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Sialyloligosaccharides content in colostrum and milk of two goat breeds☆

S. Claps[a,*], M.A. Di Napoli[a], L. Sepe[a], A.R. Caputo[a], D. Rufrano[a], A. Di Trana[b], G. Annicchiarico[a], V. Fedele[a]

[a] Consiglio per la Ricerca e la Sperimentazione in Agricoltura – Unità di Ricerca per la Zootecnia Estensiva, Via Appia, Muro Lucano, PZ 85054, Italy
[b] Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

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ABSTRACT

Milk sialyloligosaccharides (SOS) play an important role in brain development and increasing immunity in infants. The few studies on goat colostrum and milk OS have shown similar profile to human milk and the highest content of SOS in comparison to other ruminants. Considering the large importance given to OS to enrich infant formulas, this work aimed investigating the content of three SOS, 3′-sialyllactose, 6′-sialyllactose and disialyllactose in colostrum and milk in two Italian goat breeds during lactation. The results obtained showed significant effect of breed and sampling time on SOS content. The results revealed that the Garganica colostrum and milk contained levels of 3′-SL and 6′-SL higher than Maltese breed. The Maltese breed was characterised by interesting content of DSL. Concerning stage of lactation, from colostrum 0 h to 24 h the 3′-SL and DSL content increased, while no significant increase of 6′-SL was detected. In milk sampled at 7, 30 and 90 days after kidding, 6′-SL decreased significantly. A significant interaction breed × sampling time occurred for 3′-SL and DSL ($P < 0.001$). Garganica breed showed the highest content of 3′-SL (328.54 mg/L) in colostrum 24 h.

1. Introduction

The oligosaccharides (OS) are the third solid component after lactose and fat in human milk and they are important bio-functional components of milk (Urashima and Taufik, 2010).

György et al. (1954) reported OS prebiotic effects, promoting the growth of *Bifidobacterium bifidum*. Recent works have recognised that OS have several biological functions because they pass undigested through the upper intestine and arrive intact in the colon (Boehm et al., 2008; Locascio et al., 2007).

Milk is a natural example of a prebiotic diet of mammals during infancy and OS are the most relevant component for the prebiotic effect of human milk (Bode, 2006; Coppa et al., 2006; Newburg and Neubauer, 1995). Considering this beneficial effect, potential prebiotic OS different from those in human milk have been added and tested in infant formula (Boehm et al., 2002).

Several studies have reported the composition, structure and some bioactivities of OS in human milk, but little information is available about OS complex in domestic mammals (Boehm and Stahl, 2007; Ninonuevo

Abbreviations: 3′SL, 3′-sialyllactose (Neu5Ac-(α2–3)-Gal-(β1–4)-Glc)); 6′ SL, 6′-sialyllactose (Neu5Ac-(α2–6)-Gal(β1–4)-Glc)); DSL, disialyllactose ((Neu5Ac(α2–8)-Neu5Ac-(α2–3)-Gal-(β1–4)-Glc)); SOS, sialyloligosaccharides.

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* Corresponding author at: Via Appia, Bella-Scalo, 85054 Muro Lucano, PZ, Italy. Tel.: +39 0976 72915; fax: +39 0976 79930.
E-mail address: salvatore.claps@entecra.it (S. Claps).

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et al., 2008). Heterogeneous data are reported between ruminant species (Martìn-Sosa et al., 2003; Mcjarrow and Van Amelsfort-Schoonbeek, 2004). Goat milk was characterised (Viverge et al., 1997), finding a profile most comparable to human milk. It has the highest content of OS (250–300 mg/L), that is 5-fold higher than bovine milk (60–90 mg/L) and 10-fold more than sheep milk, but much lower than human milk (12–13 g/L) (Urashima et al., 2013).

The sialyloligosaccharides (SOS) play an important role in brain development and increasing immunity in infants (Boehm and Stahl, 2007; Montserrat and Alicia, 2001; Wang and Brand-Miller, 2003). Among sialyloligosaccharides, two of the most representatives SOS in human milk are 3′-SL and 6′-SL. In human mature milk, SOS range from 1 g/L in colostrum to 90–450 mg/L in milk (Martìn-Sosa et al., 2003). In goat mature milk, they range from 120 to 190 mg/L (Martinez-Ferez et al., 2006).

Only a few studies have been carried out on the effect of breed: Mcjarrow and Van Amelsfort-Schoonbeek (2004) and Sundekilde et al. (2012) reported results on bovine milk. To our knowledge no investigation has been made on the effect of goat breed and the different stage of milk production on SOS content.

Aim of the present study was to investigate the evolution during lactation, from colostrum time to 90 days in milk, of three SOS, 3′-sialyllactose (3′-SL), 6′-sialyllactose (6′-SL) and disialyllactose (DSL) in two Italian goat breeds as Garganica and Maltese.

2. Materials and methods

2.1. Study location and animals

For this trial, two Italian goat breeds were used as genetically distant: Maltese breed (M) (dairy goat) and Garganica breed (G) (autochthonous and less productive goat), reared at the experimental farm of CRA ZOE (Research Unit of Extensive Animal Husbandry), located in Bella, Basilicata region, Southern Italy. The animals were fed indoor, receiving polifita hay *ad libitum* plus concentrate supplementation at 14% of crude protein (CP), respectively 600 g/d for M and 400 g/d for G breed, in relation to their level of milk production. Within each breed, animals were grouped homogenously for live weight and milk production (1200 and 800 g/day of milk respectively, in mid lactation).

2.2. Sample collection and preparation

Individual colostrum and milk samples were collected from Maltese goats (M) ($n = 20$) and Garganica goats (G) ($n = 15$). Samples of 500 mL were taken from the morning milking at the following five stages: colostrum 0 h (immediately after kidding); colostrum 24 h; milk 7th day; milk 30th day; and milk 90th day. They were distributed into 10 mL collection tubes and immediately frozen at −20 °C until further analysis.

2.3. Chemicals and standards

Sodium hydroxide solution 50% (NaOH), zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), barium hydroxide octahydrate ($Ba(OH)_2{\cdot}8H_2O$) and 3′SL (from human milk, >98% pure), 6′SL (from human milk, >95% pure), DSL (from bovine colostrum, 99% pure) standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium acetate anhydrous (NaOAc) was purchased from Carlo Erba SpA (Rodano, MI, Italy).

Table 1
HPAEC-PAD separation method for carbohydrate analysis.

	Eluent A (%)	Eluent B (%)
Initial	99	1
0.0	99	1
0.2	99	1
10	90	10
50	55	45
50.1	0	100
55	0	100
55.1	99	1
70	99	1

Eluent A (NaOH 0.1 M).
Eluent B (0.1 M NaOH/0.5 M NaOAc).
By product Carbopac manual Dione.

2.4. Oligosaccharides isolation and HPAEC analysis

OS were isolated from individual colostrum and milk samples as described by Mcjarrow and Van Amelsfort-Schoonbeek (2004). Briefly, after centrifugation at 2000 × *g* at 4 °C for 10 min, the supernatant lipid layer was removed, and the proteins were precipitated by addition of 0.5 volumes of 1.8 g 100 mL^{-1} $Ba(OH)_2{\cdot}8H_2O$ and 0.5 volumes of 2 g 100 mL^{-1} $ZnSO_4{\cdot}7H_2O$. The blend was mixed by vortex and centrifuged at 12,000 × *g*, in a microfuge for 10 min at 4 °C. The supernatant was carefully removed and centrifuged again. The second supernatant was filtered with a nylon filter at 0.45 μm pore size.

Total OS fraction was separated using high performance anion-exchange chromatography (HPAEC) on a Dionex PA100 column (Dionex, Sunnyvale, California, USA), at reported conditions (Table 1). The eluting fractions were monitored by pulsed amperometric detection (Dionex ED40) and the gradient controlled by a Varian ProStar pump system, capable to maintain a flow rate of 1 ml/min for the duration of the run. Data were collected and analysed by Star Chromatography Workstation 6.41 (Varian, Inc. Walnut Creek, California, USA) and 6′-SL, 3′-SL, DSL external standards were used to generate standard curves for comparison.

2.5. Statistical analysis

Changes in colostrum and milk of SOS were analysed by means of ANOVA repeated measures procedure, which was performed using SYSTAT statistical package (Systat 13, 2009). Analysis included between-subjects main effect of breed (Garganica, Maltese), within-subjects main effect of sampling time (colostrum 0 h, colostrum 24 h, milk at 7th, 30th and 90th day after parturition) and interaction between breed × sampling time. The effects were considered to be significant at $P < 0.05$; differences between means were tested using least significant difference.