1 Title

2 Protein and oligosaccharide composition of colostrum and transition milk from pasture-based dairy cows

3 supplemented prepartum with inorganic selenium, organic selenium or rumen-protected choline.

4 Authors

5 Fionnuala McDermott[1,2,3,4,*], Emer Kennedy[2,3], John T. Tobin[1,3], Michael Egan[2], Tom F. O'Callaghan[3,5],

6 Lorraine Brennan[3,4] and Sean A. Hogan[1,3]

7 [1] Teagasc, Food Research Centre, Moorepark Fermoy, Co. Cork, Ireland

8 [2] Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Fermoy, Co. Cork, Ireland

9 [3] VistaMilk SFI Research Centre, Teagasc Moorepark, Fermoy, Co. Cork, Ireland

10 [4] UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

11 [5] School of Food and Nutritional Sciences, University College Cork, Co. Cork, Ireland

12 *Corresponding author: Fionnuala McDermott

13 Fionnuala.McDermott@teagasc.ie

14

15 Abstract

16 Prepartum diet and mineral supplementation can influence bovine colostrum proteins and

17 oligosaccharides (OS). The objective of this study was to evaluate the effects of prepartum

18 supplementation with 1) inorganic selenium (INORG), 2) organic selenium (ORG) or 3) rumen-protected

19 choline (RPC) on these components. Fifty-seven (12 primiparous and 45 multiparous) cows were

20 supplemented daily from 49 ± 12.9 d prior to calving until d of calving. Colostrum was collected within 1

21 h postpartum and transition milk (TM) samples 1 – 5 were collected over the following 2 – 3 d

22 postpartum. Prepartum supplementation with INORG, ORG or RPC had no significant effect on the

23 gross, OS or IgG composition of colostrum. Animals supplemented with ORG or RPC produced

24 significantly higher concentrations of colostrum α_{s2}-Casein and β-Casein. Each milking postpartum had a

25 significant effect on the protein and OS composition of milk. This research demonstrates that

supplementing these micro-nutrients prepartum does not have a deleterious effect on subsequent colostrum or TM composition.

1. Introduction

Colostrum is the initial mammary secretion produced following parturition and is crucial to the health and welfare of the neonate over the first few d of life (Playford & Weiser, 2021). Colostrum is distinguished by an elevated concentration of immunoglobulin G (IgG), which is indicative of colostrum quality, with concentrations of IgG at ≥50 mg mL^{-1} considered good quality (Weaver, Tyler, VanMetre, Hostetler, & Barrington, 2000). Previous research on bovine colostrum has tended to focus on IgG (Godden, 2008) at the expense of the remaining proteins. Milk proteins can be broadly separated into casein (CN) and whey proteins, each with distinctive biological functions. Casein proteins exist in their native form as casein micelles and size distribution of these micelles impacts metabolism and IgG absorption in the neonate (Sats, Kaart, & Jõudu, 2023). Whey proteins are soluble proteins not associated with the CN micelle (Deeth & Bansal, 2019) and possess a range of nutritional and biological properties including antimicrobial activity and roles in stimulating the immune system (Clare, Catignani, & Swaisgood, 2003; Madureira, Pereira, Gomes, Pintado, & Malcata, 2007).

Oligosaccharides (OS) are carbohydrates composed of three – 20 monosaccharide moieties (Weijers, Franssen, & Visser, 2008). Colostrum contains a variety of OS at concentrations approximately 15 to 70 times greater than mature milk (Fischer-Tlustos et al., 2020). Bovine colostrum OS exist as sugars or associated with lipids and proteins (Mehra et al., 2021) and are classified as neutral or acidic (Gopal & Gill, 2000) with prebiotics identified as the main role of OS (Jeurink, van Esch, Rijnierse, Garssen, & Knippels, 2013). The change in OS composition from colostrum to transition milk (TM) may reflect the evolution in the biological requirement for OS in the neonate over the initial days of life as the neonates own immune system develops. Research on the OS composition of bovine colostrum suggests their

potential in the prevention of pathogen adhesion in the intestines (Martín, Martín-Sosa, & Hueso, 2002) and aiding IgG absorption in the neonate (Gill, Mahmood, Nagpaul, & Mahmood, 1999).

A number of factors influence the protein and OS composition of colostrum including breed (Franzoi et al., 2019), diet (O'Callaghan et al., 2018; Durham et al., 2022), parity (Robinson et al., 2019) and genetics (Poulsen, Robinson, Barile, Larsen, & Buitenhuis, 2019; Zhou et al., 2019). The pre and postpartum periods are stressful with the cow undergoing numerous physiological and metabolic changes in preparation for parturition (Abuelo, Hernández, Benedito, & Castillo, 2019). Dairy cows are often supplemented with a combination of minerals during the prepartum period to prevent metabolic disorders occurring postpartum and to ensure a smooth transition into the subsequent lactation (Osorio et al., 2016). Dairy cows have a nutritional requirement for selenium (Se) at levels of 0.3 mg kg DM d^{-1}. It is routinely supplemented during the prepartum period as an important antioxidant and for immune system support (Zhang, Zeng, & Cheng, 2018). Selenium as a supplement is available in both inorganic and organic forms, with the former supplemented more frequently as it is less costly to produce. Selenium has a maximum supplemental level of 0.5 mg kg DM d^{-1} (EC, 2013) with organic Se supplemented at a maximum of 0.2 mg kg DM d^{-1}. Inorganic Se must undergo metabolic transformation before it is incorporated into selenoproteins, a group that includes a selenocysteine amino acid residue (Schrauzer, 2003). The organic form of Se is absorbed and incorporated directly into selenoproteins. Although milk proteins act as a biological pool of Se (Suzuki & Ogra, 2002), the effects of supplementing Se during the prepartum period on individual colostral CN and whey proteins are largely unknown.

Another nutrient of emerging interest, which is not typically supplemented to dairy cows, is choline. Although present in forage (Baldi & Pinotti, 2006), free choline is degraded in the rumen limiting its bioavailability (Jin, Li, & Wang, 2021). Therefore, it must be supplemented as rumen-protected choline (RPC), to ensure absorption in the small intestine. Choline acts as a methyl donor for the resynthesis of

methionine (Zeisel et al., 1991), an essential amino acid required for protein synthesis. Choline has the ability to spare methionine which is a possible mechanism for increased milk protein yield following choline supplementation (Pinotti, Baldi, & Dell'Orto, 2002). It has been reported that choline supplementation increased milk protein yield (Zom et al., 2011) and tended to increase milk protein content (Xu, Ye, Liu, & Yu, 2006), both during the early lactation period. The biological mechanism causing these effects on milk protein has not yet been described, nor is it fully established if RPC supplementation affects milk CN or whey proteins individually or collectively. Milk OS are composed of monosaccharide units which include glucose, galactose and *N*-acetylglucosamine (Aldredge et al., 2013). Glucose, necessary for the synthesis of milk OS, has been increased in the blood of dairy cows when Se or choline has been supplemented (Juniper, Phipps, Jones, & Bertin, 2006; Xu, Ye, Liu, & Yu, 2006). Lactose is also necessary for the production of OS as each OS contains either lactose or *N*-acetyllactosamine at its reducing end with a monosaccharide residue branching off from the non-reducing galactose (Gopal & Gill, 2000; Aldredge et al., 2013). Supplementing Se or choline to dairy cows has also been shown to increase milk lactose (Mohsen, Gaafar, Khalafalla, Shitta, & Yousif, 2011; Respati et al., 2023). Although blood glucose and milk lactose have been increased with the supplementation of Se or choline, the subsequent effect of supplementing these micro-nutrients on milk OS has not yet been investigated.

Although it is established that prepartum mineral supplementation is beneficial for cow health and neonatal vitality, previous data on the protein composition of bovine colostrum in terms of individual CN and whey proteins are rather limited (Levieux & Ollier, 1999) or have reported values semi-quantitatively (Sobczuk-Szul, Wielgosz-Groth, Wronski, & Rzemieniewski, 2013). The effects of supplementing inorganic Se, organic Se or RPC on the individual protein and OS composition of bovine colostrum or TM has not been examined. Irish dairy cows following the spring calving and winter housing system (with grass silage fed indoors during these winter months) are known in the literature as pasture-based

(O'Brien, Moran, & Shalloo, 2018; McKay et al., 2022). Therefore, cows in this study are referred to as pasture-based dairy cows due to the feeding of grass silage throughout the experimental period. The objectives of this study were to (1) determine the effect of supplementing pasture-based dairy cows with a mineral supplement containing inorganic Se (INORG), organic Se (ORG) or rumen-protected choline (RPC) during the prepartum period on the protein (both CN and whey) and OS composition of bovine colostrum and (2) to establish the change occurring in the protein and OS composition of TM.

2. Materials and Methods

This study was carried out at the Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland between December 2020 and December 2021. Ethical approval was granted by the Teagasc Animal Ethics Committee (TAEC) (TAEC263/2020). Experiments were undertaken in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC.

2.1 Experimental Design

Fifty-seven (12 primiparous and 45 multiparous) Holstein-Friesian (≥70 %; HF) (n=36) and HF × Jersey (30 % HF and >25 % Jersey; JEX) (n=21) cows were enrolled in this study and were balanced on parity (2.4 ± 0.8), expected calving date (8th February 2021 ± 12.7 d), prepartum body weight (BW) (595 ± 57.4 kg), prepartum body condition score (BCS) (3.23 ± 0.041) and Economic Breeding Index (EBI; Berry et al., 2007) (€173 ± 39.5). Each treatment group consisted of an equal number of first parity (primiparous) (n=4), second parity (n=4) and third or greater parity (n=11) (combined for multiparous) animals. Mean (±SD) length of prepartum supplementation was 49 ± 12.9 d. Sample size calculation to obtain ≥80% power was performed using SAS (version 9.4; SAS Institute Inc., Cary, NC) (PROC POWER) and was calculated using previously reported concentrations of colostrum IgG (Barry et al., 2019).

Animals were assigned to one of three treatment groups:

1. Supplemental sodium (Na) selenite at 50 mg kg^{-1} to provide 0.5 mg Se kg DM d^{-1} (INORG),
2. Supplemental Na selenite at 30 mg kg^{-1} along with Se-yeast (Selsaf®) at 20 mg kg^{-1} to provide 0.5 mg Se kg DM d^{-1} (ORG),
3. Supplemental Na selenite at 50 mg kg^{-1} with the addition of 20 g kg DM d^{-1} of rumen-protected choline (RPC). The choline was rumen-protected with hydrogenated, high melting point rapeseed oil providing choline of high bioavailability (Cargill Animal Nutrition, Naas, Co. Kildare, Ireland).

Animals were group penned by their respective treatment (n=19 per pen) within the same house. Animals were managed on 13 – 14 kg DM^{-1} of grass silage daily which was allocated each morning and fed using shared feed bunks. Grass silage was allocated based on an 8 % refusal rate to ensure intake was not limited. Feed space allowance for each treatment group was in line with current recommendations (0.6 to 0.75 m cow^{-1}; Farm Animal Welfare Advisory Council, 2018). Feed was pushed up every afternoon prior to evening milking, feed refusals were removed each morning prior to silage allocation. Animals had *ad lib* access to water for the duration of the experimental period. From dry off, animals received a standard dry cow mineral blend (Table 1) followed by the addition of one of the three micro-nutrients (Agritech, Nenagh, Co. Tipperary, Ireland) depending on their treatment group. Each dry cow mineral blend was top-dressed onto the silage twice daily at the recommended feeding rate (prior to morning milking and evening milking).

2.2 Milk sample collection

Colostrum samples were collected from all 57 cows within one h of birth in the calving pen using an individual, portable milking unit (InterPuls, Wiltshire, United Kingdom) or in the milking parlour (DairyMaster, Causeway, Co. Kerry, Ireland) when calving occurred within one h of the scheduled milking (07:30 h or 15:30 h). Cows were fully milked into the individual unit, the milk sample was mixed and then a 500 mL representative sample collected. The 500 mL of milk were transferred to a sterile

container (Sarstedt Ltd., Wexford, Ireland). Samples were transported to the laboratory within 30 min of collection and aliquoted, using transfer pipettes (Fisher Scientific, Dublin), under a fume hood, into 15 mL centrifuge tubes (Sarstedt Ltd., Wexford, Ireland) before storage at -20 °C until required for analysis. To prevent continual defrosting and refreezing, a single 15 mL centrifuge tube was used for each analysis. Transition milk samples (consecutive milking's one to five post colostrum collection; TM1 – TM5) were then collected from a random sub-set of five cows from each treatment group (n=15) with a mean (±SD) prepartum supplementation (45 ± 6.4 d), parity (2.6 ± 0.8), calving date (4th February 2021 ± 6.4 d), BW (615 ± 81.9 kg), BCS (3.31 ± 0.391), breed (HF (n=10) and JEX (n=5)) and EBI (€174 ± 35.2). Transition milk samples were collected as consecutive milking's after colostrum collection and were collected in the milking parlour at scheduled milking time. Each cow was milked into a separate individual churn to enable individual sample collection. After sample collection, each TM sample was treated in the same way as colostrum above. All analysis was carried out within eight months of sample collection.

2.3 Chemical reagents

Acetic acid, sodium acetate, trichloroacetic acid (TCA), nitric acid, sodium sulfate (Na_2SO_4), urea, bis-tris propane, 2-mercaptoethanol, acetonitrile, trifluoroacetic acid (TFA), sodium hydroxide (NaOH), sodium acetate (NaOAc), methanol (MeOH), IgG from bovine serum (I5506-50 mg) and BSA all sourced from Sigma-Aldrich (Arklow, Co. Wicklow, Ireland). The choline assay buffer (MAK056) sourced from Sigma (St Louis, MO, USA). Oligosaccharide standards used; 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), 5-*N*-acetylneuraminic acid (sialic acid), 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL) sourced from Carbosynth Ltd. (Berkshire, UK). Casein

175 (CN) and whey standards; κ-CN, α_s-CN, β-CN, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) A and
176 B supplied by Merck (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland).
177
178 2.4 Selenium and choline concentration in colostrum
179 Selenium content of colostrum was determined by ICP-MS (Teagasc Ashtown Food Research Centre,
180 Scribbletown, Dublin 15, Ireland). Colostrum samples were weighed (0.5 ± 0.0050 g) into MARS
181 XPRESS 55 mL PTFE digestion vessels and digested with 5 mL nitric acid in a MARS6 microwave
182 digestor (CEM Corporation, Matthews, NC, USA). Method parameters were as follows: ramp 20 min,
183 hold 20 min and temperature 200 °C. The power was set at 1800 W. Vessels were cooled after extraction
184 and digests transferred to 50 mL polypropylene tubes containing 15 mL ultrapure water. The vessels
185 were rinsed with a further three × 10 mL of ultrapure water, which were then added to the polypropylene
186 tubes. Extracts were further diluted by adding 2 mL extract to 8 mL ultrapure water in 15 mL
187 polypropylene tubes. Digested samples were analysed using a model 7900 inductively coupled plasma-
188 mass spectrometer (Agilent Technologies., Santa Clara, CA, USA) using hydrogen as the collision gas. A
189 Se calibration curve was prepared in the range 0.025 μg mL^{-1} to 1.0 μg mL^{-1}. The ICP-MS system was
190 controlled and the results processed by MassHunter 4.5 workstation data analysis software. Choline
191 content of colostrum was measured using a Choline/Acetylcholine Quantification Kit (MAK056, Sigma,
192 St Louis, MO) via a coupled enzymatic reaction, using a colorimetric (570 nm) assay. Colostrum samples
193 were diluted at 1:20 sample:buffer (choline assay buffer provided) prior to analysis. Colorimetric assay
194 was carried out in duplicate according to the manufacturer's instructions. Both analyses were carried out
195 in duplicate.
196
197 2.5 Raw milk composition
198 Milk samples were defrosted in a water bath for 30 min at 35 °C followed by manual inversion several
199 times to ensure sample homogeneity. The non-casein nitrogen (NCN) and non-protein nitrogen (NPN)

contents were determined using the Kjeldahl method and a nitrogen-to-milk protein conversion factor of 6.38 (IDF, 2004; IDF, 2001), using a Tecator Digestor Auto and Kjeltec 8400 distiller (Foss Electric, Hillerød, Denmark). Briefly, NCN was determined by casein precipitation with 10 g of milk sample diluted with deionized water at 40 ° C and acidified to pH 4.6 with the addition of acetic acid (10 % *v/v*) and sodium acetate (8.2 % *w/v*). Samples were cooled and filtered using Whatman No.1 filter paper. For NPN content, protein was precipitated from 8 mL of milk sample using TCA (15 % *w/v*). Precipitates were then filtered using Whatman No.1 filter paper. A Kjeldahl determination was carried out on both the NPN and NCN filtrate collected. Milk total solids (TS), true protein (TrP), total protein (TP) and lactose were analysed by infrared absorption spectroscopy using a FT6000 Milkoscan (Foss Ireland Ltd., Dublin, Ireland). Ash content was calculated by difference; TS minus fat content (data reported by McDermott et al., in review), TP and lactose.

2.6 Protein characterisation by RP-HPLC

Protein profiles of colostrum and TM samples were determined using reversed-phase HPLC (RP-HPLC) as previously described by Magan et al. (2019). Casein (CN) and whey standards used included: κ-CN, α_s-CN, β-CN, α-LA and β-LG A and B. Each sample was diluted at 1:20 sample: buffer (pH 7.5 urea buffer; 7 M urea and 20 mM bis-tris propane) followed by the addition of 20 µL of 2-mercaptoethanol (50 µL per 10 mL urea buffer). Samples were passed through 0.20 µm 17 mm polyethersulfone filter (Labquip, Clondalkin, Dublin 22, Ireland) and 0.5 µL of filtrate injected onto the column. Protein separation was carried out using an Agilent HPLC (Agilent 1220 Infinity II LC, Santa Clara, CA, USA) with an Agilent 300SB Poroshell (2.1 × 75 mm) column (Agilent Technologies, Santa Clara, CA) using aqueous (phase A) and organic (phase B) mobile phases. Phase A: acetonitrile–water–TFA (100:900:1 (*v/v/v*)) and phase B: acetonitrile–water-TFA (900:100:1 (*v/v/v*)) maintained at a flow rate of 0.5 mL min^{-1}. Protein detection was done at 214 nm using ChemStation software using known retention times of each standard (Agilent Technologies Ireland Ltd, Little Island, Cork, Ireland).

225

226 2.7 Immunoglobulin G (IgG) and Bovine Serum Albumin (BSA) quantification

227 Colostrum and TM IgG and BSA were separated and quantified by size exclusion high-performance
228 liquid chromatography (SE-HPLC) using an Acquity UPLC. Columns were fitted to a Waters Acquity H-
229 class UPLC separation module (Waters Corporation, Milford, Mass, USA) with a TSK G3000SW (300 ×
230 7.5 mm) column and two TSK-Gel columns in sequence (G3000SW, Tosoh 7.5 mm I.D. × 30 cm, 10 µm
231 followed by G3000SWXL, Tosoh 7.8 mm I.D. × 30 cm, five µm; Biosciences LLC, Tokyo, Japan). An
232 internal standard of IgG and BSA was used at a concentration of 1 mg mL^{-1}. Colostrum and TM1 were
233 diluted 1/100 and TM2 – TM5 diluted 1/10 with MilliQ HPLC-grade water. Samples were vortexed,
234 centrifuged at 14,000 *g* for 15 min at 4 °C to defat the sample. The aqueous layer was filtered through a
235 0.22 µm polyether sulfone filter (Thermo Scientific, Cardiff Valley Road, Rockwood) and 20 µL of
236 filtrate injected onto the column. The eluent used was 0.1 M Na_2SO_4 in 0.1 M phosphate buffer +10 %
237 MeOH (pH 6.7, at room temperature ~20 °C), at a flowrate of 0.5 mL min^{-1} with samples monitored
238 continuously at 214 nm using a Waters TUV wavelength detector. Chromatographic data was collected
239 and analysed using Empower data handling software (Waters Corporation, Milford, MA, USA) using
240 previously determined retention times of each standard. Mixed standards were run at concentrations of 10,
241 20, 50 and 100 µg mL^{-1} and calibration curves generated using Empower software package.

242

243 2.8 Oligosaccharide analysis

244 Milk OS were separated and quantified by high performance anion exchange chromatography with pulsed
245 amperometric detection (HPAEC-PAD) using an ICS3000 system equipped with an electrochemical
246 detector (Dionex, Sunnyvale, CA, USA) using 15 identified standards. Oligosaccharide standards
247 included 2'-FL, 3-FL, LSTc, LSTa, LNnH, 3'-SNL, DSLNT, DSL, LNH, LNT, LNnT, sialic acid, 3'-SL
248 and 6'-SL. 5-*N*-acetylneuraminic acid was quantified as free 5-*N*-acetylneuraminic acid, referred to as
249 sialic acid. Sample preparation and analysis were carried out as described by Quinn et al. (2020).

Colostrum and TM1 were diluted 1/100 and TM2 – TM5 were diluted at 1/10 with MilliQ HPLC-grade water. Samples were vortexed, centrifuged at 14,000 rpm for 15 min at 4 °C to separate the fat. The aqueous phase was then filtered through a 0.22 µm nylon syringe filter (Macherey-Nagel, Labquip Ireland Ltd. Dublin, Ireland), 1.5 mL placed in a HPLC vial and 20 µL of filtrate injected onto the column. The eluents used were; A (100 mM NaOH) and B (100 mM NaOH with 500 mM NaOAc). Elution was carried out with the following gradient: (t = 0 – 3 min, 95 % Eluent A; t = 3 – 13 min 88 % Eluent A; t = 13 – 30 min 50 % Eluent A; t = 30 – 45 min, 95 % Eluent A). A CarboPac PA100 column (250 × 4 mm) with a guard column (CarboPac PA-100, 4 × 50mm) were used for separation. Quantification of OS was carried out by comparing peak areas to the standard curves of the known standards above.

2.9 Statistical analysis

All statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc., Cary, NC). Data were checked for normality using the Shapiro-Wilk test (PROC UNIVARIATE) and as all *p*-values from tests for normality were >0.15, this provided insufficient evidence to reject the assumption of normality. A mixed effects model described by equation (1) was used to determine the effects of prepartum supplementation on colostrum gross composition (total solids (TS), protein, NPN and NCN), CN and whey protein and OS composition (mg mL^{-1}):

$$(1)\ Y_{ijkl} = \mu + P_i + B_j + T_k + b_1X_{ijkl} + b_2DOM_{ijkl} + e_{ijkl}$$

Where Y_{ijkl} represents the response of the cow l in parity i of breed j to prepartum supplementation k; μ = mean; P_i = parity ($_i$ = 1 – 3); B_j = breed ($_j$ = 1 - 2); T_k = prepartum supplementation ($_k$ = 1 – 3); b_1X_{ijkl} = milk weight (kg) and b_2DOM_{ijkl} = duration of mineral supplementation and e_{ijkl} = residual error term. The random effect was block (i.e. how the animals were blocked into each treatment group prepartum). The interactions between the fixed effects were also tested but the differences were found to be not significant.

For colostrum and transition milk analysis, a linear mixed model described by equation (2) was used:

$$(2)\ Y_{ijklm} = \mu + P_i + B_j + T_k + S_m + b_1X_{ijklm} + b_2DOM_{ijklm} + e_{ijklm}$$

Where Y_{ijkl} represents the response of the cow l in parity i of breed j to prepartum supplementation k to each milking postpartum m; μ = mean; P_i = parity ($_i$ = 1 – 3); B_j = breed ($_j$ = 1 - 2); T_k = prepartum supplementation ($_k$ = 1 – 3); S_m = each milking postpartum ($_m$ = colostrum – TM5); b_1X_{ijklm} = milk weight (kg) and b_2DOM_{ijklm} = duration of mineral supplementation and e_{ijklm} = residual error term. The random effect was block (i.e. how the animals were blocked into each treatment group prepartum). The interactions between prepartum supplementation and each milking postpartum were also tested but the differences were found to be not significant. Predicted Transmitting Ability figures (PTAs), obtained from the EBI, were used as covariates to improve the accuracy of the models. Milk weight (kg) and duration of mineral supplementation were also included as covariates. An analysis of the strength of the correlation between colostrum total protein and the casein and whey proteins was also assessed using a coefficient of determination, with values of an $R^2 > 0.25$ deemed significant.

3. Results

3.1 Colostrum

3.1.1 Gross composition

Supplementation during the prepartum period did not significantly affect concentrations of colostrum Se or choline (Table 2). Colostrum Se concentrations were 0.17 (± 0.019), 0.21 (± 0.021) and 0.19 (± 0.018) mg kg^{-1} for INORG, ORG and RPC respectively. Colostrum choline concentrations were 3.21 (± 0.643), 3.52 (±0.645) and 4.85 (± 0.657) ng μL^{-1} for INORG, ORG and RPC respectively. Neither breed nor parity had a significant effect on Se or choline concentrations (Table 3; Table 4). Neither supplementation during the prepartum period nor breed had a significant effect on the gross composition of colostrum (Table 2; Table 3). Parity had a significant effect on the gross composition of colostrum (Table 4) as third and greater parity animals produced significantly less TP than first or second parity animals (p=0.032), first or second parity animals produced similar colostrum TP. First parity animals

produced significantly less NPN than higher parity animals, second parity animals produced significantly less NPN than third and greater parity animals (p=0.001) which produced the highest NPN at 0.07 %. First parity animals produced similar NCN to second parity animals but significantly less NCN than third and greater parity animals. Second parity animals produced similar NCN to third and greater parity animals (p=0.035).

3.1.2 Proteins

Prepartum supplementation with INORG produced significantly less α_{s2}-CN (Table 2; p=0.008) in colostrum compared to animals supplemented with ORG or RPC, which produced similar concentrations of α_{s2}-CN. A similar trend was also observed for colostrum β-CN (p=0.015). Casein proteins (CNP) were combined (κ-CN, α_{s2}-CN, α_{s1}-CN and β-CN) to determine the effect of supplementation on the overall CNP. Animals supplemented with INORG produced significantly less (p=0.008) CNP compared to ORG or RPC, which produced similar CNP concentrations. The most abundant CNP in colostrum was α_{s1}-CN contributing 61.5, 64.1 and 63.9 % of the total CNP in the INORG, ORG and RPC, respectively. Prepartum supplementation had no significant effect on colostrum whey proteins (WP; (α-LA, β-LG A and B). The most abundant WP in colostrum was β-LG A contributing 47.5, 48.1 and 48.0 % of the total WP in the INORG, ORG and RPC, respectively. A moderate correlation was observed for both WP and total protein (R^2 = 0.440; p< 0.001), and CNP and total protein (R^2 = 0.860; p< 0.001) (Figure 1 – supplementary material). Breed had no significant effect on colostrum proteins (Table 3). Parity had a significant effect on colostrum IgG concentrations (Table 4; p=0.021), first and second parity animals produced similar IgG concentrations but significantly less than third and greater parity animals. In terms of the CNPs, first parity animals produced significantly less α_{s1}-CN (p=0.036), β-CN (p=0.012) and CNP (p=0.004) compared to higher parity animals. Second and third and greater parity animals produced similar α_{s1}-CN, β-CN and CNP. In terms of the whey proteins, first parity animals produced significantly less α-LA compared to third and greater parity animals (p=0.044). Second parity animals produced

similar α-LA to first and third and greater parity animals. First parity animals produced significantly less β-LG B (p=0.038) and WP (p=0.018) compared to higher parity animals. Second and third and greater parity animals produced similar β-LG B and WP.

3.1.3 Oligosaccharides

Prepartum supplementation had no significant effect on the concentrations of colostrum OS producing an average colostrum OS concentration of 2.90 (± 0.161) mg mL^{-1} (Table 2). The most abundant OS in colostrum was 3'-SL contributing 64.8, 63.7 and 65.4 % of the total colostrum OS in the INORG, ORG and RPC, respectively. Breed had a significant effect on 3-FL (Table 3; p=0.028) and 3'-SL concentrations (p=0.017), with JEX producing significantly higher concentrations compared to HF. Concentrations of 6'-SL tended to be higher in colostrum produced by JEX (p=0.068), producing 0.02 mg mL^{-1} more 6'-SL than HF. Total OS of colostrum were significantly affected by breed (p=0.033) with JEX producing colostrum with higher concentration of OS (+0.40 mg mL^{-1}) than HF. First parity animals produced significantly less 3-FL (Table 4; p=0.001) and DSLNT (p=0.001) compared to higher parity animals, second and third and greater parity animals produced similar 3-FL and DSLNT. First parity animals produced significantly less 6'-SL (p=0.035) compared to second parity animals, third and greater parity animals produced similar 6'-SL to first and second parity animals.

3.2 Transition Milk

3.2.1 Gross composition

Prepartum supplementation had no significant effect on the gross composition of TM. Each milking postpartum had a significant effect on TM gross composition (Table 5; Figure 2 – supplementary material; p=0.001). Total solids were highest in colostrum (27.6 (± 1.14) %) and decreased significantly by 23 % to TM1 with a further decrease of 22 % to TM2. Milk TS were similar for TM2 and TM3, TM3 was also similar to TM4 and TM5. With each milking, TrP significantly reduced from 15.4 (± 1.79) %

(colostrum) to 3.59 (± 0.16) % (TM5). A similar trend was observed for TP as it significantly reduced with each milking from 16.2 (± 1.11) % (colostrum) to 3.9 (± 0.09) % (TM5). Similar NPN was observed in colostrum and TM2 with significantly less NPN in TM1 compared to colostrum and TM2. Contents of NPN significantly reduced by 44.4 % from TM2 to TM3 and remained stable thereafter. With each milking, NCN significantly reduced, with highest NCN in colostrum (1.7 (± 0.13) %) and lowest observed in TM5 (0.2 (± 0.01) %). Contents of ash were highest in colostrum (3.5 (± 0.43) %) and decreased with each milking until TM3, ash was similar for TM3 and TM4 followed by a significant reduction of 26.4 % from TM4 to TM5.

3.2.2 Proteins

Prepartum supplementation had no significant effect on the protein composition of TM. Each milking postpartum had a significant effect on TM protein concentrations (Table 5; Figure 2 – supplementary material; p=0.001). Highest concentrations of IgG were observed in colostrum and significantly decreased by 84.2 % to TM1, with a further reduction of 56.6 % from TM1 to TM2. Concentrations of IgG were similar for TM2 and TM3 and significantly reduced from TM3 to TM5. Lowest IgG was observed in TM5 at 1.8 (± 0.16) mg mL^{-1}, a 98.3 % reduction in IgG was observed from colostrum to TM5. Highest concentrations of BSA were observed in colostrum with a 95.2 % reduction in BSA observed from colostrum to TM2. Concentrations of BSA were similar in TM2 and TM3 followed by a further 46.3 % reduction in BSA from TM3 to TM4 after which concentrations plateaued. Concentrations of κ-CN were highest in colostrum and decreased by 68.3 % until TM4 after which concentrations plateaued. Similar concentrations of α_{s2}-CN were observed in colostrum and TM1 with a significant reduction from TM1 to TM2. Similar concentrations of α_{s2}-CN were observed in TM2 and TM3, TM3 was also similar to TM5. Highest concentrations of α_{s1}-CN were observed in colostrum and reduced by 28.6 % in TM1 followed by a 19.9 % reduction from TM1 to TM2. Concentrations of α_{s1}-CN were similar in TM2 and TM5, TM3 and TM4 and TM3 was also similar to TM5. Concentrations of β-CN

significantly reduced by 54.8 % from colostrum to TM4 after which concentrations plateaued. Highest concentrations of CNP were observed in colostrum with a 54.1 % reduction to TM4. Concentrations of CNP in TM5 were similar in TM3 and TM4. Concentrations of α-LA were highest in colostrum and decreased with each milking, a 61.2 % reduction was observed from colostrum to TM3 after which values plateaued. A similar trend was observed for β-LG A. Concentrations of β-LG B were similar in colostrum and TM1 and significantly reduced by 61.7 % from TM1 to TM4, TM5 was similar in TM3 and TM4. Highest concentrations of WP was observed in colostrum and decreased with each milking until TM3. Concentrations of WP were similar in TM3 and TM4 followed by a further 4.3 % reduction from TM4 to TM5.

3.2.3 Oligosaccharides

Prepartum supplementation had no significant effect on the oligosaccharide composition of TM. Each milking postpartum had a significant effect on TM OS concentrations (Table 5; p=0.001) with 3'-SL being the most abundant OS throughout the TM. 3-FL was highest in colostrum and reduced by 84.8 % from colostrum to TM5. Sialic acid was lowest in colostrum and increased to TM1, TM1 was similar to TM2 and TM4, a significant reduction occurred from TM3 to TM4. Concentrations of sialic acid were similar in TM3 and TM5. Concentrations of 3'-SNL decreased with each milking until TM2 which was similar to TM3. 3'-SNL decreased from TM3 to TM4, TM4 was similar to TM5. Concentrations of 6'-SL significantly reduced by 63.6 % from colostrum to TM3 after which values plateaued. A similar trend was observed for DSLNT. Concentrations of 3'-SL significantly reduced by 88.7 % from colostrum to TM3. 3'-SL similar in TM3 and TM4, TM4 was also similar to TM5. A similar trend was observed for DSL. With each milking total OS significantly reduced, from 2.85 (± 0.067) mg mL^{-1} (colostrum) to 0.37 (± 0.002) mg mL^{-1} (TM5), an 87 % reduction in total OS from colostrum to TM5.

4. Discussion

Numerous studies have reported the effects of RPC supplementation on milk protein yield and content (Elek, Newbold, Gaal, Wagner, & Husveth, 2008; Sales, Homolka, & Koukolova, 2010). However, to the best of the authors' knowledge, no research has determined the effects of supplementing Se in different forms, or RPC on individual CN and whey proteins. This is also the first study which has examined the impact of prepartum supplementation with these three micro-nutrients has on OS in bovine colostrum and TM. Animals supplemented with INORG can be considered as a control as this mineral blend is characteristic of that typically provided in a commercial setting to pasture-based dairy cows.

4.1 Colostrum

Concentrations of Se and choline were quantified in colostrum only, as it is well established that minerals and nutrients decrease with each milking postpartum (Weiss & Hogan, 2005; Shah, Shah, Hassan, Yousif, & Wang, 2019; Van Emon, Sanford, & McCoski, 2020; Valldecabres & Silva-del-Río, 2022). As milk is the only source of Se available to the calf (Slavik et al., 2008), the levels of Se in colostrum produced by each of the prepartum supplementation groups was sufficient to meet neonatal requirements of 0.10 mg kg DM d^{-1} (NRC, 2001; Suttle, 2022). Previous research reported colostrum Se concentrations were not affected by supplementing different forms of Se (12.15 and 11.64 mg L^{-1} Se, for sodium selenite and Se-yeast, respectively) (Khalili, Chamani, Amanlou, Nikkhah, & Sadeghi, 2019). In contrast, Weiss & Hogan (2005) reported a significant increase in colostrum Se concentrations in animals supplemented with Se-yeast compared to sodium selenite (both supplemented at a rate of 0.3 mg Se kg^{-1}) for 60 d prior to calving. Ensuring calves receive colostrum with high levels of Se is critical as Mu et al. (2023) reported that Se deficiency can induce oxidative stress, inflammation and lung tissue damage in weaned calves. Variability between colostrum Se concentrations and the effects observed may be attributable to the basal level of Se in the diet or the duration and dose supplemented. An increase in colostrum Se concentrations may be observed if cows are deficient in Se (basal diet containing <0.05 mg kg DM^{-1} (Claude, 2002)) prior to supplementation.

Prepartum supplementation with RPC did not significantly affect colostrum choline concentrations. This finding is supported by Swartz et al. (2022) who reported that colostrum choline levels were not significantly affected regardless of the level of supplementation (30 g d^{-1} or 45 g d^{-1} of RPC). In contrast, Pinotti et al. (2003) reported that choline colostrum concentrations increased significantly (+39 % choline concentrations in colostrum) in animals supplemented with 20 g d^{-1} of RPC from 14 d prior to calving, these animals were also maintained on a total mixed ration (TMR) diet. The effects of RPC supplementation can be influenced by dietary protein supply and the availability of other nutrients (Hartwell, Cecava, & Donkin, 2000). As the amino acid profile of TMR and grass silage differs considerably (Phipps, Jones, Tingey, & Abeyasekera, 2005), this may alter the impact of supplemental RPC on colostrum choline concentrations which may explain the significant effect observed by Pinotti et al. (2003) which was not observed in the current study.

Colostrum quality is determined by the concentration of IgG and is considered good quality at ≥50 mg mL^{-1} (Kessler, Bruckmaier, & Gross, 2021). Each supplementation group within the current study produced colostrum greater than this threshold and greater than previously reported by Playford & Weiser (2021) whose values ranged from 30 – 87 mg mL^{-1}. Although Playford & Weiser (2021) reported colostrum IgG concentrations with no specific detail on the animals or the nutrition management, various factors influence colostrum IgG concentrations one of which is the interval between parturition and colostrum collection (Conneely et al., 2013). It is critical that colostrum is collected immediately following parturition, like animals in the current study, as delayed colostrum collection may affect the IgG concentrations (Moore, Tyler, Chigerwe, Dawes, & Middleton, 2005). Higher colostrum IgG concentrations in the current study may also be attributable to the pasture-based system, as it is well-known that pasture-based dairy cows produce lower milk yields than animals on a TMR diet (Bargo,

449 Muller, Delahoy, & Cassidy, 2002; White, Benson, Washburn, & Green Jr, 2002), causing pasture-based
450 dairy cows to produce a more concentrated colostrum than TMR cows.
451
452 Supplementation with Se in the inorganic or organic form had no significant effect on colostrum IgG
453 concentrations, this has also been reported in previous studies which have demonstrated that the source of
454 Se has no effect on colostral IgG concentrations (Koenig & Beauchemin, 2009; Juniper, Rymer, &
455 Briens, 2019). Zenobi et al. (2018) supplemented cows with 60 g d^{-1} RPC and reported an increase in
456 colostrum IgG concentrations when compared to animals receiving 0 g d^{-1} of RPC. Although animals in
457 the Zenobi et al. (2018) study were supplemented with a significantly higher dose of RPC (+40 g d^{-1}) than
458 animals in the current study (20 g d^{-1}), similar colostrum IgG concentrations were produced in both
459 studies. This may indicate that supplementing a higher dose of RPC may not cause an increase in IgG
460 concentrations as Zenobi et al. (2018) compared their results to animals receiving 0 or 60 g d^{-1} of RPC
461 whereas, the IgG results of the RPC group in the current study were compared to animals supplemented
462 with INORG or ORG.
463
464 Numerous factors affect milk protein composition these include; seasonal variability (Gellrich et al.,
465 2014), body condition of the cow (Ostersen, Foldager, & Hermansen, 1997), genetic variation
466 (Gustavsson et al., 2014) and diet (Kennedy, O'Donovan, Murphy, Delaby, & O'Mara, 2005). The impact
467 of prepartum supplementation with INORG, ORG or RPC on κ-CN, α_{s2}-CN, α_{s1}-CN and β-CN was
468 evaluated as these are the four most predominant CNs within bovine milk (Visker et al., 2011; Hinz,
469 O'Connor, Huppertz, Ross, & Kelly, 2012). In conjunction with this, limited research exists on the
470 analysis of these individual CN proteins in bovine colostrum. Selenium is involved in many cellular
471 processes, including protein folding (Steinbrenner, Speckmann, & Klotz, 2016) and is associated with
472 both the whey and CN protein fractions of milk. Mathias, Hogue, & Loosli (1967) reported that 60 % of
473 Se in milk is present in the CN fraction which may explain the significant impact of supplementing ORG

474 on the various CNP in the current study. Chabaev et al. (2022) reported that supplementing organic Se at
475 0.37 mg kg d^{-1} was not sufficient to cause a significant increase in milk CN levels. This was reflected in
476 the current study as animals supplemented with ORG at a higher rate of 0.5 mg kg d^{-1} produced colostrum
477 with significantly higher concentrations of α_{s2}-CN, β-CN and CNP, compared to animals supplemented
478 with INORG.
479
480 Numerous studies have reported the effects of RPC supplementation on milk protein yield and content
481 (Elek, Newbold, Gaal, Wagner, & Husveth, 2008; Sales, Homolka, & Koukolova, 2010). Jayaprakash,
482 Sathiyabarathi, Robert, & Tamilmani (2016) reported that increases in milk protein observed in animals
483 supplemented with RPC were also due to elevated levels of milk CN. This work, however, does not
484 provide a biological explanation. Pinotti, Baldi, & Dell'Orto (2002) suggested that RPC acts as a methyl
485 donor, which provides additional methionine for protein synthesis in the mammary gland. The present
486 study suggests, which has not been previously reported in the literature, that the increases in milk protein
487 following the prepartum supplementation with ORG or RPC may be predominantly attributable to
488 changes in the CN fraction. This was reflected in the stronger correlation observed between CNP and TP,
489 compared to WP and TP. This stronger correlation is related to CN dominating bovine milk proteins.
490
491 Breed did not have a significant impact on colostrum proteins but had a significant effect on the
492 concentrations of 3-FL, 3'-SL and total OS in colostrum. Sundekilde et al. (2012) reported a significantly
493 higher level of fucosylated OS, including 3'FL, in milk produced by Jersey compared to Friesian cows
494 which concurred with results in the current study. The most abundant OS in bovine milk is 3'-SL (Liu,
495 Auldist, Wright, Cocks, & Rochfort, 2017), which in this study was found in colostrum but not in mature
496 milk. Robinson et al. (2019) reported that milk obtained from Jersey cows contained more 3'-SL than
497 their HF counterparts, an effect also observed in this study. Although JEX exhibit greater variation in OS
498 types, it also produces milk with a greater total concentration of OS (Tao, DePeters, German, Grimm, &

499 Lebrilla, 2009) which was also observed in the current study. Targeting colostrum produced from JEX
500 animals may be beneficial as a number of OS have been shown to have beneficial, prebiotic properties
501 (Yu, Chen, & Newburg, 2013). Parity had a significant impact on numerous proteins and OS in
502 colostrum. Fahey, Fischer, Steele, & Greenwood (2020) also observed significant differences in the levels
503 of colostrum proteins between primiparous and multiparous cows. The physiology of the mammary gland
504 is different between primiparous and multiparous animals and undergoes dramatic changes during puberty
505 and the prepartum period (Lang, Iverson, & Bowen, 2012). The mammary gland of multiparous cows
506 have a higher density of secretory cells (Miller et al., 2006) responsible for protein secretion which may
507 explain the lower concentrations of proteins produced by first parity animals in the current study. Miller
508 et al. (2006) also found that the metabolic and secretory activity of mammary gland is higher in
509 multiparous animals. Fischer-Tlustos et al. (2020) observed an effect of parity on colostrum OS and
510 hypothesised that the maturity of the mammary gland may be responsible for the higher concentrations of
511 certain OS compared to primiparous cows. Results from the current study support this hypothesis as first
512 parity animals produced significantly less 3-FL and DSLNT compared to higher parity animals.
513
514 4.2 Transition Milk
515 In comparison to mature milk, colostrum contains about five times more protein with a total content of
516 around 15 % (Playford & Weiser, 2021). This corresponds to the current study in which the average
517 colostrum TP content was 14.9 (± 0.53) %. Li et al. (2011) reported NPN values ranging from 0.02 – 0.04
518 % and NCN from 0.08 – 0.16 %, which also compares well with the findings of this study. Similar
519 colostrum TS levels were observed by Kehoe, Jayarao, & Heinrichs (2007) at 27.6 % and by Fischer-
520 Tlustos et al. (2020) at 26.7 %. A similar trend in milk TS was also observed by Fischer-Tlustos et al.
521 (2020) in which the decline in milk TS was significant from colostrum to TM1 and from TM1 to TM2
522 with TS plateauing from TM3. Milk TS at TM5 of the current study (14.4 (± 0.46) %) was higher than
523 reported by Fischer-Tlustos et al. (2020) at their equivalent of TM5 (13.6 %). The higher TS at TM5 of

524 the current study may be attributed to the inclusion of JEX animals in the current study as Fischer-Tlustos
525 et al. (2020) enrolled HF alone. Ash content in colostrum (3.49 (± 0.43) %) was higher than previously
526 reported by Godden (2008) at 1.11 %. Animals in the current study produced colostrum with higher TS,
527 TP and lower lactose compared to the TS (23.9 %), TP (14.0 %) and lactose (2.7 %) reported by Godden
528 (2008) this explains the higher ash content observed. The decrease in TS, TP and ash contents with each
529 milking postpartum is well described in the literature (Godden, 2008; McGrath, Fox, McSweeney, &
530 Kelly, 2016).
531
532 As antibodies are large molecules and the permeability of the calf intestine decreases rapidly following
533 birth (Bellof & Granz, 2018), decreasing concentrations of IgG in TM may be related to such decreases in
534 permeability. The greatest decrease in IgG concentrations was observed from colostrum to TM1, previous
535 research has also reported that the greatest decrease in IgG concentrations occurs from colostrum to TM1
536 followed by another significant reduction in IgG concentrations from TM1 to TM2 (Conneely et al.,
537 2011). Concentrations of IgG at TM5 were similar to the maximum range observed by Conesa et al.
538 (2005). A further reduction in IgG concentrations may have been observed if additional milk samples
539 were collected to reflect the IgG concentrations reported by Cakebread, Humphrey, & Hodgkinson (2015)
540 as Levieux (1999) reported that milk IgG reaches its lowest concentration between the second and third
541 month of lactation. It is well established in the literature that IgG secretion into the blood markedly
542 decreases following parturition resulting in the decrease in IgG concentrations over the initial days
543 postpartum (Herr, Bostedt, & Failing, 2011). Lactose concentration in mature bovine milk is typically in
544 the region of 47 – 50 g L^{-1} (Silanikove, Leitner, & Merin, 2015). As lactose increases with each milking
545 postpartum and IgG concentrations decrease concomitantly, Dunn et al. (2017) reported the increase in
546 lactose may cause a dilution effect and result in the reduction of IgG concentrations. Low lactose
547 concentrations ensure colostrum is a concentrated IgG solution for the neonate. Zabielski, Le Huërou-
548 Luron, & Guilloteau (1999) also found low concentrations of lactase in the neonate, which from a

549 biological perspective, coincides with low lactose concentrations in colostrum. Mature milk has BSA
550 concentrations of about 0.4 mg mL^{-1} (Madureira, Pereira, Gomes, Pintado, & Malcata, 2007), similar to
551 the concentrations observed at TM5. Fuquay, McSweeney, & Fox (2011) suggested that BSA is a leakage
552 protein from blood with no significant biological function in milk.
553
554 Karman & Van Boekel (1986) reported concentrations in mature milk of 10.0, 2.6, 9.3 and 3.3 mg mL^{-1}
555 for α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN, respectively. The values observed at TM5 of the current study are
556 considerably higher and although Karman & Van Boekel (1986) did not provide information on the parity
557 of the animals, the higher protein concentration observed in the current study may be attributable to the
558 inclusion of animals of various parities or due to the lower yields and higher milk solids of these pasture-
559 based cows. It may also be the pasture-based aspect of this study as higher protein concentrations may be
560 observed as a cause of the reduced milk yields if the animals in the Karman & Van Boekel (1986) study
561 were maintained on a TMR diet. In the current study the most predominant CN protein was α_{s1}-CN and
562 the most abundant whey proteins were α-LA and β-LG which was previously observed by Ginger &
563 Grigor (1999) and Corredig, Nair, Li, Eshpari, & Zhao (2019) in mature milk. These major whey proteins
564 make up 25 and 65 % of the total whey proteins in mature milk respectively (De Kruif, Huppertz, Urban,
565 & Petukhov, 2012). Chatterton, Nguyen, Bering, & Sangild (2013) demonstrated that the variation of CN
566 in mature bovine milk to be 2~30 g L^{-1} with Tziboula-Clarke (2003) reporting mature milk having a total
567 CN of 2.4 – 2.8 %/*wt*. The limited availability of research on the CN and whey fractions of bovine
568 colostrum makes comparison difficult. Previous research has presented changes in protein fractions semi-
569 quantitatively. The current study, in contrast, provides quantitative data, with analysis of individual CN
570 and whey proteins in bovine colostrum in detail, not previously reported in the literature.
571
572 Previous research reporting the change occurring in OS concentrations as milk transitions from colostrum
573 to mature milk have been carried out under the TMR system (Fischer-Tlustos et al., 2020) or have not

574 collected milk samples consecutively following calving (Quinn et al., 2020). Bovine colostrum is a rich
575 source of OS with concentrations observed at 2.9 (± 0.16) mg mL^{-1} in the current study. This is
576 significantly higher than the range previously reported by (Nakamura et al., 2003) at 0.7 – 1.2 mg mL^{-1},
577 the differences in colostrum OS concentrations may be attributable to the inclusion of JEX animals in the
578 current study as Nakamura et al. (2003) enrolled HF animals alone. Previous research has reported lower
579 concentrations of bovine colostrum 3'FL and also, concentrations of 3-FL lower in respective to
580 concentrations of colostrum 3'SL than what is reported in the current study however, these concentrations
581 were observed in colostrum derived from cows not maintained on a pasture-based diet as in the current
582 study (Zhang et al., 2023). One such study which was used as a reference for this study was Quinn et al.
583 (2020) which also quantified numerous OS in bovine colostrum obtained from pasture-based dairy cows.
584 Quinn et al. (2020) reported similar concentrations of 3-FL (0.73 mg mL^{-1}) and 3'SL (0.79 mg mL^{-1}) in
585 bovine colostrum, a trend which was also observed in the current study (3-FL; 0.81 mg mL^{-1} and 3'SL;
586 1.03 mg mL^{-1}). Although the concentrations of both 3-FL and 3'SL were higher than that was reported by
587 Quinn et al. (2020), a possible explanation for this is the inclusion of JEX animals in the current study as
588 the afore mentioned OS were significantly higher in JEX animals compared to HF whereas, Quinn et al.
589 (2020) enrolled HF animals alone which resulted in a lower concentration of these OS.
590
591 The concentration of OS in mature milk has been reported at 0.3 – 0.5 g L^{-1} (ten Bruggencate, Bovee-
592 Oudenhoven, Feitsma, van Hoffen, & Schoterman, 2014) which is comparable to the values observed at
593 TM5 in the current study. Bacteria from the environment can enter colostrum and bind to IgG with the
594 effect of impairing intestinal absorption of IgG (Cummins, Berry, Murphy, Lorenz, & Kennedy, 2017).
595 Oligosaccharides are also capable of binding to bacteria which therefore may allow the effect of blocking
596 IgG-bacterial interactions and subsequently promoting IgG absorption (Short, Moore, & Sischo, 2016).
597 Oligosaccharides have been shown to block the entry of *Cryptosporidium, Clostridia* and *Salmonella* all
598 of which cause scour in calves (Suler, Mullins, Rudge, & Ashurst, 2016; Rukambile, Sintchenko,

599 Muscatello, Kock, & Alders, 2019; Gull, 2022). Fischer, Malmuthuge, & Steele (2018) demonstrated that
600 high concentrations of OS may promote beneficial gut bacteria in the neonate. Therefore, the improved
601 understanding of the compositional change occurring in OS from colostrum to TM, reported in the current
602 study, provides additional information on the role of OS in promoting calf immunity (Fischer-Tlustos et
603 al., 2020).
604
605 5. Conclusion
606 Supplementing INORG, ORG or RPC during the prepartum period did not effect colostrum OS.
607 Supplementing dairy cows with ORG or RPC during the prepartum period produced colostrum with
608 significantly higher concentrations of α_{s2}-CN, β-CN and total CN. Colostrum with sufficient selenium and
609 IgG to meet neonatal requirements was produced from cows across the three treatments. This study
610 quantified individual CN and whey proteins in bovine colostrum while transitioning into mature milk,
611 previously not reported in the literature. The changes observed in the protein and OS composition with each
612 milking postpartum enables a clear distinction to be made between colostrum and TM.
613
614 6. Acknowledgements
615 This research has emanated from research conducted with the financial support of Teagasc, the Irish
616 Agriculture and Food Development Authority, Science Foundation Ireland (SFI) and the Department of
617 Agriculture, Food and Marine (DAFM) on behalf of the Government of Ireland under Grant Number
618 [16/RC/3835] - VistaMilk.
619
620 7. References
621 Abuelo, A., Hernández, J., Benedito, J. L., & Castillo, C. (2019). Redox biology in transition periods of
622 dairy cattle: Role in the health of periparturient and neonatal animals. *Antioxidants, 8,* 20.

623 Aldredge, D. L., Geronimo, M. R., Hua, S., Nwosu, C. C., Lebrilla, C. B., & Barile, D. (2013).
624 Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel
625 fucosylated structures. *Glycobiology*, *23,* 664 – 676.
626 Baldi, A., & Pinotti, L. (2006). Choline metabolism in high-producing dairy cows: Metabolic and
627 nutritional basis. *Canadian Journal of Animal Science, 86,* 207 – 212.
628 Bargo, F., Muller, L., Delahoy, J., & Cassidy, T. (2002). Performance of high producing dairy cows with
629 three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science, 85,*
630 2948 – 2963.
631 Barry, J., Bokkers, E. A., Berry, D. P., de Boer, I. J., McClure, J., & Kennedy, E. (2019). Associations
632 between colostrum management, passive immunity, calf-related hygiene practices, and rates of mortality
633 in preweaning dairy calves. *Journal of Dairy Science, 102,* 10266 – 10276.
634 Bellof, G., & Granz, S. (2018). Tierproduktion: Nutztiere züchten, halten und ernähren. *Georg Thieme*
635 *Verlag.*
636 Berry, D., Shalloo, L., Cromie, A., Veerkamp, R., Dillon, P., Amer, P., et al. (2007). The economic
637 breeding index: a generation on. Technical report to the Irish Cattle Breeding Federation. *Irish Cattle*
638 *Breed Federation,* 1 – 50.
639 Cakebread, J. A., Humphrey, R., & Hodgkinson, A. J. (2015). Immunoglobulin A in bovine milk: a
640 potential functional food? *Journal of Agricultural and Food Chemistry, 63,* 7311 – 7316.
641 Chabaev, M., Petrushkin, D., Zelenchenkova, A., Tsis, E., Dovydenkova, M., & Nekrasov, R. (2022). Use
642 of Organic Selenium Supplementation in the Diet of Lactating Cows Under Conditions of Intensive Milk
643 Production. Pages 113-122 in Proc. *Agriculture Digitalization and Organic Production: Proceedings of*
644 *the Second International Conference*, ADOP 2022, St. Petersburg, Russia, June 06–08, 2022. Springer.
645 Chatterton, D. E., Nguyen, D. N., Bering, S. B., & Sangild, P. T. (2013). Anti-inflammatory mechanisms
646 of bioactive milk proteins in the intestine of newborns. *The International Journal of Biochemistry & Cell*
647 *Biology, 45,* 1730 – 1747.

Clare, D., Catignani, G., & Swaisgood, H. (2003). Biodefense properties of milk: the role of antimicrobial proteins and peptides. *Current Pharmaceutical Design, 9,* 1239 – 1255.

Claude, J. (2002). Introduction à la nutrition des animaux domestiques. Tec & Doc/EM Inter: Paris, France.

Conesa, C., Lavilla, M., Sánchez, L., Pérez, M., Mata, L., Razquín, P., et al. (2005). Determination of IgG levels in bovine bulk milk samples from different regions of Spain. *European Food Research and Technology, 220,* 222 – 225.

Conneely, M., Berry, D., Lorenz, I., Doherty, M., & Kennedy, E. (2011). The change in immunoglobulin G concentration over the first six milkings in Irish Holstein-Friesian dairy cows. *Moorepark Research Report 2011, 38.*

Conneely, M., Berry, D., Sayers, R., Murphy, J., Lorenz, I., Doherty, M., et al. (2013). Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. Animal, 7, 1824 – 1832.

Corredig, M., Nair, P. K., Li, Y., Eshpari, H., & Zhao, Z. (2019). Invited review: Understanding the behavior of caseins in milk concentrates. *Journal of Dairy Science, 102,* 4772 – 4782.

Council, N. R. (2001). NRC Requirements of Dairy Cattle Seventh Revised Edition, 2001. *The National Academies Press: Washington, DC, USA.*

Cummins, C., Berry, D., Murphy, J., Lorenz, I., & Kennedy, E. (2017). The effect of colostrum storage conditions on dairy heifer calf serum immunoglobulin G concentration and preweaning health and growth rate. *Journal of Dairy Science, 100,* 525 – 535.

De Kruif, C. G., Huppertz, T., Urban, V. S., & Petukhov, A. V. (2012). Casein micelles and their internal structure. *Advances in Colloid and Interface Science, 171,* 36 – 52.

Deeth, H., & Bansal, N. (2019). Whey proteins: an overview. *Whey proteins*, 1 – 50.

Dunn, A., Ashfield, A., Earley, B., Welsh, M., Gordon, A., & Morrison, S. (2017). Evaluation of factors associated with immunoglobulin G, fat, protein, and lactose concentrations in bovine colostrum and

colostrum management practices in grassland-based dairy systems in Northern Ireland. *Journal of Dairy Science, 100,* 2068 – 2079.

Durham, S. D., Lemay, D. G., Wei, Z., Kalscheur, K. F., Finley, J. W., Fukagawa, N. K., et al. (2022). Dietary fiber to starch ratio affects bovine milk oligosaccharide profiles. *Current Developments in Nutrition*, *6,* nzac033.

EC—European Commission. (2013). COMMISSION IMPLEMENTING REGULATION (EU) No 427/2013 of 8 May 2013 concerning the authorisation of selenomethionine produced by. Saccharomyces cerevisiae.

Elek, P., Newbold, J., Gaal, T., Wagner, L., & Husveth, F. (2008). Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. *Animal, 2,* 1595 – 1601.

Fahey, M. J., Fischer, A. J., Steele, M. A., & Greenwood, S. L. (2020). Characterization of the colostrum and transition milk proteomes from primiparous and multiparous Holstein dairy cows. *Journal of Dairy Science, 103,* 1993 – 2005.

Fischer, A. J., Malmuthuge, N., & Steele, M. A. (2018). The effect of heat treatment of bovine colostrum on the concentration of oligosaccharides in colostrum and in the intestine of neonatal male Holstein calves. *Journal of Dairy Science, 101,* 401 – 407.

Fischer-Tlustos, A., Hertogs, K., Van Niekerk, J., Nagorske, M., Haines, D., & Steele, M. (2020). Oligosaccharide concentrations in colostrum, transition milk, and mature milk of primi-and multiparous Holstein cows during the first week of lactation. *Journal of Dairy Science, 103,* 3683 – 3695.

Franzoi, M., Niero, G., Visentin, G., Penasa, M., Cassandro, M., & De Marchi, M. (2019). Variation of detailed protein composition of cow milk predicted from a large database of mid-infrared spectra. *Animals, 9,* 176.

Fuquay, J. W., McSweeney, P. L., & Fox, P. F. (2011). Encyclopedia of Dairy Sciences. Academic Press.

Gill, R. K., Mahmood, S., Nagpaul, J. P., & Mahmood, A. (1999). Functional role of sialic acid in IgG binding to microvillus membranes in neonatal rat intestine. *Neonatology, 76,* 55 – 64.

Ginger, M. R., & Grigor, M. R. (1999). Comparative aspects of milk caseins. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 124,* 133 – 145.

Godden, S. (2008). Colostrum management for dairy calves. *Veterinary Clinics of North America: Food Animal Practice, 24,* 19 – 39.

Gopal, P. K., & Gill, H. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. *British Journal of Nutrition, 84,* 69 – 74.

Gull, T. (2022). Bacterial Causes of Intestinal Disease in Dairy Calves: Acceptable Control Measures. *Veterinary Clinics: Food Animal Practice*, *38*,107 – 119.

Gustavsson, F., Buitenhuis, A. J., Johansson, M., Bertelsen, H. P., Glantz, M., Poulsen, N. A., et al. (2014). Effects of breed and casein genetic variants on protein profile in milk from Swedish Red, Danish Holstein, and Danish Jersey cows. *Journal of Dairy Science, 97,* 3866 – 3877.

Hartwell, J., Cecava, M., & Donkin, S. (2000). Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. *Journal of Dairy Science, 83,* 2907 – 2917.

Herr, M., Bostedt, H., & Failing, K. (2011). IgG and IgM levels in dairy cows during the periparturient period. *Theriogenology, 75,* 377 – 385.

Hinz, K., O'Connor, P. M., Huppertz, T., Ross, R. P., & Kelly, A. L. (2012). Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. *Journal of Dairy Research, 79,* 185 – 191.

Jayaprakash, G., Sathiyabarathi, M., Robert, M. A., & Tamilmani, T. (2016). Rumen-protected choline: A significance effect on dairy cattle nutrition. *Veterinary World*, *9*, 837.

Jeurink, P. V., van Esch, B. C., Rijnierse, A., Garssen, J., & Knippels, L. M. (2013). Mechanisms underlying immune effects of dietary oligosaccharides. *The American Journal of Clinical Nutrition, 98,* 572S – 577S.

721 Jin, Y., Li, H., & Wang, H. (2021). Dietary rumen-protected choline supplementation regulates blood
722 biochemical profiles and urinary metabolome and improves growth performance of growing lambs.
723 *Animal Biotechnology,* 1 – 11.
724 Juniper, D. T., Phipps, R. H., Jones, A. K., & Bertin, G. (2006). Selenium supplementation of lactating
725 dairy cows: effect on selenium concentration in blood, milk, urine, and feces. *Journal of Dairy*
726 *Science*, *89,* 3544 – 3551.
727 Juniper, D. T., Rymer, C., & Briens, M. (2019). Bioefficacy of hydroxy-selenomethionine as a selenium
728 supplement in pregnant dairy heifers and on the selenium status of their calves. *Journal of Dairy Science,*
729 *102,* 7000 – 7010.
730 Karman, A., & Van Boekel, M. S. (1986) Evaluation of the Kjeldahl factor for conversion of the nitrogen
731 content of milk and milk products to protein content. Netherlands Milk and Dairy Journal, 40, 315 – 336.
732 Kehoe, S., Jayarao, B., & Heinrichs, A. (2007). A survey of bovine colostrum composition and colostrum
733 management practices on Pennsylvania dairy farms. *Journal of Dairy Science, 90,* 4108 – 4116.
734 Kennedy, E., O'Donovan, M., Murphy, J. P., Delaby, L., & O'Mara, F. (2005). Effects of grass pasture
735 and concentrate-based feeding systems for spring-calving dairy cows in early spring on performance
736 during lactation. *Grass and Forage Science, 60,* 310 – 318.
737 Kessler, E., Bruckmaier, R., & Gross, J. J. (2021). Comparative estimation of colostrum quality by Brix
738 refractometry in bovine, caprine, and ovine colostrum. *Journal of Dairy Science, 104,* 2438 – 2444.
739 Khalili, M., Chamani, M., Amanlou, H., Nikkhah, A., & Sadeghi, A. (2019). The effect of feeding
740 inorganic and organic selenium sources on the performance and content of selenium in milk of transition
741 dairy cows. *Acta Scientiarum. Animal Sciences, 41.*
742 Koenig, K. M., & Beauchemin, K. A. (2009). Supplementing selenium yeast to diets with adequate
743 concentrations of selenium: Selenium status, thyroid hormone concentrations and passive transfer of
744 immunoglobulins in dairy cows and calves. *Canadian Journal of Animal Science, 89,* 111 – 122.

745 Lang, S. L., Iverson, S. J., & Bowen, W. D. (2012). Primiparous and multiparous females differ in
746 mammary gland alveolar development: implications for milk production. *Journal of Experimental*
747 *Biology, 215,* 2904 – 2911.
748 Levieux, D. (1999). Le colostrum, un lait particulièrement riche en de nombreux composants: peut-on en
749 déceler la présence dans les livraisons de lait de vache?. *Le lait, 79,* 465 – 488.
750 Levieux, D., & Ollier, A. (1999). Bovine immunoglobulin G, β-lactoglobulin, α-lactalbumin and serum
751 albumin in colostrum and milk during the early post partum period. *Journal of Dairy Research, 66,* 421 –
752 430.
753 Li, H., Ma, Y., Li, Q., Wang, J., Cheng, J., Xue, J., et al. (2011). The chemical composition and nitrogen
754 distribution of Chinese yak (Maiwa) milk. *International Journal of Molecular Sciences, 12,* 4885 – 4895.
755 Liu, Z., Auldist, M., Wright, M., Cocks, B., & Rochfort, S. (2017). Bovine milk oligosaccharide contents
756 show remarkable seasonal variation and intercow variation. Journal of Agricultural and Food Chemistry,
757 65, 1307 – 1313.
758 Madureira, A. R., Pereira, C. I., Gomes, A. M., Pintado, M. E., & Malcata, F. X. (2007). Bovine whey
759 proteins–Overview on their main biological properties. *Food Research International, 40,* 1197 – 1211.
760 Magan, J. B., Tobin, J. T., O'Callaghan, T. F., Kelly, A. L., Fenelon, M. A., Hennessy, D., et al. (2019).
761 Physicochemical properties of whole milk powder derived from cows fed pasture or total mixed ration
762 diets. *Journal of Dairy Science*, *102,* 9611 – 9621.
763 Martín, M. J., Martín-Sosa, S., & Hueso, P. (2002). Binding of milk oligosaccharides by several
764 enterotoxigenic Escherichia coli strains isolated from calves. *Glycoconjugate Journal, 19,* 5 – 11.
765 Mathias, M., Hogue, D., & Loosli, J. (1967). The biological value of selenium in bovine milk for the rat
766 and chick. *The Journal of Nutrition, 93,* 14 – 20.
767 McGrath, B. A., Fox, P. F., McSweeney, P. L., & Kelly, A. L. (2016). Composition and properties of
768 bovine colostrum: a review. *Dairy Science & Technology, 96,* 133 – 158.

McKay, Z. C., Mulligan, F. J., Brady, E. L., O'Sullivan, M., Rajauria, G., Lynch, M. B., et al. (2022). Impact of a total mixed ration or pasture/pasture silage-based feeding strategy in the initial stages of lactation of spring-calving dairy cows on milk production, composition and selected milk processability parameters.

Mehra, R., Singh, R., Nayan, V., Buttar, H. S., Kumar, N., Kumar, S., et al. (2021). Nutritional attributes of bovine colostrum components in human health and disease: a comprehensive review. *Food Bioscience, 40,* 100907.

Miller, N., Delbecchi, L., Petitclerc, D., Wagner, G., Talbot, B., & Lacasse, P. (2006). Effect of stage of lactation and parity on mammary gland cell renewal. Journal of Dairy Science, 89, 4669 – 4677.

Mohsen, M. K., Gaafar, H. M. A., Khalafalla, M. M., Shitta, A. A., & Yousif, A. M. (2011). Effect of rumen protected choline supplementation on digestibility, rumen activity and milk yield in lactating Friesian cows. *Slovak Journal of Animal Science*, *44*, 13 - 20.

Moore, M., Tyler, J. W., Chigerwe, M., Dawes, M. E., & Middleton, J. R. (2005). Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. *Journal of the American Veterinary Medical Association, 226,* 1375 – 1377.

Mu, J., Lei, L., Zheng, Y., Liu, J., Li, J., Li, D., et al. (2023). Oxidative Stress Induced by Selenium Deficiency Contributes to Inflammation, Apoptosis and Necroptosis in the Lungs of Calves. *Antioxidants, 12,* 796.

Nakamura, T., Kawase, H., Kimura, K., Watanabe, Y., Ohtani, M., Arai, I., et al. (2003). Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. *Journal of Dairy Science, 86,* 1315 – 1320.

O'Brien, D., Moran, B., & Shalloo, L. (2018). A national methodology to quantify the diet of grazing dairy cows. *Journal of Dairy Science, 101,* 8595 – 8604.

O'Callaghan, T., Hennessy, D., McAuliffe, S., Sheehan, D., Kilcawley, K., Dillon, P., et al. (2018). The effect of cow feeding system on the composition and quality of milk and dairy products. *Sustainable Meat and Milk Production from Grasslands,* 762.

Osorio, J. S., Trevisi, E., Li, C., Drackley, J. K., Socha, M. T., & Loor, J. J. (2016). Supplementing Zn, Mn, and Cu from amino acid complexes and Co from cobalt glucoheptonate during the peripartal period benefits postpartal cow performance and blood neutrophil function. *Journal of Dairy Science, 99,* 1868 – 1883.

Ostersen, S., Foldager, J., & Hermansen, J. E. (1997). Effects of stage of lactation, milk protein genotype and body condition at calving on protein composition and renneting properties of bovine milk. *Journal of Dairy Research, 64,* 207 – 219.

Phipps, R., Jones, A., Tingey, A., & Abeyasekera, S. (2005). Effect of corn silage from an herbicide-tolerant genetically modified variety on milk production and absence of transgenic DNA in milk. *Journal of Dairy Science, 88,* 2870 – 2878.

Pinotti, L., Baldi, A., & Dell'Orto, V. (2002). Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow. *Nutrition Research Reviews, 15,* 315 – 332.

Pinotti, L., Baldi, A., Politis, I., Rebucci, R., Sangalli, L., & Dell'Orto, V. (2003). Rumen-protected choline administration to transition cows: effects on milk production and vitamin E status. *Journal of Veterinary Medicine Series A, 50*, 18 – 21.

Playford, R. J., & Weiser, M. J. (2021). Bovine colostrum: Its constituents and uses. *Nutrients, 13*, 265.

Poulsen, N. A., Robinson, R. C., Barile, D., Larsen, L. B., & Buitenhuis, B. (2019). A genome-wide association study reveals specific transferases as candidate loci for bovine milk oligosaccharides synthesis. *BMC Genomics, 20,* 1 – 15.

Quinn, E. M., O'Callaghan, T. F., Tobin, J. T., Murphy, J. P., Sugrue, K., Slattery, H., et al. (2020). Changes to the oligosaccharide profile of bovine milk at the onset of lactation. *Dairy, 1,* 284 – 296.

816 Respati, A. N., Yanza, Y. R., Yano, A. A., Astuti, D., Ningsih, N., Purnamayanti, L., et al. (2023). Meta-
817 analysis of the effects of dietary sources of selenium on lactational performance and oxidative status of
818 dairy cows. *Animal Feed Science and Technology*, *305*, 115782.
819 Robinson, R. C., Poulsen, N. A., Colet, E., Duchene, C., Larsen, L. B., & Barile, D. (2019). Profiling of
820 aminoxyTMT-labeled bovine milk oligosaccharides reveals substantial variation in oligosaccharide
821 abundance between dairy cattle breeds. *Scientific Reports, 9,* 1 – 10.
822 Rukambile, E., Sintchenko, V., Muscatello, G., Kock, R., & Alders, R. (2019). Infection, colonization and
823 shedding of Campylobacter and Salmonella in animals and their contribution to human disease: A
824 review. *Zoonoses and Public Health*, *66,* 562 – 578.
825 Sales, J., Homolka, P., & Koukolova, V. (2010). Effect of dietary rumen-protected choline on milk
826 production of dairy cows: A meta-analysis. *Journal of Dairy Science, 93,* 3746 – 3754.
827 Sats, A., Kaart, T., & Jõudu, I. (2023). Bovine Colostrum Casein: Post-Partum Changes in Micelle Size,
828 Content and Related Traits. *Content and Related Traits.*
829 Schrauzer, G. N. (2003). The nutritional significance, metabolism and toxicology of selenomethionine.
830 *Advances in Food and Nutrition Research, 47,* 73 – 112.
831 Shah, A. M., Shah, A. R., Hassan, M. F., Yousif, M., & Wang, Z. (2019). Colostrum composition and its
832 importance to the health of animals-a review. *Punjab University Journal of Zoology, 34,* 197 – 206.
833 Short, D. M., Moore, D. A., & Sischo, W. M. (2016). A randomized clinical trial evaluating the effects of
834 oligosaccharides on transfer of passive immunity in neonatal dairy calves. *Journal of Veterinary Internal*
835 *Medicine*, *30,* 1381 – 1389.
836 Silanikove, N., Leitner, G., & Merin, U. (2015). The interrelationships between lactose intolerance and
837 the modern dairy industry: global perspectives in evolutional and historical backgrounds. *Nutrients, 7,*
838 7312 – 7331.

Slavik, P., Illek, J., Brix, M., Hlavicova, J., Rajmon, R., & Jilek, F. (2008). Influence of organic versus inorganic dietary selenium supplementation on the concentration of selenium in colostrum, milk and blood of beef cows. *Acta Veterinaria Scandinavica, 50,* 1 – 6.

Sobczuk-Szul, M., Wielgosz-Groth, Z., Wronski, M., & Rzemieniewski, A. (2013). Changes in the bioactive protein concentrations in the bovine colostrum of Jersey and Polish Holstein–Friesian cows. *Turkish Journal of Veterinary & Animal Sciences, 37,* 43 – 49.

Steinbrenner, H., Speckmann, B., & Klotz, L. O. (2016). Selenoproteins: Antioxidant selenoenzymes and beyond. *Archives of Biochemistry and Biophysics, 595,* 113 – 119.

Suler, D., Mullins, D., Rudge, T., & Ashurst, J. (2016). Cryptosporidium parvum infection following contact with livestock. *North American Journal of Medical Sciences, 8,* 323.

Sundekilde, U. K., Barile, D., Meyrand, M., Poulsen, N. A., Larsen, L. B., Lebrilla, C. B., et al. (2012). Natural variability in bovine milk oligosaccharides from Danish Jersey and Holstein-Friesian breeds. *Journal of Agricultural and Food Chemistry, 60,* 6188 – 6196.

Suttle, N. F. (2022). Mineral nutrition of livestock. Cabi.

Suzuki, K., & Ogra, Y. (2002). Metabolic pathway for selenium in the body: speciation by HPLC-ICP MS with enriched Se. *Food Additives & Contaminants, 19,* 974 – 983.

Swartz, T., Bradford, B., Malysheva, O., Caudill, M., Mamedova, L., & Estes, K. (2022). Effects of dietary rumen-protected choline supplementation on colostrum yields, quality, and choline metabolites from dairy cattle. *JDS Communications, 3,* 296 – 300.

Tao, N., DePeters, E., German, J., Grimm, R., & Lebrilla, C. (2009). Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. *Journal of Dairy Science, 92,* 2991 – 3001.

ten Bruggencate, S. J., Bovee-Oudenhoven, I. M., Feitsma, A. L., van Hoffen, E., & Schoterman, M. H. (2014). Functional role and mechanisms of sialyllactose and other sialylated milk oligosaccharides. *Nutrition Reviews, 72,* 377 – 389.

Tziboula-Clarke, A. (2003). Encyclopedia of Dairy Science. Vol. 2.

Valldecabres, A., & Silva-del-Río, N. (2022). First-milking colostrum mineral concentrations and yields: Comparison to second milking and associations with serum mineral concentrations, parity, and yield in multiparous Jersey cows. *Journal of Dairy Science, 105,* 2315 – 2325.

Van Emon, M., Sanford, C., & McCoski, S. (2020). Impacts of bovine trace mineral supplementation on maternal and offspring production and health. *Animals, 10,* 2404.

Visker, M., Dibbits, B., Kinders, S., Van Valenberg, H., Van Arendonk, J., & Bovenhuis, H. (2011). Association of bovine β-casein protein variant I with milk production and milk protein composition. *Animal Genetics, 42,* 212 – 218.

Weaver, D. M., Tyler, J. W., VanMetre, D. C., Hostetler, D. E., & Barrington, G. M. (2000). Passive transfer of colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine, 14,* 569 – 577.

Weijers, C. A., Franssen, M. C., & Visser, G. M. (2008). Glycosyltransferase-catalyzed synthesis of bioactive oligosaccharides. *Biotechnology Advances, 26,* 436 – 456.

Weiss, W. P., & Hogan, J. S. (2005). Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. *Journal of Dairy Science, 88,* 4366 – 4374.

White, S., Benson, G., Washburn, S., & Green Jr, J. (2002). Milk production and economic measures in confinement or pasture systems using seasonally calved Holstein and Jersey cows. *Journal of Dairy Science, 85,* 95 – 104.

Xu, G., Ye, J. A., Liu, J., & Yu, Y. (2006). Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. *Asian-Australasian Journal of Animal Sciences, 19,* 390 – 395.

Yu, Z. T., Chen, C., & Newburg, D. S. (2013). Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology, 23,* 1281 – 1292.

888 Zabielski, R., Le Huërou-Luron, I., & Guilloteau, P. (1999). Development of gastrointestinal and
889 pancreatic functions in mammalians (mainly bovine and porcine species): influence of age and ingested
890 food. *Reproduction Nutrition Development, 39,* 5 – 26.
891 Zeisel, S. H., Da Costa, K. A., Franklin, P. D., Alexander, E. A., Lamont, J. T., Sheard, N. F., et al.
892 (1991). Choline, an essential nutrient for humans. *Faseb Journal, 5,* 2093 – 2098.
893 Zenobi, M., Gardinal, R., Zuniga, J., Dias, A., Nelson, C., Driver, J., et al. (2018). Effects of
894 supplementation with ruminally protected choline on performance of multiparous Holstein cows did not
895 depend upon prepartum caloric intake. *Journal of Dairy Science, 101,* 1088 – 1110.
896 Zhang, L., Lin, Q., Zhang, J., Shi, Y., Pan, L., Hou, Y., et al. (2023). Qualitative and Quantitative
897 Changes of Oligosaccharides in Human and Animal Milk over Lactation. *Journal of Agricultural and*
898 *Food Chemistry, 71,* 15553 – 15568.
899 Zhang, L., Zeng, H., & Cheng, W. H. (2018). Beneficial and paradoxical roles of selenium at nutritional
900 levels of intake in healthspan and longevity. *Free Radical Biology and Medicine, 127,* 3 – 13.
901 Zhou, C., Li, C., Cai, W., Liu, S., Yin, H., Shi, S., et al. (2019). Genome-wide association study for milk
902 protein composition traits in a Chinese Holstein population using a single-step approach. *Frontiers in*
903 *Genetics, 10,* 72.
904 Zom, R., Van Baal, J., Goselink, R., Bakker, J., De Veth, M., & Van Vuuren, A. (2011). Effect of rumen-
905 protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy
906 cattle. *Journal of Dairy Science, 94,* 4016 – 4027.
907

8. Tables and Figures

Table 1. Dry cow mineral blend provided to each prepartum supplementation group with the addition of inorganic selenium (INORG), organic selenium (ORG), or rumen-protected choline (RPC).

Additives - Trace Minerals	**Supplying**
Manganese from Manganous Oxide	3,350 mg kg^{-1}
Zinc from Zinc chloride hydroxide monohydrate	375 mg kg^{-1}
Zinc from Zinc Oxide	7,205 mg kg^{-1}
Zinc from Zinc chelate of glycine hydrate	375 mg kg^{-1}
Copper from Copper Sulphate pentahydrate	1,000 mg kg^{-1}
Copper from Copper chelate of glycine hydrate	750 mg kg^{-1}
Copper from Tribasic hydroxyl copper chloride	1,250 mg kg^{-1}
Iodine from Potassium Iodide	250 mg kg^{-1}
Iodine from Calcium Iodate	250 mg kg^{-1}
Cobalt from Cobalt Carbonate monohydrate	100 mg kg^{-1}
Additives Vitamins	
Vitamin A	500,000 IU kg^{-1}
Vitamin D3	200,000 IU kg^{-1}
Vitamin E	5,000 IU kg^{-1}
Vitamin B1	250 IU kg^{-1}
Vitamin B12	1.5 mg kg^{-1}
Analytical Constituents for INORG and ORG	
Magnesium	25 % RDI (25.00 g)
Phosphorus	3 % RDI (3.00 g)
Sodium	11.25 % RDI (11.25 g)
Calcium (Zero added, background sources)	1.50 % RDI (1.50 g)
Analytical Constituents for RPC	
Crude oil	15 % RDI (8.06 g)
Crude fibre	10.8 % RDI (5.80 g)
Crude protein	1.5 % RDI (0.81 g)
Crude ash	1 % RDI (0.54 g)

*RDI: Recommended dietary intake or / g of the provided dose.
Composition of ingredients for INORG and ORG treatment group: Magnesium oxide, Magnesium phosphate, sodium chloride, molasses and inactivated yeast.
Feeding rate of INORG and ORG: 100 g / per head d^{-1}.
Composition of ingredients for RPC treatment group: Choline chloride, vegetable carrier, hydrogenated rape oil.
Feeding rate of RPC: 53.7 g to provide 20 g choline d^{-1}.

Table 2. Protein and oligosaccharide composition of bovine colostrum from pasture-based dairy cows supplemented with inorganic selenium (INORG), organic selenium (ORG) or rumen-protected choline (RPC) during the prepartum period.

	Treatment[1]			*p*-value[2]
	INORG	**ORG**	**RPC**	**Treatment**
Micro-nutrient composition				
Selenium concentration (mg kg^{-1})	0.17 ± 0.019	0.21 ± 0.021	0.19 ± 0.018	0.381
Choline concentration (ng μL^{-1})	3.21 ± 0.643	3.52 ± 0.645	4.85 ± 0.657	0.229
Gross composition (% *w/w*)				
Total solids (TS)	25.04 ± 0.940	27.36 ± 0.923	24.58 ± 0.936	0.069
True protein (TrP)	14.32 ± 0.662	15.42 ± 0.664	14.18 ± 0.661	0.209
Total protein (TP)	14.79 ± 0.533	15.52 ± 0.535	14.39 ± 0.532	0.265
Non-protein nitrogen (NPN)	0.06 ± 0.003	0.06 ± 0.003	0.06 ± 0.003	0.174
Non-casein nitrogen (NCN)	1.33 ± 0.089	1.48 ± 0.087	1.39 ± 0.088	0.421
Lactose	1.77 ± 0.142	1.50 ± 0.141	1.95 ± 0.145	0.079
Ash	3.12 ± 0.508	3.87 ± 0.511	2.84 ± 0.506	0.303
Globular proteins (mg mL^{-1})				
Immunoglobulin G (IgG)	81.05 ± 7.146	91.03 ± 7.021	83.99 ± 7.120	0.551
Bovine serum albumin (BSA)	6.76 ± 0.582	7.40 ± 0.572	6.85 ± 0.580	0.664
Casein and whey proteins (mg mL^{-1})				
κ-Casein (κ-CN)	10.15 ± 0.894	11.70 ± 0.879	11.40 ± 0.891	0.386
α_{s2}-Casein (α_{s2}-CN)	11.31^{b} ± 1.519	15.76^{a} ± 1.513	17.17^{a} ± 1.530	0.008
α_{s1}-Casein (α_{s1}-CN)	30.18 ± 1.788	31.98 ± 1.757	33.57 ± 1.782	0.397
β-Casein (β-CN)	26.78^{b} ± 1.294	29.65^{a} ± 1.301	30.73^{a} ± 1.313	0.015
Casein protein (CNP)	78.34^{b} ± 3.408	88.98^{a} ± 3.410	93.06^{a} ± 3.414	0.008
α-lactalbumin (α-LA)	2.60 ± 0.418	3.10 ± 0.416	3.31 ± 0.420	0.384
β-lactoglobulin A (β-LG A)	11.72 ± 1.807	13.11 ± 1.776	13.77 ± 1.801	0.701
β-lactoglobulin B (β-LG B)	7.88 ± 1.203	8.98 ± 1.182	9.30 ± 1.199	0.657
Whey protein (WP)	22.31 ± 1.834	25.24 ± 1.837	26.46 ± 1.838	0.213
Oligosaccharides (mg mL^{-1})				
3-fucosyllactose (3-FL)	0.80 ± 0.054	0.79 ± 0.053	0.85 ± 0.054	0.657
5-*N*-acetylneuraminic acid (Sialic Acid)	0.03 ± 0.002	0.03 ± 0.002	0.03 ± 0.002	0.875
3'sial-*N*-acetyllactosamine (3'-SNL)	0.12 ± 0.048	0.09 ± 0.048	0.16 ± 0.048	0.337
6'-sialyllactose (6'-SL)	0.13 ± 0.009	0.11 ± 0.009	0.13 ± 0.009	0.204
3'-sialyllactose (3'-SL)	0.99 ± 0.051	1.07 ± 0.050	1.02 ± 0.051	0.546
Disialyllacto-*N*-tetraose (DSLNT)	0.09 ± 0.016	0.11 ± 0.016	0.10 ± 0.017	0.609
Disialyllactose (DSL)	0.63 ± 0.061	0.73 ± 0.060	0.66 ± 0.061	0.414
Total oligosaccharide	2.81 ± 0.161	2.95 ± 0.160	2.95 ± 0.161	0.720

Values are the mean ± standard deviation (SD).

[a] – [b] Mean values in the same row with different subscripts differ ($p < 0.05$) for the fixed effect of treatment.

[1] The three different supplementation groups are denoted by INORG (inorganic selenium), ORG (organic selenium) and RPC (rumen-protected choline).

[2] Treatment = impact of prepartum supplementation

926 Table 3. Impact of breed (Holstein-Friesian (HF) and HF × Jersey (JEX)) on the protein and
927 oligosaccharide composition of bovine colostrum.

	Breed[1]		*p*-value
	HF	**JEX**	**Breed**
Micro-nutrient composition			
Selenium concentration (mg kg^{-1})	0.19 ± 0.023	0.20 ± 0.021	0.646
Choline concentration (ng μL^{-1})	3.85 ± 0.549	3.87 ± 0.552	0.977
Gross composition (% *w/w*)			
Total solids (TS)	25.48 ± 0.736	25.84 ± 0.854	0.748
True protein (TrP)	14.56 ± 0.609	14.71 ± 0.611	0.836
Total protein (TP)	14.93 ± 0.470	14.87 ± 0.472	0.918
Non-protein nitrogen (NPN)	0.06 ± 0.002	0.06 ± 0.002	0.901
Non-casein nitrogen (NCN)	1.44 ± 0.069	1.36 ± 0.081	0.407
Lactose	1.65 ± 0.122	1.83 ± 0.126	0.300
Ash	3.54 ± 0.434	3.01 ± 0.430	0.381
Globular proteins (mg mL^{-1})			
Immunoglobulin G (IgG)	88.70 ± 5.593	82.01 ± 6.495	0.429
Bovine Serum Albumin (BSA)	7.20 ± 0.456	6.80 ± 0.529	0.567
Casein and whey proteins (mg mL^{-1})			
κ-Casein (κ-CN)	10.11 ± 0.701	12.06 ± 0.814	0.068
α_{s2}-Casein (α_{s2}-CN)	14.57 ± 1.248	14.92 ± 1.489	0.836
α_{s1}-Casein (α_{s1}-CN)	30.65 ± 1.400	33.17 ± 1.625	0.236
β-Casein (β-CN)	28.76 ± 1.121	29.34 ± 1.331	0.654
Casein protein (CNP)	83.94 ± 2.766	89.64 ± 2.936	0.156
α-lactalbumin (α-LA)	2.83 ± 0.342	3.18 ± 0.408	0.460
β-lactoglobulin A (β-LG A)	11.88 ± 1.414	13.85 ± 1.643	0.356
β-lactoglobulin B (β-LG B)	9.43 ± 0.943	8.01 ± 0.921	0.315
Whey protein (WP)	24.31 ± 1.615	25.04 ± 1.618	0.731
Oligosaccharides (mg mL^{-1})			
3-fucosyllactose (3-FL)	0.74^{b} ± 0.043	0.89^{a} ± 0.050	0.028
5-*N*-acetylneuraminic acid (Sialic Acid)	0.03 ± 0.001	0.03 ± 0.002	0.846
3'sial-*N*-acetyllactosamine (3'-SNL)	0.13 ± 0.042	0.12 ± 0.049	0.799
6'-sialyllactose (6'-SL)	0.11 ± 0.008	0.13 ± 0.009	0.068
3'-sialyllactose (3'-SL)	0.95^{b} ± 0.040	1.10^{a} ± 0.047	0.017
Disialyllacto-*N*-tetraose (DSLNT)	0.09 ± 0.013	0.11 ± 0.016	0.186
Disialyllactose (DSL)	0.65 ± 0.048	0.70 ± 0.060	0.505
Total oligosaccharide	2.70^{b} ± 0.131	3.10^{a} ± 0.156	0.033

928 Values are the mean ± standard deviation (SD).
929 [a] – [b] Mean values in the same row with different subscripts differ ($p < 0.05$) for the fixed effect of breed.
930 [1] HF denotes Holstein-Friesian cows; JEX denotes HF × Jersey cows
931

Table 4. Impact of parity on the protein and oligosaccharide composition of bovine colostrum.

	Parity[1]			*p*-value
	P1	**P2**	**P≥3**	**Parity**
Micro-nutrient composition				
Selenium concentration (mg kg^{-1})	0.16 ± 0.029	0.21 ± 0.032	0.21 ± 0.034	0.472
Choline concentration (ng μL^{-1})	3.13 ± 0.657	2.93 ± 0.627	5.53 ± 0.649	0.076
Gross composition (% *w/w*)				
Total solids (TS)	23.99 ± 1.358	25.76 ± 1.153	27.23 ± 0.110	0.152
True protein (TrP)	14.32 ± 0.643	15.42 ± 0.678	14.18 ± 0.665	0.312
Total protein (TP)	14.79^{a} ± 0.664	15.52^{a} ± 0.661	14.39^{b} ± 0.663	0.032
Non-protein nitrogen (NPN)	0.05^{c} ± 0.004	0.06^{b} ± 0.004	0.07^{a} ± 0.002	0.001
Non-casein nitrogen (NCN)	1.22^{b} ± 0.128	1.38^{ab} ± 0.109	1.60^{a} ± 0.072	0.035
Lactose	2.11 ± 0.139	1.57 ± 0.156	1.55 ± 0.148	0.107
Ash	3.12 ± 0.598	3.87 ± 0.610	2.84 ± 0.576	0.061
Globular proteins (mg mL^{-1})				
Immunoglobulin G (IgG)	72.14^{b} ± 10.325	80.74^{b} ± 8.769	103.19^{a} ± 5.857	0.021
Bovine Serum Albumin (BSA)	5.64 ± 0.841	7.31 ± 0.714	8.05 ± 0.477	0.086
Casein and whey proteins (mg mL^{-1})				
κ-Casein (κ-CN)	8.67 ± 1.295	12.25 ± 1.101	12.33 ± 0.735	0.086
α_{s2}-Casein (α_{s2}-CN)	14.40 ± 2.368	14.36 ± 2.068	15.48 ± 1.359	0.860
α_{s1}-Casein (α_{s1}-CN)	26.22^{b} ± 2.584	35.50^{a} ± 2.194	34.01^{a} ± 1.466	0.036
β-Casein (β-CN)	23.63^{b} ± 2.164	32.07^{a} ± 1.866	31.46^{a} ± 1.254	0.012
Casein protein (CNP)	72.42^{b} ± 3.845	94.50^{a} ± 3.954	93.45^{a} ± 3.988	0.004
α-lactalbumin (α-LA)	2.05^{b} ± 0.648	2.99^{ab} ± 0.565	3.98^{a} ± 0.372	0.044
β-lactoglobulin A (β-LG A)	11.89 ± 2.611	12.37 ± 2.218	14.33 ± 1.481	0.639
β-lactoglobulin B (β-LG B)	5.19^{b} ± 1.744	9.89^{a} ± 1.484	11.08^{a} ± 0.990	0.038
Whey protein (WP)	19.04^{b} ± 2.241	25.74^{a} ± 2.416	29.23^{a} ± 2.249	0.018
Oligosaccharides (mg mL^{-1})				
3-fucosyllactose (3-FL)	0.49^{b} ± 0.079	0.90^{a} ± 0.067	1.05^{a} ± 0.045	0.001
5-*N*-acetylneuraminic acid (Sialic Acid)	0.03 ± 0.002	0.03 ± 0.002	0.02 ± 0.001	0.173
3'sial-*N*-acetyllactosamine (3'-SNL)	0.10 ± 0.082	0.12 ± 0.069	0.15 ± 0.048	0.850
6'-sialyllactose (6'-SL)	0.10^{b} ± 0.147	0.15^{a} ± 0.013	0.12^{ab} ± 0.008	0.035
3'-sialyllactose (3'-SL)	1.09 ± 0.074	0.99 ± 0.063	1.01 ± 0.042	0.631
Disialyllacto-*N*-tetraose (DSLNT)	0.01^{b} ± 0.025	0.12^{a} ± 0.021	0.15^{a} ± 0.014	0.001
Disialyllactose (DSL)	0.73 ± 0.089	0.72 ± 0.076	0.56 ± 0.051	0.112
Total oligosaccharide	2.57 ± 0.248	3.12 ± 0.216	3.03 ± 0.142	0.258

Values are the mean ± standard deviation (SD).

[a] – [b] Mean values in the same row with different subscripts differ ($p < 0.05$) for the fixed effect of parity.

[1] P1 denotes first parity (primiparous) cows; P2 denotes second parity cows and P≥3 denotes third and greater parity cows.

937 Table 5. Protein and oligosaccharide composition of bovine colostrum and transition milk one to five collected at each consecutive milking following parturition.

	Each milking postpartum[1,2]						*p*-value[3]		
	Colostrum	**TM1**	**TM2**	**TM3**	**TM4**	**TM5**	**Treatment**	**Time**	**Treatment *Time**
Gross composition (% *w/w*)									
Total solids (TS)	$27.61^{a} \pm 1.140$	$21.27^{b} \pm 0.996$	$16.57^{c} \pm 0.402$	$15.72^{cd} \pm 0.923$	$15.08^{d} \pm 0.406$	$14.35^{d} \pm 0.455$	0.675	0.001	0.513
True protein (TrP)	$15.38^{a} \pm 1.794$	$9.59^{b} \pm 0.732$	$7.32^{c} \pm 0.284$	$5.29^{d} \pm 0.193$	$4.46^{e} \pm 0.158$	$3.59^{f} \pm 0.162$	0.791	0.001	0.581
Total protein (TP)	$16.17^{a} \pm 1.112$	$9.66^{b} \pm 0.773$	$7.62^{c} \pm 0.538$	$5.59^{d} \pm 0.235$	$4.72^{e} \pm 0.187$	$3.90^{f} \pm 0.092$	0.674	0.001	0.510
Non-protein nitrogen (NPN)	$0.08^{a} \pm 0.016$	$0.06^{b} \pm 0.003$	$0.09^{a} \pm 0.034$	$0.05^{c} \pm 0.002$	$0.05^{c} \pm 0.003$	$0.05^{c} \pm 0.002$	0.341	0.001	0.258
Non-casein nitrogen (NCN)	$1.71^{a} \pm 0.132$	$0.92^{b} \pm 0.105$	$0.44^{c} \pm 0.039$	$0.24^{d} \pm 0.014$	$0.20^{e} \pm 0.014$	$0.16^{f} \pm 0.005$	0.234	0.001	0.105
Lactose	$1.43^{e} \pm 0.035$	$2.45^{d} \pm 0.069$	$3.10^{c} \pm 0.091$	$3.62^{b} \pm 0.112$	$3.75^{b} \pm 0.115$	$4.14^{a} \pm 0.413$	0.378	0.001	0.286
Ash	$3.49^{a} \pm 0.426$	$2.12^{b} \pm 0.313$	$1.89^{c} \pm 0.262$	$1.35^{d} \pm 0.189$	$1.21^{d} \pm 0.192$	$0.89^{e} \pm 0.131$	0.767	0.001	0.231
Globular proteins (mg mL^{-1})									
Immunoglobulin G (IgG)	$107.46^{a} \pm 10.028$	$16.96^{b} \pm 1.115$	$7.36^{c} \pm 1.179$	$5.88^{c} \pm 0.683$	$3.15^{d} \pm 0.433$	$1.79^{e} \pm 0.157$	0.122	0.001	0.296
Bovine serum albumin (BSA)	$8.50^{a} \pm 0.891$	$1.01^{b} \pm 0.071$	$0.41^{c} \pm 0.071$	$0.41^{c} \pm 0.043$	$0.22^{d} \pm 0.024$	$0.18^{d} \pm 0.018$	0.503	0.001	0.697
Casein and whey proteins (mg mL^{-1})									
κ-Casein (κ-CN)	$11.75^{a} \pm 0.634$	$6.59^{b} \pm 0.536$	$5.23^{c} \pm 0.407$	$4.11^{d} \pm 0.195$	$3.72^{e} \pm 0.143$	$3.50^{e} \pm 0.124$	0.721	0.001	0.914
α_{s2}-Casein (α_{s2}-CN)	$15.45^{a} \pm 1.946$	$13.12^{a} \pm 0.910$	$9.59^{b} \pm 0.553$	$8.60^{bc} \pm 0.296$	$8.01^{d} \pm 0.255$	$8.27^{cd} \pm 0.366$	0.860	0.001	0.339
α_{s1}-Casein (α_{s1}-CN)	$32.93^{a} \pm 2.089$	$23.52^{b} \pm 1.521$	$18.84^{c} \pm 0.955$	$16.62^{de} \pm 0.465$	$16.14^{d} \pm 0.277$	$17.59^{ce} \pm 0.392$	0.772	0.001	0.848
β-Casein (β-CN)	$31.89^{a} \pm 1.388$	$24.21^{b} \pm 1.377$	$18.61^{c} \pm 0.952$	$15.41^{d} \pm 0.455$	$14.41^{e} \pm 0.402$	$14.50^{e} \pm 0.290$	0.239	0.001	0.468
Casein protein (CNP)	$92.08^{a} \pm 5.732$	$67.43^{b} \pm 4.385$	$52.25^{c} \pm 1.384$	$44.70^{d} \pm 0.842$	$42.26^{e} \pm 0.623$	$43.83^{de} \pm 0.440$	0.549	0.001	0.517
α-lactalbumin (α-LA)	$3.79^{a} \pm 0.606$	$2.10^{b} \pm 0.155$	$1.69^{c} \pm 0.089$	$1.47^{d} \pm 0.072$	$1.43^{d} \pm 0.056$	$1.38^{d} \pm 0.063$	0.359	0.001	0.634
β-lactoglobulin A (β-LG A)	$15.38^{a} \pm 2.126$	$8.10^{b} \pm 0.697$	$4.02^{c} \pm 0.401$	$3.17^{d} \pm 0.164$	$2.94^{d} \pm 0.141$	$3.26^{d} \pm 0.180$	0.497	0.001	0.705
β-lactoglobulin B (β-LG B)	$11.49^{a} \pm 2.028$	$7.70^{a} \pm 0.712$	$4.63^{b} \pm 0.296$	$3.27^{c} \pm 0.191$	$2.95^{d} \pm 0.162$	$3.00^{cd} \pm 0.178$	0.741	0.001	0.980
Whey protein (WP)	$30.89^{a} \pm 2.104$	$17.84^{b} \pm 0.913$	$10.31^{c} \pm 0.487$	$7.88^{d} \pm 0.341$	$7.63^{d} \pm 0.267$	$7.30^{e} \pm 0.398$	0.338	0.001	0.541
Oligosaccharides (mg mL^{-1})									
3-fucosyllactose (3-FL)	$0.92^{a} \pm 0.068$	$0.66^{b} \pm 0.062$	$0.31^{c} \pm 0.030$	$0.21^{d} \pm 0.026$	$0.17^{e} \pm 0.022$	$0.14^{f} \pm 0.023$	0.266	0.001	0.424
5-*N*-acetylneuraminic acid (Sialic Acid)	$0.02^{a} \pm 0.002$	$0.03^{b} \pm 0.003$	$0.03^{b} \pm 0.004$	$0.04^{c} \pm 0.006$	$0.03^{b} \pm 0.003$	$0.04^{c} \pm 0.003$	0.487	0.001	0.377
3'sial-*N*-acetyllactosamine (3'-SNL)	$0.09^{a} \pm 0.035$	$0.06^{b} \pm 0.009$	$0.02^{c} \pm 0.003$	$0.02^{c} \pm 0.003$	$0.01^{d} \pm 0.002$	$0.01^{d} \pm 0.059$	0.478	0.001	0.915
6'-sialyllactose (6'-SL)	$0.11^{a} \pm 0.008$	$0.09^{b} \pm 0.006$	$0.06^{c} \pm 0.005$	$0.04^{d} \pm 0.003$	$0.04^{d} \pm 0.003$	$0.04^{d} \pm 0.002$	0.469	0.001	0.865
3'-sialyllactose (3'-SL)	$0.97^{a} \pm 0.059$	$0.65^{b} \pm 0.057$	$0.22^{c} \pm 0.015$	$0.11^{d} \pm 0.011$	$0.10^{de} \pm 0.009$	$0.09^{e} \pm 0.007$	0.723	0.001	0.977
Disialyllacto-*N*-tetraose (DSLNT)	$0.15^{a} \pm 0.023$	$0.05^{b} \pm 0.006$	$0.03^{c} \pm 0.006$	$0.01^{d} \pm 0.003$	$0.01^{d} \pm 0.002$	$0.01^{d} \pm 0.002$	0.906	0.001	0.577
Disialyllactose (DSL)	$0.58^{a} \pm 0.050$	$0.38^{b} \pm 0.044$	$0.10^{c} \pm 0.015$	$0.05^{d} \pm 0.006$	$0.04^{de} \pm 0.007$	$0.04^{e} \pm 0.005$	0.706	0.001	0.307

Total oligosaccharide	$2.85^{a} \pm 0.067$	$1.94^{b} \pm 0.027$	$0.77^{c} \pm 0.020$	$0.49^{d} \pm 0.003$	$0.42^{e} \pm 0.001$	$0.37^{f} \pm 0.002$	0.873	0.001	0.807

Values are the mean ± standard deviation (SD).

[a-f] Mean values in the same row with different subscripts differ ($p < 0.05$) for the fixed effect of each milking postpartum.

[1] As there was no significant interaction observed between treatment and time, values for each milking postpartum are given as averages of the three prepartum supplementation treatment groups

[2] TM = Transition milk (TM1.. TM5; Transition milk one to Transition milk five)

[3] Treatment = impact of prepartum supplementation; Time = each milking postpartum; Treatment*Time = interaction between treatment × time

944