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Original Research Article

Composition and enrichment of caprine milk oligosaccharides from New Zealand Saanen goat cheese whey

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ABSTRACT

Goat milk contains oligosaccharides that are structurally similar to human milk, which suggests that caprine milk oligosaccharides (CMO) could mimic the beneficial physiological effects described for human milk oligosaccharides for infant health. This study aimed to characterise the nutrient composition of New Zealand Saanen goat colostrum, regular milk and whey samples and to develop an easily scalable approach to produce an enriched CMO product for use in *in vivo* experimentation. Goat milk whey was processed by a combination of ultrafiltration, enzymatic hydrolysis of the lactose, solid-phase extraction and rotary vacuum evaporation. An 80% recovery of the oligosaccharide fraction with an enrichment of 24-fold was obtained when compared to the starting whey. Lactose was reduced to 2.5% of its initial concentration by enzymatic treatment. From 8 batches (approximately 1200 mL per batch) of whey, 19 g of product were generated of which around 8% were oligosaccharides, 44% monosaccharides, 44% lactose and 4% galacto-oligosaccharides.

1. Introduction

Goat milk production is a dynamic and growing industry that is fundamental to the wellbeing of millions of people worldwide and is an important part of the economy in many countries (Silanikove et al., 2010; Yangilar, 2013). In New Zealand, the NZ dairy goat co-operative has 50 exclusive breeders with a total herd size of 30,000 animals, predominantly represented by Saanen breed. Most of the milk (20 million litres per year) is used to manufacture infant formula which is exported to around 20 countries (Co-operative, 2014). The increasing demand of goat milk for infant nutrition is evidenced by widespread reports of the use of raw goat milk and homemade formulas for infants (Grant et al., 2005; Silanikove et al., 2010; Zhou et al., 2014).

Infant formulas are used when there is not enough breast milk or breast-feeding is not possible. Goat milk provides an alternative base for the production of infant formulas, as it is known for its relatively lower allergenic burden, easier digestion and physiological benefits,

Abbreviations: CMO, caprine milk oligosaccharides; HMO, human milk oligosaccharides; BMO, bovine milk oligosaccharides; GOS, galacto-oligosaccharides; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry.

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compared to bovine milk (Jenness, 1980; Lara-Villoslada et al., 2006; Park and Haenlein, 2008; Prosser et al., 2004). Differences in milk composition have been linked to those physiological effects. The amount of lactose in bovine and goat milk is similar, but Alpha-s1 casein (only present in ruminant milk) is found in much lower concentrations in goat compared to bovine milk (Jenness, 1980; Park and Haenlein, 2008). Goat milk contains higher concentrations of nucleotides, polyamines and some of the essential amino acids (Ceballos et al., 2009; Scano et al., 2014). Goat, bovine and human milk also contain non-nutritional components, such as oligosaccharides, in different concentrations and structures that possible play a role in the different effects seem from formula-feeding.

Infants cannot digest milk oligosaccharides, which remain intact until they reach the large intestine where they are fermented and stimulate the growth of health-promoting bacteria. Oligosaccharides are highly concentrated in human milk (5–23 g L^{-1}), and structurally diverse with more than 200 different oligosaccharides reported (Zivkovic et al., 2011). Consumption of human milk oligosaccharides (HMO), for example, are known to improve immune function (Newburg, 2009), prevent adhesion of pathogens to intestinal epithelial tissues (Newburg et al., 2005), increase absorption of minerals (Scholz-Ahrens et al., 2007) and improve glucose homeostasis (Laitinen et al., 2009). Lower rates of diarrhoea, respiratory diseases, allergies and inflammatory diseases have been reported, among breast-fed infants, compared with formula-fed infants (Le Huerou-Luron et al., 2010) which may be related to the consumption of HMO (Bertino et al., 2012).

The oligosaccharide composition of goat and bovine milk has been intensely studied in the past decade (Albrecht et al., 2014; Tao et al., 2008). The number of oligosaccharides reported thus far in bovine milk (up to 40) (Marino et al., 2011; Tao et al., 2008), bovine colostrum (over 50) (Aldredge et al., 2013) and goat milk (40) (Albrecht et al., 2014) is less than the 200 different structures reported in human milk (Aldredge et al., 2013; German et al., 2008). Some of the predominant caprine milk oligosaccharides (CMO) and bovine milk oligosaccharides (BMO) are structurally similar to those in HMO suggesting that, to some extent, they may have identical or similar functions as described for HMO (Mehra and Kelly, 2006).

The aim of the present study was to investigate the oligosaccharide composition and develop a method to provide an enriched, protein-free and partially characterised oligosaccharide fraction from New Zealand Saanen goat milk on a scale sufficient for subsequent *in vivo* experimentation.

2. Materials and methods

2.1. Milk and whey origin and chemical characterisation

New Zealand Saanen goat colostrum, pasteurised regular milk and whey (from camembert-type cheese) were obtained from Over the Moon Dairy Company Limited (Putaruru, New Zealand). Colostrum samples were collected in the first 24 h after doe delivery. Regular milk and whey samples were collected during any specific period of lactation. Colostrum, regular milk and whey where obtained from pasture fed animals, milked in the morning. Colostrum, milk and whey samples were refrigerated during transportation and stored frozen at −20 °C if not processed immediately. Aliquots of each sample type were analysed for proximate composition, carbohydrate and oligosaccharide composition, as detailed below.

2.2. Proximate composition

Dry matter, protein and lipid contents of colostrum, whey and regular milk samples were determined according to the Association of Official Analytical Chemists (AOAC International) methods 930.15 (930.15, 2000), 968.06 (968.06, 2005) and 954.02 (954.02, 2005) respectively.

2.3. High performance liquid chromatography

The carbohydrate composition of colostrum, regular milk and whey samples were determined by high performance liquid chromatography (HPLC) for galactose, glucose and lactose and liquid chromatography–mass spectrometry (LC–MS) for oligosaccharides. Samples of goat colostrum, regular milk, whey and hydrolysed permeate were ultra-filtered using a 10 KDa membrane in an Amicon ultrafiltration cell (Model 8200, Millipore, Danvers, USA) to remove proteins prior to analysis. Freeze-dried oligosaccharide-enriched product was reconstituted with reverse osmosis water to a final concentration of 5 g L^{-1}. The samples were analysed for sugars using a Shimadzu LC10A HPLC (Shimadzu Oceania Ltd., Auckland, New Zealand) fitted with a Bio-Rad Aminex HPX 87H HPLC column (maintained at 45 °C) and a Shimadzu refractive index detector, RID10A. The mobile phase was sulphuric acid (5 mM) using an isocratic elution with a flow rate of 0.8 mL min^{-1}. Injection volumes were 50 μL with a run time of 15 min. Monosaccharides were quantified using calibration curves prepared for D-glucose, D-galactose, and lactose (BDH, Prolabo, UK) (0.1–2.5 g L^{-1}).

2.4. Liquid chromatography–mass spectrometry

Oligosaccharides and galactooligosaccharides (GOS) were characterised and quantified by LC–MS (Q-Exactive, Thermo Fisher Scientific, Waltham, MA, USA). Samples (20 μL) were prepared for HPLC analysis and separated on a Thermo Hypercarb column (100 mm × 2.1 mm, 5 μm particle size, Thermo Fisher Scientific, Auckland, NZ) at a flow rate of 0.4 mL min^{-1} with a gradient elution programme as follows: initial 1% B (0.1% formic acid in LiChroSolv acetonitrile, Merck, Palmerston North, New Zealand) held for 0.5 min, to 10% B after 1 min, to 16% B at 6 min, to 20% B at 12 min, to 30% B at 16 min, to 90% B at 18 min, held for 2 min, and then returned to initial conditions for a 5 min equilibration. Water was used as mobile phase A (Fraser et al., 2012).

Oligosaccharide characterisation and quantification was performed on a Thermo Scientific LTQ XL-Linear Ion Trap Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with electrospray ionisation in negative mode. Data were collected in profile data acquisition mode over the mass range from 300 to 2000 mass/charge (m/z) and processed using the Xcalibur software package provided by the manufacturer. 3- and 6-sialyl-lactose and GOS were identified based on purified standards and the other oligosaccharides were putatively identified using both retention time and m/z data. Calibration curves were; 4-galactosyllactose (Glentham Life Sciences, London, UK), 3′- and 6′-sialyl-lactose milk oligosaccharide; α-3′-and α-2′-fucosyllactose, lacto-N-hexaose, disialyllactose, lactose 1P (Carbosynth, Compton, UK) and GOS (kindly donated by Yakult, Tokyo, Japan) (0.01–0.5 g L^{-1}). While it was recognised that the various oligosaccharides will have different ionisation efficiencies during MS analysis the lack of purified oligosaccharide calibration standards meant that for this study, oligosaccharide quantification was estimated using the response curves of the purified 3- and 6-sialyl-lactose where no OS standards were available.

2.5. Goat whey oligosaccharide enrichment process

Ten litres of goat whey were processed in 8 batches (approximately 1200 mL per batch). Each batch was subject to lactose