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Changes to the Oligosaccharide Profile of Bovine Milk at the Onset of Lactation

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Abstract: Numerous bioactive components exist in human milk including free oligosaccharides, which represent some of the most important, and provide numerous health benefits to the neonate. Considering the demonstrated value of these compounds, much interest lies in characterising structurally similar oligosaccharides in the dairy industry. In this study, the impacts of days post-parturition and parity of the cows on the oligosaccharide and lactose profiles of their milk were evaluated. Colostrum and milk samples were obtained from 18 cows 1–5 days after parturition. Three distinct phases were identified using multivariate analysis: colostrum (day 0), transitional milk (days 1–2) and mature milk (days 3–5). LS-tetrasaccharide c, lacto-*N*-neotetraose, disialyllacto-*N*-tetraose, 3′-sial-*N*-acetyllactosamine, 3′-sialyllactose, lacto-*N*-neohexaose and disialyllactose were found to be highly affiliated with colostrum. Notably, levels of lactose were at their lowest concentration in the colostrum and substantially increased 1-day post-parturition. The cow's parity was also shown to have a significant effect on the oligosaccharide profile, with first lactation cows containing more disialyllacto-*N*-tetraose, 6′-sialyllactose and LS-tetrasaccharide compared to cows in their second or third parity. Overall, this study identifies key changes in oligosaccharide and lactose content that clearly distinguish colostrum from transitional and mature milk and may facilitate the collection of specific streams with divergent biological functions.

Keywords: bovine milk oligosaccharides; lactation; parity; profiles; phases

1. Introduction

The diversity and abundance of complex sugars known as human milk oligosaccharides (HMOs) remain some of the most remarkable features of breast milk. Oligosaccharides are indigestible to the infant, and therefore reach the colon intact [1]. HMOs are the preferred substrates for several health-promoting bacteria in the colon, including bifidobacteria, thereby acting as prebiotics [2]. Furthermore, HMOs can directly modulate the host's epithelial responses by blocking the binding of pathogens to intestinal epithelial cells, or by acting as decoy receptors, inhibiting the binding of enteric pathogens [3]. Metabolic products from HMOs, such as sialic acid, have also been suggested to play important roles in brain development, neural transmission and synaptogenesis [4,5]. However, the health-promoting effects associated with

HMOs have been unavailable to formula-fed infants until recently. HMOs have now become available in quantities and at prices accessible for a broad range of applications. Two HMOs, 2′ fucosylactose and lacto-*N*-neotetraose, have recently been added to infant formula in more than 30 countries and non-infant products are beginning to emerge [6,7]. Despite this, the complexity of HMOs (with over 200 HMOs structures identified to date [8,9]) makes it almost impossible for their associated functions to be duplicated in formulas, even as more structures become available. Infant milk formulas are mainly based on bovine milk, and at least ten bovine milk oligosaccharides (BMOs) do share the same structures as certain HMOs, which could imply common functionalities [2,10–12].

Although much lower in concentration when compared to human milk (estimated concentrations in mature human milk lie in the range 5–15 g/L [1,13]), oligosaccharides are particularly rich in bovine colostrum, occurring at 0.7–1.2 g/L [14] in comparison to 0.1 g/L in mature bovine milk [15,16]. In terms of commercial availability, bovine colostrum accounts for 0.5% of the annual production of bovine milk; thus, colostrum offers a potentially viable source of milk oligosaccharides [17]. However, the cow's genetics, breed, parity, diet, length of the dry period and days post-partum all contribute to colostrum variability [18], making it difficult to identify specific pools and their suitability for use in specific industrial processes. A study comparing the oligosaccharide profiles of Holstein Dairy and Angus herds found that there were increased abundances of several oligosaccharides structures in the milk of Angus herds, highlighting breed to breed oligosaccharide variations [19]. Furthermore, another study, comparing milk samples from two Danish breeds, Jersey and Holstein–Friesian, demonstrated that sialylated and the more complex neutral fucosylated oligosaccharides were present in the milk of the Jersey cattle. Smaller molecular weight and simpler neutral oligosaccharides were present in the milk obtained from the Holstein–Friesians [20]. Some groups indicate that colostrum production occurs immediately post-parturition (day 0) [14,21,22], while others indicate colostrum occurs for up to 2 days post-parturition [23–25], 4 days post-parturition [26–29] or even as late as 7 days post-parturition [30–32]. To date, the lack of clarity surrounding the definitions of colostrum and early lactation milks has contributed to uncertainty in relation to its suitability for many industrial processes. Recently, our group demonstrated that changes in the fatty acid composition of colostrum collected from Irish Holstein–Friesian cows occurred as it transitioned from colostrum (day 0) to mature milk [33]. In this study, three key phases in the fatty acid profile were identified: colostrum (day 0), transition milk (days 1 and 2) and mature milk (days 3–5). In the current study, we aimed to explore the impacts of days post-parturition and parity of the cow on the oligosaccharide composition as it transitioned from colostrum to mature milk. In doing so, we aimed to gain a better understanding of the duration for which bovine mammary secretions should be classified as colostrum, and highlight the potential of colostrum as a key source of bioactive oligosaccharides. Uncovering such profile changes may indicate the economicvalue of colostrum and transitional milk streams that would otherwise be unsuitable for industrial processes.

2. Materials and Methods

2.1. Materials

The oligosaccharide standards used included 2′-fucosyllactose (2′-FL), 3-fucosyllactose (3-FL), LS-tetrasaccharide c (LSTc), LS-tetrasaccharide a (LSTa), lacto-*N*-neohexaose (LNnH), 3′sial-*N*-acetyllactosamine (3′-SNL), disialyllacto-*N*-tetraose (DSLNT), disialyllactose (DSL), lacto-*N*-hexaose (LNH), lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT), *N*-acetylneuraminic acid (Sialic Acid), 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL). All oligosaccharide standards were purchased from Carbosynth Ltd. (Berkshire, UK) and lactose was obtained from VWR (Dublin, Ireland).

2.2. Experimental Design and Sample Collection

Eighteen Holstein–Friesian cows representing an evenly distributed spread of 1st lactation (n = 6), 2nd lactation (n = 6) and 3rd lactation (n = 6) were included in this study and selected from the spring

calving dairy herd located at the Teagasc Moorepark Dairy Research Farm, Fermoy, Co. Cork, Ireland, as previously described by O'Callaghan et al. [33]. Cattle were fed straw (30% of DM), grass silage (40% of DM) and a blended concentrate (30% of DM) (maize gluten meal and rolled barley at a 40:60 ratio) prior to calving. Cows had unrestricted access to feed and fresh clean water 24 h per day. A total of six milk samples were obtained from each cow including colostrum (day 0) taken on the day of calving, and consecutive morning milkings 1, 2, 3, 4 and 5 days after parturition. Each cow was milked into a separate stainless-steel churn at the time of milking to enable sample collection. An approximate sample of 400 mL was collected from each cow and stored at refrigeration temperature immediately. Once all samples were obtained, they were frozen at −20 °C until the time of analysis. For constancy, all analysis was performed sequentially once the entire sample set was collected.

2.3. Oligosaccharide Analysis

The filtered milks were separated and analysed to quantify levels of 15 standards, as previously described [34] with minor modifications. In brief, the milk samples were first warmed to 35 °C for 30 min to defrost the samples. The samples were mixed thoroughly by inversion several times, and from each, an aliquot was taken and mixed with water at dilution levels of 1/10 for mature milk (day 1 to day 5) and at dilution levels of 1/100 for the colostrum samples. Each milk sample was diluted in duplicate. The diluted samples were mixed using a vortex, centrifuged at 14,000 rpm and the supernatant filtered through a 0.22 µm nylon syringe filter (Macherey-Nagel, Labquip Ireland Ltd. Dublin, Ireland). A Dionex ICS-3000 series system (Dionex Corporation, Sunnyvale, CA, USA), which was fitted with an electrochemical detector, was used to separate and quantify oligosaccharides. Samples were separated, quantified and detected on a CarboPac PA100 column (250 × 4 mm) which was equipped with a guard column using an electrochemical detector with pulsed amperometric detection (PAD). Samples were separated using the gradient: 95% 100 mM NaOH (Eluent A) and 5% 100 mM NaOH with 500 mM NaAc (Eluent B) for 3 min, increasing to 12% eluent B over 10 min and to 16% eluant B in a further 2 min. The eluant was then increased to 50% B over 10 min and held for 10 min to clean the column. The column was re-equilibrated for 15 min with 95% eluent A and 5% eluent B after each separation.

2.4. Statistical Analysis

SPSS v24.0 (IBM Statistics Inc., Armonk, NY, USA) was used to complete statistical analysis. Between and within-subjects repeated measures ANOVA with post hoc Tukey test were implemented to correlate the oligosaccharide and lactose contents of colostrum and milk samples with "days" post-parturition (colostrum and day 1–5) and with varying numbers of lactation "parity" (1st, 2nd, and 3rd). p-values < 0.05 were considered significant. The strengths of significant results were also recorded as the partial eta^2 effect size (η^2), where effect sizes are small ($0.01 \leq \eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$) or large ($\eta^2 \geq 0.14$).

Multivariate analysis of the oligosaccharide composition was also conducted to determine the impact of days post-parturition and parity. A supervised multivariate model was constructed using partial least squared discriminant analysis (PLS-DA). A permutation test with 2000 repetitions was conducted to check that the model differed from a random model. The variable importance in projection analysis (VIP) demonstrates which variables have larger influences on the latent variables of the constructed model. These tests and subsequent figures were performed using Metaboanalyst (www.metaboanalyst.ca) [35,36].

The variations in the carbohydrate contents of colostrum and milk as a result of days post-parturition are illustrated as the means ± standard deviations (mg/L) of individual samples, unless otherwise stated.

3. Results and Discussion

During the initial days of lactation, rapid decreases occur in the concentrations of fats, proteins, peptides, oligosaccharides, ash, non-protein nitrogen, vitamins and minerals, growth factors, hormones, cytokines and nucleotides, while lactose production in contrast rapidly increases [37,38]. Both human and bovine milk experience a decrease in total oligosaccharide content and changes in their specific oligosaccharide profiles during its transition to mature milk [15,39,40]. Variation in oligosaccharide profiles over the course of lactation may be linked to varying biological activities, which may reflect the co-evolution between maternal milk and the changing needs of the nursing off-spring. Identification of hallmark changes in the oligosaccharide profile of milk as it evolves from colostrum to mature milk could help food manufacturers identify specific phases in lactation most suitable for specific processes and products such that optimum value can be obtained from bovine milk.

In this study, changes in the relative abundance of bovine oligosaccharide and lactose concentrations were recorded in colostrum and samples 1–5 days post-parturition (Table S1). We identified and quantified the levels of 3-FL, LNnT, LNnH, LSTc, 3′-SNL, 6′-SL, 3′-SL, DSLNT, DSL, LNT, sialic acid and lactose, which were present in all samples, but 2′-FL, LSTa and LNH were not detected. It should be noted, however, that 2′-FL may have been obscured by the presence of lactose, as both have a similar retention time, and further analysis is required to confirm the presence or absence of 2′-FL in the samples. Lactose was not removed, as other low molecular weight oligosaccharides may have been lost in the process, and thus, we wanted to maintain the authenticity of the raw milk. Future analysis should include a separate method designed for the detection of 2′-FL. Furthermore, parallel sample analysis on both the raw milk samples and samples which have had lactose removed could be conducted to ensure the presence of lactose does not obscure other oligosaccharide structures through comparative analysis. Overall, days post-parturition was shown to have a substantial effect (p-value < 0.001) on the concentration of every oligosaccharide detected and on the level of lactose present. Significant decreases in the concentrations of 3-FL, LNnT, LSTc, 6′-SL, 3′-SL, DSLNT and DSL were observed after day 0 (colostrum) and between day 1 and 5 post-parturition; lactose, sialic acid and LNT increased in concentration post-parturition (Table S1).

Partial least squares discriminant analysis (PLS-DA) demonstrated the changing oligosaccharide profiles over time, and three distinct phases were evident as the milk transitioned from colostrum to mature milk. These phases included colostrum (day 0), transition milk (days 1 and 2) and mature milk (days 3–5) (Figure 1A). The colostrum (red) was distinctly different from that of all other sampling days. Transitional milks on day 1 (green) and 2 (blue) were somewhat similar to each other; however, distinct differences were observed, and these appeared to separate well, emerging as individual clusters. All samples between days 3 and 5 were shown to cluster together, with subtle differences occurring between days 3 and 5, indicating the milk continued to evolve progressively over time. Notably, in a previous study using the same batch of milk, three distinct phases of fatty acid profile were identified over the same lactation period, including colostrum (day 0), transition milk (days 1 and 2) and mature milk (days 3–5) [33].

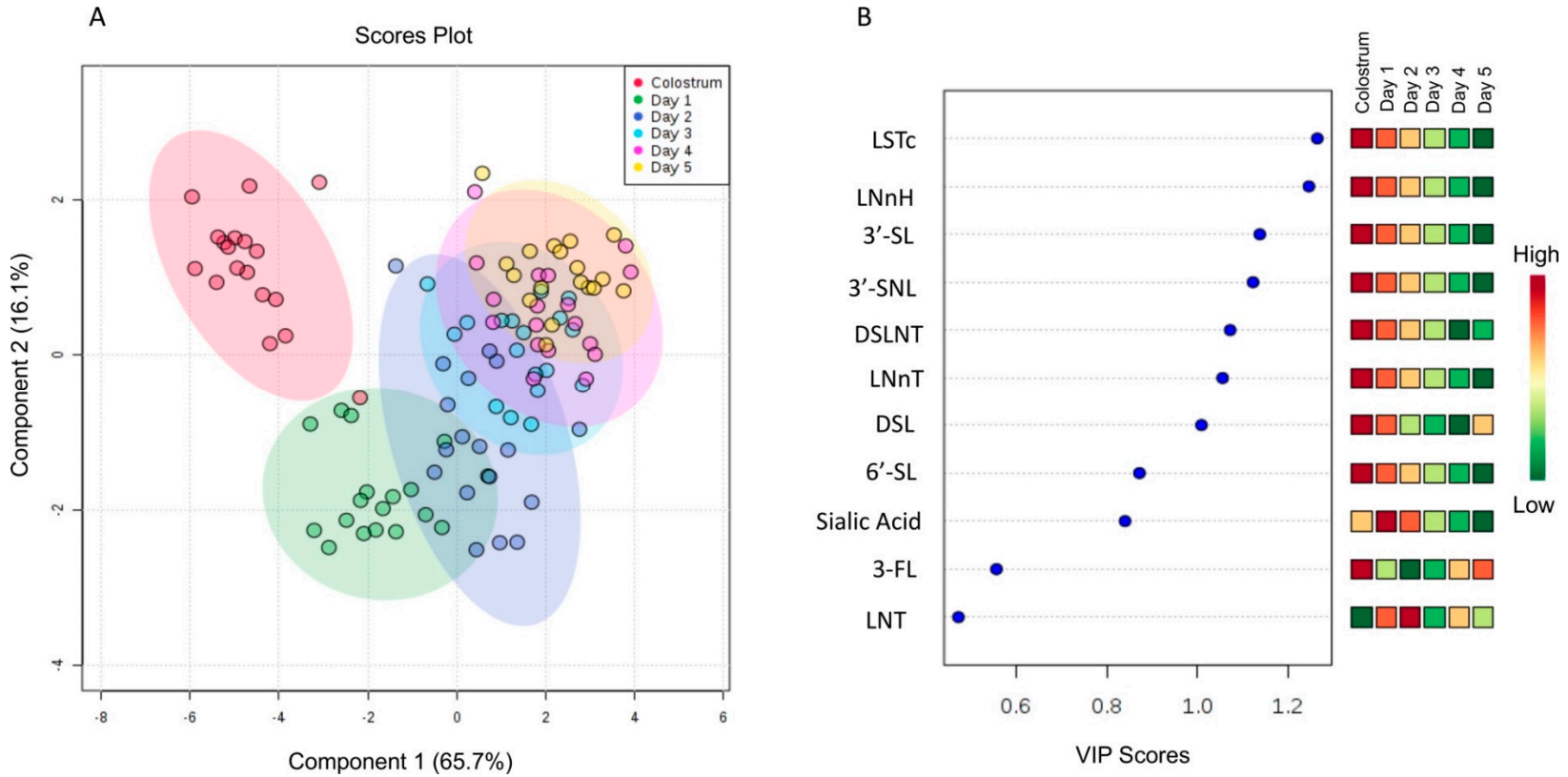

Figure 1. (**A**) Partial least square discriminant analysis (PLS-DA) demonstrating the changes in oligosaccharide profiles appearing as milk transitions from colostrum to mature milk over five days post-parturition (0, colostrum (red); 1–5 post-parturition (green, blue, light blue, violet, yellow respectively)). (**B**) Variable importance in projection plots of the oligosaccharides most implicated in the observed separations in PLS-DA; the boxes in colour on the right depict the relative concentrations of the corresponding oligosaccharides in each group under study. 2′-fucosyllactose (2′-FL), 3-fucosyllactose (3-FL), LS-tetrasaccharide c (LSTc), LS-tetrasaccharide a (LSTa), lacto-*N*-neohexaose (LNnH), 3′sial-*N*-acetyllactosamine (3′-SNL), disialyllacto-*N*-tetraose (DSLNT), disialyllactose (DSL), lacto-*N*-hexaose (LNH), lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT), *N*-acetylneuraminic acid (Sialic Acid), 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL).

Variable importance in projection (VIP) (Figure 1B) demonstrated that there were seven oligosaccharides (VIP ≥ 1) implicated in the observed separation, including 3′-SL, LSTc, LNnT, DSLNT, 3′-SNL, LNnH and DSL. Each of these structures was highly affiliated with colostrum rather than with post-parturition samples collected on later days (Figure 1B). The current understanding of why specific structural changes in the oligosaccharide profile occur days post-partition is limited. These changes may reflect unique roles for certain milk oligosaccharides as lactation advances and requirements for neurodevelopment, growth, immunity and gut microbiota are reprioritised. Sialylated oligosaccharides account for 70% of those present in bovine colostrum and 50% of the oligosaccharides in mature bovine milk [41]. Total sialylation is known to decrease as milk evolves from colostrum to mature bovine milk [42], and this decrease has been suggested to be associated with a shift from di-sialylated oligosaccharides in colostrum to mono-sialylated glycans, which are present as sialic acid, which is thought to be a key stabiliser of glycoproteins and can be attributed to its calcium-binding abilities [43,44]. Specifically, sialylated oligosaccharides such as 3′-SL, 6′-SL and 6′-SLN from bovine colostrum have been shown to decrease dramatically during the first 24 h of lactation [14]. In the current study, 3′-SL was one of the most abundant oligosaccharides detected (Table S1) and was highest in colostrum at a concentration of 786 ± 252.87 mg/L (Table S1), but significantly decreased (p-value < 0.001) with each day post-parturition, and was lowest in the day 5 samples at a concentration of 63.98 ± 20.94 mg/L. The concentrations of 6′-SL and 3′-SNL were also at their highest levels in the colostrum sample, and decreased from day 1, reaching the lowest concentration on day 5. Notably, levels of sialic acid were highest on day 1 of lactation (57.37 ± 12.8 mg/mL) and were higher on day 2 (43.12 ± 10.68 mg/mL) when compared to day 0 (32.52 ± 7.34 mg/mL) (Table S1). Between days 3 and 5, the concentration decreased, with the lowest concentration being observed on day 5 (22.16 ± 5.2 mg/mL). Sialylated HMOs and sialic acid can resemble human epithelial cell receptors and thus can become recognised by sialic acid-dependent pathogens thereby acting as decoys, and this can result in inhibition of their adhesion to the intestinal epithelial cells of newborns and infants. Low levels of intact sialylated oligosaccharides can be absorbed in the gastrointestinal tract and remain in the infant's circulation at concentrations that are capable of modulating the immune system at the cellular level and can also contribute to brain development during infancy [45]. For this reason, the fact that bovine colostrum is rich in sialylated oligosaccharides may prove particularly useful if developing bovine milk oligosaccharides for applications in infant formula. It should be noted that while few studies have detected the presence of 3′-SNL in human milk, it can be found in human biofluids and is a dominant oligosaccharide in the urine of breastfed infants [46]. Another consideration relating to certain bovine sialylated oligosaccharides is that humans lack the ability to synthesize the common sialic acid, *N*-glycolylneuraminic acid (Neu5Gc), which is commonly produced in bovine milk [47]. However, these structures are present in the human diet in foods such as red meat and dairy, and humans synthesise polyclonal antibodies against Neu5Gc-glycans [48,49]. Health implications are associated with Neu5Gc, as it can be metabolically incorporated into newly synthesized glycans and present on human cells [50,51]. The presence of Neu5Gc-containing epitopes and circulating anti-Neu5Gc antibodies is suggested to play a role in chronic inflammation-mediated diseases [52]. Moreover, the inclusion of bioactive ingredients in infant formula is highly regulated. Thus, the inclusion of individual or pools of specific bioactive bovine milk-derived oligosaccharides in such formulations would require purification as a prerequisite to ensure no potentially harmful components are present. It may be necessary to remove certain oligosaccharides from the stream using membrane filtration technology or enzymatic digestions.

Recently, LNnT and 2′-FL have been added to certain infant formulas [53,54]. In this study, LNnT was implicated in the observed separation in VIP, depicting the changes in oligosaccharide profiles appearing as milk transitions from colostrum to mature milk over five days post-parturition (Figure 1B). LNnT was rich in colostrum but decreased in subsequent days (Table S1). LNnT may help protect the developing offspring from pathogenic infection, as indicated in a number of studies [55,56], and thus, high levels present in colostrum may be useful as a source of such bioactive therapeutics.

In the current study, 3-FL was present at high concentrations throughout the lactation period and was highest in colostrum (Table S1). Furthermore, there was a sharp decrease in 3-FL concentration by day 1, after which it remained relatively constant with a slight increase detected between days 3 and day 4 (p-value = 0.02) (Table S1). Fucosylation is a feature associated with human milk oligosaccharides which accounts for up to 70% of OS species in human milk [57]. HMOs containing Fuc linkages by α1,2-glycosidic bonds have been indicated to promote the growth of bifidobacteria which are capable of hydrolysing fucosylated HMOs [58]. Increased levels of fucosylated BMOs may facilitate a prebiotic effect on days 3 and 4. Additionally, fucosylated oligosaccharides are associated with anti-infective abilities [59]. While production of 3-FL in an engineered *Escherichia coli* using α-1,3-fucosyltransferase isolated from *Helicobacter pylori* has been performed [60], isolation of fucosylated oligosaccharides from bovine colostrum may be a more attractive to consumers in comparison.

LNT has been previously reported to be found in bovine milk [61]. Notably, in human colostrum LNT has been indicated to increase in concentration during the first 24 h of lactation [62] in agreement with the current study, suggesting that this may be a key evolutionary feature of transitional milk. LNT is a neutral oligosaccharide which is also present in human colostrum and milk. LNT, with its anti-inflammatory capabilities [16], anti-adhesive effects against *Streptococcus pneumoniae* [63] and its ability to bind *Clostridium difficile* toxins [64], has also been indicated to promote the growth of intestinal bifidobacteria in the newborn [65,66]. Thus, bovine colostrum may prove a valuable source of such components.

In this study, lactose was found to be at its lowest concentration in colostrum and increased on sequential days in agreement with previous studies [37,38]. In human milk, a rapid increase in infant intestinal lactase activity following the first breastfeeding has been indicated [67–69]. This increase in lactose concentration may reflect an evolutionary adaptation between the maternal milk and the infants gut. In fact, hydrolysis of lactose via lactase in the human gut typically exceeds 98% efficiency within the first five days of breastfeeding [67,70], and this may also occur in other mammalian species.

In this study, the cow's lactation number was also shown to have a significant effect on the oligosaccharide profile as demonstrated by PLS-DA which showed the differences in the oligosaccharide profiles between cows in their first (1), second (2) and third (3) lactations (Figure 2A). It is clear that the oligosaccharide profile from the 1st lactation differs from the 2nd and 3rd lactation, however, there is much overlap between the groups. The oligosaccharides contributing to the observed separation of the PLS-DA are presented in Figure 2B. DSLNT, 6'-SL and LSTc represent the most important structures in this observed separation (VIP > 1). Notably, first lactation cows were observed to have the lowest abundance of these components, with higher levels being observed in second and third lactation cows (Figure 2B). This is in agreement with a previous study where abundances of several oligosaccharides were shown to increase in second lactation cows [71]. DSLNT, which was highest in 3rd lactation cows can reduce the risk of preterm infants developing necrotising enterocolitis [72]. Notably, 6'-SL, which was highest in 2nd lactation cows, can inhibit the adhesion of pathogenic bacteria, their toxins and some viruses [64,73,74]. It can also increase the adhesion of *B. infantis* to intestinal cells in vitro [75]. These components may be very important in the early establishment of a healthy gut microbiota, thus 2nd and 3rd-lactation cows may prove useful as a source of specific bioactive components capable of reducing the risk of necrotising enterocolitis in preterm infants and establishing a healthy gut microbiota.

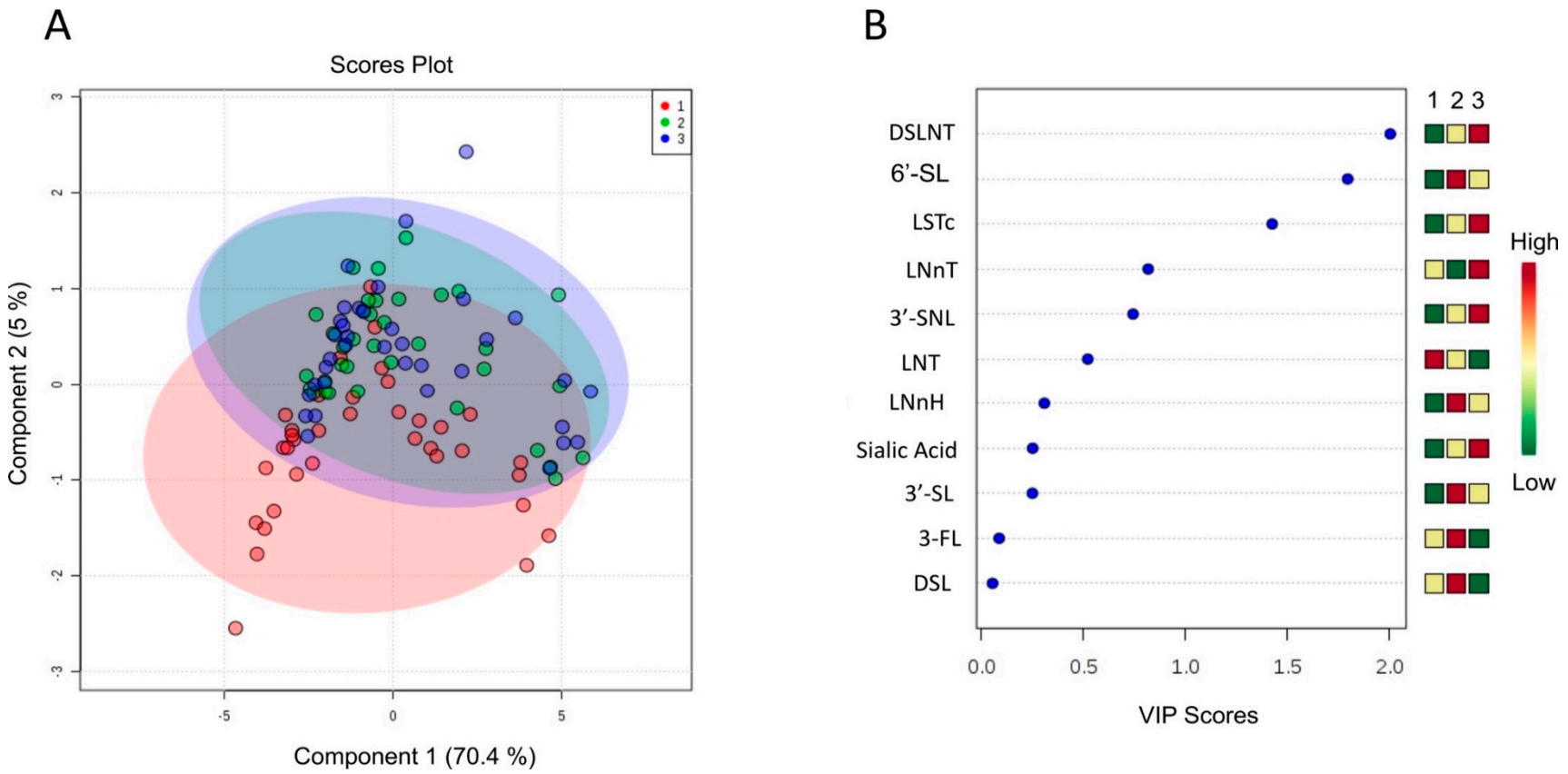

Figure 2. (**A**) Partial least square discriminant analysis (PLS-DA) demonstrating the impact of parity (i.e., 1st, 2nd and 3rd lactation cows, denoted 1 (red), 2 (green) and 3 (blue), respectively) on the oligosaccharide profile. (**B**) Variable importance plot highlighting the oligosaccharides contributing the most to the observed separations in PLS-DA based on parity; the coloured boxes on the right indicate the relative concentrations of the corresponding oligosaccharides in each group under study. 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), LS-tetrasaccharide c (LSTc), LS-tetrasaccharide a (LSTa), lacto-*N*-neohexaose (LNnH), 3'sial-*N*-acetyllactosamine (3'-SNL), disialyllacto-*N*-tetraose (DSLNT), disialyllactose (DSL), lacto-*N*-hexaose (LNH), lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT), *N*-acetylneuraminic acid (Sialic Acid), 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL).

It should be noted that there may be discrepancies in the annotation of certain structures in this study. For example, 3′-SNL may correspond to 6′-SNL, which was not included as a standard. Similarly, DSLNT may not be correctly annotated as this has not been previously identified in bovine colostrum and milk [12]. Given the similar molecular weight of 3-FL and isoglobotriose, miss annotation may have occurred in the absence of an isoglobotriose standard. Thus, future studies should seek to also annotate such structures using mass spectrometry. Mass spectrometry was outside the scope of the current study as the method is inherently restrictive for quantitation because of differences in the ionization efficiency and/or detectability. In the case of 6′SL and 3′SL, both oligosaccharides are dominant in bovine milk and they present as a pattern in the profile which is comparable with the standard profile and therefore it is highly likely these structures were accurately annotated. Supporting this, accuracy and precision of the oligosaccharide peaks were obtained as per manufacturer's instructions [76]. The mixed standard was run throughout the sequence several times, in a process known as bracketing the standard, with the first two injections being omitted. The standard concentration was then averaged and the averaged amount for each standard was used to quantify the unknown peak. The standard deviation of the averaged standards was kept below ±5%.

Overall, value may lie in extracting and concentrating oligosaccharides from bovine colostrum with a view to their addition as an active ingredient to infant formulas. De Moura Bell et al. [77] recently developed a novel pilot-scale approach for the recovery of highly pure oligosaccharides, from colostral bovine whey permeate. Although membrane filtration is the most commonly investigated technique for producing dairy-derived oligosaccharides at large-scale, there has been some recent success using scalable chromatography approaches to produce bovine oligosaccharides from whey streams by our group (European Patent Application number EP18214230.7), an area which we continue to explore.

4. Conclusions

As HMOs are supplied through breastfeeding, their valuable effects have been largely missing for formula-fed infants. Substitution of infant formula with bovine oligosaccharides to impart HMOs functions is a potential solution, in addition to the benefits already observed by supplementation of formulas with 2′-FL. Distinct oligosaccharide and lactose profile changes that occur as bovine milk transitions from colostrum to mature milk at the onset of lactation were identified in this study. These phases included colostrum (day 0), transition milk (days 1 and 2) and mature milk (days 3–5). Such phases may facilitate the healthy growth and development of the newborn, and these benefits could be harnessed for use in the food and ingredient formulation industry. In this study, while colostrum milk (day 0) had the most distinct and nutrient-dense profile, transitional milk may also prove useful as a source of specific components that can be used as targeted therapeutics. Overall, this study identified key oligosaccharide phases that may facilitate the collection of specific streams that may have divergent biological properties.

Supplementary Materials: The following are available online at http://www.mdpi.com/2624-862X/1/3/19/s1. Table S1: Impacts of days post-parturition on the oligosaccharide contents of colostrum and milks.

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