There are no reports about bovine CFG size available except for results which are published and discussed in the context of fatty acid profile and CFG size distribution in our previous paper (Sats et al., 2022). The first milking CFG D[4; 3] is in a similar range as that of average milk fat globules reported in earlier work (Fleming et al., 2017; Jaakamo et al., 2019; Lopez et al., 2011). Our findings of increasing colostrum fat globule size with decreasing fat content disagree with previous reports showing a positive relationship between the size of fat globules and fat content in mature milk (Carroll et al., 2006; Wiking, Stagsted, Björck, & Nielsen, 2004). However, Argov, Lemay, and German (2008) suggested that larger fat globules are secreted due to mammary epithelial cells enveloping the fat globules, which could explain the increase of CFG during post-partum milkings found in this study.

### 3.3. *Casein micelles sizes*

The colostrum CM size decreased significantly ($p < 0.001$) during the four sequential milkings post-partum (Table 2; the sizes were 227.2, 220.1, 207.1 and 201.9 nm, SE = 5.43, respectively). The colostrum CM size was lower in the primiparous cows than in the multiparous cows. Also, the decrease in colostrum CM size over milkings was more rapid in primiparous cows (Fig. 1). Neither parity effect nor milking-by-parity interaction effect had a significant influence on CM size (both $p > 0.05$). The random effect of cow explained 39.5% of the variability of colostrum CM size, indicating a high dependence on the individual animal. The first post-partum milking CM diameter measured in this study is almost identical to first post-partum day CM size (227 ± 19.7 nm) reported by Tsioulpas et al. (2007) who however reported faster decrease; on the second day, the colostrum CM diameter was 189 nm and the following three months showed no significant ($p > 0.05$) changes. Considering that our study demonstrated that CM diameter varied highly between cows, the discrepancy between the results found in this study and that by Tsioulpas et al. (2007) can be explained by the different sample sizes, which were 46 and 8, respectively.

The results of colostrum CM size decrease during four post-partum milkings are consistent with previous reports on κ-casein. Sobczuk-Szul, Wielgosz-Groth, Wroński, and Rzemieniewski (2013) reported a decrease in the proportion of colostrum's κ-casein over post-partum milkings. Conversely, there are reports suggesting that smaller mature milk CMs contain more κ-casein (Day, Williams, Otter, & Augustin, 2015; Zhang et al., 2020). However, earlier work by De Kruif and Huppertz (2012) did not provide sufficient evidence for such a relationship. This lack of sufficient evidence is likely explained by genetic variants of κ-casein and the amount of glycosylated κ-casein as a fraction of total κ-casein as described by Bijl et al. (2014). Furthermore, earlier studies (Bijl et al., 2014; Mootse et al., 2014) reported that the average size of CM in mature milk is around 190 nm, while in colostrum, the CM size is around 220 nm. This difference in size may be attributed to the specific nutritional requirements and metabolic peculiarities of new-borns, as the gut epithelium's ability to absorb macromolecules diminishes within approximately 24 h post-partum. Additionally, Huppertz et al. (2021) observed a negative correlation between CM size in mature milk and non-sedimentable casein. The reduction in non-sedimentable casein and the subsequent improvement in whey syneresis may facilitate the efficient transfer of immunoglobulins (whey proteins). However, it is important to note that these assumptions warrant further research and investigation.

### *3.4. CM size relationships with colostrum composition and fat globules size*

The colostrum CM size, as well as TS, fat, protein, casein, and Brix decreased similarly over milkings (Fig. 1) and were all significantly ($p < 0.005$) positively correlated with CM size (Fig. 2). In contrast, the sizes of CFG and the proportion of casein from total protein (CFTP) showed a significant ($p < 0.001$) negative correlation with colostrum CM size. The results suggest that studied relationships are nonlinear, as evidenced by the presence of Lowess smoothing lines in Fig. 2 and the higher Spearman rank correlation coefficients (0.04–0.05 higher) compared with Pearson linear correlation coefficients. This indicates that colostrum with smaller CM size tends to have lower TS, fat, protein, casein contents, and Brix values, while milk with larger CM size can have both lower and higher TS, fat, protein, casein contents, as well as Brix values. From a practical standpoint, this correlation suggests that although milk with smaller CM coagulates faster and forms stronger gels (Glantz et al., 2010; Zhang et al., 2020), the overall product outcome might be limited due to lower TS content. The negative

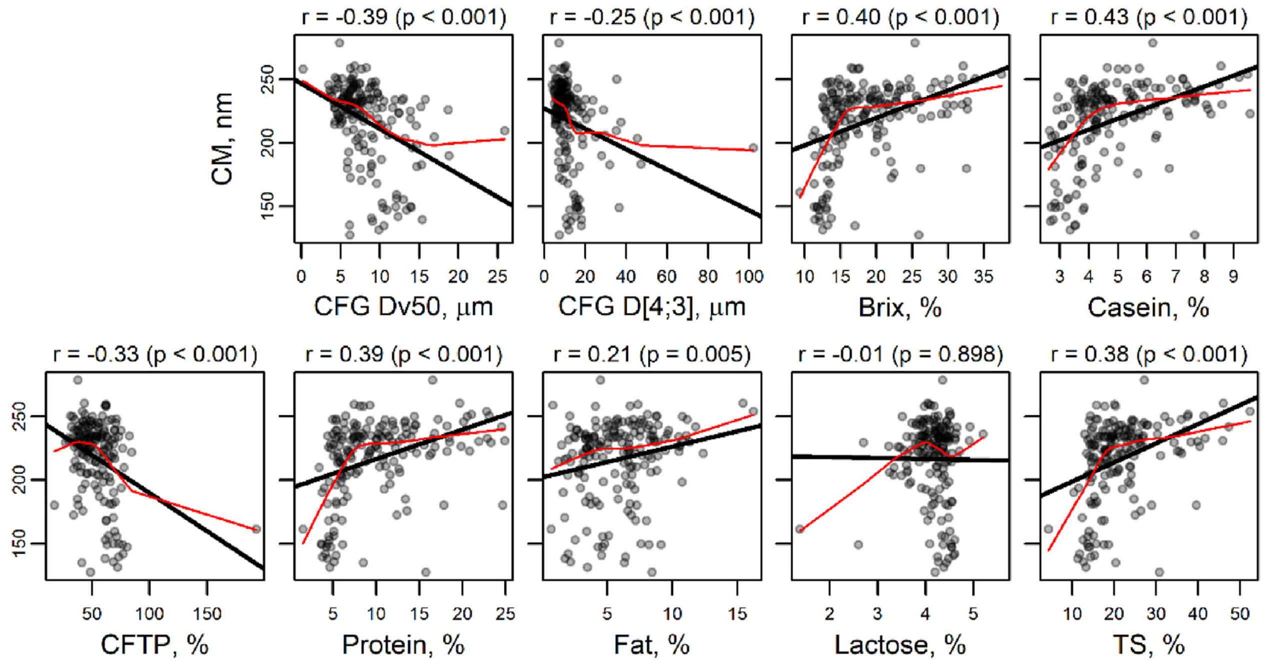

**Fig. 2.** Relationships between colostrum casein micelles (CM; y-axis in all subfigures) diameter and other studied traits (CFG Dv50: colostrum fat globules diameter median; CFG D [4; 3]: colostrum fat globules diameter volume moment mean; CFTP: casein of the total protein; TS: total solids). Each sample is marked with a dot, straight black line and numerical values above the figures indicate a linear relationship, the red line indicates a possible nonlinear relationship estimated with Lowess smoothing.

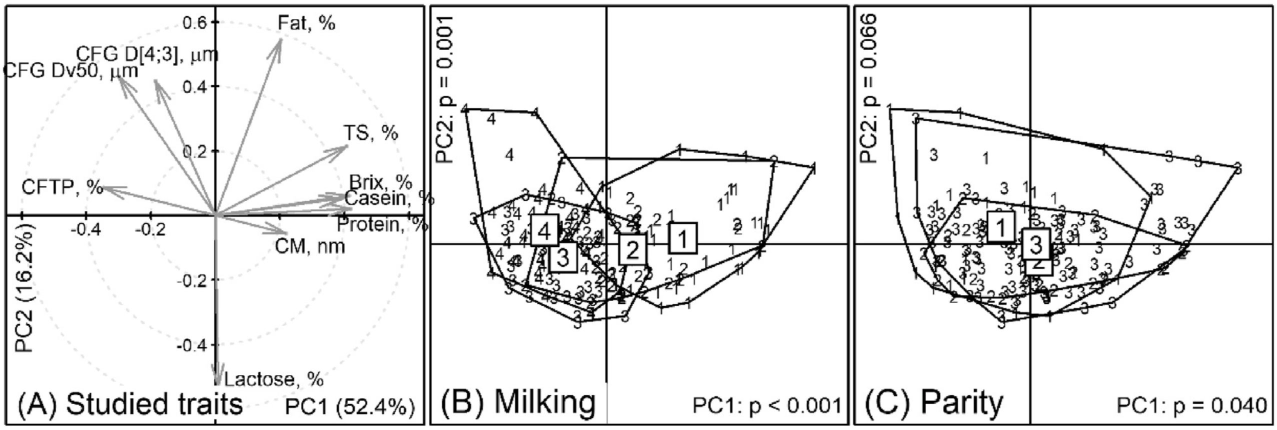

**Fig. 3.** Results of principal component analysis. Panel A, the weights (eigenvectors) of studied traits in the first two principal components (PC; CM: casein micelles hydrodynamical diameter; CFG Dv50: colostrum fat globules diameter median; CFG D[4; 3]: colostrum fat globules diameter volume moment mean; CFTP: casein in total protein; TS: total solids). Panels B and C, location of samples according to their first two PC scores sorted by milking (B) and parity group (C). Each sample is marked with its group symbol and samples from the same group are surrounded by a line; higher values in boxes mark the groups' centroids and *p*-values denote the significance of the factors according to the linear mixed model considering fixed effects of milking and parity, and random effect of cow; for both PCs separate models were fitted.

relationships between colostrum CM size and CFG size, as well as the proportion of casein to total protein (CFTP), were linear.

The PCA confirms relatively strong overall patterns among the studied colostrum traits. PC1, which explains 52.4% of the total variance, mainly describes similar changes in TS, protein and casein contents, Brix, and CM sizes, with opposite changes in the casein proportion of the total protein (CFTP) (Fig. 3A). Comparing PC1 values between post-partum milkings and parity groups indicates that TS, protein and casein contents, Brix, and CM sizes decrease over the four milkings and have slightly lower values in primiparous cows, while CFTP follows an opposite pattern (Fig. 3B and C). These PC1 patterns align with the results reported and discussed above.

PC2, explaining 16.2% of the total variance, describes similar changes in colostrum fat globule sizes and fat contents (Fig. 3A), along with an opposing pattern of changes in lactose content. PC2 values, representing colostrum fat globule size and fat content, were slightly higher in the fourth milking and in primiparous cows, while lactose content showed an opposing pattern of change (Fig. 3B and C). Alessio et al. (2016) concluded that lactose content in milk is unrelated to fat levels.

The effect of individual cows accounted for 58.3% and 31.8% of the variability in PC1 and PC2, respectively. This suggests that colostrum traits associated with PC1 (TS, protein, casein, Brix, CM size, and CFTP) are more specific to individual animals, while traits within PC2 (colostrum fat globule sizes, and fat and lactose contents) vary more widely.

## 4. Conclusions

This study investigated the size of bovine colostrum CM across four post-partum milkings and explored their relationships with the main components of colostrum. The results revealed a decrease in colostrum casein content and CM size over the four post-partum milkings. However, the CM size remained larger than that observed in mature milk. Parity had minimal impact on CM size and the other studied colostrum characteristics. It was observed that colostrum with smaller CM size exhibited lower TS, fat, protein, casein contents, and Brix values, while milk with larger CM size showed variations in TS, fat, protein, and casein contents. This diversity suggests that although milk with smaller CM size can coagulate faster and form stronger gels, the product yield may be low due to a lower TS content. Further research is needed to better understand the effect and role of casein content and CM size in colostrum whey syneresis and the transfer of immunoglobulins in neonates.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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