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Differential effects of sheep and cow skim milk before and after fermentation on gastrointestinal transit of solids in a rat model

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ABSTRACT

Fermentation of milk is considered to improve ease of digestion. The protein composition of sheep milk differs from cow milk. We hypothesized that sheep milk would produce bioactive properties with different effects on gastrointestinal (GI) motility compared with cow milk and that this would also differ following fermentation. We compared the effect of sheep and cow milk drinks, pre and post fermentation, fed to rats over two weeks, on the rate of GI transit of beads over 12-hours using X-ray imaging. Stomach emptying in animals fed sheep yoghurt was more complete than that for cow yoghurt. GI transit was increased for sheep milk treated animals than for cow milk, and colonic transit was increased, with a similar pattern observed for the yoghurts. The increased colonic transit for sheep milk compared with cow milk reveals prominent species differences, regardless of whether or not the milk was fermented.

1. Introduction

Milk and dairy products are considered healthy protein sources associated with maintaining muscle, bone and digestive health. Gastrointestinal (GI) dysmotility can be a symptom of functional GI disorders such as Irritable Bowel Syndrome resulting in faster or slower GI transit (Mayer, Labus, Tillisch, Cole, & Baldi, 2015). Because dairy proteins can alter GI transit, they have potential as functional foods. Dairy protein may also help to reduce the risk of metabolic disorders such as Type 2 diabetes and obesity (Bendtsen, Lorenzen, Bendsen, Rasmussen, & Astrup, 2013; McGregor & Poppitt, 2013) as well as cardiovascular disease (Marcone, Belton, & Fitzgerald, 2017). The composition and processing of dairy protein has an impact on digestion and absorption (Barbé et al., 2013; Barbé, Ménard et al., 2014; Claeys et al., 2014), therefore manipulation of dairy protein composition through combinations of specific protein components in milk or fermented milk may provide a way to maximize benefits for specific health outcomes.

Milk is used to produce a variety of dairy products including fermented milk products such as yoghurt or drinking yoghurt. Fermentation of milk is thought to improve cardiovascular function via angiotensin-converting-enzyme (ACE) inhibitors, used to treat high blood pressure (Hideaki et al., 1990; Kohmura et al., 1989; Pihlanto-Leppälä, Rokka, & Korhonen, 1998). Effects of fermented milk on digestion are largely attributed to a combined effect of the culture bacteria together with the bioactive peptides released during the fermentation process (Beermann & Hartung, 2013; McKinley, 2005), which occurs due to the activity of lactic acid bacteria (Chaves-López et al., 2014; Hafeez et al., 2014; Hayes, Ross, Fitzgerald, & Stanton, 2007). In addition, milk proteins are digested at various points in the human GI tract to give rise to an array of bioactive peptides that can elicit a variety of physiologic effects in humans (Silva & Malcata, 2005). The rate of digestion and transit, however, could depend on the format of dairy products (e.g. milk vs. yoghurt) and types of dairy proteins (e.g. caseins vs. whey proteins) because processing alters protein structure and aggregation, thus leading to different peptides being released (Boutrou et al., 2013; Chabance et al., 1998).

Although sheep milk production worldwide is small compared with cow milk, it is a fast emerging dairying industry (Broadhurst, Samuelsson, & Day, 2016). The health benefits and nutritional value of sheep milk are far from being fully understood. Not only is the protein content higher in sheep milk than cow milk but the proteins differ in their composition resulting in different physiochemical properties (Park, Juárez, Ramos, & Haenlein, 2007). This difference may affect

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how proteins behave during processing and their biological actions once ingested.

The main proteins in cow and sheep milk are casein and whey proteins from which most bioactive peptides are derived (Nielsen, Beverly, Qu, & Dallas, 2017). Sheep milk is considered more easily digested than cow milk and of lower allergenicity, but the precise reasons for these putative differences are unknown. Sheep milk has a different casein protein composition from cow milk, being low in α-casein and high in β-casein (Park et al., 2007). This compositional change could lead to differences in micelle size and structure and soluble caseins, which could make it more easily digested providing greater potential for improving GI comfort and transit.

How fermentation of dairy protein affects transit of contents from the stomach to the colon during digestion has not been thoroughly investigated. Previous research has focussed on the probiotic effect of fermentation altering the microbiome (Veiga et al., 2014) which may, in turn, affect GI transit rather than the possibility of direct effects of the peptides themselves. Fermented infant formulas are examples of fermented milk drinks that do not contain significant amounts of viable bacteria yet can improve digestive symptoms (Szajewska, Skórka, & Pieścik-Lech, 2015). These observations might be indicative of direct peptide action.

Understanding the biological effects of cow and sheep milk pre and post fermentation may suggest possible long-term approaches to self-management of mild dysmotility, for example through dietary intervention.

The aim of this study was to investigate differences in milk from different species, and the effects of fermentation, on food function and physiology. In it, we compared the effect of the milk and yoghurt drinks from cow and sheep (standardised to 3% protein) on peptide profile and correlated this with GI transit in a rat model. Due to the sequence differences between sheep and cow milk proteins, we hypothesized that sheep milk would produce different bioactive properties from cow milk following fermentation with the same bacterial cultures, resulting in different GI transit rates. We freeze-dried the yoghurt to reduce the influence of the culture and studied the peptides resulting from fermentation. The technique used to track GI transit has been used in previous rodent studies and approximates that in humans for semi-solid contents (Dalziel, Fraser et al., 2017; Dalziel, Young et al., 2016). Understanding how milk peptide composition affects GI transit at specific GI locations will help determine the health attributes they may impart as functional foods.

2. Materials and methods

2.1. Yoghurt drinks

Cow skim milk powder (SMP 001 (111115)) was kindly provided by NZ Food Innovation (Waikato) Ltd, Hamilton, New Zealand, and sheep skim milk powder (031215 Cypher number KY03) was kindly provided by Blue River Dairy, Invercargill, New Zealand.

The fermentation of cow and sheep milk was carried out using a standard laboratory preparation procedure for set yoghurt production using thermophilic cultures that were freeze-dried then rehydrated to a drinking yoghurt. Cow skim milk powder (38% protein, < 0.1% fat, 45% lactose) (2.1 kg/15 L water) and sheep skim milk powder (52% protein, 1% fat, 37% lactose) (1.575 kg/15 L water) were rehydrated to liquid milk over 2 h using a stick blender. They were then heated to 85 °C slowly over 2 h and held at this temperature for 30 min with constant stirring. The milk was then cooled to 43 °C (over 60 min) and a commercial starter culture containing a 1:1 ratio of *Streptococcus thermophilus* and *Lactobacillus debrueckii* subsp. *bulgaricus* (CHR Hansen YF-L811 – Yo Flex®) was added to the milk at a concentration of 0.26 U/L. The inoculated milk was incubated at 43 °C for 5–6 h until the pH dropped to 4.5. The yoghurt was then frozen at −20 °C in shallow trays (in 3–4 L batches). To improve the freeze-drying process and to also reduce bacterial viability, the yoghurt was annealed by partially thawing to −5 °C and then re-freezing to −20 °C before freeze-drying.

Four dairy drinks (3% protein) were studied for cow and sheep milk, pre and post fermentation. The milk and yoghurt drinks were prepared by reconstituting the milk or yoghurt powder (at 3% protein) with water and blended for 30 s in a Waring blender. Drinks were made up daily and provided as two feeds with half kept at 4 °C prior to use.

The viscosity of the drinks (20 mL sample) was measured using a Paar Physica controlled-stress rheometer (Model MCR 301, PHYSICA Mebtechnik GmbH, Stuttgart, Germany) equipped with a cup and bob geometry (the inner diameter of the cup was 28.9 mm and the diameter of the bob was 26.6 mm) giving a gap of 1.15 mm. Samples were allowed to rest for 5 min before applying a shear rate sweep between 0.1 and 100 s^{-1}. Measurements were performed in triplicate at a constant temperature of 20 °C.

2.2. Bacterial quantification

The viable strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ss bulgaricus* were assessed in freeze-dried powder prior to the animal study. All dairy samples were reconstituted in sterile Milli-Q water at 3% protein by blending for 30 s, serially diluted in phosphate-buffered saline and grown on the appropriate medium. This system also sterile filters the water to ensure no microbial contamination and the milk provides the mineral content for the animal.

Streptococcus thermophilus was grown on Mitis-Salivarius Agar with 5% CO_2 at 37 °C for 24–48 h, and *L. delbrueckii ss bulgaricus* was grown on MRS (Fort Richard Laboratories Ltd, Auckland, NZ) pH 5.2 Agar and incubated in an anaerobic jar with Anaerobic GasPak at 45 °C for 72 h. Following fermentation and annealing the yoghurt drinks contained no *Lactobacillus bulgaricus* from the starter culture, while a *Streptococcus thermophilus* count of only 4.5×10^6 CFU/mL remained for cow yoghurt and 1.5×10^4 CFU/mL for sheep yoghurt. The reconstituted milk samples were negative for both strains.

2.3. Animal care

This study was conducted following ethical approval (AE13501) by the AgResearch Grasslands Animal Ethics Committee (Palmerston North, New Zealand) in accordance with the Animal Welfare Act, 1999 (NZ). Male Sprague-Dawley rats, 400 g, 12 weeks old, were bred at the AgResearch Small Animal Breeding Unit (Hamilton, New Zealand). The animals were housed individually at a constant temperature of 21 °C and maintained under a 12/12 h light/dark cycle. At 10 weeks of age, they were fed a solid diet of AIN-93M OpenStandard Rodent Diet (Research Diets, Inc. New Brunswick, NJ, USA) in which the protein source was egg white. This was supplemented with dairy drinks: cow milk, cow yoghurt, sheep milk or sheep yoghurt, provided *ad libitum* for two weeks. To be able to assess the effect of dairy drinks on GI transit the animals were fed a dairy-free nutritionally balanced diet in which egg white was the protein source. The animals were monitored three times weekly for weight, food intake, and General Health Score (1–5; NZ Animal Health Care Standard). At the end of the study, the rats were euthanized using carbon dioxide inhalation overdose followed by cervical dislocation.

2.4. GI transit procedures and measurements

The methods used have been described previously (Dalziel, Fraser et al., 2017; Dalziel, Young et al., 2016; Dalziel, Young, McKenzie, Haggarty, & Roy, 2017). Each rat received six solid stainless steel beads, d = 1.4 mm (Bal-tec, Los Angeles, CA, USA) via oral gavage in 2 mL of 15% barium sulfate (E-Z-HD 98% w/w, Cat. No. 764, E-Z-EM Canada Inc., kindly provided by Palmerston North Hospital, New Zealand). Isoflurane anesthesia was induced in a chamber and persisted for 5 min during which gavage was performed upon recovery of the swallow