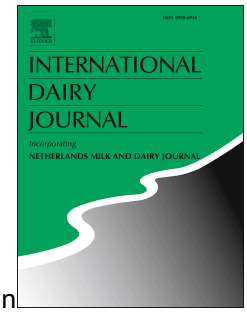

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Evolution of the bovine milk fatty acid profile - From colostrum to milk five days post parturition

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ABSTRACT

Milk was collected from each of 18 cows (presenting an even spread of 1st, 2nd and 3rd lactation): colostrum on the day of calving and subsequent morning milk 1–5 days post parturition. Days post parturition significantly affected the fatty acid profile of colostrum and transition milk samples. The colostrum fatty acid profile was distinctly different from that of mature milk, with significantly higher levels of polyunsaturated and saturated fatty acids. Parity of the cow had a significant effect on the fatty acid profile of colostrum and transition milk samples; conjugated linoleic acid was significantly higher in cows entering their 1st lactation than in those in their 3rd lactation, while multiparous cows produced significantly higher concentrations of C16:0. The changing composition of the fatty acid profile can be classed into three distinct phases: colostrum (D0), transition milk (D1 and D2 post parturition) and mature milk (D3 to D5).

1. Introduction

Colostrum is the initial milk secreted by mammals post parturition, the composition of which differs significantly from that of mature milk. Colostrum has an evolutionary design providing an initial source of essential nutrients for the new born mammal. A number of factors can affect the composition of bovine colostrum, including breed, lactation number, diet (Zarcula et al., 2010), length of dry period prepartum and time post-partum. In addition, the biological function of colostrum and its composition, changes in the days post parturition as it transitions from being colostrum to mature milk (Tsioulpas, Grandison, & Lewis, 2007). The definition of time periods associated with each of these stages varies considerably in the literature from classification as colostrum immediately after parturition, to also include between 5 and 7 days post-partum (McGrath, Fox, McSweeney, & Kelly, 2016). As a result of the differences in macro components of colostrum compared with mature milk, greater knowledge of its composition relative to the transition periods would be beneficial to minimise undesirable mixing of raw milk with colostrum prior to processing. Such information may avoid encountering processing related issues that have previously been reported with colostrum (Tsioulpas et al., 2007); furthermore, the segregation of colostrum from mature milk can also be important in instances where antibiotic dry cow treatments are used.

Accounting for approximately 0.5% of a cows annual milk production (Scammell, 2001), research on bovine colostrum has focused on its role in the initial development of the calf. Colostrum has a significant effect on the development of the calf through the provision of passive immunity (Korhonen, Marnila, & Gill, 2000), influencing metabolism, endocrine systems, and development of the gastrointestinal tract (Blum & Hammon, 2000). It is essential that the new-born calf receives an adequate supply of colostrum in the early stages

of life, as although colostrum is a rich source of immunoglobulins both their concentration and the permeability of the gut decreases rapidly in the first 24 h post-partum (Weaver, Tyler, VanMetre, Hostetler, & Barrington, et al., 2000). Indeed Fischer et al. (2018), found that delaying colostrum feeding within 12 h of life decreases the passive transfer of IgG, and may delay the colonisation of bacteria in the intestine, increasing the risk of infection to the calf.

Although surplus colostrum was previously thought of as unmarketable (Foley & Otterby, 1978), in recent years the bioactive components in bovine colostrum have attracted interest as a potentially beneficial food ingredient for the future (Sacerdote et al., 2013). As mentioned previously, with levels accounting for 0.5% of cows' annual milk production, this quantity does represent a viable stream for further processing into high value products. Colostrum has been sold in tablet form, in powder form or as colostrum based drinks (Boland, 2010). Mizelman, Duff, Kontulainen, and Chilibeck (2017) on review of the topic, highlighted how supplementation of the diet with bovine colostrum appears to improve immune function and prevent inflammation after exercise. In rodent models, the consumption of colostrum has been demonstrated to prevent gastrointestinal injury as a result of taking non-steroidal inflammatory drugs (Playford et al., 1999). Another consideration in the commercial production of bovine colostrum is that, while the cow produces far in excess of the amount required by the calf, the availability of colostrum can be dependent on the type of lactational system being practised at farm level. Seasonal calving systems, such as that in Ireland and New Zealand, result in colostrum only being available for a short period at the beginning of the lactational cycle, whereas a year round calving system would result in a consistent supply of colostrum.

There is an abundance of information available relative to the changes that occur to the macro components of colostrum in the first days post parturition (El-Fattah, Rabo, El-Dieb, & El-Kashef, 2012; Tsioulpas et al., 2007), with in-depth research focusing on the

protein fraction (Senda, Fukuda, Ishii, & Urashima, 2011; Tsioulpas et al., 2007). However, knowledge of the changes occurring in relation to fat composition during the different transition stages is currently limited. The objective of this study was to examine the influence of days post parturition and parity of cow on the fatty acid (FA) profile of bovine colostrum in an Irish context. This study provides a robust overview of the changes taking place to better define the stages of transition, as colostrum evolves into mature milk over the first 5 days of lactation relative to the fatty acid profile.

2. Materials and methods

2.1. Reagents

Heptane, sodium hydrogen monohydrate and 25% sodium methoxide were purchased from Sigma Aldrich (Dublin, Ireland). Diethyl ether was purchased from Fisher Scientific (Dublin, Ireland). The internal standard trinonadecanoin (C19:0 TAG) [part number T-165] which was used for sample prep and a standard of CLAc9t11 were purchased from Nu Chek-prep, Inc (Elysian, MN, USA). Fatty acid methyl ester (FAME) standard mix containing C4:0 to C24:0 (Part number 35077) was purchased from Thames Restek UK Ltd (Buckinghamshire, UK). C19 FAME was purchased from Sigma Aldrich.

2.2. Experimental design

Eighteen Holstein Friesian cows consisting of an even spread of 1st lactation (n = 6), 2nd lactation (n = 6) and 3rd lactation (n = 6) were selected from the spring calving dairy herd based at the Teagasc Moorepark Dairy Research Farm, Fermoy Co. Cork, Ireland. Prior to

calving, animals were fed grass silage (40% of DM), straw (30% of DM) and a blended concentrate (30% of DM) (rolled barley and maize gluten meal at a 60:40 ratio). Animals had access to feed 24 h per day and fresh clean water. Animals were feed to 100% ULF requirement plus a 10% refusal, and feeding was adjusted in accordance with month of gestation. Animals remained on this diet from dry off until one week post calving when animals were turned out to grazed grass.

In total, 6 milk samples were collected from each cow consisting of colostrum taken on the day of calving, and subsequent morning milk 1, 2, 3, 4, and 5 days post parturition. Each cow was milked into a separate stainless steel churn at milking time to enable sample collection. Approximately 400 mL of milk was collected from each cow and immediately refrigerated at 4 °C. Once aliquoted for respective testing, samples were frozen at –20 °C prior to analysis. For continuity, all analysis was carried out sequentially once the entire sample set was collected.

2.3. Fat content analysis

Fat content of the colostrum and milk samples was analysed using the Röse–Gottlieb method (IDF, 1996).

2.4. Fatty acid analysis

Lipid extraction was performed as per the procedure outlined by De Jong and Badings (1990) similarly to that of O'Callaghan et al. (2016, 2019). Briefly, 10 mL of ethanol (98% purity), and 1 mL of 2.5 M H_2SO_4 was added to 10 mL of each sample and mixed. This mixture was extracted three times with 15 mL diethyl ether/heptane (1:1) and each time the

141 solution was clarified by centrifugation at 1500 × *g* for 5 min. The collected extracts were
142 pooled and dried down at 55 °C under N_2 gas.

143 For methyl ester derivatisation of triglycerides (TAG), a volume of 4.8 mL of C19:0
144 TAG (500 ppm) in heptane was added to ~60 mg of the extracted lipid sample, following this
145 200 µL of 2 M sodium methoxide solution was added and the sample was mixed vigorously
146 for about 30 s. Then, 1g of sodium hydrogen sulfate monohydrate (Sigma Aldrich) was added
147 to the solution and the mixture was again shaken vigorously. After the salt had settled, the
148 upper layer containing the methyl esters was decanted into a clean test tube and diluted with
149 8 mL of heptane. FAMEs were stored at –20 °C prior to gas chromatography analysis in 2
150 mL amber vials which were capped with PTFE/white silicone septa.

151 FAME analysis was performed on an Agilent 7890A gas chromatograph system,
152 equipped with an Agilent 7693 autosampler (Agilent Technologies, Cork, Ireland) and flame
153 ionisation detector (FID). The column was a Select FAME capillary column (100 m × 250
154 µm I.D., 0.25 µm phase thickness, part number: CP7420) (Agilent Technologies, Little
155 Island, Cork, Ireland). The injector was held at 250 °C for the entire run and was operated in
156 split mode using a split ratio of 1:10. The inlet liner was a split gooseneck liner (Part no:
157 8004-0164, Agilent Technologies). The column oven was held at 80 °C for 8 min and raised
158 to 200 °C at 8.5 °C min^{-1} and held for 55 min. The total runtime was 77.12 min. The FID was
159 operated at 300 °C. The carrier gas was hydrogen and was held at a constant flow of 1.0 mL
160 min^{-1}. Results were processed using OpenLab CDS Chemstation edition software version
161 Rev.C.01.04 (35) (Agilent Technologies).

162 All standard mixtures were prepared in heptane and stored at −18 °C until analysis.
163 The maximum allowable storage time was 6 months. Quantitation of FAMEs was carried out
164 by establishing calibration curves using the C4:0–C24:0 FAME reference mix and CLA
165 standard. 5 point curves with concentrations from 10 to 900 ppm were used with a coefficient

of determination (R^2) of no less than 0.99 being accepted. The necessary dilutions with heptane were carried out using the sample prep Workbench (Agilent Technologies). C19 FAME was added as in internal standard (ISTD), to give a final concentration of 200 ppm, during the dilution step prior to GC-FID analysis. Quantitation of individual FAMEs was based on their correction factors against the ISTD.

The FAME reference mix was also used as an in-run quality control sample, with the FAMEs present at 60–180 ppm concentration, to ensure accurate quantitation was being achieved throughout sample analysis. When setting up a sample batch for GC-FID analysis, the FAME mix was analysed once every 10 samples in the sequence. Accuracy was monitored by comparing the measured concentration of this FAME mix against its true concentration.

2.5. Statistical analysis

Statistical analysis was performed using SPSS v24.0 (IBM Statistics Inc., Armonk, NY). A between- and within-subjects repeated measures ANOVA with post hoc Tukey test was used to compare the FA content of colostrum and milk samples over the days post parturition (D0, Day 1, Day 2, Day 3 Day 4 and Day 5) from herds on different number of lactations (1^{st}, 2^{nd}, and 3^{rd}); *p*-values < 0.05 were considered significant. The strength of statistically significant results are also reported as the partial eta^2 effect size (η^2) where effect sizes are small ($0.01 \leq \eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$), and large ($\eta^2 \geq 0.14$).

Multivariate analysis of the fatty acid profile was also performed to examine the impact of day and parity. A supervised multivariate model was built using PLS-DA. To validate the model, a permutation test with 2000 repetitions was performed to check that the model differed from a random model. Also, the R^2 and Q^2 parameters were obtained to assess

the performance of the model using 10 fold cross validation approach. The variable importance plot (VIP) shows which variables have a larger influence on the latent variables of the built model. Each of these tests and generation of subsequent Figures were carried out using Metaboanalyst (www.metaboanalyst.ca) (Chong et al., 2018; Xia & Wishart, 2016).

The atherogenicity index (AI) and thrombogenicity index have been calculated as described by Ulbricht and Southgate (1991). The desaturase index (DI) is calculated as the [sum of delta 9 desaturase products]/[sum of delta 9-desaturase products+substrates], (Kay, Roche, Kolver, Thomson, & Baumgard, 2005). Results presented in the text are mean ± standard deviation unless otherwise stated.

3. Results and discussion

Colostrum is a nutrient-dense and bioactive rich feed source for the new born calf. The bioactive composition of colostrum has resulted in increased interest for its use as a potentially beneficial food ingredient (Mizelman et al., 2017). While much of the literature to date has focused on the protein fraction of colostrum and its immune components (Stelwagen, Carpenter, Haigh, Hodgkinson, & Wheeler, 2009), comparatively limited information is known about the lipid fraction. The purpose of this study was to examine and document the fatty acid profile of colostrum and transition milk as affected by days post parturition and parity of cows.

The overall fat content of colostrum was higher than that of the transition milks, statistical analysis of within subjects effects demonstrated that days post parturition had a significant effect on the fat content of milk; however, follow up pairwise comparison test did not find any significant differences. There was a larger variation in total fat content between cows in the Day(D) 0 samples (7.17 ± 2.97%) compared with D1(5.24 ± 1.10%), D2 (4.72 ±

1.10%), D3 (5.08 ± 1.28%), D4 (5.34 ± 1.29%) and D5 milks (5.23 % ± 0.84%). Tsioulpas et al. (2007), on examination of colostrum and milks between day 1 and day 90 of lactation, reported that that there was no particular trend observed in the fat content, which varied over the sampling period. McGrath et al. (2016), on review of the topic, discussed how colostrum fat content is typically, but not always, higher than that of milk, coupled with variation in fat composition. Fat content of colostrum and milk samples reported herein are similar to those reported by El-Fattah et al. (2012) for Holstein cows.

Days post parturition was demonstrated to have a significant effect on the majority of fatty acids measured with the exception of C11:0. C13:0, C20:0, and C21:0 (Table 1) as determined by the repeated measures ANOVA analysis. PLS-DA demonstrated the evolution of the fatty acid profile from colostrum through the transition milk stages to mature or regular milk on Day 5 (Fig. 1A). While the colostrum fatty acid profile appears distinct at each time point it is evident that changes are also taking place in the fatty acid profile between Days 1 and 2 post parturition. While Days 3, 4, and 5 profiles contain some subtle differences, these samples appear to be more similar than the previous days. The fatty acids contributing most to the observed separation of the PLS-DA are presented in Fig. 1B.

Days post parturition had a significant effect on the de novo fatty acid index (C4:0 to C15:0) (p = 0.028). De novo fatty acids, which are the fatty acids synthesised in the cows mammary gland from the volatile fatty acids acetate and butyrate, have increasingly been used as an indicator of rumen function on commercial dairy farms (Woolpert et al., 2017). Butyric acid (C4:0) increased significantly between D0 and D5 samples. This was attributed to significant increases between D0 and D1 (p = < 0.001) and D1 and D2 (p = 0.001) after which the levels stabilised with no significant changes thereafter. Butyric acid or butyrate supplementation in calves has received much attention in recent years as a result of its hypothesised ability to enhance calf growth and intestinal development.

O'Hara et al. (2018) demonstrated that supplementation of calves with butyrate in the form of sodium butyrate changes the abundance of SCFA producing and health-associated bacteria in the hindgut of milk-fed calves. Górka, Kowalski, Zabielski, and Guilloteau (2018) and Guilloteau et al. (2009) also discussed the beneficial effects of sodium butyrate supplementation on maturation of gastrointestinal function, while Guilloteau et al. (2009) highlighted how this may also be applied to other mammal species. From a human perspective butyric acid has been highlighted as a modulator of gene function (Smith, Yokoyama, & German, 1998), and may play a role in cancer prevention (German, 1999). Caproic acid (C6:0) and octanoic acid (C8:0) significantly increased between D0 and D1 ($p < 0.001$); following this increase there was no significant change in C6:0 between D1 and D5. The concentration of lauric acid (C12:0) remained steady across D0, D1 and D2 samples after which it dropped significantly between D2 and D3 ($p = 0.021$). Myristic acid (C14:0) and myristoleic acid (C14:1) were highest in colostrum samples (D0) and decreased significantly until D3 ($p = 0.002$) after which they remained constant. Days post parturition also had a significant effect on pentadecanoic acid (C15:0) concentrations which decreased between D1 and D5 ($p = 0.007$). Palmitic acid (C16:0) the most abundant saturated fatty acid (SFA) in milk, and palmitoleic acid (C16:1) were highest in colostrum samples and significantly decreased between D0 and D4 ($p < 0.001$). Negative effects associated with SFA consumption, include increased levels of total and low density lipoprotein (LDL) cholesterol in blood, which is considered an important risk factor for cardiovascular disease (CVD), with C12:0, C14:0, and C16:0 attributed to this effect (Ohlsson, 2010). However, the true effects of lauric acid on cholesterol has been questioned recently as a result of its ability to increase the levels of beneficial high density lipoprotein (HDL) (Lordan, Tsoupras, Mitra, & Zabetakis, 2018). Nevertheless, studies have consistently demonstrated that there is no clear evidence that dairy food consumption is consistently associated with a higher risk of CVD

(German et al., 2009; Guo et al., 2017; Lordan et al., 2018). Heptadecanoic acid (C17:0) significantly increased between D0 and D3 post-partum ($p = 0.003$) after which it remained stable. Stearic acid (C18:0) was significantly affected by time post-partum and increased between D0 and D5 ($p < 0.001$); however, there was no significant difference between D4 and D5. The supplementation of lactating cows with C16:0 and C18:0 has been carried out in the past with a variety of observed benefits. Both fatty acids have specific roles and functions in metabolism including provision of energy (Loften et al., 2014). However, from a calf perspective, Azad-Shahraki, Khani, Ahmadi, Ariana, and Beiranvand (2019) demonstrated that pre-ruminant calves would not benefit from palmitic acid inclusion in their starter diet. Oleic acid (C18:1n9c) is one of the most abundant unsaturated fatty acids in milk. The concentration of oleic acid was lowest in colostrum samples which significantly increased in concentrations until D3 ($p < 0.001$). Okada, Goto, Furukawa, Ikuta, and Yasuda (2009) investigated the impact of supplementing milk replacer and prevalence of white scour which can cause significant economic losses at farm level. The authors concluded that increases in saturated long chain fatty acids in milk were closely related to the onset of white scour in calves potentially as a result of poor absorption rates.

Linoleic acid (C18:2n6c) is the most abundant Ω 6 fatty acid in milk and was significantly higher in colostrum but dropped significantly between D0 and D1, after which it remained constant ($p = 0.004$). α-Linolenic acid (C18:3n3), the most abundant Ω 3 fatty acid in milk, was highest on D0 and D1 samples, after which its concentrations dropped significantly until D3 and remained constant until D5. Both linoleic and linolenic acid are classed as essential fatty acids, which act as substrates for fatty acids important for neural development and production of hormones, such as 20:5n3, 22:6n-3. 18:3n6, 20:3n6, 20:4n6 (Klein, 2002). As such the aforementioned fatty acids are important for both calf and human nutrition. Results have demonstrated that supplementation of calf starter with C18:2 and

C18:3n3 had a beneficial effect on average daily weight gain and feed efficiency (Hill, Aldrich, Schlotterbeck, & Bateman, 2007; Hill, Bateman, Aldrich, & Schlotterbeck, 2009). Both Ω3 and Ω6 fatty acids are precursors to signalling molecules with opposing effects that modulate the membrane microdomain composition, receptor signalling and gene expression (Schmitz & Ecker, 2008). Garcia et al. (2015) noted that the balance of these fatty acids is important, while linoleic acid consumption could help the calf in terms of inflammatory response when exposed to environmental pathogens, serving as a precursor to pro-inflammatory mediators such as, cytokines and eicosanoids (Calder, 2006). Thus, the anti-inflammatory properties of linolenic acid could also aid in calf inflammatory conditions, known to impair calf health. CLA c9t11 was lowest in colostrum samples and significantly increased up to 2 days post-partum ($p < 0.001$), after which it remained constant. CLA c9t11 is produced in the rumen as a product of the biohydrogenation of dietary linoleic acid to stearic acid by rumen microorganisms (Dhiman, Seung-Lee, & Ure, 2005). Previous studies have demonstrated that animal diet has a significant impact on the content of CLAc9t11 in milk. Milk derived from pasture fed cows, for example, have been demonstrated to have significantly higher content of CLA than that from cows on a total mixed ration diet; this has been linked to high levels of α-linolenic acid content in fresh forage that is subsequently converted to CLA (O'Callaghan et al., 2016). However, vaccenic acid can also be converted to CLA c9t11 by the action of delta9-desaturase in the mammary gland (Griinari et al., 2000). In recent years, CLA has received much attention as a result of its interesting biological functions and apparent benefits to human health as demonstrated in human and animal models. Such effects include reduction of carcinogenesis, atherosclerosis, inflammation, obesity, and diabetes (Yang et al., 2015).

The number of days post parturition also had a significant effect on a variety of fatty acid indices derived from the milk fatty acid profiles, as shown in Table 2. In ruminants a key

316 enzyme influencing the milk fatty acid profile is stearoyl-CoA desaturase 1, this enzyme is
317 responsible for the conversion of saturated fatty acids with 10–18 carbon atoms into their
318 monounsaturated fatty acid (MUFA) counterparts and plays a significant role in the synthesis
319 of CLA in the mammary gland (Kgwatalala, Ibeagha-Awemu, Mustafa, & Zhao, 2009). The
320 activity of this enzyme is classed as the desaturation index whereby increased activity results
321 in higher levels of desirable MUFAs with concomitant reduction in SFA concentrations (Reh
322 et al., 2004). The desaturase index values in the present study were positively correlated with
323 concentrations of MUFAs ($p < 0.001$, r = 0.979) and CLA ($p = 0.001$, r = 0.969) and
324 significantly negatively correlated with SFA content ($p < 0.001$, r = –0.979). Days post
325 parturition had a significant effect on the desaturase index ($p < 0.001$), which increased
326 significantly from D0 to D3 post-partum and remained stable thereafter. Such results appear
327 to indicate a shift in the enzyme activity of the mammary gland after D0 (colostrum) resulting
328 in production of more MUFAs. Both the thrombogenicity index (TI) and atherogenicity index
329 (AI) were highest in colostrum samples and decreased significantly ($p \leq 0.001$) in days post-
330 partum and were lowest at D5. Theses higher values for AI would be resultant of higher
331 levels of SFAs in the colostrum samples than in the latter samples including C14:0 and
332 C16:0. While it was not possible due to logistics to examine the fatty acid profile of these
333 milks in mid lactation, previous studies from the same farm using seasonal calving systems
334 have also demonstrated that the fatty acid profile of milks from cows on pasture or total
335 mixed ration diets continues to evolve throughout lactation (O'Callaghan et al., 2016).
336 Contarini et al. (2014) also demonstrated that the fatty acid composition of milks after 5 days
337 post parturition and 5 months were significantly different.

338 The lactation number of the cows was also demonstrated to have a significant effect
339 on some of the fatty acids measured including C14:1, C15:0, C16:0, C18:0, C18:2n6t,
340 C18:3n3 C20:0, CLA, and C21:0 (Table 1) as determined by the repeated measures ANOVA.

Partial least square discriminant analysis (PLS-DA) demonstrates the differences of the fatty acid profile between cows in their first (1), second (2), and third (3) lactation (Fig. 2A). It can be noted that the fatty acid profiles of milk from 1st lactation and 3rd lactation cows are different from each other, while that from 2nd lactation cows appears to fall between both groups.

The fatty acids contributing to the observed separation of the PLS-DA are presented in Fig. 2B, with the major compounds being CLA, C18:3n3, C15:0, C21:0, C17:0, and C16:0. The current understanding of the mechanisms for these changes in fatty acids is limited. One potential hypothesis could be that changes in the colostrum and milk fatty acid profiles are dependent upon the composition and functionality of the rumen microbiome, which in itself is linked to the cow based factors including the number of lactations. Buitenhuis et al. (2019) demonstrated that the rumen microbiome has a pronounced impact on the content of odd chain fatty acids and polyunsaturated fatty acids, including C15:0, C17:0, C18:2n6, C18:3n3, and CLA. Each of these fatty acids originates through biohydrogenation of feed derived C18 fatty acid by rumen microorganisms or from odd chain fatty acids that are synthesised by rumen microbes (Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006). Interestingly, Kumar, Indugu, Vecchiarelli, and Pitta (2015), also demonstrated that the bacterial community was different between primiparous and multiparous cows, indicating that the microbiome continues to evolve as the cow progresses from first to multiple lactations thereafter. Other considerations include nutrient/feed uptake relative to the cow's size, which is normally smaller during the first lactation cycle coupled with immaturity of the mammary gland, both of which may influence fatty acid synthesis. These results are similar to those reported by Contarini et al. (2014) who concluded that differences in the fatty acid composition observed between the multiparous and primiparous

365 colostrum samples could be linked to the physiological responses to increased energy
366 requirements due to the onset of lactation between younger and older cows.

367 In summary, colostrum is a nutritionally dense material with polyunsaturated, Ω3 and
368 Ω6 fatty acids, and other components which are beneficial for development. In the
369 subsequent days post-partum the concentrations of these fatty acids are depleted with
370 concomitant increases in CLA and other fatty acids beneficial to health. While consumption
371 of products with increased concentrations of Ω3 fatty acids would be beneficial to health, the
372 high levels of Ω6 and palmitic acid, however, may be undesirable, considering the excessive
373 levels of Ω6 already present in the current western diet, coupled with the cholesterol-raising
374 effects of C16:0. In this regard, from a nutritional perspective the fat profile of milk from day
375 3 post parturition onwards could be better for human consumption with decreased
376 concentrations of C16:0, Ω6 fatty acids and concomitant lower indices for AI and TI, coupled
377 with increased concentrations of unsaturated fatty acids, CLA, C18:0 and C18:1n9c. Such
378 differences in the fatty acid profile of colostrum in the days post parturition will be an
379 important future consideration should the material need to be processed and stabilised for
380 human consumption.

381 To date the majority of beneficial factors in colostrum have been associated with the
382 protein fraction. Nevertheless, it remains to be seen what valorisation strategies could be
383 applied to the fat portion of colostrum to allow its conversion into attractive products that are
384 beneficial to the consumer. Therefore, future work on processing characteristics, rheological
385 and sensory properties of products derived from the fat fraction of colostrum is warranted.
386 Concentrated fat streams such as cream and anhydrous milk fat would offer potential
387 mechanisms for the purification of the fat, for subsequent incorporation into formulated
388 products. However, as the results highlight changes occurring in the fatty acid profile may
389 have a significant effect on the processing characteristics, functionality and nutritional

properties of the products that should be considered when choosing best use strategies for colostrum and transitionary milk post parturition.

4. Conclusion

Our study has demonstrated the impact of days post parturition on the fatty acid profile of colostrum and transition milk. Days post parturition has a significant effect on the fatty acid profile; that of colostrum is distinctly different from that of milk produced in subsequent days, with significantly higher levels of polyunsaturated fatty acids and palmitic acid. Parity of the cow also has a significant effect on the fatty acid profile of colostrum and milk samples, with CLA being one of the major compounds impacted, with significantly higher levels in cows entering their 1st compared with those in their 3rd lactation cycle. Multiparous cows (lactation number 2 and 3) produced significantly higher concentrations of C16:0 than primiparous cows. It is clear that the changing composition of the fatty acid profile can be classed into three distinct phases as it evolves, including colostrum (D0), transition milk (D1 and D2 post parturition) and mature milk (D3 and D5).

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Figure legends

Fig. 1. Panel A, partial least square discriminant analysis (PLS-DA) depicting the changes occurring as milk transition from colostrum to milk over five days post parturition [0, colostrum (red); 1–5, days 1–5 post parturition (green, blue, light blue, violet, yellow, respectively)] (R^2, 0.79; Q^2, 0.75). Panel B, variable importance plot highlighting the fatty acids most responsible for observed separations in PLS-DA; the coloured boxes on the right indicate the relative concentrations of the corresponding fatty acid in each group under study. Panels C–N, fatty acids changing significantly over time (C18:0, C14:0, C16:1, C16:0, C20:3n6, C6:0, C4:0, C14:1, C20:2, C23:0, C181n9c and C17:0, respectively; different letters denote significant differences.

Fig. 2. Panel A, partial least square discriminant analysis (PLS-DA) depicting the impact of parity [i.e., 1^{st}, 2^{nd} and 3^{rd} lactation cows, denoted 1 (red), 2 (green) and 3 (blue), respectively) on the fatty acid composition of colostrum and transition milk (R^2, 0.52; Q^2, 0.41). Panel B, variable importance plot highlighting the fatty acids most responsible for observed separations in PLS-DA based on parity; the coloured boxes on the right indicate the relative concentrations of the corresponding fatty acid in each group under study.

Table 1

Fatty acid content of colostrum and milk samples up to 5 days post parturition from Spring calving cows. [a]

Fatty acid	Colostrum	Days post parturition					*p*-Value					
		Day1	Day 2	Day 3	Day 4	Day 5	Day	η^2	Day*Parity	η^2	Parity	η^2
C4:0	3.01 ± 0.79	4.25 ± 0.61	5.05 ± 0.72	5.3 ± 0.63	5.47 ± 0.72	5.64 ± 0.91	<0.001	0.767	0.660	0.093	0.095	0.269
C6:0	1.42 ± 0.26	2.06 ± 0.32	2.25 ± 0.32	2.19 ± 0.4	2.21 ± 0.49	2.25 ± 0.54	<0.001	0.652	0.861	0.041	0.756	0.037
C8:0	0.75 ± 0.15	1.01 ± 0.19	1.09 ± 0.21	1.02 ± 0.24	1.02 ± 0.29	1.03 ± 0.32	<0.001	0.467	0.875	0.039	0.734	0.040
C10:0	1.67 ± 0.47	2.08 ± 0.51	2.12 ± 0.54	1.88 ± 0.53	1.84 ± 0.61	1.86 ± 0.66	0.003	0.309	0.779	0.590	0.488	0.910
C11:0	0.08 ± 0.08	0.08 ± 0.12	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.03	0.251	0.088	0.147	0.203	0.917	0.011
C12:0	2.83 ± 0.86	2.65 ± 0.59	2.6 ± 0.63	2.3 ± 0.6	2.21 ± 0.67	2.21 ± 0.74	0.001	0.376	0.219	0.170	0.392	0.118
C13:0	0.06 ± 0.02	0.08 ± 0.08	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.108	0.159	0.399	0.117	0.366	0.125
C14:0	13.65 ± 3.49	10.32 ± 1.57	9.45 ± 1.6	8.57 ± 1.53	8.23 ± 1.62	8.26 ± 1.79	<0.001	0.752	0.191	0.188	0.088	0.277
C14:1	0.96 ± 0.41	0.61 ± 0.13	0.51 ± 0.1	0.42 ± 0.13	0.42 ± 0.1	0.42 ± 0.12	<0.001	0.625	0.145	0.218	0.001	0.633
C15:0	0.97 ± 0.15	0.95 ± 0.17	0.89 ± 0.16	0.85 ± 0.16	0.82 ± 0.15	0.85 ± 0.17	0.002	0.359	0.730	0.084	0.041	0.347
C16:0	40.36 ± 5.3	34.61 ± 3.33	31.21 ± 2.91	29.2 ± 2.05	28.26 ± 1.99	28.46 ± 2.22	0.001	0.933	0.000	0.527	0.008	0.472
C16:1	2.67 ± 0.86	2.11 ± 0.33	1.97 ± 0.36	1.92 ± 0.4	1.81 ± 0.45	1.79 ± 0.54	<0.001	0.510	0.017	0.372	0.416	0.110
C17:0	0.79 ± 0.2	0.9 ± 0.11	0.91 ± 0.1	0.95 ± 0.11	0.94 ± 0.14	0.94 ± 0.13	<0.001	0.474	0.009	0.382	0.097	0.267
C18:0	8.02 ± 2.34	10.8 ± 1.27	12.51 ± 1.32	14.38 ± 1.05	15.38 ± 1.44	15.83 ± 2.63	<0.001	0.848	0.146	0.195	0.007	0.481
C18:1 n9c	20.92 ± 5.79	23.86 ± 4.19	25.77 ± 4.14	27.45 ± 4.11	27.79 ± 4.49	26.41 ± 7.35	<0.001	0.713	0.005	0.403	0.405	0.113
C18:2 n6c	1.95 ± 0.47	1.58 ± 0.27	1.5 ± 0.24	1.53 ± 0.25	1.51 ± 0.23	1.53 ± 0.24	<0.001	0.535	0.586	0.079	0.490	0.091
C18:2 n6t	0.37 ± 0.29	0.14 ± 0.09	0.21 ± 0.23	0.21 ± 0.21	0.31 ± 0.36	0.73 ± 0.55	0.001	0.331	0.168	0.180	0.043	0.342
C20:0	0.14 ± 0.04	0.14 ± 0.04	0.15 ± 0.03	0.15 ± 0.02	0.16 ± 0.03	0.17 ± 0.04	0.136	0.129	0.604	0.080	0.001	0.588
C18:3 n3	0.79 ± 0.21	0.79 ± 0.18	0.76 ± 0.15	0.73 ± 0.14	0.71 ± 0.13	0.7 ± 0.12	0.004	0.303	0.132	0.010	0.010	0.456
CLA c9t11	0.5 ± 0.22	0.61 ± 0.21	0.67 ± 0.19	0.68 ± 0.18	0.68 ± 0.16	0.65 ± 0.16	<0.001	0.652	0.152	0.003	0.003	0.531
C21:0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.02 ± 0.01	0.126	0.120	0.103	0.004	0.004	0.522
C20:2	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0.01	<0.001	0.407	0.342	0.365	0.365	0.126
C20:3n6	0.25 ± 0.06	0.14 ± 0.07	0.09 ± 0.02	0.01 ± 0.03	0.01 ± 0.03	0.06 ± 0.02	<0.001	0.837	0.105	0.711	0.711	0.044
C23:0	0.28 ± 0.08	0.2 ± 0.08	0.16 ± 0.03	0.11 ± 0.03	0.1 ± 0.02	0.1 ± 0.03	<0.001	0.764	0.171	0.052	0.052	0.325

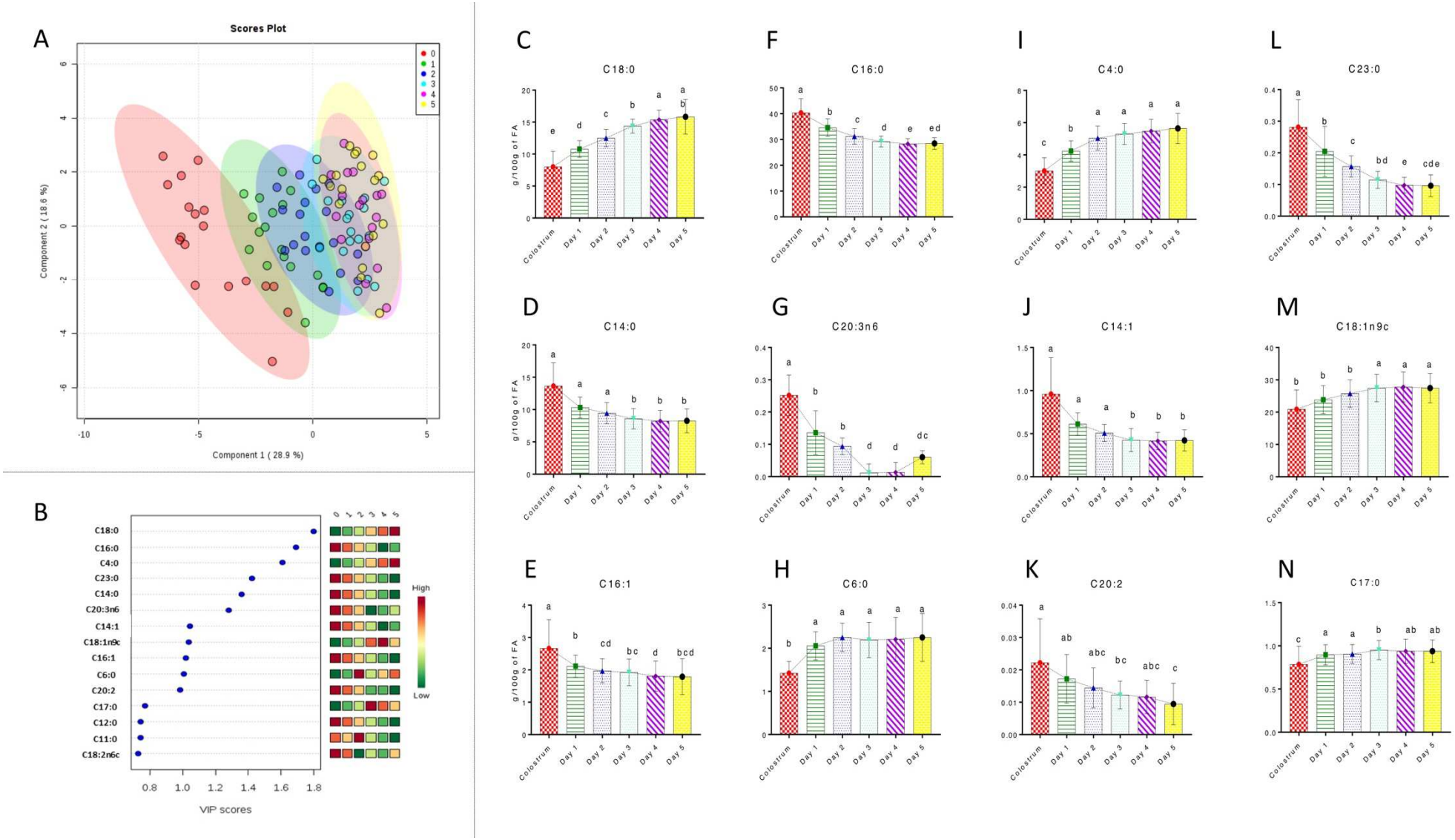
[a] Values are the mean ± standard deviation (g 100 g^{-1} total fatty acids); in total, 108 samples were collected and analysed, n = 6 for each lactation number on each day of collection post partum; η^2, partial eta^2 effect size where effect sizes are small ($0.01 \leq \eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$), and large ($\eta^2 \geq 0.14$).

Table 2

Fatty acid indices of colostrum and milk samples up to 5 days post parturition from Spring calving cows. [a]

Fatty acid	Colostrum	Days post parturition					*p*-Value					
		Day1	Day 2	Day 3	Day 4	Day 5	Day	η^2	Day*Parity	η^2	Parity	η^2
Saturated	74.05 ± 7.84	70.14 ± 4.67	68.5 ± 4.84	67.03 ± 4.66	66.75 ± 4.94	67.7 ± 7.63	<0.001	0.443	0.093	0.231	0.347	0.132
Unsaturated	28.4 ± 6.36	29.84 ± 4.66	31.48 ± 4.84	32.96 ± 4.66	33.23 ± 4.94	32.29 ± 7.63	<0.001	0.486	0.072	0.259	0.476	0.094
MUFA	24.54 ± 5.74	26.58 ± 4.29	28.25 ± 4.42	29.79 ± 4.51	30.01 ± 4.93	28.62 ± 7.78	<0.001	0.561	0.033	0.305	0.511	0.086
PUFA	3.88 ± 0.69	3.28 ± 0.54	3.25 ± 0.56	3.18 ± 0.58	3.23 ± 0.57	3.68 ± 0.52	<0.001	0.329	0.576	0.096	0.043	0.342
Omega 3	0.79 ± 0.21	0.79 ± 0.18	0.76 ± 0.15	0.73 ± 0.14	0.71 ± 0.13	0.7 ± 0.12	0.004	0.303	0.357	0.132	0.010	0.456
Omega 6	2.57 ± 0.46	1.86 ± 0.31	1.8 ± 0.33	1.75 ± 0.39	1.83 ± 0.41	2.32 ± 0.51	<0.001	0.453	0.352	0.132	0.937	0.009
Omega 9	20.92 ± 5.79	23.86 ± 4.19	25.77 ± 4.14	27.45 ± 4.11	27.79 ± 4.49	26.41 ± 7.35	<0.001	0.713	0.005	0.403	0.405	0.113
n3/n6	0.31 ± 0.08	0.43 ± 0.1	0.43 ± 0.08	0.43 ± 0.08	0.4 ± 0.09	0.32 ± 0.1	<0.001	0.482	0.195	0.158	0.061	0.311
De novo (C4–C15)	25.4 ± 5.48	24.1 ± 3.24	24.05 ± 3.11	22.63 ± 3.46	22.31 ± 4.25	22.61 ± 4.85	0.028	0.218	0.341	0.136	0.438	0.104
LA/ALA	2.56 ± 0.69	2.1 ± 0.57	2.02 ± 0.41	2.13 ± 0.38	2.16 ± 0.36	2.23 ± 0.38	<0.001	0.538	0.031	0.301	0.231	0.177
Atherogenicity index	3.7 ± 1.06	2.8 ± 0.72	2.43 ± 0.68	2.12 ± 0.6	2.03 ± 0.56	2.03 ± 0.58	<0.001	0.844	0.003	0.426	0.313	0.144
Thrombogenicity index	4.01 ± 0.96	3.37 ± 0.68	3.1 ± 0.68	2.91 ± 0.59	2.89 ± 0.56	2.94 ± 0.64	<0.001	0.768	0.001	0.780	0.339	0.134
Desaturase index	0.28 ± 0.06	0.32 ± 0.05	0.35 ± 0.05	0.36 ± 0.05	0.37 ± 0.05	0.36 ± 0.05	<0.001	0.829	0.001	0.459	0.363	0.126

[a] Values are the mean ± standard deviation (g 100 g^{-1} total fatty acid); in total 108 samples were collected and analysed, n = 6 for each lactation number on each day of collection parturition. η^2, partial eta^2 effect size where effect sizes are small ($0.01 \leq \eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$), and large ($\eta^2 \geq 0.14$). Definitions are: saturated, ∑(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C23:0); unsaturated, ∑(C14:1, C16:1, C18:1n9c, C18:2n6c, C18:2n6t, C18:3n3, CLA c9t11, C20:3n6); MUFA, ∑(C14:1, C16:1, C18:1n9c); PUFA, ∑(C18:2n6c, C18:2n6t, C18:3n3, CLAc9t11, C20:2, C20:3n6); omega 3, C18:3n3; omega 6, ∑(C18:2n6c, C18:2n6t, C20:3n6); omega 9, C18:1n9c; atherogenicity index = $\frac{C12{:}0+(4\ x\ C14{:}0)+C16{:}0}{\text{Omega 6 PUFA+Omega 3 PUFA+MUFA}}$; thrombogenicity index = $\frac{C14{:}0+C16{:}0+C18{:}0}{(0.5\text{ x MUFA})+(0.5\ x\ Omega\ 6\ PUFA)+(3\ x\ Omega\ 3\ PUFA)+(\frac{Omega\ 3\ PUFA}{Omega\ 6\ PUFA})}$; desaturase index = $\frac{(C14{:}1+C16{:}1+C18{:}1n9c)}{(\text{C14:0+CC16:0+C18:0})+(C14{:}1+C16{:}1+C18{:}1n9c)}$.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Scores Plot
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Component 1 (28.9 %)
B
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C14:1
C18:1n9c
C16:1
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C17:0
C12:0
C11:0
C18:2n6c
High
Low
VIP scores
C
C18:0
F
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C23:0
D
C14:0
G
C20:3n6
J
C14:1
M
C18:1n9c
E
C16:1
H
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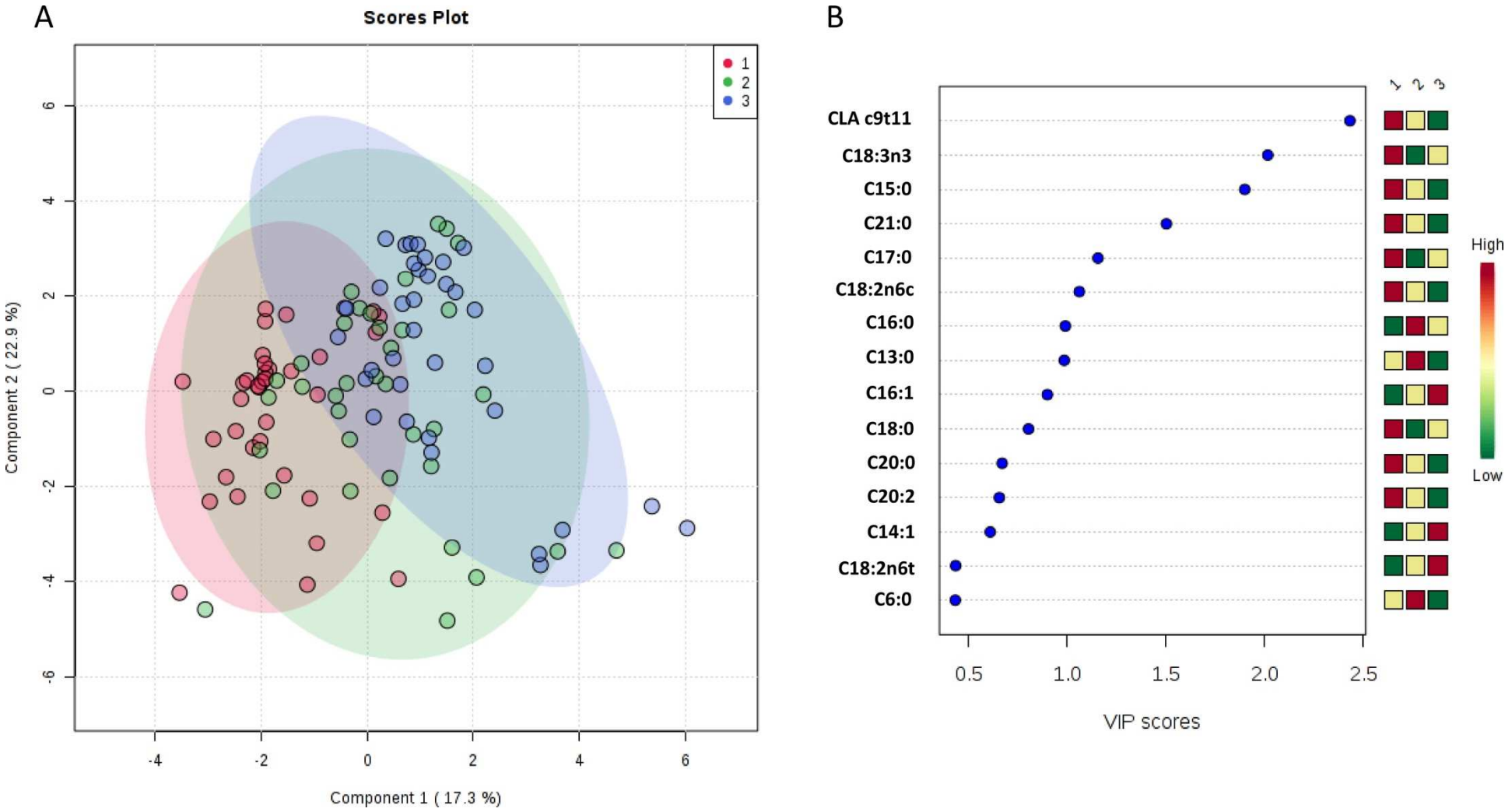
C17:0
g/100g of FA
Colostrum
Day 1
Day 2
Day 3
Day 4
Day 5

Figure 1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Scores Plot
1
2
3
Component 2 (22.9 %)
Component 1 (17.3 %)
B
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C14:1
C18:2n6t
C6:0
High
Low
VIP scores

Figure 2