Short communication: Odd-chain and branched-chain fatty acid concentrations in bovine colostrum and transition milk and their stability under heating and freezing treatments

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ABSTRACT

Although odd-chain fatty acids (OCFA) and branched-chain fatty acids (BCFA) are found in bovine milk and have some positive influences on human and animal health, their concentrations in bovine colostrum and transition milk have not been reported. In this study, we reported the OCFA and BCFA concentrations in colostrum and transition milk and their stability after heating or freezing treatments (or both), which are processes commonly applied in dairy calf management. Milk samples were collected from 12 Holstein dairy cows (6 primiparous and 6 multiparous) at the first milking (colostrum), fifth milking (transition milk), and ninth milking (mature milk) after calving, respectively, and were used for fatty acid analysis using gas chromatography. The sum concentration of OCFA and BCFA (termed OBCFA) was 134 mg/100 g of milk in the colostrum, which was 24% and 35% lower than that in the transition milk and mature milk, respectively. Among these fatty acids detected, C15:0 and C17:0 were the top 2 abundant fatty acids in all milk types, accounting for 20 to 25% and 21 to 24% of the total concentration of OBCFA, respectively. Additionally, *anteiso*-C17:0 was the most abundant BCFA, followed by *iso*-C17:0, *anteiso*-C15:0, *iso*-C16:0, *iso*-C15:0, *iso*-C18:0, and *iso*-C14:0 in 3 types of milk. Significant interactions between milk type and cow parity were observed for all OCFA and BCFA concentrations. The milk samples were also treated with heating (at 65°C for 60 min), freezing (at –20°C for 30 d), and heating and freezing (at 65°C for 60 min and then at –20°C for 30 d), and milk OCFA and BCFA concentrations were similar between these treatments. In conclusion, the OBCFA concentration was lower in colostrum,

compared with transition and mature milks, and it remained stable after heating and freezing treatments.

Key words: odd-chain and branched-chain fatty acids, colostrum, transition milk, stability

Short Communication

Odd-chain fatty acids (OCFA) are a group of straight-chain fatty acids having an odd number of carbon atoms, whereas branched-chain fatty acids (BCFA) are isomer fatty acids with a methyl branch on the penultimate or ultimate carbon. The odd- and branched-chain fatty acids include tetradecanoic acid (*iso*-C14:0), pentadecanoic acid (C15:0, *iso*-C15:0 and *anteiso*-C15:0), hexadecanoic acid (*iso*-C16:0), heptadecanoic acid (C17:0, *iso*-C17:0 and *anteiso*-C17:0), and stearic acid (*iso*-C18:0) (Vlaeminck et al., 2006; Ran-Ressler et al., 2014). Recently, OCFA and BCFA (termed OBCFA) have gained significant attention as bioactives, especially for their biological impact on the gut health of mammals. For example, C17:0, the most abundant OCFA in the colon lumen, has been reported to promote gastrointestinal movement in rats and concentration of C17:0 had a positive association with abundances of bacterial genera *Prevotella* and *Lactobacillus* (Zhao et al., 2018). These 2 bacteria displayed significantly higher relative abundances in healthy rodents than those with gut disorder (Liu et al., 2018a; Wang et al., 2019). As for BCFA, Ran-Ressler et al. (2008) identified that they were constituents in newborn infants' guts and speculated that these BCFA might play a role in gut development. In support of this hypothesis, the same researchers found that replacing 20% of milk fat with BCFA dramatically reduced necrotizing enterocolitis incidence in neonatal rats (Ran-Ressler et al., 2011). Additionally, our preliminary study illustrated remarkably different BCFA profiles in feces between diarrheic and healthy dairy calves, and some BCFA (e.g., *anteiso*-C15:0, *iso*-C16:0, *iso*-C17:0, *iso*-C18:0, and total even-chain BCFA) might potentially

Received May 31, 2020.

Accepted July 27, 2020.

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be used as classifiers or predictors of gut health status in neonatal calves (H. Xin, T. Ma, Y. Xu, G. Chen, Y. Chen, C. Villot, D. L. Renaud, M. A. Steele, and L. L. Guan; Northeast Agricultural University, Harbin, China; University of Alberta, Edmonton, Canada; Chinese Academy of Agricultural Science, Beijing, China; University of Guelph, Guelph, Canada; unpublished data). These results suggest that OBCFA could benefit human and animal health through potential effects on gut functions.

Bovine colostrum, the first mammary secretion at the onset of lactation, is rich in bioactive compounds and immunoregulatory factors that are crucial to newborn calf survival and health (Fahey et al., 2020; Fischer-Thustos et al., 2020). Some fatty acids, as part of bioactives, have been reported to have antimicrobial functions (Hristov et al., 2004) and be essential for immune system development and activation in calves (Hill et al., 2011). However, research on colostrum fatty acids has been primarily focused on UFA, such as *trans*-isomers (e.g., *trans* 18:1, 18:2, and 18:3 isomers) and conjugated linoleic acid (Leiber et al., 2011; Contarini et al., 2014; O'Callaghan et al., 2020). Compared with colostrum, the subsequent 2 to 6 milk secretions (referred to as transition milk) exhibit remarkable changes in bioactive amounts (Blum and Hammon, 2000). To date, there are considerable publications reporting the presence of OCFA and BCFA in milk (Vlaeminck et al., 2006; Rodríguez-Alcalá et al., 2014; Bainbridge et al., 2016), but they have not been reported in bovine colostrum and transition milk.

In the dairy industry, colostrum and transition milk are usually pasteurized using heat treatment with low temperature (usually at 60–65°C for 30–60 min) to eliminate potential pathogenic microorganisms (Godden et al., 2006) and then stored frozen for further use. In addition, bovine raw milk is also pasteurized (e.g., 63°C for 30 min) before human consumption, which is an effective way to kill harmful bacteria that can cause disease. It has been known that milk lipid is stable, especially SFA, which do not easily react with most chemicals, even under different heating conditions (Rodríguez-Alcalá et al., 2014). It has been reported that pasteurization could significantly reduce relative abundance of *Pseudomonas* spp. (from 26% to 4%; Quigley et al., 2013), one of microorganisms causing raw milk spoilage (Ternström et al., 1993) and containing OCFA and BCFA in their cell membranes (Kaneda, 1991; Chao et al., 2010), suggesting that such a process may alter OBCFA concentrations in the milk. In addition, formation of ice crystals intercellularly during freezing storage could damage bacterial cell membranes, which would cause lipid loss (Ray and Speck, 1973). Moreover, freezing can also destroy milk fat globules and

lead to reduced milk quality, as previously reviewed by Muir (1984). These results suggest that milk OCFA and BCFA concentrations could change after heating or freezing treatments (or both). Therefore, the aims of the present study were to (1) characterize the OCFA and BCFA concentrations in bovine colostrum, transition milk, and mature milk, and (2) investigate whether heating or freezing treatments (or both) affect the OCFA and BCFA concentrations in these 3 types of milk.

Three types of milk samples used in this study were collected from 12 healthy Holstein dairy cows (6 primiparous cows and 6 multiparous cows) co-housed with the same ration management (Table 1) at the Dairy Research and Technology Centre, University of Alberta. After parturition, milk samples (50 mL/sample) were collected from each cow as (1) colostrum (the first milking), (2) transition milk (the fifth milking), and (3) mature milk (the ninth milking). All samples were separated into 4-aliquot sets and were randomly assigned to 1 of 4 treatments: (1) raw, (2) heating treatment: incubated at 65°C in a water bath (Grant Optima T100, Cambridge Ltd., Cambridge, UK) for 60 min, (3) freezing treatment: frozen for 30 d at –20°C, and (4) heating and freezing treatment: incubated at 65°C in a water bath for 60 min and then frozen at –20°C for 30 d. The OCFA and BCFA analyses were performed on the same day of sample collection for the raw and heat-treated samples, and on d 30 for the frozen and heated and frozen samples. Twenty microliters of 50 mg/mL nonadecanoic acid (C19:0; Larodan Fine Chemicals, Malmö, Sweden) was added as an internal standard to each nylon-cap glass tube following being dried under N₂. Then, around 0.2 g of milk samples was added into the same glass tube for lipid extraction according to the procedures described by Erickson and Dunkley (1964). Subsequently, the extracted milk lipid was methylated with 3 M methanolic HCl and incubated at 80°C for 1 h (Raes et al., 2001). Then the mixture was neutralized with 0.9% NaCl and re-extracted with hexane. Finally, the upper organic phase was collected, dried under N₂, and reconstituted in 1 mL of isooctane for analysis on an Agilent 6890N Gas Chromatograph (Agilent Technologies, Santa Clara, CA). The temperature procedure for the GC oven was set according to Dingess et al. (2017). Individual fatty acid was identified according to peak and retention time of standards including *iso*-C14:0, *iso*-C15:0, *anteiso*-C15:0, C15:0, *iso*-C16:0, *iso*-C17:0, *anteiso*-C17:0, C17:0, and *iso*-C18:0 (Larodan Fine Chemicals, Malmö, Sweden). Fatty acid quantification was double confirmed on an Agilent 6890N Gas Chromatograph equipped with a 5975 inert XL Mass Selective Detector (Agilent Technologies). The data were analyzed using PROC MIXED model in SAS

Table 1. Ingredient and chemical compositions of the prepartum and postpartum diets

Item	Value
Prepartum	
Ingredient, TMR, %	
Barley straw	21.75
Barley silage	40.92
Bypass soy	5.23
Barley grain	2.61
Corn grain	5.97
Canola meal	6.45
Beet pulp	5.82
Corn gluten meal	0.90
Corn distillers	2.09
Soybean hulls	3.73
Yeast	0.03
Premix ¹	4.5
Chemical composition, % DM	
CP	14.67
Ether extract	3.24
NDF	43.54
ADF	28.12
peNDF ²	31.92
Starch	16.39
Postpartum	
Ingredient, TMR, %	
Alfalfa	7.15
Barley silage	42.81
Bypass soy	7.41
Barley grain	13.72
Corn grain	13.86
Canola meal	10.73
Beet pulp	1.11
Corn gluten meal	0.74
Premix ³	2.5
Chemical composition, % DM	
CP	17.39
Ether extract	3.15
NDF	33.91
ADF	22.6
peNDF	21.7
Starch	24.1

¹The premix contained 0.26% dry cow plus PMX, 1.49% limestone (40%), 0.15% MgO (55%), 1.49% Animate, 0.30% MgSO₄ (10%), 0.34% CaHPO₄, 0.01% monensin sodium (200 mg/kg), 0.07% vitamin E (50,000 IU/kg), and 0.37% choline. This product was obtained from Nutrisource, Strathmore, AB, Canada.

²peNDF = physically effective NDF.

³The premix contained 0.82% limestone (40%), 0.17% dairy plus PMX, 0.34% 0.19% MgO (55%), 0.41% NaHCO₃, 0.45% calcium magnesium mixture, 0.34% red salt, 0.02% yeast, and 0.09% methionine. This product was obtained from Nutrisource.

(version 9.4, SAS Institute Inc., Cary, NC). The model included the fixed effects of milk types (colostrum, transition milk, and mature milk), parity (primiparous and multiparous), and treatments (raw, heated, frozen, and heated and frozen), interaction effects (milk type × parity and milk type × treatment), and random effect of dairy cows. Statistical significances were declared at $P < 0.05$.

In total, 9 OBCFA were identified in 3 types of milk (Table 2), including 2 OCFA (C15:0 and C17:0), 5 *iso*-BCFA (*iso*-C14:0, *iso*-C15:0, *iso*-C16:0, *iso*-C17:0,

and *iso*-C18:0) and 2 *anteiso*-BCFA (*anteiso*-C15:0 and *anteiso*-C17:0). The concentrations of total OBCFA ranged from 89 to 268 mg/100 g of milk in all milk samples which are within the ranges summarized by Vlaeminck et al. (2006). Among them, OCFA of C15:0 and C17:0 were the top 2 abundant fatty acids, accounting for 20 to 25% and 21 to 24% of the total concentration of OBCFA, respectively. These are consistent with their abundances in the milk of Holstein cows taken from d 5 after parturition (Bainbridge et al., 2016) and in the milk samples collected over 40 wk of lactation (Craninx et al., 2008), as previously reported. In the present study, total OCFA concentrations ranged from 44.4 to 120.9 mg/100 g of milk, accounting for 45 to 50% of total concentration of OBCFA in milk samples. The OCFA are mainly formed by repeated condensation of malonyl-CoA with propionyl-CoA as the precursor (Fulco, 1983). In addition to that, Emmanuel (1978) found that OCFA could also be produced by rumen bacteria in a pure culture system through α -oxidation from even-chain fatty acids (e.g., C16:0 or C18:0) which are originated from acetate or butyrate (Kaneda, 1991). Therefore, the proportion of OCFA observed in this study could probably reflect the availability of these fatty acid precursors in ruminants. On the other hand, total concentrations of BCFA ranged from 69.2 to 147.6 mg/100 g of milk in the milk samples. Among them, *anteiso*-C17:0 was the most predominant fatty acid in all milk samples, followed by *iso*-C17:0, *anteiso*-C15:0, *iso*-C16:0, *iso*-C15:0, *iso*-C18:0, and *iso*-C14:0. Meanwhile, the concentrations of total *iso*-BCFA and total *anteiso*-BCFA ranged from 25 to 79 mg/100 g of milk and 20 to 69 mg/100 g of milk, respectively, in different milk samples. The amount of total *iso*-BCFA and total *anteiso*-BCFA in mature milk obtained from primiparous cows in our study is greater than the findings of Bainbridge et al. (2016) who reported the total concentrations of *iso*- and *anteiso*-BCFA were 38 and 30 mg/100 g of milk in the milk obtained from primiparous Holstein dairy cows 5 d after calving. Generally, BCFA are produced by short branched-chain fatty acids which are related to branched-chain fatty acids (e.g., valine, leucine, and isoleucine; Kaneda, 1991). Specifically, the *iso*-BCFA (e.g., *iso*-C14:0 and *iso*-C16:0) are originated from isobutyrate and isovalerate (e.g., *iso*-C15:0 and *iso*-C17:0), whereas *anteiso*-BCFA (e.g., *anteiso*-C15:0 and *anteiso*-C17:0) originate from 2-methylbutyrate in ruminants (Vlaeminck et al., 2006). The generation of these short BCFA is also highly associated with the breed (Bainbridge et al., 2016), dietary strategy, ruminal environment, and physiological conditions of cows (Liu et al., 2018b; Roman-Garcia, 2019). The varied management, diets, and sampling time might partly explain the inconsistent concentrations of *iso*- and

anteiso-BCFA between these 2 studies. To date, limited information is available regarding abundance of *iso*- and *anteiso*-BCFA in colostrum and transition milk. Thus, future studies are needed to determine whether cow nutrition, breed, or other factors affect their concentrations in these 2 types of milk.

All individual OCFA and BCFA concentrations were significantly affected by the interactions of milk type and cow parity ($P < 0.05$; Table 2). In general, for each fatty acid, the highest level was found in the mature milk from primiparous cows, whereas the colostrum milk from multiparous cows had the lowest concentrations ($P < 0.05$). Meanwhile, the concentrations of OCFA and BCFA in colostrum obtained from either primiparous or multiparous cows were lower than those in transition and mature milks. Consequently, similar tendencies were observed in the concentrations of total OCFA, total BCFA, total *iso*-BCFA, total *anteiso*-BCFA, as well as total OBCFA in all milk samples. Similarly, a previous study has reported that the sum concentrations of C15:0 and C17:0 were increased from 5 to 275 lactation days in both Holstein and Jersey cows (Bainbridge et al., 2016). In addition, Leiber et al. (2011) concluded that total BCFA concentration in milk linearly increased from 1.20% to 1.67% (% of total fatty acids) during the first 40 d of early lactation. It is known that OCFA and BCFA are particularly prominent in ruminant products, as they are mainly synthesized by microorganisms in the rumen (Kaneda, 1991). After calving, the cows usually have a strong decline in DMI, especially during the first few hours postpartum (Grummer, 1995), which could lead to the lower availability of substrates for fatty acid synthesis. This may in part explain the decrease in sum production of OCFA and BCFA in colostrum. In addition, we measured fatty acid concentrations in a commercial milk replacer (MR) and found there was only 0.70 and 0.61 mg/g of OCFA and BCFA, respectively, in this MR product (Supplemental Table S1; <https://doi.org/10.3168/jds.2020-18994>). Given MR is formulated with water (MR: water = 1:7; wt/wt) when feeding the calves, the total OBCFA content in 100 g of MR liquid is estimated to be 16.4 mg, which is only 9.8% and 8.2% of that found in 100 g of bovine transition milk and mature milk, respectively. This suggests that the MR feeding may not have the health benefit of bovine milk due to low contents of OCFA and BCFA and future MR manufacturing should consider OCFA and BCFA as part of its nutritional component. In addition, our results showed that individual milk OCFA and BCFA concentrations in primiparous cows were 1.0 to 2.6 times higher than those of multiparous cows. In a previous study, Craninx et al. (2008) observed significantly higher levels of OCFA and BCFA (e.g.,

Table 2. The concentrations of odd-chain fatty acids (OCFA) and branched-chain fatty acids (BCFA) in colostrum, transition milk, and mature milk obtained from primiparous and multiparous dairy cows

Fatty acid, ¹ mg/ 100 g of milk	Primiparous (n = 6)			Multiparous (n = 6)				P-value		
	Colostrum	Transition milk	Mature milk	Colostrum	Transition milk	Mature milk	SEM	Type	Parity	Type × parity
OCFA										
C15:0	45.58	42.09	56.47	25.33	35.82	26.22	4.367	0.06	0.01	<0.0001
C17:0	37.51	43.97	64.40	19.02	38.20	33.98	3.249	<0.0001	0.0004	<0.0001
Total OCFA	83.10	86.06	120.87	44.36	74.01	60.20	7.269	<0.0001	0.001	<0.0001
BCFA										
<i>iso</i> -C14:0	3.58	4.46	6.99	1.81	3.00	2.64	0.432	<0.0001	0.001	<0.0001
<i>iso</i> -C15:0	6.84	8.11	12.30	5.95	8.03	6.43	1.311	0.01	0.17	0.004
<i>anteiso</i> -C15:0	13.48	17.01	26.09	6.39	11.57	10.70	1.428	<0.0001	0.0004	<0.0001
<i>iso</i> -C16:0	11.36	13.68	20.84	6.24	10.92	8.93	1.285	<0.0001	0.002	<0.0001
<i>iso</i> -C17:0	16.73	20.35	30.09	8.22	14.81	13.54	1.327	<0.0001	<0.0001	<0.0001
<i>anteiso</i> -C17:0	24.66	28.58	42.48	13.42	24.39	22.02	2.052	<0.0001	0.0003	<0.0001
<i>iso</i> -C18:0	5.46	5.83	8.84	2.59	4.71	4.96	0.536	<0.0001	0.0004	0.02
Total BCFA	82.11	98.02	147.62	44.63	77.44	69.22	7.307	<0.0001	0.0003	<0.0001
Total <i>iso</i> -BCFA	43.97	52.43	79.05	24.82	41.47	36.50	4.075	<0.0001	0.0004	<0.0001
Total <i>anteiso</i> -BCFA	38.14	45.59	68.57	19.81	35.96	32.72	3.342	<0.0001	0.0002	<0.0001
OBCFA	165.21	184.08	268.49	88.98	151.45	129.42	14.435	<0.0001	0.001	<0.0001

¹OCFA = C15:0 + C17:0; BCFA = *iso*-BCFA + *anteiso*-BCFA; *iso*-BCFA = *iso*-C14:0 + *iso*-C15:0 + *iso*-C16:0 + *iso*-C17:0 + *iso*-C18:0; *anteiso*-BCFA = *anteiso*-C15:0 + *anteiso*-C17:0; OBCFA = OCFA + BCFA.

Table 3. The odd-chain fatty acid (OCFA) and branched-chain fatty acid (BCFA) concentrations in raw and treated milk samples

Fatty acid, ¹ mg/ 100 g of milk	Raw	Heated	Frozen	Heated and frozen	SEM	P-value
OCFA						
C15:0	38.84	36.56	40.34	38.42	4.404	0.693
C17:0	39.91	36.59	41.14	39.42	4.091	0.686
Total OCFA	78.75	73.15	81.51	77.85	8.251	0.668
BCFA						
<i>iso</i> -C14:0	3.87	3.55	3.93	3.65	0.516	0.707
<i>iso</i> -C15:0	7.18	6.82	8.93	8.60	1.084	0.166
<i>anteiso</i> -C15:0	14.25	13.38	14.94	14.17	1.888	0.748
<i>iso</i> -C16:0	12.04	11.05	12.76	12.01	1.463	0.566
<i>iso</i> -C17:0	17.52	16.33	17.83	17.19	2.025	0.826
<i>anteiso</i> -C17:0	25.99	23.99	27.19	26.05	2.658	0.632
<i>iso</i> -C18:0	6.00	5.07	5.33	5.12	0.622	0.429
Total BCFA	86.87	80.20	90.94	86.79	9.638	0.631
Total <i>iso</i> -BCFA	46.62	42.83	48.80	46.57	5.203	0.608
Total <i>anteiso</i> -BCFA	40.24	39.70	40.36	40.22	4.364	0.998
OBCFA	165.61	153.35	172.46	164.63	17.775	0.642

¹OCFA = C15:0 + C17:0; BCFA = *iso*-BCFA + *anteiso*-BCFA; *iso*-BCFA = *iso*-C14:0 + *iso*-C15:0 + *iso*-C16:0 + *iso*-C17:0 + *iso*-C18:0; *anteiso*-BCFA = *anteiso*-C15:0 + *anteiso*-C17:0; OBCFA = OCFA + BCFA.

iso-C14:0, *iso*-C15:0, *iso*-C16:0, and C17:0) in milk taken from cows with parity ≤ 2 , compared with milk from older cows, which is in agreement with our findings. It is known that multiparous cows usually have higher milk production potential (Nielsen et al., 2003), and higher incidence of metabolic diseases with lipid metabolism disorder (e.g., ketosis) compared with primiparous cows (Markusfeld, 1987; Suthar et al., 2013). These suggest that the physiological and metabolic differences between multiparous and primiparous may affect OCFA and BCFA concentrations in milk, which warrants further investigation in the future.

The OCFA and BCFA concentrations in colostrum, transition milk, and mature milk did not change after heated, frozen, or heated and frozen treatments (Table 3). Similar to our results, Rodríguez-Alcalá et al. (2014) did not observe alternations in OCFA and BCFA concentrations in 2 different batches of milk samples after heating with low (72°C, 30 s) and high temperatures (135°C, 30 s or 150°C, 5 min). It is well known that OCFA and BCFA are SFA, which have single bonds through all carbon chains. Therefore, they are highly stable and not readily broken in various environments. The freezing treatment in our study also did not compromise OCFA and BCFA concentrations in bovine milk, which is consistent with observations of unaltered fatty acid concentrations in sheep milk samples frozen for 1 to 6 mo (Zhang et al., 2006). The stability of OBCFA concentrations in heated, frozen, and heated and frozen milks suggest that these processes would not affect their potential bioactivities.

In summary, OCFA and BCFA concentrations were affected by milk type, parity, as well as their interaction. The total concentration of OCFA and BCFA

was lower in colostrum, compared with transition milk and mature milk samples. In addition, these fatty acid concentrations did not change after heating, freezing, or heating and freezing treatments, suggesting the commonly used pasteurization and storage practices on farm do not affect the stability of OCFA and BCFA. The identification of OCFA and BCFA in different milk types suggest that these fatty acids could play a role in calf and human infant gut health in addition to other known bioactives. In addition, the identified OCFA and BCFA in milk highlight the importance of formulating MR or starter with supplementation of these fatty acids that are needed to provide better nutritional values to dairy calves.

ACKNOWLEDGMENTS

This study was supported by the Natural Sciences and Engineering Research Council of Canada Discovery grants to G.C. and L.L.G. Westgen, BC Dairy Association, Alberta Milk, SaskMilk, and Dairy Farmers of Manitoba and by the National Natural Science Foundation of China (grant no. 31702135) and Chinese Scholarship Council (CSC). We are grateful to C. Felton (Dairy Research and Technology Centre, Edmonton, Canada) for her assistance with milk sample collections. The authors have no conflicts of interest to declare.

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