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Short communication: The biological value of transition milk: analyses of immunoglobulin G, IGF-I and lactoferrin in primiparous and multiparous dairy cows

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ABSTRACT

Colostrum (the first mammary gland secretion after calving) is known to contain high concentrations of nutrients as well as bioactive substances (including immunoglobulins, growth factors, and antimicrobial factors) to ensure neonatal survival. Due to its immunomodulatory, antibacterial, and antiviral activities, bovine colostrum has been used not only in calves but also in the prevention and treatment of human gastrointestinal and respiratory infections. Transition milk is the mammary secretion from the second milking to the sixth, which may contain these bioactive compounds to a lesser extent. The objective of the present study was to measure IGF-I, immunoglobulin G (**IgG**), and lactoferrin (**LTF**) concentrations in colostrum and transition milk of primiparous and multiparous cows to further assess its potential use in veterinary and nutraceutical applications. The results demonstrated that the concentrations of these three bioactive molecules decrease from the first milking to the tenth. Concentrations of IGF-I and LTF were greater in multiparous than in primiparous cows. Also, lactation number interacted with milking number in IGF-I, since primiparous cows had a smoother decline of IGF-I concentrations than multiparous ones. Overall, transition milk from the second milking showed a 46% decrease in the analysed colostrum bioactive molecules. Therefore, further studies are needed to apply this knowledge in neonate farm management practices or in developing pharmaceutical supplements from farm surpluses.

Implications

After the first milking of colostrum, the mammary secretion from the second milking to the sixth is considered transition milk. Although it is sometimes treated as waste milk, it contains at least half of the immunoglobulin G, IGF-I, and lactoferrin found in colostrum. An appreciation of the biological value of transition milk is needed to exploit its potential for future applications on animal and human health. To maximise the industrial process of obtaining the molecules, the parity and milking number of the cow should be considered to evaluate the economic viability of the process.

Introduction

Transition milk (**TM**) is the mammary gland secretion from the second milking after calving until the sixth when it becomes saleable whole milk (Godden et al., 2019). Colostrum is well known to contain high concentrations of nutrients as well as bioactive compounds (such as immunoglobulins, growth factors, cytokines, and antimicrobial factors) to ensure calf survival. These compounds decrease throughout the upcoming milkings, still being in large amounts in the second and third milking (Blum and Hammon, 2000).

Recently, bovine colostrum (**BC**) has been used in the prevention and treatment of human gastrointestinal and respiratory infectious diseases, showing an alternative or combined therapy to the antimicrobials (Ulfman et al., 2018). The main antibacterial and antiviral activities of BC lie with the ability of immunoglobulin G (**IgG**) to bind to many pathogens, but also with some other colostrum components such as lactoferrin (**LTF**) and proinflammatory cytokines (Ulfman et al., 2018). In the dairy industry, BC may be an effective tool to reduce the use of antibiotics, as calves with an adequate transfer of passive immunity (serum IgG at 24 h after birth higher than 10 g/L) had lower mortality and morbidity and fewer antibiotics were used to raise them (Godden et al., 2019). Also, when the administration of colostrum is prolonged in calves, it promotes the survival of intestinal epithelial cells and reduces mucosal-epithelial cell turnover and consequently might reduce diarrhoea susceptibility and enhance growth performance

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(Blättler et al., 2001). Chamorro et al. (2017) supplemented milk replacers with colostrum replacers obtained from dairy farm surpluses to enhance preweaned calves' health. In their study, colostrum supplementation reduced the incidence of diseases as well as the associated use of antibiotics compared with non-supplemented calves. In addition, the parity effect has been studied for IgG in BC but to a lesser extent for IGF-I or LTF (Blum and Hammon, 2000; Cheng et al., 2008; Tortadès et al., 2022).

Alternatively, TM is an underrated by-product from the dairy cow given that is less concentrated in constituents (total solids, fat, immunoglobulins, and lactose) than colostrum and is often considered waste milk by the industry. However, TM contains many bioactive components in greater amounts than in whole milk that might be useful in fighting neonatal and infectious diseases either in calves or human medicine. Thus, the objective of the present study was to determine the biological value of TM compared to BC for its use in veterinary and human medicine. Herein, we present interesting results from evaluating IGF-I, IgG, and LTF concentrations in primiparous and multiparous cows which encourages further research in this topic.

Material and methods

Collection and sample analysis

One hundred mL of BC (first milking-**M1**), TM from the second (**M2**) and third (**M3**) milkings, and milk from the tenth milking (**M10**) were collected by farm personnel from a total of 45 primiparous and 45 multiparous Holstein-Friesian cows randomly selected from three different commercial farms (2 × 15 cows from each farm) in the North-East of Spain and frozen at −20 °C until its use. The samples were obtained after cleaning and disinfecting cows' teats and collected in sterilised 50 mL Falcon tubes. In all farms, cows were dried at 60 days before parturition, but Farms B and C vaccinated dry cows against Rotavirus, Coronavirus, and *Escherichia coli* K99, and Farm A did not. All cows were fed a unique dry cow diet, but it slightly differed among farms (Table 1). Furthermore, cows in Farms B and C were milked three times per day, and twice in Farm A. Bulk milk tank from each farm was sampled every 2 days during the study and analysed for somatic cell count (**SCC**) and total bacteria count at the Central Laboratory for Milk Recording (ALLIC, Catalonia, Cabrils, Spain). The mean of SCC and total bacteria values was calculated to obtain a general farm overview of milk quality.

After thawing at room temperature, IgG, IGF-I, and LTF concentrations were quantified.

Table 1
Nutrient composition of the dry cows' rations and the mean quality of milk tank based on SCC and total bacteria in Farms enrolled in the study.

Item	Farm A	Farm B	Farm C
Nutrient, g/kg DM			
DM	440	522	633
CP	125	125	125
EE[1]	21	26	27
NDF	450	562	522
ADF	293	354	288
Ash	77	80	55
NE_l[2], MJ/kg DM	5.52	5.76	5.44
Milk tank quality[3]			
Milk SCC[4], cells/L	215	207	229
Total bacteria count, cfu/L	11	26	14

[1] EE = Ether extract.
[2] NE_l = Net energy of lactation.
[3] Mean of SCC and cfu/L values measured in tank milk samples every two days during the study.
[4] SCC = Somatic Cell Count.

Immunoglobulin G quantification

Samples were analysed for IgG concentrations using a single radial immunodiffusion kit (Radial Immunodiffusion Test, Triple J Farms, Bellingham, WA, USA) according to the manufacturer's recommendations. Bovine colostrum and TM were centrifuged for 15 min at 2 000*g* at 4 °C to remove the upper-fat layer of the sample. Whey fraction was collected and diluted with Phosphate-buffered saline (**PBS**) 1:10 (M1), 1:5 (M2), and 1:2 (M3), respectively. Five µL of the sample was added per well and incubated at room temperature for 24 h until plate reading.

Insulin growth factor-I quantification

Samples were analysed for IGF-I concentrations using an IGF-I ELISA kit (Mediagnost, Reutlingen, Germany). According to the kit manual, samples were initially diluted with PBS 1:20 but afterwards, some of them were repeated at 1:40 (M1 and M2), 1:21 (M3), or 1:10 (M10) as they were out of the detection range. Plates were read at 450 nm using EMS Reader MF V.2.9-0 from Labsystems (Vantaa, Finland).

Lactoferrin quantification

Samples were analysed for LTF concentration using Bovine Lactoferrin ELISA kit from Cloud-Clone Corp. (Katy, TX, USA). Following the manufacturer's instructions, samples were centrifuged for 15 min at 10 000*g* at 4 °C. The aqueous fraction was collected and centrifuged twice more for a total of three cycles. After that, samples were diluted with PBS 1:100 000 (M1 and M2), 1:20 000 (M3), and 10 000 (M10). Plates were read at 450 nm using Model 680 Microplate Reader from Bio-Rad (Hercules, CA, US).

Statistical analyses

Data were examined using descriptive statistics, and Tukey outlier boxplots were performed to detect outliers using JMP®, version 16.0.0 (SAS Institute Inc., Cary, NC, USA). Outlier exclusion criterion was a 1.5-fold interquartile range considering each biomolecule, milking number, and lactation, which was indicated by the length of the boxplot's whiskers. Finally, 16, 8, and 24 observations of IgG, IGF-I, and LTF data, respectively, were excluded, leaving 315, 267, and 311 observations for IgG, IGF-I, and LTF analysis, respectively. The remaining missing values were mainly from M10 samples because they were below the technique detection range. Furthermore, data from one cow were removed due to clinical mastitis.

The final dataset obtained was statistically analysed using SAS software (version 9.4, Institute, Cary, NC, USA). Data were analysis with a mixed-effect model accounting for the random effects of cow and farm, as well as the fixed effects of parity (primiparous or multiparous), milking number, and their interaction. To explore the effect of the farm on the parameters studied, a mixed-effect model accounting for cows as a random effect, and milking number, farm, and their interaction as fixed effects were done. In all cases, milking entered the model as a repeated measure using an autoregressive covariance matrix (the structure with the lowest Bayesian criterion), except for the IGF-I, in which was used an unstructured covariate matrix. Additionally, on the LTF analysis, the plate was included as a block in the fixed effects. Data were previously transformed into logarithm to achieve a normal distribution.

Results and discussion

Although this study was not designed to elucidate the effects of farm practices on milk bioactive compounds, it was observed that

Farm A had lower ($P < 0.05$) IgG concentrations than the two other Farms. Dry cow vaccination against calf diarrhoea in Farm B and C, but not in Farm A might have influenced this parameter. Although Baumrucker et al. (2022) found a positive correlation of IgG_1 with LTF, in this study, IgG and LTF were not correlated. Contrarily, LTF was lower ($P < 0.05$) in Farm C compared with Farms A and B. Cheng et al. (2008) suggested the stage of lactation, daily milk production, and SCC as the main factors associated with milk LTF concentration. Individual SCC and milk volume of the different milkings may explain differences among the three Farms in LTF concentrations. Since different on-farm management practices like vaccination protocols or milk volume can affect the concentration of these bioactive compounds, the Farm was included as a random effect in the statistical model. Despite IGF-I concentrations being similar in all Farms, it was observed a positive correlation with IgG concentrations ($R^2 = 0.388$, $P < 0.001$), which might suggest a common mechanism of secretion of both components.

The results of the current study showed that the concentration of IgG, IGF-I, and LTF decreased ($P < 0.001$) from the first milking to the tenth. Multiparous cows produced colostrum/transition milk significantly richer in IGF-I and LTF than the primiparous cows ($P < 0.01$, $P < 0.05$, respectively, Table 2), and parity affected the change in IGF-I between the M1 and M2 milkings ($P < 0.001$, Table 2). As the milking progressed, multiparous cows, despite having initially greater concentrations of M1 and M2, had similar IGF-I dilution rates than primiparous cows until M3 (Fig. 1).

Focusing on the IgG concentration, M1 and M2 met the quality standard of 50 g IgG/L to ensure calf survival (Godden et al., 2019). After parturition, more than half of the IgG concentration was maintained between the first two milkings. Particularly, a total of 54, 36, and 5% of the IgG BC content remained in the M2, M3, and M10 milking, respectively. This decrease could be explained not only because a reduction in production but also because of the osmotic pressure in the mammary gland caused by molecules like lactose, which incorporates more water and reduces the concentration of the biomolecules accumulated in the mammary gland entirely before parturition. In the present study, primiparous and multiparous cow's milk IgG followed a similar disappearance rate along successive milking. Regarding IGF-I concentrations, M2, M3, and M10 conserved 58, 25, and 3% of the IGF-I concentrations of M1, respectively, and values were within the wide range of IGF-I described in Meyer et al. (2017)'s review. Blum and Hammon (2000) also found similar IGF-I concentration conservation from first to second (63%) and from first to third milking (34%), with very low concentrations in mature milk. There is limited literature reporting LTF concentration in TM. This study shows greater LTF concentration conservation than Blum and Hammon (2000), obtaining LTF conservation values from M1 to M2 of 72 vs 47% and from M1 to M3, 38 vs 25%, for this study vs Blum & Hammon (2000), respectively. Most literature concerning LTF concentration is focused on mature milk, with the stage of lactation and SCC positively correlated, and milk yield negatively correlated with LTF concentration in milk (Cheng et al., 2008).

This study revealed that TM from M2 contains at least 54% of the concentration of colostrum bioactive molecules. When the concentration of colostrum components in M2 vs M1 samples was compared, LTF was conserved the most (72.3%), followed by IGF-I (58%) and finally, IgG (54%). Parity also affected the concentration of biomolecules in M2 samples. Overall, multiparous cows produced more bioactive molecules than primiparous cows (26, 31, and 34 % for IgG, IGF-I, and LTF, respectively). At present, surplus colostrum is processed and transformed into different by-products for multiple purposes (Kelly, 2003), such as IgG or LTF supplements for humans (BioNatIn BV, Son en Breugel, The Netherlands) or calves (Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada) to enhance immune and digestive health or to promote sports nutrition by improving performance and recovery. Even a TM replacer is currently available on the market (Transformula, Bonanza Calf Nutrition, Dundalk, Ireland). These commercial examples demonstrate the industry's potential to exploit these nutraceutical surplus by-products from dairy farms.

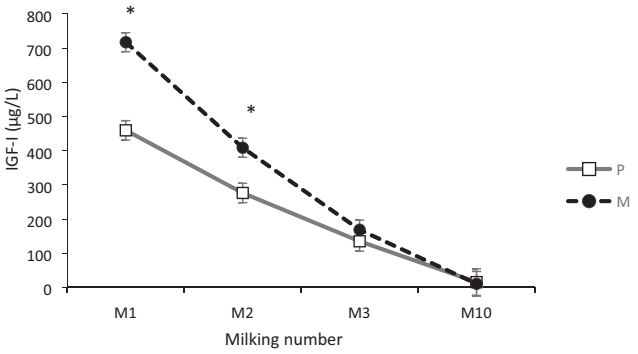

Fig. 1. Bovine milk IGF-I concentration evolution by milking number and parity, primiparous (P) and multiparous (M). Statistical differences in milking number are indicated as follows: * denotes $P < 0.001$.

To conclude, our study demonstrates that TM from the second milking contains more than half of the concentration of bioactive molecules present in colostrum. This may indicate a potential future application of this by-product. However, further studies are required to investigate the effect of parity to ensure that the biological value of TM is maintained.

Ethics approval

This is an observational study, and no ethical approval is required. Milk samples were obtained during the routine milking manipulation of animals in the milking parlour, without any extra animal contact. No ethical approval was needed.

Data and model availability statement

The model was not deposited in an official repository. The data analysed during the current study are available from the corresponding author upon reasonable request.

Table 2
Mean of bioactive peptides in bovine milk by parity.

Item	Primiparous				Multiparous				SEM		*P*-value[1]	
	1	2	3	10	1	2	3	10		LN	M	LNxM
IgG, g/L	98	50	20	1	122	68	22	1	6	0.139	<0.001	0.263
IGF-I, µg/L	465^{b}	280^{c}	133^{d}	21^{e}	717^{a}	407^{b}	166^{d}	13^{e}	32	0.006	<0.001	<0.001
LTF, g/L	0.72	0.57	0.34	0.18	1.28	0.87	0.42	0.19	0.14	0.022	<0.001	0.264

[1] LN = effect of lactation number (Primiparous vs Multiparous); M = effect of milking number; LNxM = effect of the interaction between lactation and milking number. Means within a row with different superscript letters differ ($P < 0.05$) in the effect of the interaction between lactation and milking number.

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Authors' contributions

AA, EGF, and **M. Terré** conceptualised the study and designed the methodology. **M. Tortadès** performed the experiments. **M. Terré** and **M. Tortadès** conducted the data analysis and wrote the original draft. **AA, EGF,** and **M. Terré** reviewed and edited the final version of the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interest

None.

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