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OLIGOSACCHARIDES IN GOAT MILK: STRUCTURE, HEALTH EFFECTS AND ISOLATION

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Abstract

Oligosaccharides have been widely recognized for their prebiotic and anti-infective properties. Among the different types of mammalian milk, the one of humans is the richest source of naturally derived oligosaccharides. However, their use as a basis for functional foods is hampered, due to their structural complexity, which in turn makes their re-synthesis extremely difficult. Thus, oligosaccharides from other sources have to be used. In this sense, goat milk constitutes a very appealing candidate, as it contains the highest amount of oligosaccharides among domestic animals, while goat milk oligosaccharides show significant similarities to human milk oligosaccharides from a structural point of view. Studies on goat milk oligosaccharides are scant, and more data is required in order to provide solid clinical evidence of their beneficial effects on humans. The aim of this review is to collect and present the main research findings on goat milk oligosaccharides structure, health effects and isolation.

Key words: Goat milk, oligosaccharides, health, structure, isolation.

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INTRODUCTION

The goat is one of the first domesticated animals, present in all continents (14). Although the goat farming sector is not well supported, compared to the corresponding cow or bovine sectors, it is particularly well organized in the Mediterranean region, especially in France, Italy, Spain, and Greece (46, 51), where it constitutes one of the most vital sectors of the national economy (15).

Goat milk is consumed probably more than any other kind of milk. In some regions, its production has become more commercialized, thus allowing for a wider profit margin. For example, in France and in Italy, goat cheese is marketed as gourmet food, and is therefore traded at relatively high prices (50). Furthermore, scientific and commercial interest in goat milk and goat products has risen over the last two decades, due to the increasing evidence of goat milk's beneficial effects on health (51). In short, some of these beneficial effects are associated with its lower allergenic burden, its higher content of short and medium chain fatty acids and medium chain triglycerides (which have been used in the treatment of many physiological disorders in humans), also with its ability to improve both the iron bioavailability and the nutritive utilization of protein, magnesium, calcium, phosphorus, zinc, selenium, and finally with its ability to lower cholesterol (1, 2, 29, 30, 45, 51). For more details on the therapeutic properties of goat milk in general, readers can refer to the review of Slacanac and colleagues (51). Given these properties, goat milk may be used as a basis for a number of new innovative health promoting products or functional foods.

A very interesting feature of goat milk, which distinguishes it from other types of milk produced by domestic animals, is its relatively high concentration in oligosaccharides. Oligosaccharides are defined as "molecules containing a small number (2 to about 10) of monosaccharide residues, connected by glycosidic linkages" according to the IUB–IUPAC nomenclature and which, in the case of milk, are lactose derived (21). Goat milk contains 250-300 mg/L oligosaccharides, which account for 4-5 times more than the concentration in cow milk, and 10 times more than the one in sheep milk (34). Although goat milk is the richest source of oligosaccharides among the different types of milk from farm animals, its content is significantly lower compared to human milk (21-24 g/L for human colostrum; 12-13 g/L for mature human milk) (32, 41). However, from a structural point of view, among the oligosaccharides found in different types of milk, goat milk oligosaccharides (GOs) are the most similar to human milk oligosaccharides (HOs) (50).

In the case of human milk a number of biological functions have already been ascribed to oligosaccharides. In short, HOs are considered to have mainly prebiotic and anti-infective properties, thus being beneficial for humans, and especially for the human-milk-fed neonate (26). However, due to HOs structural complexity, synthesizing oligosaccharides with a profile similar to HOs is very difficult. Thus, there is no infant formula supplemented with oligosaccharides similar to HOs currently on the market (34).

Goat milk seems to be a very appealing candidate for a natural source of human-like oligosaccharides, due to its concentration and structure. However, research data on the bioactive oligosaccharides of goat milk is still scant. The aim of this review is to collect and present the main research findings on GOs structure, health effects and isolation.

CHEMICAL STRUCTURE

Milk oligosaccharides are mainly derived from lactose, and therefore almost all carry the lactose unit at the reducing end. They are classified into two classes: The neutral ones, which do not contain any charged monosaccharide residues, and the acidic ones, which contain one or more negatively charged residues of sialic acid (17, 37). D-glucose (Glc), D-galactose (Gal), fucose (Fuc), N-acetylglucosamine (GlcNAc), and N-acetylgalactosamine (GalNAc) can be found in milk oligosaccharides. The main sialic acid of milk oligosaccharides is N-acetylneuraminic acid (Neu5Ac), while N-glycolylneuraminic acid (Neu5Gc) and 4-, 7-, or 8-O-acetyl-N-acetylneuraminic acids have also been identified (20).

In goat milk the neutral oligosaccharides that have already been described are: three different isomers (α'3-, β'3-, β'6-) of galactosyllactose (1-2-3, Table 1), 2'-fucosyllactose (4, Table 1), N-acetylglucosaminyllactose (5, Table 1), a lacto-N-neotetraose carbohydrate with a β1-6 linkage between the lactose and the N-acetylglusamine units (6, Table 1), two different isomers of a lacto-N-fucosylpentaose (7-8, Table 1), a lacto-N-tetraose carbohydrate with a galactosyl unit attached by a β1-4 linkage to the core structure (9, Table 1), and a lacto-N-hexaose (10, Table 1).

The acidic oligosaccharide profile in goat's milk is rich in structures containing both one and two molecules of sialic acid. The main acidic oligosaccharides that have already been characterized are: 6'-N-acetylneuraminyllactose (11, Table 1), 3'-N-acetylneuraminyllactose (12, Table 1), 6'-N-glycolylneuraminyllactose (13, Table 1), 6'-N acetylneuraminyllactosamine (14, Table 1), di-(N-acetylneuraminyl)lactose (15, Table 1), 6'-N-acetylneuraminyl-β3'-galactosyllactose (16, Table 1) and 3'-N-acetylneuraminyl-β6'-galactosyllactose (17, Table 1).

As stated earlier, among the oligosaccharides found in different domestic animals' milks, GOs share the most structural similarities with HOs (50). In fact, although the complexity in HOs structure is well documented, there are still some common structures between GOs and HOs. Four out of ten neutral (β3'-galactosyllactose, β6'-galactosyllactose, 2'-fucosyllactose, lacto-N-hexaose) and two out of seven acidic oligosaccharides (6'-N-acetylneuraminyllactose, 3'-N-acetylneuraminyllactose) that are found in goat milk are also found in human milk. Moreover, lacto-N-biose unit - the building block of type I HOs - has also been identified in GOs.

Presently available in the scientific literature are a number of articles linking specific structures found in HOs to their health-promoting effects. Direct pathogen inhibition and bifidogenicity are well-studied examples of health-related structure-function relationships of HOs (6, 19, 40, 43, 48, 52, 58, 60). Special attention has to be paid to (α1-2)-fucosylated HOs, which showed inhibition of campylobacter, norovirus and toxin-producing Escherichia coli in vitro and lower incidence of diarrhea in breast fed infants in vivo (19, 40, 43). Moreover, it has been recently suggested that 2'-fucosyllactose and lacto-N-fucopentaose have an overall immunomodulatory effect (52). Fucose and sialic acids residues on the oligosaccharides' structure are also known to bind to bacterial walls, thereby preventing them from binding to the receptors on the surface of epithelial cells (39). It has been shown that oligosaccharides containing sialic acid (NeuAc) residue reduce the adhesion of leukocytes to endothelial cells, an indication for an immune regulatory effect of certain HOs (7). Lacto-N-biose (Gal-(β1-3)-GlcNAc) unit also seems to be associated with the health-promoting effects of HOs, as it has been described as essential bifidogenic factor of breast milk (22).

Given the above description of the structure-related health-promoting effects of HOs and their structural similarities to GOs, it can be suggested that goat milk may be a first choice of a natural source of human-like oligosaccharides. However, it has been also shown that oligosaccharides (e.g. galactooligosaccharides and fructooligosaccharides) with a structure different from those found in human milk are able to mimic functions of human milk, as reviewed by Boehm and Stahl (9). Hence, indentifying animal-derived oligosaccharides with biological functions similar to those of HOs, though different in structure compared to HOs, might be an interesting approach.

HEALTH EFFECTS

Based on the above, GOs have gained lately much attention as potential nutritional supplements or therapeutic agents. Thus, they have been used in a number of studies in order to evaluate their health-promoting effects.

In cell level, Martinez-Ferez *et al.* (33) suggested an anti-inflammatory action of GOs. Their research aimed at investigating whether GOs could inhibit the adhesion of monocytes to human umbilical vein endothelial cells. The results of this research indicated that GOs may in fact act as anti-inflammatory agents in the newborn infant, an effect that has already been shown for HOs, and can be attributed to the structural similarities between GOs and HOs.

In vitro, Lara-Villoslada *et al.* (28) studied the effect of GOs in the early recovery of an experimental (dextran sodium sulfate - DSS) induced colitis, using a rat model. The results from this study suggested that GOs are actively involved in the repairing process after a DSS-induced colitis. Although the mechanism of action is not very clear, the authors suggested that this may be either due to a competitive interaction of GOs with specific pathogens, or due to a prebiotic action of GOs. Thus, it is possible that GOs bind to certain pathogens, thus depriving them from their ability to bind to their targets, i.e., to the epithelial surface cell receptors on the mucosa. Furthermore, oligosaccharides may beneficially affect the host by selectively stimulating growth and/or activity of a number of bacteria in the colon, and thus ameliorate host's health (27). In fact, the development of the intestinal flora can be stimulated by oligosaccharides containing N-acetylglucosamine that enhance the growth of *Bifidobacterium bifidum* (27).

The use of GOs as prebiotic anti-inflammatory agents has also been proposed by Daddaoua *et al.* (13). Their research group studied the effect of GOs in an animal model of experimental colitis induced by the hapten trinitrobenzenesulfonic acid. GOs pre-treated rats showed decreased colonic inflammation and fewer necrotic lesions compared to the respective controls. The authors suggested that the observed upregulation of the trefoil factor 3, which is involved in tissue repair, could indicate a possible mechanism of action. Although, further research is needed in order to validate this approach, GOs seem promising as a therapeutic strategy against inflammatory bowel disease.

Table 1. Summary of oligosaccharides from goat milk, and their corresponding presence in human milk.

	Structures	Caprine milk *(mg L^{-1})*	Human milk *(mg L^{-1})*
	Neutral oligosaccharides		
1.	Gal(α1-3)Gal(β1-4)Glc (54)*	1.6 (54)	-
2.	Gal(β1-3)Gal(β1-4)Glc (54)*	36.25 (54)	ND (25)
3.	Gal(β1-6) Gal(β1-4)Glc (54)	48.5 (54)	2-3 (24)
4.	Fuc(α1-2)Gal(β1-4)Glc (54)	0.95 (54)	1580-2490 (5) 2590-4130 (53)
5.	GlcNAc(β1-6)Gal(β1-4)Glc (10)	20-40 (34)	-
6.	Gal(β1-4) GlcNAc(β1-6)Gal (β1-4)Glc (10)	nd	-
7.	Gal(β1-4)GlcNAc(β1-6)Gal(β1-4)Glc | Fuc (α1-3) (11)	nd	-
8.	Gal(β1-3)GlcNAc(β1-6)Gal(β1-4)Glc | Fuc (α1-3) (8)	nd	-
9.	Gal(β1-4) | GlcNAc(β1-3)Gal(β1-4)Glc | Gal(β1-3) (10)	nd	-
10.	Gal(β1-4)GlcNAc(β1-6) | Gal(β1-4)Glc | Gal(β1-3)GlcNAc(β1-3) (30)	1-5 (34)	50-170 (12)
	Acidic oligosaccharides		
11.	Neu5Ac(α2–6)Gal(β1–4)Glc (55)	50-70 (34)	1140-1310 (37)
12.	Neu5Ac(α2–3)Gal(β1–4)Glc (55)	30-50 (34)	100-300 (37)
13.	Neu5Gc(α2-6)Gal(β1-4)Glc (55)	40-60 (34)	-
14.	Neu5Ac(α2-6)Gal(β1-4)GlcNAc (55)	nd	-
15.	Neu5Ac(α2-8)Neu5Ac(α2-3)Gal(β1-4)Glc (34)	1-5 (34)	-
16.	Gal(β1-3)Gal(β1-4)Glc | Neu5Ac (α2-6) (59)	nd	-
17.	Gal(β1-6)Gal(β1-4)Glc | Neu5Ac (α2-3) (59)	nd	-

nd: not determined, ND: not detectable **Note*: Gal(1-3)Gal(β1-4)Glc: 30-50 mg L^{-1} in caprine milk (34).

It follows from the previous discussion that although, *in vitro* and *in vivo* studies to test GOs clinical efficacy are scant, there is some evidence indicating that they may be appealing candidates for producing either infant formulas or nutraceuticals for specific target groups, which is mainly due to their structural similarities compared to HOs.

However, GOs have also specific structural differences compared to HOs, which in turn may pose a potential impediment to their use. In fact, GOs contain both Neu5Ac and Neu5Gc, as opposed to HOs that contain only Neu5Ac.

The latter is due to the fact that humans are genetically deficient in synthesizing Neu5Gc. Given that an alternative pathway in humans to synthesize this specific sialic acid is not known, it is assumed that Neu5Gc in human tissues derives only from exogenous sources (8). Moreover, anti-Neu5Gc-antibodies have been found in humans and are associated with several diseases (18, 38). Thus, epidemiological studies are necessary in order to address whether oligosaccharide-derived Neu5Gc are correlated with tissue-incorporated Neu5Gc, and also whether accumulated Neu5Gc could alter humans' physiology and/or predispose them to develop any diseases in the future (8).

ISOLATION

In order to identify the biological functions of milk oligosaccharides in *in vitro* studies, they first need to be isolated from the complex milk-mixture, which contains proteins and fats. Some of the currently used methods include selective precipitation, chromatography, electrophoresis, and membrane technology (34, 49).

The first steps for obtaining oligosaccharides from milk are the removal of lipids and proteins. The lipids are easily removed by centrifugation, followed by filtration through a loosely packed glass-wool column (11, 23). Proteins are typically removed by precipitation by the addition of ethanol up to a final concentration of 68% (11, 31, 34). In this step, part of the lactose is also precipitated. However, the efficiency of lactose removal may depend on the temperature used (34, 61). The remaining lactose can be removed by passing through a Sephadex G-25 column connected to a fast protein liquid chromatography system (FPLC) (34). The oligosaccharides obtained in this way can be further fractionated on a Bio-Gel P-4 column and the fractions can be further purified by reverse- phase high-performance liquid chromatography (HPLC) (11).

An alternative method to remove lactose is hydrolysis by β-galactosidase (49). In the protocol described by Sarney *et al.*, after hydrolysis, the enzyme was removed by ultrafiltration and the obtained solution, containing glucose, galactose and the oligosaccharides, was used for nanofiltration. The process was run at 30 bars and was repeated 2-3 times, resulting in an enriched final retentate containing more than 90 % oligosaccharides.

More recently, membrane technology, is being more and more used for oligosaccharide isolation from milk. Martinez- Ferez and his co-workers described the use of membrane technology for the isolation of oligosaccharides from pasteurized skimmed goat milk. A two-stage tangential filtration process was used. In the first stage, the milk was filtered with molecular-weight cut-off of 50 kDa to remove part of the proteins. The permeate was then filtered over a membrane with a molecular-weight cut-off of 1 kDa to remove lactose and salts. At the end of the process, 95% of the oligosaccharides have been obtained in the final retentate (34-36). However, due to the choice of the first membrane still 3 %(w/v) protein remained in the final retentate.

One limiting factor in the use of membranes in an industrial scale is the cost of purchasing and replacing them. In this sense, cleaning of the membranes is essential in order to maintain their functionality and lower the variable costs (3). According to Almecija *et. al.* (3) the use of consecutive cleaning agents (alkaline, acid, disinfectant) is the most effective cleaning protocol, although it cannot reassure the complete recovery of the membranes. Furthermore, the cleaning temperature can also influence the permeability of ceramic membranes. A cleaning temperature of 50°C is suggested as optimal, as far as membrane permeability restoration and separation yield is concerned (4).

Following the isolation and purification of the oligosaccharides, a number of techniques can be applied for the determination of their structural features. In case of GOs, nuclear magnetic resonance (NMR) spectroscopy (10, 11, 54), fast atom bombardment (FAB-MS) (34) and a combination of them (55, 59) have successfully been applied.

Concluding, as already described above, there is a variety of methods and techniques which could be used in GOs isolation. However, the optimal selection should be based both on the cost and the productivity of each of them. Additionally, the selection of the raw material is crucial. Given that colostrum contain higher amounts of oligosaccharides, goat milk from the first four days after parturition may be a better source for recovering higher amounts of oligosaccharides.

CONCLUSIONS

Research data suggest that goat milk may be a very appealing source of human-like oligosaccharides. It is both GOs structural profile and higher content, as opposed to other domestic mammals, that places GOs in the first ranks of animal derived oligosaccharides. However, there is still lack of data concerning the variation of GOs profile depending on the season, the diet, the lactation stage, the breed, and the number of milkings. Potential variations in GOs composition and concentration could be crucial for their further use. Moreover, more clinical and epidemiological studies are needed, in order to validate whether GOs exhibit health-promoting effects comparable to the ones already proven for HOs.

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