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Fatty acid profile of milk from Saanen and Swedish Landrace goats

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ABSTRACT

Recent years have had an increased demand for goat milk and its products. The quality of goat milk is determined, in part, by the fatty acid (FA) profile, but there is little information about breed influence on the FA profile of goat milk. The aim of this study was to describe and compare FA profiles of goat milk produced by Saanen and Swedish Landrace breeds. FA profiles were analysed by gas chromatography with a flame ionisation detector using 100 m capillary column coated with ionic liquids of extreme polarity (SLB-IL111). The amounts of 19 FAs were measured. Analyses indicated that FA profile in the milk of Saanen goats differs from that of Swedish Landrace goats with the first having higher proportions of most SFA-s and the second having lower proportions of C16:0, C16:1 and C18:1. This knowledge enables the improvement of the quality of goat milk and goat milk-derived products.

1. Introduction

Increased awareness of health has led to a higher demand for products made from goat's milk. These products are an especially highly valued part of the diet of small children, elderly people and people with nutritional allergies (Yangilar, 2013). Compared to cow milk fat, goat milk fat has higher digestibility. This is related to the lower mean milk fat globule size (Tatar et al., 2015) and the higher content of short- and medium-chain fatty acids (FA) (Ceballos et al., 2009). The fatty acid profile and contents in milk influence the quality, texture, aroma and flavour of milk and milk products (Markiewicz-Kęszycka, Czyżak-Runowska, Lipińska, & Wójtowski, 2013; Yangilar, 2013; Cossignani, Giua, Urbani, Simonetti, & Blasi, 2014). It is well documented that consumption of SFA increases the risk of cardio vascular disease (CVD), while PUFA have been found to have protective effects against CVD (Simopoulos, 2008).

Increased intakes of PUFA are also associated with health benefits such as improved brain function, and reduced risk of dementia (Ruxton, Reed, Simpson, & Millington, 2004). A recent development in FA-related research is using the milk fatty acid profile as a diagnostic biomarker for prediction of subacute ruminal acidosis (Colman, Waegeman, De Baets, & Fievez, 2015).

The fatty acid profile of goat milk is influenced by a number of factors such as diet - mainly the lipid supplementation (Matsushita et al., 2007; Park, Juàrez, Ramos, & Haenlein, 2007; Martínez Marín et al., 2012; Tripathi, 2014; Ayeb et al., 2015), season (Czarniawska-Zajac et al., 2006; Toyes-Vargas, Cordoba-Matson, Espinoza-Villavicencio, Palacio-Espinosa, & Murillo-Amador, 2013), stage of lactation (Strzałkowska et al., 2009; Haile et al., 2016), storage (Sumarmono, Sulistyowatia, & Soenarto., 2015), individual differences of goats and the environment (Yangilar, 2013). There are fatty acid profile differences between goat and cow milk, with goat milk having a higher content of medium-chain and conjugated linoleic acids (CLA), and a lower amount of C18:0 and C18:1 (Ceballos et al., 2009; Markiewicz-Kęszycka et al., 2013). However, only two studies have examined if the FA profiles in the milk of different goat breeds differ, with Talpur, Bhanger, and Memon (2009) reporting differences and Mayer & Fiechter (2012) not. This disagreement might be caused by differences in the regions where the studies were preformed (Talpur et al. 2009 in Pakistan and Mayer & Fiechter 2012 in Austria). More

Abbreviations: C4:0, butanoic acid; C6:0, hexanoic acid; C8:0, octanoic acid; C10:0, decanoic acid; C11:0, undecanoic acid; C12:0, dodecanoic acid; C14:0, tetradecanoic acid; C14:1n-5, (Z)-tetradec-9-enoic acid; C15:0, pentadecanoic acid; C15:1n-5, (Z)-pentadec-10-enoic acid; C16:0, hexadecanoic acid; C16:1n-9, (Z)-hexadec-9-enoic acid; C17:0, heptadecanoic acid; C17:1n-7, (Z)-heptadec-10-enoic acid; C18:0, octadecanoic acid; C18:1n-9c, (Z)-octadec-9-enoic acid; C18:1n-9t, (E)-octadec-9-enoic acid; C18:2n-6c, (9Z,12Z)-octadeca-9,12-dienoic acid; C18:2n-6t, (9E,12E)-octadeca-9,12-dienoic acid; C18:3n-3, (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid; C18:3n-6, (6Z,9Z,12Z)-octadeca-6,9,12-trienoic acid; C20:0, icosanoic acid; C20:1n-9, (Z)-icos-11-enoic acid; C20:2n-6, (11E,14E)-icosa-11,14-dienoic acid; C21:0, henicosanoic acid; C22:0, docosanoic acid; I.S./C13:0, internal standard/tridecanoic acid/; FA, fatty acid; FAME, fatty acid methyl ester; GC, gas chromatography; GC-FID, gas chromatography with flame ionization detector; LOD, limit of detection; LOQ, limit of quantitation; SE, standard error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; ω-6/ω-3, ratio of ω-6 and ω-3 fatty acids; AI, atherogenic index; DI, desaturase index

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studies from different regions, conducted with uniform methodology, are therefore needed to clarify breed as a factor in the FA profile of goat milk.

Recently goat farming in Estonia has increased. In 2016 there were 2869 female goats in 386 farms in Estonia (Statistics Estonia, 2017). Only seven farms operated with milking herds of over 60 head. The main goat breeds used in Estonia are the Saanen, Swedish Landrace, Thuringian and Anglo Nubian.

Gas chromatography (GC) with a polar polymer-type column as polyethylene glycol and cyanopropyl polysiloxane stationary phases is most frequently used to analyse FA profiles. The use of these columns enables the separation of fatty acids with a wide range of carbon numbers, but the separation is not sufficient to resolve some compounds and isomers (Nakamura, Shimizu, & Ando, 2014). To solve this problem, a new type of capillary column, SLB-IL, was developed. In the SLB-IL series, use of ionic liquid as the stationary phase has enabled high-temperature GC on polar stationary phases. The polar column SLB-IL111 has been found to be suitable for analysing fatty acid isomers (Delmonte et al., 2012; De la Fuente, Rodríguez-Pino, & Juarez, 2015), also in goat milk (Pittau, Panzalis, Spanu, Scarano, & De Santis, 2013).

The aim of this study was to describe and compare FA profiles of goat milk produced by Saanen and Swedish Landrace breed goats kept under identical feeding conditions on two Estonian goat farms. Additional goal was to validate the SLB-IL 111 column operating conditions for analysing FA isomers in goat milk.

2. Materials and methods

2.1. Milk sampling

Milk samples were collected from two farms in inland Estonia, located 160 km from each other (58°05′53.2″N 25°18′08.5″E and 58°11′33.8″N 27°15′00.2″E). In the context of Estonia, both farms can be considered big scale farms with 65 milking goats and with an average yearly milk production of 1000–1400 kg per goat. The current research included eight Swedish Landrace goats from the first and seven Saanen goats from the second farm. One to four milk samples per goat (average 3.6 samples per goat) were collected, as some of the goats entered the dry period during the experiment. Goats which entered the dry period were removed from the sampling flock and replaced. All selected goats were in the second half of their second or higher lactation and in good health. Machine milked samples from each goat in both flocks were collected using in-line milk-meters and 50 mL of sample were frozen (−20 °C) until analysis. Samples were collected twice in October of 2015 and once in January, February and March of 2016. All samples ($n = 28$ from Swedish Landrace goats and $n = 26$ from Saanen goats) were analysed separately and then averaged. Throughout the study, goats on both farms were kept indoors on deep litter and hay, and were fed identically. The feed consisted of grass silage, hay, a concentrate mix and a mineral lick. Silage, hay and the mineral lick were available *ad libitum* and the concentrate mix was fed twice a day (200 g per day) in the milking parlour. Silage and hay, fed in both farms, had similar botanical compositions. The concentrate, with ingredients of the same origin and proportions, was mixed on-site on both farms. The concentrate mix consisted of barley, wheat, rapeseed cake (purchased as the same batch from manufacturer – Scanola Baltic), and a mineral and vitamin premix.

2.2. Chemicals and standards

Methanol (purity 99.9%), heptane (purity 99%), and a boron trifluoride – methanol complex solution (13–15%) were purchased from Sigma-Aldrich (Germany). Sodium hydroxide (purity 98%) and sodium chloride (purity 99.8%) were obtained from Reachim (Russia). Sodium sulphate (purity 99%) originated from the Mikhailovsky Plant of Chemical Reagent (Russia). Identification of the individual FAME was carried out with the use of FAME standards C4-C22 from Dr. Ehrenstorfer GmbH (Germany). A standard mixture of common fatty acid methyl esters CRM47885 (Supelco 37 Component FAME Mix) was obtained from Supelco (USA). Tridecanoic acid methyl ester (C13:0) as internal standard (I.S.) was purchased from Sigma Aldrich (Germany).

2.3. Calibration and standards preparation

The Supelco CRM47885 standard mixture was used as stock solution for GC calibration. Calibration curves were produced from six working standards, which were prepared daily from the stock solution by diluting with heptane. The stock solution of the internal standard was prepared by dissolving 60 mg methyl tridecanoate in 25 mL heptane. All the solutions of the working standards and IS solution were stored at −20 °C until analysis.

2.4. Sample preparation

Extraction of fatty acids was performed according to the boron trifluoride method described previously (Yurchenko, Sats, Poikalainen, & Karus, 2016). Approximately 1 mL of obtained extract was transferred into a test tube, dissolved in 1 mL of heptane, and 200 µL of I.S. was added. Finally, 1 µL of dilution was injected manually into the GC injector port for analysis.

2.5. Gas chromatography of FAMEs

GC analysis was carried out with a Varian 3900 gas chromatograph equipped with Supelco capillary column of SLB-IL111 (100 m × 0.25 mm i.d., 0.20 µm film thickness) and a flame ionization detector (FID). This ionic liquid-coated capillary column has the highest polarity and consists of 1,5-Di(2,3-dimethylimidazolium) pentane bis (trifluoromethylsulphonyl)imide phase. For chromatographic separation of FAMEs the following oven time-temperature programme was used: start at temperature 80 °C (held for 2 min), set at 15 °C/min from 80 °C to 168 °C (held isothermally at 168 °C for 18 min), and set at 5 °C/min from 168 °C to 186 °C (held at 186 °C for 23 min). Analysis was performed by manual injection of 1 µL of each sample at a split ratio of 10:1. The injector temperature was set at 250 °C. Split cup design wool-packed liner with 4 mm i.d. was chosen for analyses. Helium (99.9996%) was used as the carrier gas at a linear velocity of 1.0 mL/min. Hydrogen (99.95%) and monitoring zero synthetic air 4.0 (mixture of oxygen (20%) and nitrogen (80%)) 80% were used for FID working. The settings for these gases were as follows: make-up (25 mL/min), hydrogen (30 mL/min), and air (300 mL/min). The detector temperature was 250 °C. The total run time for each analysis was 52.47 min. The Star Chromatography Workstation Version 6.3 (Varian, USA) was used for data collection.

2.6. Calculation of fatty acid content

The peak areas of FAMEs in the chromatogram were integrated applying Star Chromatography Workstation software, and the amounts of individual fatty acids were expressed as weight percentages (g/100 g total fatty acids).

The following equation was applied:

$$wt\%_i = \frac{A_i}{A_T - A_{I.S.}} \cdot f_i \cdot RRF_i \cdot 100\%,$$

where $wt\%_i$ – weight percentage of individual FA, A_i – the peak area of individual FAME i in the sample chromatogram, A_T – the total integrated peak area of FAME from C4:0 to C20:2n-6 in the sample chromatogram, $A_{I.S.}$ – the peak area of C13:0 internal standard in the sample chromatogram, RRF_i – relative response factor for individual FAME i (RRF_i was calculated from triplicate analyses of CRM47885