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The bovine colostrum and milk metabolome at the onset of lactation as determined by ^{1}H-NMR

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ABSTRACT

The purpose of this study was to characterise the metabolome of bovine colostrum and milk in the initial days of lactation. Colostrum and milk samples were collected from 18 cows representing an even spread of 1st, 2nd and 3rd lactation (n = 6) over the first 6 days of lactation. Samples were subsequently analysed using ^{1}H-NMR. The metabolome of defatted colostrum and milk in the days immediately post parturition was demonstrated to be complex and changed significantly over time. The colostrum was rich in nutrients beneficial for growth and development of the new-born mammal with significantly higher levels of essential branched chain amino acids and choline and orotic acid that decreased in the subsequent days as milk composition evolved to that of regular milk. Multivariate analysis of metabolome profiles using PLS-DA was demonstrated to clearly distinguish between colostrum and subsequent milk samples.

1. Introduction

Colostrum is classified as the initial secretion of milk post parturition, providing a complex mixture of essential nutrients and growth factors for the development of the newborn mammal (McGrath, Fox, McSweeney, & Kelly, 2016). There are numerous factors that can affect the composition and quality of colostrum, namely individual and environmental factors, including parity of the animal, diet, season, breed, dry period length, time post parturition and health status of the cow (Puppel et al., 2019). The length of period of which milk is classified as colostrum varies significantly in literature, as its composition of both macro and micronutrients, significantly evolves in the initial days of lactation from colostrum to that of mature milk.

On examination of the lipid fraction of colostrum and milk O'Callaghan et al. (2020) concluded that this evolution can be classified into 3 stages in the initial 5 days post parturition. Colostrum (Day 0), transition milk Day 1 and 2 post parturition and mature milk Day 3 – Day 5 post parturition (O'Callaghan et al., 2020). Metabolomics is the measure of small molecule metabolites in cells, tissues and biofluids and is widely used in biomedical, nutritional, crop and livestock research (Goldansaz et al., 2017). The application of metabolomics in livestock has for the most part been directed towards disease detection, production and bio product assessment (Goldansaz et al., 2017).

The majority of research to date on bovine colostrum has focused on the protein (Fahey, Fischer, Steele, & Greenwood, 2020; Tacoma et al., 2017) and immunoglobulin (Hurley & Theil, 2011) fractions and changes to its physical properties (Tsioulpas, Grandison, & Lewis, 2007). However, to date, data on the colostrum metabolome and factors affecting it are currently lacking. Curtasu, Theil, and Hedemann (2016) examined the metabolome of colostrum and milk from lactating sows and reported several changes occurring in the days post parturition to nutrients beneficial to the development of the piglets. Furthermore, Picone et al. (2018) on examination of the sow colostrum metabolome

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demonstrated its composition is affected by breed and environmental factors and that such changes may have consequences on piglets performance. Sundekilde et al. (2016), demonstrated that the metabolome of human milk was influenced by gestational and lactation stage. Levels of valine, leucine, betaine and creatinine were higher in colostrum than in mature milk from term mothers (Sundekilde et al., 2016). Li et al. (2020) used HPLC MS/MS to demonstrate changes in the amino acid profiles in bovine colostrum and mature milk. O'Callaghan et al. (2018), using ^{1}H-NMR examined the impact of bovine diet on the milk metabolome and highlighted ^{1}H-NMR as a potential tool for verification of pasture derived milks identifying several metabolites associated with pasture based diets.

However, at present there is still limited information available on the bovine colostrum metabolome or the changes that occur to low molecular weight metabolites of colostrum and milk in the initial days of lactation. The purpose of this study was to characterise the composition of the bovine colostrum and changes that occur in the initial days post parturition.

2. Materials and methods

2.1. Experimental design

The experimental design for this study and animal feed was previously reported by O'Callaghan et al. (2020). Briefly, 18 Holstein Friesian cows consisting of a spread of 1st, 2nd and 3rd lactation ($n = 6$) were selected from the spring calving dairy herd based on the Teagasc Moorepark Dairy Research Farm, Fermoy Co. Cork, Ireland. Milk samples were collected from individual cows on the day of calving, and subsequent mornings, 1, 2, 3, 4, and 5 days post parturition. Cows were milked into individual stainless steel churns at milking time for sample collection. Approximately 400 mL of sample was collected from each cow and immediately stored at refrigerated temperatures. 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.

2.2. Metabolomic analysis

Preparation of colostrum and milk samples was carried out similarly as was described by O'Callaghan et al. (2018). Briefly, 3 kDa filters (Amicon Microcon YM-3; Sigma–Aldrich, St. Louis, MO, USA) were washed five times using 500 μL HPLC-grade water and centrifuged at 9520 ×*g* for 10 min. Samples were defrosted and thawed, and 2.8 mL aliquots were centrifuged at 3500 ×*g* at 4 °C for 15 min to separate fat from skimmed milk layer. The defatted milk was removed and filtered through washed 3 kDa filters at 11520 ×g for 35 min at 4 °C; 540 μL of filtrate was mixed with 10 μL sodium trimethylsilyl [2,2,3,3-2H_4] proprionate (TSP) and 60 μL deuterium oxide (D_2O).

Spectra were acquired on a 600 MHz Varian Spectrometer (Varian Limited, Oxford, UK) using the first increment of a nuclear Overhauser enhancement spectroscopy pulse sequence at 25 °C. Spectra were acquired with 16,384 data points and 128 scans. Water suppression was achieved during the relaxation delay (2 s) and the mixing time (500 ms). All ^{1}H-NMR spectra were referenced to TSP at 0.0 parts per million (ppm) and processed manually with the Chenomx NMR Suite (version 8.3) using a line broadening of 0.2 Hz, followed by phase and baseline correction. Metabolite identification was performed by comparison with the Chenomx library and O'Callaghan et al. (2018). Example annotated spectra from a day 3 milk sample is presented in Fig. 1.

2.3. Statistical analysis

Statistical analysis was performed using SPSS v18.0 (IBM Statistics Inc., Armonk, NY, USA). A between- and within-subjects repeated measures ANOVA with post hoc Tukey test was used to compare metabolites in colostrum and milk samples over time post parturition (Colostrum, Day 1, Day 2, Day 3 Day 4 and Day 5) from herds on different number of lactations (1st, 2nd, and 3rd). *P*-values < 0.05 were considered significant. The strength of statistically significant results is 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 metabolite profile was also performed to examine the impact of "Day" and "Parity". Log transformation and data scaling was applied to the data before performing the multivariate analyses. A supervised multivariate model was built using partial least squared discriminant analysis (PLS-DA). To validate the model, a permutation test with 2000 repetitions was performed to check that the model differed from a random model. The variable importance in projection (VIP) score shows which variables have a larger influence on the latent variables of the built model. Each of these tests and subsequent figures were carried out using Metaboanalyst (www.metaboanalyst.ca/) (Chong et al., 2018; Xia & Wishart, 2016).

3. Results and discussion

The purpose of this study was to examine the metabolome of bovine colostrum and milk samples in the days immediately post parturition, and characterise the changes occurring in the initial days of lactation. In total 28 metabolites were profiled in colostrum and milk samples using ^{1}H-NMR, all of which have been previously reported in milk (Foroutan et al., 2019; O'Callaghan et al., 2018). Each of the compounds and their relative levels are presented in Table 1.

The colostrum and milk metabolome was demonstrated to be complex and changed significantly over the initial days of the cows lactation. Partial least square discriminant analysis demonstrates the apparent evolution of the milk metabolome as the samples transition from the colostrum stage to mature milk five days post parturition. While the colostrum metabolome appears distinct from other time points, day 1 and day 2 samples appear to be quite similar to each other, as do days 4 and 5 (Fig. 2A). The hierarchal clustering analysis heatmap (Fig. 2B), also demonstrates that the colostrum metabolome is distinctly different from that of the latter samples. The metabolites most contributing to this separation were determined using VIP analysis (Fig. 3A) and were found to be valine, UDP-galactose, glycerophospohocholine, orotic acid, leucine, betaine, isoleucine, isobutyrate and uridine (Fig. 3B–J), which is in agreement with significant changes outlined in Table 1.

Amino acids in milk are the building blocks of protein; however, they also play important roles in metabolism, growth and development (Li et al., 2020).

Colostrum had significantly higher levels of branched chain amino acids leucine, isoleucine and valine. Leucine was highest in colostrum samples and decreased significantly thereafter and was lowest in day 4 and 5 milk samples. Isoleucine was also highest in the colostrum samples and decreased significantly ($P < 0.05$) thereafter. Valine was highest in colostrum samples and decreased significantly thereafter to its lowest levels on days 4 and 5. Branched chain amino acids play an important role in nutrition, development and intestinal health (Zhou, Yu, Gao, Htoo, & Chen, 2018); in particular leucine has been demonstrated to stimulate protein synthesis and upregulate protein synthesis in skeletal muscle by enhancing the activity and synthesis of proteins involved

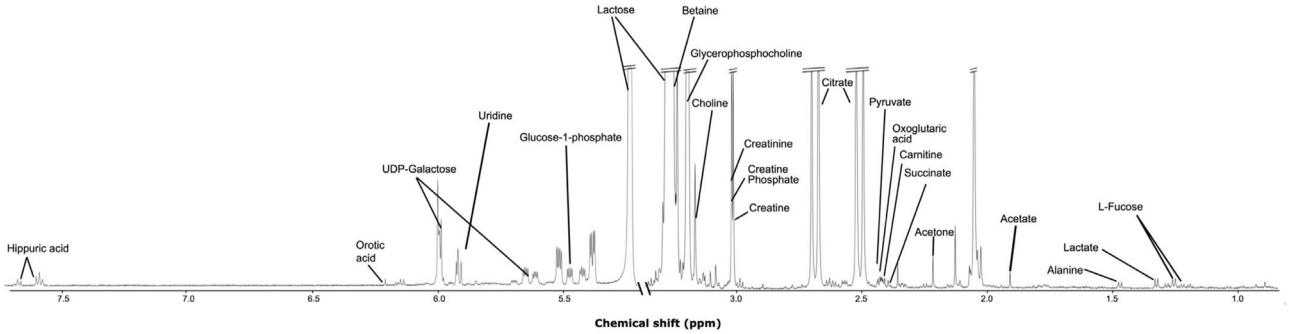

Fig. 1. Annotated ^{1}H-NMR spectra of a day 3 milk sample demonstrating compounds detected.

Table 1
Metabolite levels in colostrum and milk samples 5 days post parturition.[a]

Metabolite	Sample						*p*-Value					
	Colostrum	Day 1	Day 2	Day 3	Day 4	Day 5	Day	η^2	Day*Parity	η^2	Parity	η^2
Acetic acid	0.082 ± 0.06	0.052 ± 0.01	0.033 ± 0.02	0.035 ± 0.02	0.031 ± 0.01	0.055 ± 0.02	0.003	0.393	0.273	0.176	0.11	0.288
Acetone	0.042 ± 0.02	0.074 ± 0.03	0.064 ± 0.03	0.075 ± 0.03	0.087 ± 0.03	0.085 ± 0.04	$p < 0.001$	0.355	0.01	0.277	0.12	0.246
Acetylcarnitine	1.116 ± 0.58	0.969 ± 0.39	0.986 ± 0.39	0.812 ± 0.33	0.737 ± 0.22	0.723 ± 0.26	0.001	0.309	0.097	0.207	$p < 0.001$	0.656
Alanine	0.062 ± 0.03	0.044 ± 0.01	0.044 ± 0.02	0.043 ± 0.02	0.035 ± 0.01	0.033 ± 0.02	0.001	0.346	0.51	0.102	0.075	0.293
Betaine	0.566 ± 0.17	0.684 ± 0.23	0.545 ± 0.18	0.38 ± 0.09	0.303 ± 0.07	0.259 ± 0.06	$p < 0.001$	0.772	0.142	0.19	0.194	0.196
Carnitine	0.087 ± 0.07	0.215 ± 0.11	0.166 ± 0.09	0.135 ± 0.07	0.126 ± 0.07	0.122 ± 0.06	$p < 0.001$	0.402	0.161	0.166	$p < 0.001$	0.739
Choline	0.62 ± 0.46	0.126 ± 0.09	0.106 ± 0.08	0.133 ± 0.09	0.139 ± 0.07	0.153 ± 0.07	$p < 0.001$	0.57	0.31	0.145	0.565	0.073
Citrate	4.538 ± 1.32	6.395 ± 1.57	8.141 ± 1.81	7.962 ± 1.25	7.602 ± 1.06	7.077 ± 1.01	$p < 0.001$	0.685	0.22	0.153	0.084	0.281
Creatine	0.795 ± 0.25	1.131 ± 0.48	0.885 ± 0.24	0.736 ± 0.17	0.696 ± 0.12	0.647 ± 0.13	$p < 0.001$	0.495	0.425	0.118	0.902	0.014
Creatine phosphate	0.262 ± 0.2	0.661 ± 0.28	0.481 ± 0.2	0.397 ± 0.13	0.396 ± 0.12	0.366 ± 0.11	$p < 0.001$	0.537	0.135	0.172	0.184	0.202
Creatinine	0.217 ± 0.07	0.181 ± 0.07	0.206 ± 0.08	0.218 ± 0.06	0.205 ± 0.06	0.192 ± 0.04	0.217	0.096	0.375	0.128	0.492	0.09
Fucose	0.034 ± 0.01	0.036 ± 0.02	0.044 ± 0.03	0.047 ± 0.03	0.029 ± 0.01	0.032 ± 0.01	0.146	0.11	0.798	0.076	0.472	0.095
Glucose-1-phosphate	0.458 ± 0.27	0.692 ± 0.44	0.57 ± 0.39	0.415 ± 0.17	0.391 ± 0.12	0.344 ± 0.1	0.007	0.264	0.311	0.142	0.456	0.099
Glycerophosphocholine	35.971 ± 13.94	15.358 ± 4.38	10.283 ± 4.31	7.891 ± 2.77	7.3 ± 2.31	6.94 ± 1.8	$p < 0.001$	0.827	0.529	0.087	0.811	0.028
Hippuric acid	0.086 ± 0.05	0.12 ± 0.03	0.094 ± 0.02	0.098 ± 0.05	0.08 ± 0.02	0.082 ± 0.03	0.025	0.206	0.307	0.143	0.015	0.428
Isobutyrate	0.003 ± 0	0.001 ± 0	0.001 ± 0	0.001 ± 0	0.001 ± 0	0.001 ± 0	$p < 0.001$	0.344	0.778	0.066	0.678	0.05
Isoleucine	0.024 ± 0.01	0.011 ± 0.01	0.007 ± 0	0.014 ± 0.01	0.006 ± 0	0.006 ± 0	$p < 0.001$	0.441	0.828	0.046	0.986	0.002
Lactate	0.294 ± 0.25	0.104 ± 0.08	0.064 ± 0.07	0.058 ± 0.04	0.042 ± 0.02	0.035 ± 0.02	$p < 0.001$	0.542	0.554	0.092	0.539	0.091
Lactose	39.425 ± 5.77	51.677 ± 11.59	55.221 ± 7.59	55.325 ± 8.31	53.115 ± 6.99	51.496 ± 7.14	$p < 0.001$	0.389	0.699	0.088	0.978	0.003
Leucine	0.031 ± 0.02	0.009 ± 0.01	0.006 ± 0	0.01 ± 0.01	0.005 ± 0	0.004 ± 0	$p < 0.001$	0.596	0.877	0.034	0.849	0.022
Orotic acid	0.252 ± 0.16	0.741 ± 0.25	0.969 ± 0.36	1.189 ± 0.76	1.227 ± 0.42	1.411 ± 0.47	$p < 0.001$	0.624	0.479	0.106	0.589	0.068
Oxoglutaric acid	0.036 ± 0.01	0.049 ± 0.02	0.058 ± 0.02	0.048 ± 0.02	0.04 ± 0.02	0.032 ± 0.01	$p < 0.001$	0.326	0.355	0.13	0.281	0.156
Pyruvic acid	0.01 ± 0.01	0.009 ± 0	0.009 ± 0.01	0.014 ± 0.01	0.008 ± 0.01	0.009 ± 0.01	0.095	0.115	0.364	0.129	0.061	0.312
Succinate	0.02 ± 0.01	0.02 ± 0.01	0.016 ± 0.01	0.018 ± 0.01	0.016 ± 0	0.016 ± 0	0.164	0.112	0.718	0.068	0.071	0.297
UDP-galactose	1.005 ± 0.79	1.077 ± 0.5	0.837 ± 0.4	0.43 ± 0.34	0.225 ± 0.21	0.161 ± 0.16	$p < 0.001$	607	0.596	0.083	0.901	0.014
Uridine	0.404 ± 0.15	0.36 ± 0.11	0.343 ± 0.11	0.317 ± 0.08	0.233 ± 0.07	0.2 ± 0.07	$p < 0.001$	0.425	0.513	0.107	0.112	0.253
Valerate	0.021 ± 0.01	0.022 ± 0.01	0.016 ± 0.01	0.025 ± 0.02	0.015 ± 0.01	0.014 ± 0.01	0.15	0.12	0.84	0.044	0.312	0.144
Valine	0.067 ± 0.04	0.018 ± 0.01	0.014 ± 0.01	0.017 ± 0.01	0.008 ± 0	0.008 ± 0	$p < 0.001$	0.679	0.37	0.126	0.802	0.029

[a] Relative metabolite levels (in mM) are means ± standard deviation; *p*-values obtained by between and within subjects repeated measures ANOVA, values < 0.05 are considered significant.

in mRNA translation (Anthony, Anthony, Kimball, & Jefferson, 2001). A similar trend for free amino acids was reported by Li et al. (2020) when comparing colostrum and mature milk.

Choline and glycerophosphocholine (GPC) were highest in colostrum samples after which their levels dropped significantly ($P < 0.05$), and remained constant thereafter. Klein et al. (2012) examined the relationship between levels of GPC and its ratio to phosphocholine for the selection of healthy and metabolically stable cows for breeding purposes. The authors reported that high levels of GPC were related to low ketosis incidence and hypothesised that selection of animals with high levels of GPC should yield animals that can better cope with negative energy balance during the early weeks of lactation. Choline is an important dietary component essential for normal function of all cells (Zeisel et al., 2000), and for optimal growth and performance of animals (de Veth et al., 2016). Furthermore, it has several important roles in the synthesis of membrane phospholipids, biosynthesis of the neurotransmitter acetylcholine and as a source of labile methyl groups (Zeisel, 2000).

Carnitine was lowest in colostrum samples, its levels sharply increased by day 1 ($P < 0.05$) after which it significantly decreased again thereafter to day 5, to similar levels as that of colostrum. Acetylcarnitine was highest in colostrum samples and remained high until day 2 after which it decreased significantly. Furthermore, carnitine and acetylcarnitine were two of only three metabolites affected by parity of the animals, whereby, cows entering their 1st lactations were significantly different from cows in their second and third lactation. Carnitine serves an important role in the metabolism of fat for energy and transport of fatty acids across the walls of the mitochondria where they undergo oxidation (Mendelson, 2008). Unlike adults, infants have very little ability to synthesise choline or carnitine as such both are routinely added to infant formulations (Zheng et al., 2018).

Betaine and creatine were highest in samples one day post parturition, significantly higher than that of colostrum and Day 2 samples ($P < 0.05$) and decreased to its lowest levels on Day 5. Betaine is formed from the mitochondrial oxidation of free choline, which along with GPC act as organic osmolytes within

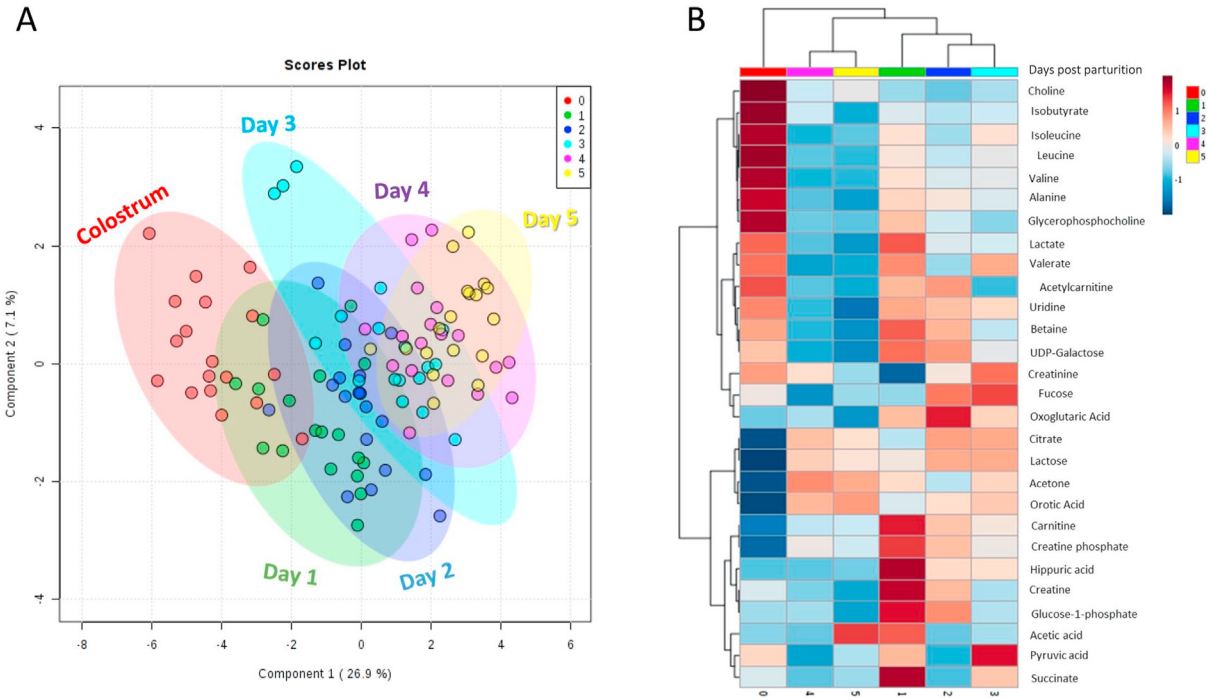

Fig. 2. PLS-DA depicting the changes occurring to the metabolome as samples transition from colostrum to milk over five days post parturition (A) [0, colostrum; 1, 2, 3, 4, and 5: D1, D2, D3, D4, and D5 post parturition, respectively (R^2, 0.83; Q^2, 0.78) and heatmap of milk metabolites in colostrum and milk samples 1, 2, 3, 4 and 5 days post parturition (B). The degree of positive and negative correlation of metabolites to specific days post parturition is indicated by +1 (red) and −1 (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

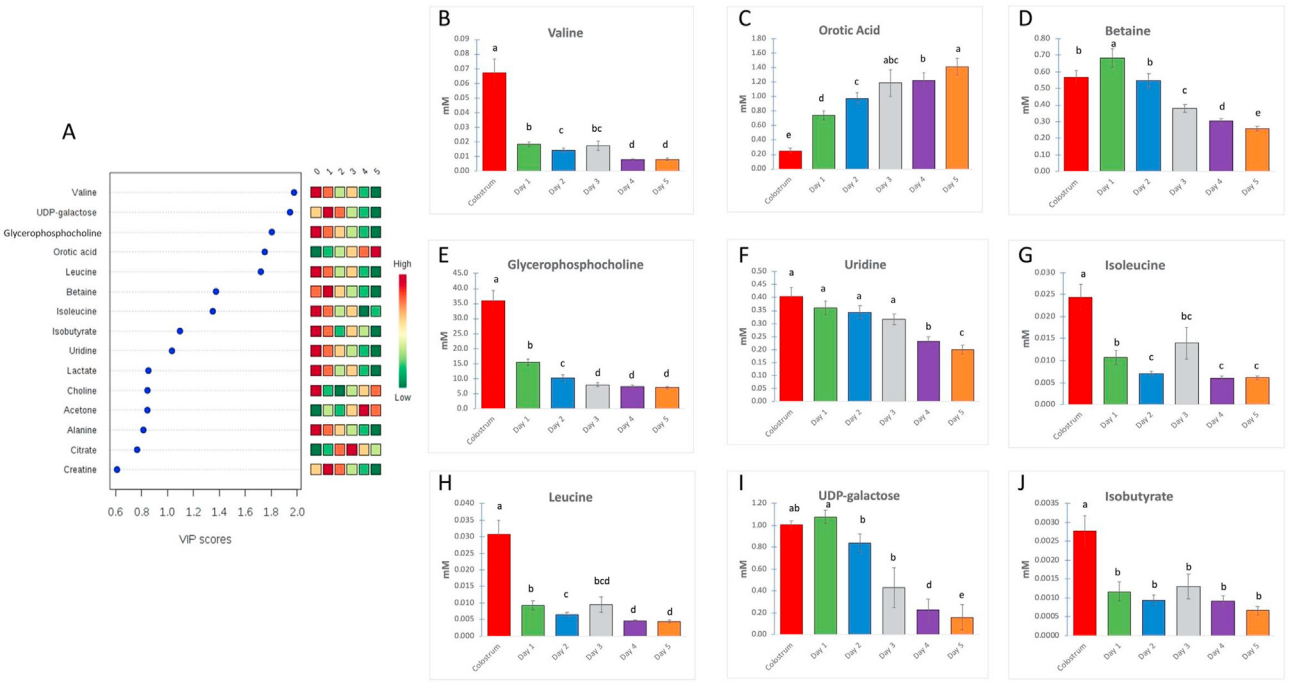

Fig. 3. Panel A, variable importance in projection (VIP) highlighting the metabolites most responsible for observed separations in PLS-DA (Fig. 2A); the coloured boxes on the right indicate the relative levels of the corresponding metabolite in each group under study; panels B–J show the metabolites changing significantly over time with highest VIP scores >1; a, b, c, and d denote significance between days, $P < 0.05$.

cells (de Veth et al., 2016). Betaine also plays a key role in one carbon metabolism in the cell through provision of methyl groups for conversion of homocysteine to methionine (Eklund, Bauer, Wamatu, & Mosenthin, 2005). Through its ability to provide labile methyl groups, betaine also plays a role in the synthesis of several metabolically active substances such as creatine and carnitine (Eklund et al., 2005). Creatine is essential for normal brain development (Edison, Brosnan, Aziz, & Brosnan, 2013).

Creatine phosphate was lowest in colostrum samples and increased significantly to its highest level on Day 1 post parturition and decreased significantly again thereafter. Both creatine and creatine phosphate have important roles in buffering ATP levels, vectorial transport of high energy phosphates and production of reactive oxygen species by mitochondria (Edison et al., 2013).

Citrate was lowest in colostrum samples and increased thereafter up to days 2 and 3 after which it dropped and the citrate levels in samples on day 5 were significantly lower than those of days 2 and 3. Citrate is an important component of milk with important implications on the quality and processability of milk (Akkerman, Larsen, Sørensen, & Poulsen, 2019). Citrate plays a key role in cellular energy metabolism as an intermediate in the tricarboxylic acid cycle and plays an indirect role in synthesis of fat (Garnsworthy, Masson, Lock, & Mottram, 2006) particularly for the de novo synthesis of fatty acids (Faulkner & Peaker, 1982), whereby if de novo synthesis of fatty acids increases, citrate concentration decreases (Garnsworthy et al., 2006). Garnsworthy et al. (2006) documented that citrate levels in milk vary across lactation and are highest in the early stage.

Hippuric acid levels varied significantly throughout the study and were highest in day 1 samples. Hippuric acid has been identified as a constituent of the non-protein nitrogen fraction of milk (Patton, 1953). The levels of hippuric acid in milk have previously been proposed as a potential biomarker of cow feeding system whereby higher levels of hippuric acid in milk represent a pasture based diet (O'Callaghan et al., 2018). As such, levels in this study are similar to that of milk hippuric acid from indoor total mixed ration cows as reported by O'Callaghan et al. (2018).

Glucose-1-phosphate and UDP-galactose were highest in the initial days in colostrum and day 1 samples, after which they decreased significantly to lowest levels on day 5. Lactose was lowest in colostrum samples after which it increased significantly and its levels remained stable. Lactose is the major carbohydrate in milk and is synthesised from free glucose and UDP-galactose (Lin et al., 2016). Lactose contributes significantly to the energy value of milk and as such plays an important role in development of newborn mammals. Lactose synthesis and level in milk are affected by a number of factors including udder health, cows energy balance and metabolism (Costa et al., 2019).

Orotic acid was lowest in colostrum samples and increased significantly thereafter to its highest levels in day 5 samples. Orotic acid had previously been classified as vitamin B13, it is produced and secreted into milk by cells in the mammary gland, and serves as an intermediate in the synthesis of pyrimidine nucleotides (Zaalberg, Buitenhuis, Sundekilde, Poulsen, & Bovenhuis, 2020). Levels of orotic acid have previously been suggested as one of several potential biomarkers of the metabolic difference between lactation and non-lactation in cows (Sun et al., 2017). Furthermore, Löffler, Carrey, and Zameitat (2015) on review have hypothesised it may have a role in regulating gene transcription. Subsequently, Löffler, Carrey, and Zameitat (2016), concluded that orotic acid is required for the regulation of genes important in development of cell, tissues and organisms. Uridine was highest in colostrum samples and its levels remained high until day 3 and decreased significantly to day 5 post parturition. Orotic acid is subsequently converted to uridine for use in the pyrimidine salvage pathway (Löffler et al., 2016).

Acetone was lowest in colostrum samples and increased thereafter to its highest levels in days 4 and 5 post parturition. Acetone levels in milk are widely used as a biomarker of subclinical ketosis (Enjalbert, Nicot, Bayourthe, & Moncoulon, 2001) and also milk yield and reproductive efficiency (Gustafsson & Emanuelson, 1996). Increasing levels of ketone bodies in the milk in the days immediately post parturition could be indicative of cows being in a state of negative energy balance. A negative energy balance is common in dairy cows particularly in the early stages of lactation as milk yield increases dramatically at the onset of lactation and consumption of food to meet these requirements can be limited (Gustafsson & Emanuelson, 1996).

From data generated herein, it is clear that the colostrum and milk metabolome is complex and undergoes significant changes over a short period at the onset of lactation. As the provision of colostrum and its composition and quality is vital to the development and growth of the new-born mammal, future research focusing on external factors such as diet, dry cow period, breed etc, on the composition of beneficial nutrients and metabolites and their subsequent effects on development and performance could be beneficial and warrants attention.

4. Conclusions

This study provides novel information on the bovine colostrum metabolome. The metabolome of the colostrum and milk is complex and undergoes significant changes in the days immediately post parturition. Multivariate analysis demonstrated the ability to distinguish between the metabolome of colostrum and milk in the subsequent days post parturition, whereby the colostrum metabolome was distinctly different from subsequent milk samples.

The colostrum metabolome appears to provide a number of nutrients beneficial for growth and development of the new-born mammal, such as branched chain amino acids and choline. In the days post-parturition, the levels of these beneficial nutrients are reduced as it evolves to a regular milk composition with significant increases in lactose content. Acetone, a metabolite widely used as a biomarker of subclinical ketosis, increased in the days post parturition potentially signifying cows being in a state of negative energy balance in this period as physiological demands on the cow from calving and the onset of lactation increased. Overall parity of the cow did not appear to have a drastic effect on the metabolome of the colostrum and milk samples. The impact of external factors such as dry cow period and feeding regimen prior to parturition on the colostrum metabolome and subsequent effects on microbiota development and performance could yield novel insights and warrants further investigation.

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