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Genetic and environmental factors shaping goat milk oligosaccharide composition

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ABSTRACT

Oligosaccharides (OS) in milk have been suggested to influence the health and development of the newborn by promoting growth of beneficial gut bacteria, stimulating brain development, and enhancing immune functions. Goat milk is a natural source of specific OS, which could be a potential beneficial ingredient for infant formula. In this study, goat milk OS (*g*MOS) content from ~1,000 dairy goats across 18 commercial farms was studied. A genomic relationship matrix was used to unravel genetic and environmental factors shaping *g*MOS content. The most abundant *g*MOS identified was 3′-N-glycolyl-neuraminyl-lactose (NGL), with a concentration of 32.05 mg/kg, whereas 3-fucosyllactose (FL) exhibited the lowest concentration at 1.85 mg/kg. Acidic OS had a notably higher content (81.67 mg/kg) than neutral OS (24.88 mg/kg). High variability in *g*MOS content was observed among individual goats, which could for a large extent be attributed to genetic differences. Heritability estimates ranged from 31% for 3′-galactosyllactose (GL) to 85% for 3-FL. High positive genetic correlations (>0.57) were estimated between 3′-sialyllactose (SL) and 6′-SL, and between 6′-GL and 3′-GL. The contribution of differences between farms to variation in milk OS content varied from 3% for 3′-NGL to 45% for 6′-SL. Although *g*MOS such as 3′-GL, 6′-GL, and 6′-NGL, were significantly influenced by systematic environmental factors such as the lactation stage, the effect of these factors was relatively minor compared with the importance of genetic and farm effects. This research, which stands out due to its relatively large sample size, underscores the pivotal role of genetics, and to a smaller extent farm practices such as feed ration, in determining *g*MOS composition.

Key words: oligosaccharides, goat milk, infant formula, genetic variability, environmental factors

INTRODUCTION

Milk provides the necessary elements needed by newborns, including primary nutrients such as fat, protein, and lactose. Additionally, it contains components such as minerals and oligosaccharides (**OS**) that offer additional health benefits. Oligosaccharides are complex carbohydrates with ~200 identified structures in human milk (Bode, 2012). Generally, they can be classified into 2 categories: (1) neutral OS that can be divided in nonfucosylated OS having a N-acetylhexosamine residue, and OS with fucose residues (fucosylated); and (2) acidic OS, containing a sialic acid residue (Wang et al., 2020). Because infants cannot digest OS and these compounds reach the gut intact, where they promote the growth of beneficial bacteria such as bifidobacteria and lactobacilli (Marcobal et al., 2010), inhibit the adhesion of pathogens (van der Toorn et al., 2023), and stimulate the immune system (Bode, 2012; Wiciński et al., 2020).

Exclusive breastfeeding in the first 6 mo of life is considered as the most optimal source of nutrition for infants. However, if breastfeeding is not an option, goat milk-based infant formula is considered a good alternative (He et al., 2022) due to its better digestibility (Haenlein, 2004) and higher concentrations of OS in both colostrum (up to 2.4 g/L) and in mature milk (ranging between 60 and 350 mg/L), as well as for the greater variety of OS structures when compared with milk from cows and sheep (van Leeuwen et al., 2020). Therefore, goat milk can be a natural source of OS to be included in infant formula. However, considerable differences are present in OS composition between human and goat milk, such as higher levels of fucosylated OS in human as compared with goat milk (e.g., 2′-fucosyllactose [**FL**] and 3-FL; Goehring et al., 2016; van Leeuwen et al., 2020). Enhancing the diversity and content of specific goat milk oligosaccharides (***g*MOS**) might improve health benefits of goat milk for the production of infant formula. If sufficient genetic variation in *g*MOS composition exists, selective breeding might be an option to achieve a *g*MOS

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composition more similar to human milk. Additionally, the influence of herd management and feeding strategies should be evaluated as alternative or complementary method for changing goat milk OS. Collectively, these strategies may offer new opportunities for the production of specialized goat based infant formula that resembles OS levels and composition/diversity found in human milk.

Despite documented variation in OS content in species as goat, human, and other mammals (Martinez-Ferez et al., 2006; Goehring et al., 2016; McGuire et al., 2017; van Leeuwen et al., 2020; Wang et al., 2023), the role of environmental factors such as diet on OS content has been poorly studied. It has been suggested that the levels of goat milk OS are affected by factors such as breed, age of the goat, parity, (Claps et al., 2014, 2016; Martín-Ortiz et al., 2017), and lactation stage (Claps et al., 2014; de Sousa et al., 2015; Martín-Ortiz et al., 2017). These studies were based on relatively small numbers of animals ranging between 5 and 56. Studies in bovine initially indicated no significant effect of feed ration on bovine milk OS composition (Liu et al., 2014, 2019; Vicaretti et al., 2018). In particular, Liu et al. (2014) could not draw definitive conclusions regarding the role of diet in milk OS composition. However, Durham et al. (2022) highlighted the potential effect of a low-starch, high-fiber diet on milk OS production. This evolving perspective underscores the current uncertainties related to factors affecting milk OS biosynthesis.

A comprehensive understanding of the genetic and environmental factors affecting the milk OS content in goat milk is crucial for developing effective strategies to enhance the OS profile in goats. In this study, we have analyzed a population of almost 1,000 goats on 18 commercial dairy farms in the Netherlands with the aim of investigating genetic and environmental factors that influence *g*MOS content.

MATERIALS AND METHODS

Ethics Statement

This research adhered to the animal experimentation guidelines of Wageningen University & Research. Blood collection was performed by a licensed veterinarian and milk samples were obtained during the regular milking process with written consent of the animal owners. Aspects concerning the welfare and treatment of animals in this study did not require approval from an Ethics Committee.

Sample Collection

Milk samples were collected from 996 goats located on 18 commercial farms in the Netherlands. The Dutch commercial dairy goat can be considered a synthetic population with contributions from several breeds such as the Dutch White goats, Saanen, Alpine, Nubian, and the Toggenburg. The contribution of each of these breeds to the genetic makeup of individual goats in the study population is unknown. The average number of goats on a Dutch dairy goat farm in 2023 was 752 (Agrimatie, 2024). Goats on farms included in this study did not have access to pasture but were kept inside year-round, such as is common on most commercial goat farms in the Netherlands. Goats were fed on a diet consisting of grass silage and concentrates and, depending upon the farm, this base ration was supplemented with for example maize silage, sugarbeet pulp, or brewers' grain. The sampling took place from May till October 2021 and a total of 50 to 60 goats from each farm were sampled on the same day. Milk samples were collected during routine morning milking, using a "quarter milker." In this way the total amount of milk produced during a morning milking by each goat was quantified. Subsequently, a representative milk sample of 250 mL was taken from each animal, stored on ice after collection, and transported to the laboratory. Cream was separated by centrifuging 50 mL of milk at 960 × *g* for 20 min at 4°C. Skim milk was stored at −80°C, and 15 mL was used for OS measurements. After the milking, blood from the goat was extracted by a veterinarian from the jugular vein and stored in EDTA tubes of 5 mL at −20°C.

Fat, protein, and lactose content of fresh whole milk was determined in all milk samples at the certified laboratory for milk control (Qlip, the Netherlands) using Fourier Transform Infrared analyses.

Genotyping. At commercial goat farms in the Netherlands, natural mating is the most common breeding system. Therefore, pedigree information is often incomplete or absent for most of the animals. The DNA analyses and SNP genotypes can be used to reconstruct family relationships enabling quantitative genetic analyses (VanRaden, 2007). The DNA was isolated using the Gentra Blood kit (Qiagen N.V.) on EDTA blood samples. The quality and quantity of the obtained DNA were evaluated using Qubit (Qiagen N.V.) and goats were genotyped using the GGP (Geneseek Genomic Profiler chip) Goat 70K array. The SNP on this chip were selected based on information obtained from several goat breeds, including fiber, meat, and milk breeds. Therefore, several of the SNP were not informative in the study population. The SNPs were screened and filtered using PLINK v. 1.07 (Purcell et al., 2007). This included removal of SNPs that did not map to the goat reference genome (ARS1), as well as those located on the sex chromosomes. Additionally, 16,290 SNPs that had a minor allele frequency lower than 5%, or a missing genotype rate higher than 10% were excluded. Finally, 47,974 SNPs were used in this study for constructing the genomic relationship matrix.

gMOS Quantification. Goat milk OS where analyzed at Eurofins (Heerenveen, the Netherlands), according to the Eurofins Complex Carbohydrates and Chemistry protocol (Austin and Bénet, 2018) using a ultra-high pressure liquid chromatography with fluorescence detection method. Briefly, absolute concentrations were estimated using the detector area ratio of each *g*MOS and the internal standard laminaritriose for each of the following *g*MOS: 2′-FL, 3-FL, 3′-sialyllactose (**SL**), 6′-SL, 3′-galactosyllactose (**GL**), 6′-GL, 3′-N-glycolyl-neuraminyllactose (**NGL**), and 6′-NGL. Area ratios were multiplied with molar mass of *g*MOS and divided by molar mass of laminaritriose and then multiplied with the amount of added laminaritriose to calculate the amount of *g*MOS in the milk sample.

Statistical Analyses

Statistical analyses of the *g*MOS were performed using the following mixed linear model:

$$\mathbf{y}_{ijklg} = \mu + farm_i + parity_j + lactation_k + \beta_1 age_l + \beta_{2,j}\left(age_l \times parity_j\right) + animal_g + e_{ijklg}, \quad [1]$$

where $\mathbf{y}_{ijklg}$ represents a vector of goat milk OS; μ is the overall mean. Other fixed effects in the model are $farm_i$ (with 18 levels), $parity_j$ (with 2 levels, parity 1 and parity >1), lactation stage ($lactation_k$), categorized into 5 levels: 7–100 d, 101–200 d, 201–300 d, 301–400 d, and >400 d. The age of the animal at last kidding (age_l) is included as a covariable (with regression coefficient β_1), and the interaction term between parity and age of the animal at last kidding ($parity_j \times age_l$) is included as a covariable (with regression coefficients $\beta_{2,j}$). The random additive genetic effect of each goat ($animal_g$) was assumed to be distributed as $N\left(\mathbf{0}, \mathbf{G}\sigma_g^2\right)$, with **G** being the genomic relationship matrix estimated based on 47,974 SNP genotypes as described at Yang et al. (2010), and σ_g^2 the additive genetic variance explained by the SNPs. The error term ($\mathbf{e}_{ijklg}$) was assumed to be distributed as $N\left(\mathbf{0}, \mathbf{I}\sigma_e^2\right)$, with **I** being the identity matrix and σ_e^2 representing the residual variance.

The h^2 was estimated as

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} = \frac{\sigma_g^2}{\sigma_p^2},$$

where σ_g^2 is the genetic variance, σ_e^2 is the residual variance, and σ_p^2 is the phenotypic variance as estimated using model [1]. The significance of fixed effects was determined based on the conditional Wald *F*-test. Statistical analyses were performed using Asreml-R version 4.1.0.126 (Gilmour et al., 2021)

Bi-variate analyses were performed using model [1] and used to estimate genetic (r_g), environmental (r_e) and phenotypic (r_p) correlations between 2 different *g*MOS (e.g., x and y):

$$r_g = \frac{Cov\left(g_x, g_y\right)}{\sqrt{\sigma_{g_x}^2 \cdot \sigma_{g_y}^2}},$$

$$r_e = \frac{Cov\left(e_x, e_y\right)}{\sqrt{\sigma_{e_x}^2 \cdot \sigma_{e_y}^2}},$$

$$r_p = \frac{Cov\left(p_x, p_y\right)}{\sqrt{\sigma_{p_x}^2 \cdot \sigma_{p_y}^2}},$$

where subscripts *x* and *y* denote the *g*MOS.

RESULTS

Descriptive Statistics

The descriptive statistics of the *g*MOS are reported in Table 1. The 3′-NGL was the most abundant *g*MOS with a mean content of 32.05 mg/kg, whereas the *g*MOS with the lowest concentration was 3-FL with a mean content of 1.85 mg/kg. The total content of acidic OS (81.67 mg/kg) was higher than that of neutral OS (33.76 mg/kg). The content of acidic OS ranged from 15.94 mg/kg (6′-SL) to 32.05 mg/kg (3′-NGL) and the content of neutral OS ranged from 1.85 mg/kg (3-FL) to 12.46 mg/kg (3′-GL). Coefficient of variation for all *g*MOS was larger than 41% indicating large differences between individual goats in *g*MOS content. In general, *g*MOS with a higher concentration tend to have a higher CV. Exceptions are 2′-FL which has a low concentration (2.02 mg/kg) but is highly variable (CV = 164%) and 3′-NGL, which has a high concentration (32.05 mg/kg) but showed the least variation (CV = 45%).

Genetic Parameters for gMOS

The proportion of the phenotypic variance that can be attributed to genetic factors (h^2) is shown in Table 2 (and Supplemental Table S1, see Notes). The h^2 ranged from moderate ($h^2 = 0.31$) for 3′-GL to very high ($h^2 = 0.85$) for 3-FL. Standard errors for h^2 estimates range from 0.07 to 0.09. Highly heritable *g*MOS included 3-FL (0.85), 3′-SL (0.78), and 3′-NGL (0.71).

Table 1. Descriptive statistics of oligosaccharide composition (mg/kg) of 996 goat samples

Item	Abbreviation	Mean (mg/kg)	SD	CV%
Neutral oligosaccharides				
2′-fucosyllactose	2′-FL	2.02	3.31	164
3′-fucosyllactose	3-FL	1.85	0.87	47
3′-galactosyllactose	3′-GL	12.46	5.12	41
6′-galactosyllactose	6′-GL	8.55	5.47	64
Acidic oligosaccharides				
3′-sialyllactose	3′-SL	17.28	13.40	78
6′-sialyllactose	6′-SL	15.94	21.12	132
3′-N-glycolyl-neuraminyl-lactose	3′-NGL	32.05	14.44	45
6′-N-glycolyl-neuraminyl-lactose	6′-NGL	16.40	14.92	91

Phenotypic and genetic correlations between *g*MOS are shown in Table 2 (Supplemental Table S2 and Supplemental Figure S1A and S1B, see Notes). Estimated genetic correlations between *g*MOS showed high SE (ranging from 0.10 to 0.25). Estimates for phenotypic correlations were more accurate, with SE lower than 0.04. Strong and positive phenotypic and genetic correlations were found between 3′-GL and 6′-GL (r_p = 0.75 and r_g = 0.57) and between 3′-SL and 6′-SL (r_p = 0.56 and r_g = 0.76). Moderately negative phenotypic correlations were estimated between 3′-NGL and 3′-SL (r_p = −0.37), and between 3′-NGL and 6′-SL (r_p = −0.33). Phenotypic correlations among several *g*MOS were weak to moderate with values ranging from −0.26 (3′-SL and 6′-NGL) to 0.42 (6′-NGL and 3′-NGL).

Systematic Environmental Factors. The systematic environmental effects of herd, parity, days in lactation, age at last kidding and the interaction between lactation days and age at last kidding were accounted for in the statistical analyses. Figure 1 shows the significance (−$\log_{10}$[*P*-value]) of the evaluated systematic environmental effects on the different *g*MOS. The estimates for effects are indicated in Supplemental Table S3A (see Notes). To make estimated effects on different OS comparable, Supplemental Table S3B presents the standardized effects. Standardization is based on standard deviations reported in Table 1. The content of 3′-GL and 6′-GL were significantly affected by lactation stage and the interaction between parity and age at last kidding. Moreover, 6′-NGL was significantly affected by lactation days. Interestingly, the other OS analyzed in this study were not significantly affected by parity, age, the interaction between parity and age, or lactation days.

The effect of farm was significant ($P < 0.05$) for all evaluated *g*MOS (Figure 1). Highly significant differences between farms were observed for 6′-SL, 6′-NGL, 6′-GL and 3′-GL ($P < 0.001$). Significant but smaller differences between farms were observed for 3′-NGL ($P < 0.05$) and 2′-FL ($P < 0.001$). Estimated farm effects are presented in Supplemental Table S3. The effects of the farms were expressed relative to farm one (which was fixed at 0). Therefore, differences between the farms rather than the absolute values are relevant. The difference in 6′-SL between the highest and the lowest farm is ~59.3 mg/kg. The estimated farm effects show that the highly significant effect on 6′-SL can be attributed for a large extent to high and deviating values for 2 farms: farm 6 and farm 13 (Supplemental Table S3). These 2 farms not only have high levels of 6′-SL but also have high levels of 6′-NGL, as compared with other farms. To gain further insight in how farm effects simultaneously influence different *g*MOS, Pearson correlation coefficients were calculated between estimated farm effects (Supplemental Table S4, see Notes). These results suggest that farm effects increasing levels of 6′-SL also tend to increase levels of 6′-NGL (r = 0.81) and 3′-SL

Table 2. Estimated genetic correlations (above diagonal), h^2 (bold diagonal), phenotypic correlation (below diagonal), and SE (in parentheses) for goat milk oligosaccharides composition

Item	Category	2′-FL	3-FL	3′-GL	6′-GL	3′-SL	6′-SL	3′-NGL	6′-NGL
2′-fucosyllactose	2′-FL	**0.36 (0.09)**	0.13 (0.13)	−0.04 (0.20)	0.02 (0.17)	0.01(0.13)	−0.12(0.17)	0.08 (0.14)	−0.07 (0.17)
3′-fucosyllactose	3-FL	0.01 (0.04)	**0.85 (0.07)**	0.24 (0.13)	0.16 (0.11)	0.14(0.09)	0.18(0.12)	0.03 (0.09)	0.23 (0.12)
3′-galactosyllactose	3′-GL	0.12 (0.03)	0.08 (0.04)	**0.31** (0.09)	0.57 (0.11)	0.21(0.13)	0.28(0.17)	−0.25 (0.14)	−0.31 (0.19)
6′-galactosyllactose	6′-GL	0.05 (0.03)	0.02 (0.04)	0.75 (0.02)	**0.46** (0.09)	0.22(0.11)	0.26 (0.14)	−0.13 (0.12)	−0.18 (0.15)
3′-sialyllactose	3′-SL	0.08 (0.03)	0.08 (0.04)	0.18 (0.03)	0.15 (0.03)	**0.79**(0.07)	0.76 (0.07)	−0.42 (0.08)	−0.41 (0.11)
6′-sialyllactose	6′-SL	0.06 (0.03)	0.02 (0.04)	0.32 (0.03)	0.26 (0.03)	0.56(0.02)	**0.46** (0.09)	−0.59 (0.10)	−0.02 (0.16)
3′-N-glycolyl-neuraminyl-lactose	3′-NGL	0.08 (0.03)	0.10 (0.04)	0.04 (0.03)	0.04 (0.04)	−0.37(0.03)	−0.33 (0.03)	**0.71** (0.08)	0.36 (0.11)
6′-N-glycolyl-neuraminyl-lactose	6′-NGL	0.01 (0.03)	0.09 (0.04)	0.16 (0.03)	0.15 (0.03)	−0.26(0.03)	0.10 (0.03)	0.42 (0.03)	**0.43** (0.09)

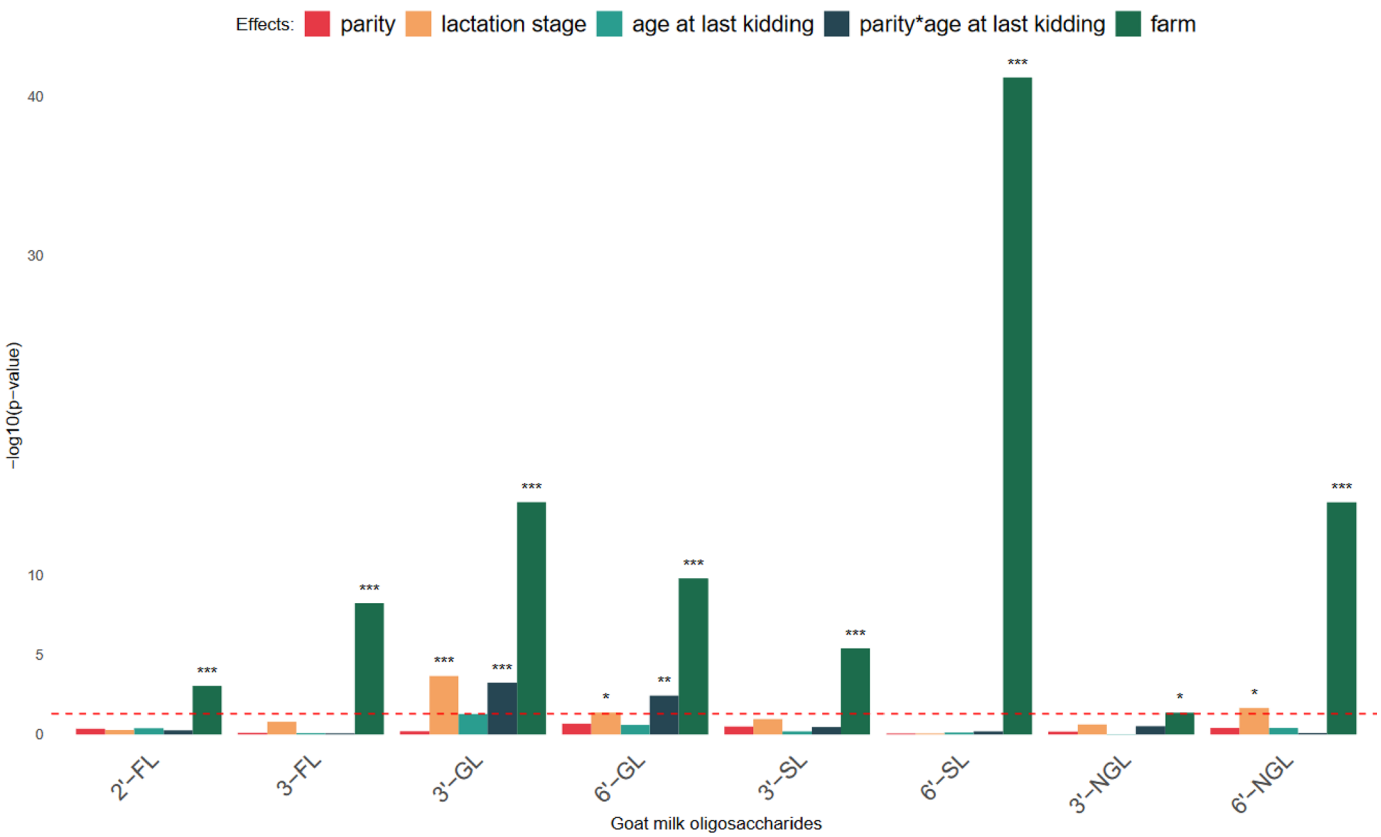

Figure 1. Effect of parity, lactation stage, age at the last kidding, interaction between lactation stage and age at the last kidding, and farm on goat milk oligosaccharide composition. The horizontal red line indicates the significance threshold, which is set at −log10(0.05). The y-axis displays the −log10(*P*-value), and the x-axis represents the 8 studied oligosaccharides. Each bar corresponds to a fixed effect included in the model. Significant effects are represented as **P*-value < 0.05, ***P*-value < 0.01, and ****P*-value < 0.001.

(r = 0.76). Moreover, farm factors that increase 6′-GL increase levels of 3′-GL (r = 0.67). Higher levels of 3-FL lead to a reduction in the content of 3′-GL (r = −0.58) and 6′-GL (r = −0.54); and an increase in 2′-FL increases 3′-GL (r = 0.64); Additionally, higher levels of 3′-NGL tend to increase 6′-NGL.

To compare the contribution of farm and genetic effects to the variation in *g*MOS, alternative analyses were performed in which farm was modeled as a random instead of a fixed effect (Equation [1], Supplemental Table S5, see Notes). Results are in line with the significances presented in Figure 1 and show that a relatively small percentage of the total variation in 2′-FL (6%) and 3′-NGL (3%) content can be attributed to differences between farms. However, a large percentage of the total variation in 6′-SL (45%), 3′-GL (21%) and 6′-NGL (18%) can be attributed to differences between farms.

Correlations of gMOS with Milk Yield and Composition. Descriptive statistics of the morning milk samples with respect to milk yield, fat-, protein- and lactose content can be found in Supplemental Table S6A (see Notes). Average lactose content was 4.21% and the CV was 6%. Estimated heritabilities for milk yield and composition and phenotypic and genetic correlations between *g*MOS and milk yield and composition are shown in Supplemental Table S6A and S6B. In general, phenotypic correlations were weak and ranged from −0.27 (between 3′-GL and milk yield) to 0.38 (between 3′-GL and protein%). Phenotypic correlations between *g*MOS and lactose content, which is one of the building blocks of *g*MOS, ranged between −0.09 and 0.10. Standard errors for phenotypic correlations were ≤0.04. Estimated genetic correlations were also weak and have SE ranging from 0.09 to 0.23.

DISCUSSION

The *g*MOS content of ~1,000 dairy goats from 18 commercial farms was analyzed with the aim to quantify the importance of genetic and environmental factors on *g*MOS content and composition. Large differences were present between individual goats in *g*MOS content. A major part of these differences could be related to genetic differences between goats. Heritability estimates ranged from moderate for 3′-GL (31%) to very high for 3-FL

(85%). Strong phenotypic and genetic correlations were identified between specific *g*MOS, such as 3′-GL and 6′-GL, and 3′-SL and 6′-SL. Interestingly, all evaluated *g*MOS were significantly affected by differences between farms, however, the importance of farm effects differed considerably among *g*MOS: 3% of the differences in 3′-NGL could be attributed to differences between farms whereas this was 45% for 6′-SL. Some *g*MOS were significantly affected by systematic environmental factors such as lactation stage, but these effects were relatively small when compared with effects of genetics and farm.

gMOS Composition

The high variability in the studied *g*MOS is in line with results from other research (Claps et al., 2016; van Leeuwen et al., 2020; Chatziioannou et al., 2021). van Leeuwen et al. (2020) highlighted large *g*MOS variability, both within and across studies. In the current study the total *g*MOS content was 106.55 mg/kg, which is within the previously reported range of 60 to 350 mg/L (Claps et al., 2014; Martín-Ortiz et al., 2016, 2017; van Leeuwen et al., 2020). Acidic *g*MOS levels (81.67 mg/kg) were higher than those of the neutral *g*MOS (24.88 mg/kg), corroborating earlier studies that reported 149.3 mg/L acidic *g*MOS and 28.8 mg/L neutral *g*MOS in mature goat milk (van Leeuwen et al., 2020). This supports results from other studies suggesting that acidic *g*MOS have a higher concentration in goat milk than neutral *g*MOS (Urashima et al., 2013; Albrecht et al., 2014; Claps et al., 2014; Chatziioannou et al., 2021). Similar to the current study, the 3′-NGL was identify as the most abundant *g*MOS in Saanen breed (Chatziioannou et al., 2021). Our results show similar 3′-SL concentrations in mature milk (17.28 mg/kg) to those reported in Saanen (n = 5, 18.51 mg/L) and Guanzhong (n = 5, 17.17 mg/L) breeds (Lu et al., 2020; van Leeuwen et al., 2020). Comparing the results obtained in a larger Saanen population (n = 57), the level of 6′-GL (8.05 mg/L) are in line with our findings (8.55 mg/kg) although concentrations of 2′-FL, 3′-SL, 6′-SL, 3′-NGL, and 6′-NGL were lower in our population (Chatziioannou et al., 2021).

The differences between *g*MOS content in the current study and other studies (Claps et al., 2016; van Leeuwen et al., 2020; Chatziioannou et al., 2021; e.g., lower amount of 2′-FL) 3′-SL, 6′-SL, 3′-NGL, and 6′-NGL, could be attributable to several factors. First, our results underscore the importance of genetic factors on *g*MOS content. Therefore, samples from different breeds or populations may naturally exhibit differences in *g*MOS content. Second, several studies were based on limited sample sizes, ranging from 5 to 57 goats, and are often sourced from a single or just a few farms (Claps et al., 2014, 2016; van Leeuwen et al., 2020; Chatziioannou et al., 2021). Our results show that *g*MOS levels can differ substantially between farms. Third, the analytical method used for quantifying *g*MOS can introduce differences between studies. In line with recommendations for the study of milk OS in human and goats species (Thurl et al., 2017; van Leeuwen, 2019; van Leeuwen et al., 2020), a cross-laboratory study evaluating various methodologies for *g*MOS quantification could clarify the extent to which methodological differences contribute to observed differences between studies.

Contribution of Genetics to gMOS Variability

To the best of our knowledge, this study is the first to estimate the h^2 of OS in goat milk. The estimated h^2 for the evaluated *g*MOS were all higher than 31% (Table 2). These results indicate that a substantial genetic component contributes to the variability of *g*MOS. In line with our results, studies in other species such as cows, demonstrate that differences in milk OS can be partly explained by genetic differences. For instance, Liu et al. (2019), reported h^2 that ranged between 50% and 84% in a population of 360 Holstein cows. Similarly, Poulsen et al. (2019) reported moderate to high h^2 (>40%) for 7 of the evaluated bovine OS in 334 Danish Holsteins and 300 Danish Jerseys cows. Poulsen et al. (2019) reported lower h^2 for acidic OS, ranging from $h^2 = 0$ to $h^2 = 25\%$ (6′-SL), than for neutral OS, with differences between both breeds. It is worth noting that the SE of h^2 estimates was large for several OS (0.13 to 0.26; Poulsen et al., 2019). Analytical methods used for quantifying OS could influence these h^2 estimates as inaccuracies in analytical methods can lower h^2 estimates due to measurement errors, and issues with overlapping peaks can result in biased h^2 estimates.

Genetic and Phenotypic Correlations Between gMOS

Genetic relationships between *g*MOS provide insight in the genetic background of *g*MOS. To date, studies have not reported genetic correlations among milk OS. We detected strong and positive phenotypic and genotypic correlations between 3′-GL and 6′-GL, and between 6′-SL and 3′-SL. This suggests that a substantial part of the biochemical pathway for synthesizing these milk OS is shared. However, the specific mechanisms by which these milk OS are produced in the mammalian gland remains largely unknown. For example, the biosynthesis of galactosyl-lactose in human milk seems distinct from other milk OS, as evidenced by a lack of further elongation with Fuc or NeuAc. The enzymes responsible for their synthesis, as well as their cellular locations, are yet

to be identified (reviewed by Sprenger et al., 2022). It can be hypothesized that the synthesis of galactosylated OS (3′-GL and 6′-GL) and sialylated OS (6′-SL and 3′-SL) might vary due to polymorphisms in specific transferase genes or polymorphism in genomic regions affecting expression levels of genes such as galactosyltransferases for 3′-GL and 6′-GL, and sialyltransferases for 6′-SL and 3′-SL (Poulsen et al., 2019). Variation in galactosyltransferases or sialyltransferases, might influence the levels of both galactosylated OS (e.g., 3′-GL and 6′-GL) and sialylated OS (e.g., 3′-SL and 6′-SL), leading to concurrent variations in their concentrations (Liu et al., 2019; Poulsen et al., 2019; Williams et al., 2021).

Systematic Environmental Effects Shaping the gMOS Content

Our findings revealed that lactation days significantly affected the level of 3′-GL, 6′-GL, and 6′-NGL. Additionally, the interaction between parity and age at last kidding affected the 3′-GL and 6′-GL content. This aligns with several studies that highlighted the effect of lactation stage on OS levels. Some studies reported large differences between OS levels in colostrum and mature goat milk (Claps et al., 2014; de Sousa et al., 2015; Martín-Ortiz et al., 2017; van Leeuwen et al., 2020) where colostrum has considerably higher concentrations of OS. In this study, we did not evaluate the content of OS in colostrum. Our results suggest a decline in OS content as lactation progresses, which is consistent with literature. Previous studies in goats found effects of age and parity on levels of specific OS (e.g., 6′-GL, 3′-NGL and 6′-NGL) in mature milk (Chatziioannou et al., 2021). These effects have also been observed in human studies, where age influenced protein-bound glycosylation and the levels of certain OS structures in human milk (Ruhaak et al., 2011; Austin and Bénet, 2018). Differences between first and subsequent parities have been reported prior in goats (Claps et al., 2016), dairy cows (Sundekilde et al., 2012; Robinson et al., 2019), and humans (Xun et al., 2022).

The results in this study demonstrated significant effects of both parity and lactation stage on specific *g*MOS levels. It is, however, important to note that these effects were relatively small in comparison to the effects of genetics and farm. For the interpretation of parity and lactation stage effects it is important to know that Dutch dairy goat farmers commonly make use of extended lactations (i.e., a practice where the interval between pregnancies is increased to extend the milking period). This resulted in goats with >400 d in lactation and a smaller number of animals with multiple parities (n = 47), thereby limiting the statistical power to detect significant effects of parity on *g*MOS composition in the current study. Furthermore, no information was available on multiple births and therefore we could not study its effect on *g*MOS composition.

Farm Effects on gMOS Content

Our results demonstrate that farm has a significant effect on the levels of *g*MOS. Farm effects might be due to differences in, for example, milking system, housing, ventilation, and particularly, feeding regimen. Furthermore, the effect of farm is confounded with test-day: all goats on a farm were sampled on the same day. Sampling took place during a limited period of 5 mo and during this sampling period no systematic changes were present in management on the farms; goats had no access to pasture, were kept inside and fed a similar diet throughout the sampling period. We did not observe any systematic differences in *g*MOS content between farms that were sampled first and those that were sampled later.

We observed marked differences in the concentration of specific *g*MOS such as 6′-SL, 6′-NGL, and 3′-GL between farms. These differences most likely can be attributed to distinct differences in feeding regimens between farms, contradicting previous results indicating that nutrition does not affect OS levels in bovine milk (Liu et al., 2014, 2019). Although the exact mechanism by which OS are produced remains largely unknown (Lebrilla and Vinjamuri, 2023), it has been proposed that the elements in the food may directly influence the activity of glycosyltransferases, thereby affecting the milk OS concentration in the human milk (Quin et al., 2020; Li et al., 2022). Indeed, Quin et al. (2020) investigated how maternal diet affects the production of human milk OS. In particular, maternal fruit intake positively correlated with 15 human milk OS types and with unsaturated fatty acids in human milk. This OS increase in human milk could be attributed to the fiber and simple sugars in fruit, which notably correlated with the milk's galactose and fucose monosaccharides (Quin et al., 2020).

Recent research by Durham et al. (2022) highlighted the effect of a low-starch, high-fiber diet on bovine milk OS content. Vicaretti et al. (2018) and Liu et al. (2014), however, found inconclusive results in their investigations into dietary effects on bovine milk OS, possibly due to sample size and intragroup variability. Farm effects on *g*MOS content might be due to differences in milk volume: a higher milk volume can dilute *g*MOS. Therefore, we estimated the proportion of the total variation that can be attributed to herd when adjusting for differences in (morning) milk yield between individual goats. These analyses showed that differences between farms in *g*MOS content cannot be explained by a dilution effect. Further investigating into farm-specific factors, and especially dietary factors, on milk OS content are needed.

Application

The current study offers novel insights regarding the extent to which genetic and environmental factors influence *g*MOS composition. Our results show that specific *g*MOS, such as 2′-FL and 3-FL, are strongly affected by genetic factors, rendering them interesting candidates for selective breeding programs designed to change fucosylated OS levels in a desired direction. Such breeding programs could be of interest for goat farmers who supply milk to dairies specialized in the production of infant formula. This is particularly relevant given that human milk is richer in fucosylated OS such as 2′-FL or 3-FL (Goehring et al., 2016; van Leeuwen et al., 2020). Increasing these OS in goat milk, although maintaining or reducing acidic OS levels, could drive its composition more closely to human milk. This could make goat milk to serve as a natural source of 2′-FL and 3-FL in for example infant formula. Changing *g*MOS composition by means of selective breeding could be based on quantifying milk OS content of selection candidates. However, as *g*MOS measurements are expensive and only available on females, this might not be the most efficient selection strategy. Selection of bucks based on genotypic information might be a more cost-effective, especially if such as in humans a limited number of major genes affect *g*MOS composition (Bode, 2012). Before selection can be implemented the effects of this selection strategy on other traits need to be quantified, including the effect on colostrum and consequences this might have on the offspring. Results from the current study suggest that correlations between *g*MOS and milk yield or milk composition are moderate, offering perspective for simultaneous changing these traits in the desired direction. Additionally, we found that farm factors, exert a significant influence on specific *g*MOS. Collectively, these findings offer interesting possibilities to change *g*MOS composition and the production of specialized dairy products.

CONCLUSIONS

Considerable variation in *g*MOS composition exists. Genetic and farm factors contribute significantly to this variability. High positive genetic correlations are present (>0.57) between certain *g*MOS (e.g., 3′-SL and 6′-SL, and 6′-GL and 3′-GL) and negative (<−0.31) genetic correlations between gMOS, such as 6′-SL, 3′-SL, 3′-GL with 6′-NGL and 3′-NGL. This suggests that these traits can be concurrently improved to produce milk with enhanced health benefits. Farm-specific factors significantly affect *g*MOS content, indicating that most likely diet plays a role in *g*MOS composition. However, the biological mechanisms behind the synthesis of gMOS remains largely unknown but the current study provides several interesting starting points for further research.

NOTES

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Nonstandard abbreviations used: FL = fucosyllactose; GL = galactosyllactose; *g*MOS = goat milk oligosaccharides; NGL = N-glycolyl-neuraminyl-lactose; OS = oligosaccharides; SL = sialyllactose.

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