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Chemical characterisation of oligosaccharides in commercially pasteurised dromedary camel (Camelus dromedarius) milk

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ABSTRACT

It has been suggested that bactrian camel milk and colostrum may be a good source of biologically significant oligosaccharides but, although the oligosaccharides found in bactrian camel milk and colostrum have been characterised, those in dromedary camel milk have not. In this study, seven oligosaccharides from commercially available pasteurised dromedary camel milk were characterised using 1H nuclear magnetic resonance spectroscopy. The following oligosaccharides were detected: $Gal(\beta1-3)Gal(\beta1-4)Glc$ (3'-galactosyllactose), $Gal(\beta1-4)Glc$ (3'-sialyllactose), $Gal(\beta1-4)Glc$ (3'-sialyllactose), $Gal(\beta1-4)Glc$ (3'-sialyllactose), $Gal(\beta1-4)Glc$ (6'-sialyllactose), $Gal(\beta1-4)Glc$ (3'-sialyllactose), $Gal(\beta1-4)Glc$ (3'-sialyllactose), Gal(

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1. Introduction

There are two species of camels: the dromedary or Arabian camel (*Camelus dromedarius*, one hump) and the bactrian camel (*Camelus bactrianus*, two humps) (Al haj & Al Kanhal, 2010). In arid and semi-arid areas where heat and lack of water and feed severely affect dairy cows, camelids play an important role in providing milk for the population (Yagil, 1982). As the result, establishment of modern camel farms for large scale milk production is currently underway. It is expected that, in the future, modern large-scale camel farms can also become sources for the production of biofunctional milk components.

The lactose and protein contents of dromedary and bactrian camel milks are similar but their fat contents differ (Medhammar et al., 2012). The composition of bactrian camel colostrum obtained 2 h post-partum has been estimated to be 14.23% protein, 0.27% fat, 4.44% carbohydrate, and 0.77% minerals. That of mature milk collected 90 days post-partum was 3.55% protein, 5.65% fat, 4.24% carbohydrate, and 0.87% minerals (Zhang et al., 2005). Aljumaah et al. (2011) reported that the mean (±standard error)

levels of milk constituents from healthy lactating dromedary camel were 2.91 \pm 0.04% fat, 3.52 \pm 0.02% protein, 5.13 \pm 0.03% lactose and 9.40 \pm 0.05% solids-non-fat (SNF).

Al haj and Al Kanhal (2010) reported that dromedary camel milk has been acknowledged to have many functional properties due to its bioactive components. However, most of the reports on these components and their functionalities were concerned with protein-related components. Reports on functionality related to oligosaccharides are very limited.

It is generally believed that milk oligosaccharides are biologically significant as receptor analogues that inhibit the attachment of pathogenic microorganisms to the colonic mucosa, as prebiotics, which stimulate the growth of colonic bifidobacteria, and as nerve growth factors (Urashima, Kitaoka, Asakuma, & Messer, 2009). Unlike the milk oligosaccharides of domesticated dairy animals such as cows, sheep and goats, those of camels have received little attention. To obtain information on the functionality of camel milk oligosaccharides, their variety, composition and chemical structures must be investigated. The oligosaccharides of the milk and colostrum of bactrian camels were studied by Fukuda et al. (2010), who showed that bactrian camel colostrum contains higher concentration of various sialyl oligosaccharides than mature milk. Sialyl oligosaccharides from many mammalian sources have been reported in many studies, such as those of Hakkarainen et al. (2005), Matrosovich and Klenk (2003), Matrosovich et al. (1993),

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and Wang et al. (2007), to possess anti-adhesive effects against certain pathogens, as well as important nutrients for brain development.

According to the Agriculture Statistical Year Book (2009) of Saudi Arabia, as cited by Aljumaah et al. (2011), the population of dromedary camels in Saudi Arabia consists of more than 800,000 animals. Of an estimated 18 million camels in the world, only 2 million are bactrian camels (Alhadrami, 2003). Thus, dromedary species supply more milk for human consumption than bactrian. The present study focused on the structural characterisation of commercially available pasteurised dromedary camel milk marketed in Riyadh city, Saudi Arabia. Thus, the chemical structures of some neutral and acidic oligosaccharides from pasteurised dromedary camel milk were characterised using ¹H nuclear magnetic resonance spectroscopy (¹H NMR).

2. Materials and methods

2.1. Milk samples

Three litres of commercially available pasteurised (at 75 °C for 15 s) bulk whole camel milk sample was purchased from the local market of Riyadh city (central region of Saudi Arabia), in February (winter season). This milk is a pasteurised product processed by Alwatania Co. Ltd. (Riyadh, Saudi Arabia), one of the food companies in Saudi Arabia that is ISO 9001 certified and applies HACCP in their production. Compositional analysis results of the sample showed that fat and solids-non-fat levels were 3% and 8.5%, respectively, and pH and acidity were 6.54 and 0.15%, respectively. The sample was then freeze-dried using a Virtis freeze dryer, model Unitop 600SL (The Virtis Co., Gardiner, NY, USA), stored in a sealed nylon pack in -20 °C and then shipped to Obihiro University of Agriculture and Veterinary Medicine, Japan, for further analysis.

2.2. Materials

Lacto-N-neohexaose [LNnH; $Gal(\beta1-4)GlcNAc(\beta1-3)[Gal(\beta1-4)GlcNAc(\beta1-6)]Gal(\beta1-4)Glc]$ was purchased from Seikagaku Co. (Tokyo, Japan), and 3'-sialyllactose [3'-NAc-SL; Neu5Ac($\alpha2-3$) $Gal(\beta1-4)Glc]$ and 6'-sialyllactose [6'-NAc-SL; Neu5Ac($\alpha2-6$)Gal($\beta1-4$)Glc] were obtained from Sigma Co. (St. Louis, MO, USA). 3'-galactosyllactose [3'-GL; $Gal(\beta1-3)Gal(\beta1-4)Glc]$ was isolated from caprine colostrum (Urashima, Bubb, Messer, Tsuji, & Taneda, 1994), while sialyl 3'-galactosyllactose [sialyl 3'-GL; Neu5Ac($\alpha2-3$)Gal($\beta1-3$)Gal($\beta1-4$)Glc], sialyllacto-N-novopentaose a [sialyl LNP a;

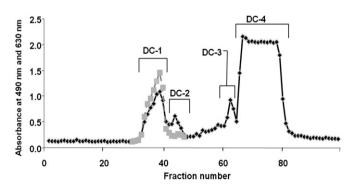


Fig. 1. Size-exclusion chromatogram of the carbohydrate fraction from dromedary camel milk. Elution from a BioGel P-2 column (2.5 \times 100 cm) was with distilled water at a flow rate of 15 mL h $^{-1}$, and of 5.0 mL fractions were collected. Each fraction was monitored by the phenol-sulphuric acid method at 490 nm (as shown by the solid line and black dots) and the periodate-resorcinol method at 630 nm (as shown by the dotted line and grey dots).

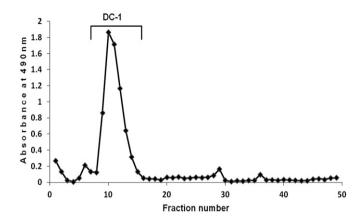


Fig. 2. Anion exchange chromatogram of fraction DC-1 separated from dromedary camel milk by gel chromatography on BioGel P-2. A DEAE—Sephadex A-50 column (1.5 \times 20 cm) equilibrated with 50 mm Tris—HCl buffer solution (pH 8.7) was used. Elution was with 250 mL of the same solution. The flow rate was 15 mL h⁻¹ and 5 mL fractions were collected. Each fraction was monitored by the phenol-sulphuric acid method.

Neu5Ac(α 2-3)Gal(β 1-3)[Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc] and monosialyllacto-N-neohexaose [MSLNnH; Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc[Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc] were isolated from bactrian camel colostrum (Fukuda et al., 2010).

2.3. Isolation of milk oligosaccharides and lactose

Milk powder (2 g) was dissolved in 18 mL of water and the solution was centrifuged at $4000 \times g$. The supernatant was extracted with 4 volumes of chloroform/methanol (2:1, v/v). The emulsion was centrifuged at $4000 \times g$ for 30 min at 4 °C, and the lower chloroform layer and the denatured protein were discarded. The methanol was removed from the upper layer by rotary evaporation, and the residue was called the carbohydrate fraction.

The carbohydrate fraction was separated into four fractions (DC-1 to DC-4, see Fig. 1) by gel filtration with a BioGel P-2 column as previously described (Fukuda et al., 2010). The above procedures were repeated eight times. The saccharides in the fractions constituting peaks DC-2, DC-3 and DC-4 (Fig. 1) were separately pooled, freeze-dried and subjected to ¹H NMR spectroscopy to characterise their chemical structures. The components in peak DC-

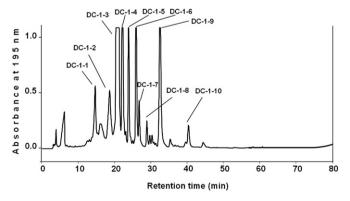


Fig. 3. High performance liquid chromatography (HPLC) chromatogram of fraction DC-1 separated from dromedary camel milk. HPLC was performed using a Shimadzu LC-10 AT VP pump on a TSK-gel Amide-80 column (4.6×250 mm, pore size 80 Å, particle size 5 µm). The mobile phases were 80% and 50% acetonitrile in 15 mM potassium phosphate buffer solution, denoted buffer A and buffer B, respectively. Elution was with a linear gradient of acetonitrile from 80% to 50% at 60 °C at a flow rate of 1 mL min⁻¹. Peaks were detected by UV absorption at 195 nm.

Table 1¹H NMR chemical shifts of fractions DC-2, DC-3 and DC-4 separated from dromedary camel milk.

Reporter group	Residue	Chemical shifts, δ (coupling constants, Hz) ^a			
		DC-2	DC-3	DC-4	
H-1	Glc α	5.222 (3.4)	5.225 (4.0)	5.221 (4.0)	
	Glc β	4.665 (8.0)	4.668 (8.0)	4.664 (8.0)	
	Gal(β1-4)	4.427 (8.0)	4.512 (8.0)	4.448 (8.0)	
		4.471 (8.0)	_	_	
		4.480 (8.0)	_	_	
	Gal(β1-3)	_	4.614 (7.4)	_	
	GlcNAc(β1-3)	4.701 (8.2)	_	_	
	GlcNAc(β1-6)	4.638 (8.5)	_	_	
		4.643 (7.4)	_	_	
H-4	Gal(β1-4)	4.145 (3.4)b	4.199 (3.4) ^b	_	
NAc	GlcNAc(β1-3)	2.030	_	_	
	GlcNAc(β1-6)	2.060	_	_	

^a The numbers in the brackets are coupling constants (*J*). Coupling constant is a measure of the interaction between neighbouring resonances or a pair of protons. ^b $J_{4,3}$.

1 (Fig. 1), which gave positive reactions with both periodate-resorcinol (630 nm) and phenol-sulphuric acid (490 nm), were subjected to anion exchange chromatography with DEAE—Sephadex A-50 as previously described (Fukuda et al., 2010). The fractions in the peak redesignated as DC-1 (see Fig. 2) were pooled, lyophilised, dissolved in 2 mL of water, and passed through a column (2.0 \times 35 cm) of BioGel P-2 to remove salts. The components in DC-1 were further subjected to high performance liquid chromatography (HPLC) on a TSK gel Amide-80 column as previously described (Fukuda et al., 2010) (chromatogram in Fig. 3). The peak fractions of oligosaccharides were subjected to $^1\mathrm{H}$ NMR spectroscopy.

2.4. ¹H NMR spectroscopy

Nuclear magnetic resonance spectra were recorded in D₂O (100.00 atom D%; Sigma–Aldrich, Milwaukee, WI, USA) at 500 or

600 MHz for 1 H NMR with a JEOL ECP-500 Fourier transform-NMR (JEOL, Tokyo, Japan) or a Varian INOVA 600 spectrometer (Varian Inc., Palo Alto, CA, USA), respectively, operated at 293.1 K. Chemical shifts are expressed as change relative to internal 3-(trimethylsilyl)-1-propane sulphuric acid, sodium salt, but measured by reference to internal acetone ($\delta = 2.225$).

3. Results

3.1. Separation of oligosaccharides from pasteurised dromedary camel milk

The carbohydrate fraction from pasteurised dromedary camel milk was subjected to gel chromatography as shown in Fig. 1. The peak that reacted positively for sialic acid (DC-1) was subjected to anion-exchange chromatography. The unadsorbed fraction, redesignated as DC-1 (Fig. 2), was pooled and passed through a BioGel P-2 column. The components contained in this fraction were then separated by HPLC (Fig. 3).

3.2. Structural characterisation of neutral oligosaccharides

As the 1 H NMR spectra of DC-4, DC-3 and DC-2 (chemical shifts in Table 1) were identical to those of authentic lactose, 3'-GL and LNnH, respectively, the oligosaccharides in these fractions were characterised as Gal(β 1-4)Glc, Gal(β 1-3)Gal(β 1-4)Glc and Gal(β 1-4)GlcNAc(β 1-3)[Gal(β 1-4)Glc, respectively.

3.3. Structural characterisation of acidic oligosaccharides

As the 1 H NMR spectra of DC-1-3 and DC-1-4 (chemical shifts in Table 2) were identical to those of authentic 3'-NAc-SL and 6'-NAc-SL, respectively, the oligosaccharides in these peaks were characterised as Neu5Ac(α 2-3)Gal(β 1-4)Glc and Neu5Ac(α 2-6)Gal(β 1-4)Glc. The 1 H NMR spectra of DC-1-5, DC-1-8 and DC-1-9 (chemical shifts in Table 2, Fig. 4) showed that their characteristic resonances

Table 2 ¹H NMR chemical shifts of fractions DC-1-3, DC-1-4, DC-1-5, DC-1-8 and DC-1-9 separated from dromedary camel milk.

Reporter group	Residue	Chemical shifts, δ (coupling constants, Hz) ^a					
		DC-1-3	DC-1-4	DC-1-5	DC-1-8	DC-1-9	
H-1	Glc α	5.221 (4.0)	5.224 (4.0)	5.225 (4.0)	5.223 (3.9)	5.219 (3.4)	
	Glc β	4.663 (8.0)	4.668 (8.0)	4.668 (7.5)	4.670 (8.1)	4.667 (8.0)	
	Gal(β1-4)	4.527 (7.4)	4.427 (8.0)	4.514 (8.0)	4.471 (7.9)	4.433 (7.4)	
		_ ` ` `	_ ` `	_ ` '	4.504 (7.9)	4.455 (8.6)	
		_	_	_	_ ` '	4.472 (8.0)	
	Gal(β1-3)	_	_	4.689 (8.0)	4.687 (7.7)	_ ` '	
	GlcNAc(β1-3)	_	_	_ ` '	_ ` '	4.731 (8.0)	
	GlcNAc(β1-6)	_	_	_	4.644 (7.7)	4.638 (8.0)	
	· · · /	_	_	_	_ ` ,	4.646 (8.0)	
H-3	Gal(β1-4)	4.113 (2.9 ^b)	_	_	_	_ ` '	
	Gal(β1-3)	_	_	4.115 (3.4 ^b)	4.114 (2.9 ^b)	_	
H-3ax	Neu5Ac(α2-3)	$1.797 (12.0^{\circ}, -12.0^{\circ})$	_	$1.802 (12.0^{\circ}, -12.0^{\circ})$	$1.802 (12.1^{\circ}, -11.9^{\circ})$	_	
	Neu5Ac(α2-6)	_	$1.744 (12.0^{\circ}, -12.0^{d})$	_	_	$1.721 (12.6^{\circ}, -12.6^{\circ})$	
H-3eq	Neu5Ac(α2-3)	2.756 (4.6 ^e)	_ ` ` ` ` ` `	2.764 (4.9 ^e)	2.762 (4.4 ^e)	_	
•	Neu5Ac(α2-6)	_ ` `	2.713 (4.6 ^e)	_ ` `	_ ` `	2.668 (4.9 ^e)	
H-4	Gal(β1-4)	_	_ , ,	4.193 (2.9 ^f)	4.173 (2.7 ^f)	4.146 (3.4 ^f)	
NAc	Neu5Ac(α2-3)	2.030	_	2030	2.029	_ ` ´	
	Neu5Ac(α2-6)	_	2.028	_	_	2.027	
	GlcNAc(β1-3)	_	_	_	_	2.051	
	GlcNAc(β1-6)	_	_	_	2.061	2.061	

^a The numbers in the brackets are coupling constants (J). Coupling constant is a measure of the interaction between neighbouring resonances or a pair of protons.

^b J_{3,4}.

^c J_{3ax,4}.

J3ax,3eq.

e J_{3eq,4}.
f J_{4,3}.

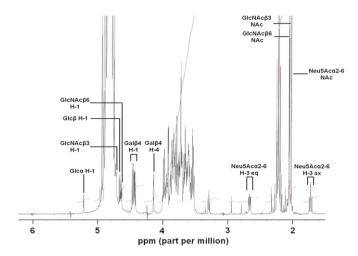


Fig. 4. The NMR spectrum of DC-1-9 (MSLNnH, Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-3) [Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc) from dromedary camel milk.

were identical to those in each spectrum of sialyl-3′-gal-actosyllactose, sialyllacto-N-novopentaose a and MSLNnH characterised from bactrian camel colostrum (Fukuda et al., 2010). Therefore, the oligosaccharides from the fractions in these peaks were characterised as Neu5Ac(α 2-3)Gal(β 1-3)Gal(β 1-4)Glc, Neu5Ac(α 2-3)Gal(β 1-3)[Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc and Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-3)[Gal(β 1-4)GlcNAc(β 1-6)] Gal(β 1-4)Glc.

The 1H NMR spectrum of DC-1-6 was identical with our unpublished data for authentic sialyllactulose, (Neu5Ac(α 2-3) Gal(β 1-4)Fru, 3'-sialyllactulose), which was probably produced from Neu5Ac(α 2-3)Gal(β 1-4)Glc by isomerisation under an alkaline conditions during anion-exchange chromatography.

Fraction DC-1-1 contained lactose, which had contaminated DC-1. The oligosaccharides in the other peaks were not characterised, because it was not possible to assign their ¹H NMR signals.

4. Discussion

Commercial processed milk is normally produced from bulk milk that is collected from many animals at different lactation stages, and the milk quality is guaranteed by food quality and safety systems during processing and along the supply chain. During the pasteurisation step, a small portion of sialic acid might have been removed from the sialyl oligosaccharides. However, as this study focused on characterisation of each oligosaccharide, such small release of sialic acid has negligible effect on the characterisation. Moreover, Bertino et al. (2008) reported that pasteurisation does not affect the concentration or pattern of analysed oligosaccharides in human milk.

The structures of the oligosaccharides of dromedary camel milk characterised in this study are shown in Table 3, which also provides a comparison of the neutral and acidic oligosaccharides of dromedary camel milk and of bactrian camel colostrum and milk. The following oligosaccharides, 3'-GL and 3'-SL, which were found in this study, also exist in bactrian camel milk (Fukuda et al., 2010). LNnH was detected only in this study, while $Gal(\beta 1-3)[Gal(\beta 1-4)]$ $GlcNAc(\beta 1-6)[Gal(\beta 1-4)Glc (lacto-N-novopentaose I) was found$ only in bactrian camel milk (Fukuda et al., 2010). However, as sialyllacto-N-novopentaose a was found in dromedary camel milk, it is possible that lacto-N-novopentaose I existed at a low concentration in this milk, too. In addition, 3'-GL, 3'-SL, 6'-SL, sialyl-3'galactosyllactose, sialyllacto-N-novopentaose a and MSLNnH, which were found in this study, were also detected in bactrian camel colostrum. Although the oligosaccharides in dromedary colostrum were not characterised in this study, the colostral oligosaccharide profile is probably similar to that found in dromedary milk; i.e., the oligosaccharides, which were found in dromedary milk in this study, might be found in dromedary colostrum. too. The oligosaccharide 3'-GL is a prebiotic molecule, which may suggest that dromedary camel milk oligosaccharides are prebiotic and could be used as a food additive with this function (Urashima et al., 2009). On the other hand, the biological function of another neutral oligosaccharide, LNnH, is still unclear.

Sialyl -3'-galactosyllactose and sialyllacto-N-novopentaose a have not been found in human milk/colostrum. However, other oligosaccharides, present in dromedary milk, including 3'-GL, LNnH, 3'-SL, 6'-SL and MSLNnH, are also present in human milk. Sialyllacto-N-novopentaose a, LNnH and MFLNnH contain Gal(β 1-4)GlcNAc (N-acetyllactosamine); these oligosaccharides are categorised as type II oligosaccharides. In camels, only type II oligosaccharides were found in this study and our previous study. The type II oligosaccharides, LNnH, MFLNnH, as well as Neu5Ac(α 2-6) Gal(β 1-4)GlcNAc(β 1-3)Gal(β 1-4)Glc (LST c), which was found in bactrian colostrum in our previous study (Fukuda et al., 2010), have

Table 3
Comparison of the characterised oligosaccharides from pasteurised dromedary camel milk (this study) with bactrian camel colostrum and milk (from the study of Fukuda et al., 2010).

Type of oligosaccharide	Milk oli	gosaccharide	Dromedary camel	Bactrian camel ^b	
	Code ^a	Structure	mature milk (fraction) ^b	Colostrum	Mature milk
Neutral	1	Gal(β1-3)Gal(β1-4)Glc	√ (DC-3)	$\overline{}$	
2 3 4 5	2	Gal(β1-6)Gal(β1-4)Glc	_	√	_
	3	$Gal(\beta 1-4)[Fuc(\alpha 1-3)]Glc$	_	√	_
	4	$Gal(\beta 1-3)[Gal(\beta 1-4)GlcNAc(\beta 1-6)]Gal(\beta 1-4)Glc$	_	_	\checkmark
	5	$Gal(\beta 1-4)GlcNAc(\beta 1-3)[Gal(\beta 1-4)GlcNAc(\beta 1-6)]Gal(\beta 1-4)Glc$	√ (DC-2)	_	_
Acidic	1	Neu5Ac(α2-3)Gal(β1-4)Glc	√ (DC-1-3)	\checkmark	\checkmark
	2	Neu5Ac(α2-6)Gal(β1-4)Glc	√ (DC-1-4)	√	_
	3	Neu5Ac(α 2-3)Gal(β 1-3)Gal(β 1-4)Glc	√ (DC-1-5)	√	_
	4	Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-3)Gal(β 1-4)Glc		√	_
	5	Neu5Ac(α 2-3)Gal(β 1-3)[Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc	√ (DC-1-8)	√	_
	6	$Gal(\beta 1-3)[Neu5Ac(\alpha 2-6)Gal(\beta 1-4)GlcNAc(\beta 1-6)]Gal(\beta 1-4)Glc$		√ √	_
	7	Neu5Ac(α 2-6)Gal(β 1-4)GlcNAc(β 1-3)[Gal(β 1-4)GlcNAc(β 1-6)]Gal(β 1-4)Glc	√ (DC-1-9)	V	_

^a For the neutral oligosaccharides, the codes refer to: 1, 3′-galactosyllactose (β3′-GL); 2, 6′-galactosyllactose (β6′-GL); 3, 3-fucosyllactose (3-FL); 4, lacto-*N*-novopentaose I (novoLNP I); 5, Lacto-*N*-neohexaose (LNnH). For acidic oligosaccharides, the codes refer to: 1, 3′-sialyllactose (3′-NAc-SL); 2, 6′-sialyllactose (6′-NAc-SL); 3, sialyl-3′-galactosyllactose (S-3′-GL); 4, sialyllacto-*N*-neotetraose c (LST c); 5, sialyllacto-*N*-novopentaose a (S-novoLNP a); 6, sialyllacto-*N*-novopentaose b (S-LNP b); 7, monosialyllacto-*N*-neotetraose (MSI.NnH).

^b The tick ($\sqrt{\ }$) indicates the oligosaccharide was isolated, the dash (-) indicates was not isolated.

been reported in human milk, too (Urashima et al., 2009). However, in human milk or colostrum, type I oligosaccharides, which contain $Gal(\beta 1-3)GlcNAc$ (lacto-N-biose I), predominate over the type II.

As mentioned previously, milk oligosaccharides are thought to have biological significances. It can therefore be expected that oligosaccharides from camel milk may in future be incorporated into human infant formulae. Mature bovine milk contains only low concentrations of milk oligosaccharides (Gopal & Gill, 2000: Martin-Sosa, Martin, Garci-Pardo, & Hueso, 2003; McJarrow & Amelsfort-Schoonbeek, 2004; Nakamura et al., 2003). The addition of bovine milk oligosaccharides to infant formulae has therefore been limited. Although fifteen neutral and twenty four sialyl oligosaccharides have been characterised in bovine colostrum (Marino et al., 2011; Urashima et al., 2009), the bulk composed of only four oligosaccharides, i.e., 3'-SL, 6'-SL, Neu5Ac(α 2-6)Gal(β 1-4) (6'-sialyl-N-acetyllactosamine) and Neu5Ac(α 2-8) Neu5Ac(α 2-3)Gal(β 1-4)Glc (disiallyllactose). The others, including neutral and sialyl saccharides, are present in only trace amounts (Fong, Ma, & McJarrow, 2011; Tao et al., 2008; Tao, DePeters, German, Grimm, & Lebrilla, 2009). However, the concentrations of sialyl oligosaccharide, including 3'-SL, 6'-SL and 6'-sialyl-N-acetyllactosamine, dramatically decrease after 48 h post-partum (Nakamura et al., 2003). Furthermore, the total concentration of oligosaccharides in bovine mature milk is very low (Fong et al., 2011), although processed bovine milk and 'waste streams' from such processing (e.g., cheese whey) should be easy sources from which to separate sialvl as well as neutral oligosaccharides. In a previous study, the profiles of sialyl oligosaccharides from colostrum and mature bovine milk were obtained (Fukuda et al., 2010). and these profiles could be compared with the oligosaccharides profile of dromedary camel milk (Fig. 3) in this study. The ratios of other sialyl oligosaccharides to 3'-SL and 6'-SL were lower than that in dromedary milk, suggesting that dromedary milk may be a better commercial source for the isolation or enrichment of these types of sialyl oligosaccharides than bovine milk and colostrum.

Camel milk oligosaccharides, especially sialyl oligosaccharides, could be isolated and added to infant formulae, as it has been reported that sialic acid may exhibit a number of health benefits for human infants, including the promotion of infant brain development (Nakamura & Urashima, 2004). There are technologies for the production of oligosaccharides, such as Neu5Ac(α 2-3)Gal(β 1-4)Glc (3'-SL, 3'-NAc-SL) by bacterial coupling (Endo, Koizumi, Tabata, & Ozaki, 2000) or GlcNAc(β1-3)Gal(β1-4)Glc (Lacto-N-triose 2, LNT $Gal(\beta 1-4)GlcNAc(\beta 1-3)Gal(\beta 1-4)Glc$ (lacto-N-neotetraose, LNnT), and 3'-NAc-SL by metabolically engineered bacteria (Priem, Gilbert, Wakarchuk, Heyraud, & Samain, 2002). However, in the food industry, the use of such technologies for production of oligosaccharides has some constraints, such as very high costs and the un-"natural" origin factor. The production of oligosaccharides that originate from natural sources, such as colostrum and milk/ milk components of dairy animals, supported by relatively low cost and simple technology such as the use of ultrafiltration membranes (Oliveira, Wilbey, Grandison, Duarte, & Roseiro, 2012), might be preferred by the food industry.

5. Conclusion

Two neutral oligosaccharides and five acidic oligosaccharides were characterised from commercially pasteurised dromedary camel milk. It is concluded that dromedary camel milk is likely to be a good source of oligosaccharides that could be utilised on industrial scale for the manufacture of functional foods, including human infant formulae. Apart from supplying milk for consumption, in the future, camel dairy farming can be developed to utilise the biofunctional properties of camel milk as an industrial resource.

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References

- Agriculture Statistical Year Book. (2009). Agricultural Research and Development Affairs, Department of Studies, Planning and Statistics. Saudi Arabia: Ministry of Agriculture, Issue 22, 163–165.
- Al haj, O. A., & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects of dromedary camel milk. *International Dairy Journal*, *20*, 811–821.
- Alhadrami, G. A. (2003). Camel. In J. W. Fuquay, P. F. Fox, & P. L. H. McSweeney (Eds.), Encyclopedia of dairy science (pp. 616–622). London, UK: Academic Press.
- Aljumaah, R. S., Almutairi, F. F., Ayadi, M., Alshaikh, M. A., Aljumaah, A. M., & Hussein, M. F. (2011). Factors influencing the prevalence of subclinical mastitis in lactating Dromedary camels in Riyadh Region, Saudi Arabia. *Tropical Animal Health and Production*, 43, 1605–1610.
- Bertino, E., Coppa, G. V., Giuliani, F., Coscia, A., Gabrielli, O., Sabatino, G., et al. (2008). Effect of holder pasteurization on human milk oligosaccharides. *International Journal of Immunopathology and Pharmacology*, 2, 381–385.
- Endo, T., Koizumi, S., Tabata, K., & Ozaki, A. (2000). Large-scale production of CMP-NeuAc and sialylated oligosaccharides through bacterial coupling. Applied Microbiology and Biotechnology, 53, 257–261.
- Fong, B., Ma, K., & McJarrow, P. (2011). Quantification of bovine milk oligosaccharides using liquid chromatography-selected reaction monitoring-mass spectrometry. *Journal of Agricultural and Food Chemistry*, 59, 9788–9795.
- Fukuda, K., Yamamoto, A., Ganzorig, K., Khuukhenbaatar, J., Senda, A., Saito, T., et al. (2010). Chemical characterization of the oligosaccharides in Bactrian camel (Camelus bactrianus) milk and colostrum. Journal of Dairy Science, 93, 5572— 5587
- Gopal, P. K., & Gill, H. S. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. British Journal of Nutrition, 84, S69–S74.
- Hakkarainen, J., Toivanen, M., Leinonen, A., Frangsmyr, L., Stromberg, N., Lapinjoki, S., et al. (2005). Human and bovine milk oligosaccharides inhibit Neisseria meningitidis pili attachment in vitro. Journal of Nutrition, 135, 2445— 2448
- Marino, K., Lane, J. A., Abrahams, J. L., Struwe, W. B., Harvey, D. J., Marotta, M., et al. (2011). Methods for milk oligosaccharide profiling by 2-aminobenzamide labeling and hydrophilic interaction chromatography. *Glycobiology*, 21, 1317– 2011.
- Martin-Sosa, S., Martin, M. J., Garcia-Pardo, L. A., & Hueso, P. (2003). Sialyloligo-saccharides in human and bovine milk and in infant formulas: variations with the progression of lactation. *Journal of Dairy Science*, 86, 52–59.
- Matrosovich, M. N., Gambaryan, A. S., Tuzikov, A. B., Byramova, N. E., Mochalova, L. V., Golbraikh, A. A., et al. (1993). Probing of the receptor-binding sites of the H1 and H3 influenza A and influenza B virus hemagglutinins by synthetic and natural sialosides. *Virology*, *196*, 111–121.
- Matrosovich, M., & Klenk, H. D. (2003). Natural and synthetic sialic acid-containing inhibitors of influenza virus receptor binding. *Reviews in Medical Virology*, 13, 85–97.
- McJarrow, P., & Amelsfort-Schoonbeek, J. (2004). Bovine sialyl oligosaccharides: seasonal variations in their concentrations in milk and a comparison of the colostrums of Jersey and Friesian cows. *International Dairy Journal*, 14, 571–579.
- Medhammar, E., Wijesinha-Bettoni, R., Stadlmayr, B., Nilsson, E., Charrondiere, U. R., & Burlingame, B. (2012). Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. *Journal of the Science of Food and Agriculture* 92, 445–474
- 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.
- Nakamura, T., & Urashima, T. (2004). The milk oligosaccharides of domestic farm animals. Trends in Glycoscience and Glycotechnology, 16, 135–142.
- Oliveira, D. L., Wilbey, R. A., Grandison, A. S., Duarte, L. C., & Roseiro, L. B. (2012). Separation of oligosaccharides from caprine milk whey, prior to prebiotic evaluation. *International Dairy Journal*, 24, 102–106.
- Priem, B., Gilbert, M., Wakarchuk, W. W., Heyraud, A., & Samain, E. (2002). A new fermentation process allows large-scale production of human milk oligosaccharides by metabolically engineered bacteria. *Glycobiology*, 12, 235–240.
- Tao, N., DePeters, E. J., Freeman, S., German, J. B., Grimm, R., & Lebrilla, C. B. (2008). Bovine milk glycome. *Journal of Dairy Science*, 91, 3768–3778.
- Tao, N., DePeters, E. J., German, J. B., Grimm, R., & Lebrilla, C. B. (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.
- Urashima, T., Bubb, W. A., Messer, M., Tsuji, Y., & Taneda, Y. (1994). Studies of the neutral trisaccharides of goat (*Capra hircus*) colostrums and of the one and two dimensional ¹H and ¹³C-NMR spectra of 6'-N-acetylglucosaminyllactose. *Carbohydrate Research*, 262, 173–184.

- Urashima, T., Kitaoka, M., Asakuma, S., & Messer, M. (2009). Milk oligosaccharides. In P. L. H. McSweeney, & P. F. Fox (Eds.), *Advanced dairy chemistry. Lactose, water, salts and minor constituents, Vol.* 3 (pp. 295–349). New York, NY USA: Springer Science + Business Media.
- Wang, B., Yu, B., Karim, M., Hu, H., Sun, Y., McGreevy, P., et al. (2007). Dietary sialic acid supplementation improves learning and memory in piglets. *American Journal of Clinical Nutrition*, 85, 561–569.
- Yagil, R. (1982). Camels and camel milk. FAO Animal Production and Health Paper [Online]. Available at http://www.disasterriskreduction.net/fileadmin/user_upload/drought/docs/FAO%20camels%20and%20camel%20milk.pdf.
- Zhang, H., Yao, J., Zhao, D., Liu, H., Li, J., & Guo, M. (2005). Changes in chemical composition of Alxa Bactrian camel milk during lactation. *Journal of Dairy Science*, 88, 3402–3410.