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UHPLC-QTOF/MS Untargeted Lipidomics and Caffeine Carry-Over in Milk of Goats under Spent Coffee Ground Enriched Diet

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Abstract: Supplementing the diet of ruminants with agro-industrial by-products is a common practice. In this study, we applied an untargeted lipidomics approach to study the changes in the milk lipid metabolite profiles linked to the addition of different doses of spent coffee grounds (SCG) to the diet of lactating goats. The carryover of caffeine from feed to milk was also studied. Compared to controls, the milk of goats on the SCG diet showed higher levels of cholesteryl esters, sphingomyelins, and phospholipids, while nonesterified fatty acids were downregulated. After 12 h from the last SCG dose, the carry-over of caffeine was, on average, 3%. Collectively, our results establish that SCG supplementation induces changes in the milk levels of complex lipid molecules and causes the transfer of caffeine and caffeine metabolites from feed to milk.

Keywords: lipid profile; lipidomics; animal feeding; lactating goats; mass spectrometry; caffeine; coffee by-products

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1. Introduction

Spent coffee grounds (SCG) are the residue of the brewing process of coffee beverages. The production of instant coffee and coffee brewing generates worldwide annually approximately six million tons of spent coffee grounds [1]. A large part of this by-product is underutilized and disposed of in landfills, representing a source of environmental issues such as a high biological oxygen demand [2]. For sustainable valorization and reuse, SCG addition to an animal's diet has been proposed and positively tested: (1) when fed with coffee by-products, dairy cows showed no negative effects on milk yield, milk composition, or grass intake [3,4]; (2) Kawai and colleagues [5] reported that cows with subclinical mastitis fed with coffee ground silage increased the antioxidant and immune activity, leading to a decrease in somatic cell counts in milk; (3) SCG supplemented diets in lactating goats showed a positive effect on the blood antioxidant status, while no effects on the milk antioxidant status, milk production, or animal health were observed [6].

The composition of SCG is largely variable depending on the type of coffee beans, roasting conditions, and extraction process [7]. It includes tannins [8], polyphenols, and methylxanthines [6,9]. Caffeine levels have been measured at 0.001–0.5% of SCG [6,7]. Polyphenols, of which coffee was found rich, exhibit health-promoting properties for humans, including antioxidant and neuroprotective properties [10]. Caffeine is one of the most consumed psychoactive substances in the world and is known as a cognitive and physical performance enhancer [11]. For many years, it has been known that caffeine and coffee can stimulate, in humans, energy expenditure and lipid mobilization to varying degrees [12]. Different in vitro studies showed that caffeine affects lipid metabolism [13,14], enhancing the fatty acid mobilization of adipose tissue. Changes in plasma-free fatty acids and triacylglycerols were reported in dairy cattle after the intraruminally administration of

caffeine [15]. Many substances, such as caffeine and its metabolites, ingested by lactating mothers pass through the epithelial cells of the mammary gland to the milk. The concentration of these metabolites in the milk depends on the dose ingested, the duration of the assumption, the amount of milk excreted daily, the mother's health, and genotype [16].

From a chemical and structural point of view, the lipid profile of milk is quite complex. In milk, lipids exist as an emulsion, organized in fat globules (MFG) of different sizes. They consist of an inner core of triacylglycerols surrounded by a membrane of different layers of proteins and complex lipids, mainly of an amphipathic nature (MFGM). Milk lipids are predominantly composed of triacylglycerols (approximately 98%), followed by phospholipids, sphingolipids, sterols, and analogs, which potentially play an important role in the health of human consumers [17]. The composition of the milk fat globule membrane can be modified by the diet [18].

Lipidomics is an omics science largely used to explore and characterize lipids in biological specimens. To study milk fat, due to its structural and compositional complexity, highly sophisticated analytical techniques are needed. In hyphenated techniques, to separate and identify complex lipids, chromatography is paired in-line with mass spectrometry. Current MS-based lipidomics can achieve the detection of all lipid classes in one run, helping us to fully characterize most lipids in the target sample [19]. The huge amount of data produced by these analytical tools is submitted to multivariate statistical analysis (MVA) to extract meaningful information and highlight lipid compositional changes due to different factors. We recently reported the use of lipidomics and metabolomics approaches to analyze the influence of ovine milk in different diet regimens [20,21] and the metabolic effects of supplementing the diet with cocoa husk by-products [22]. The aim of this research was to gain an understanding of the impact of a diet supplemented with SCG on the milk lipid profile in lactating goats using a lipidomics approach based on a liquid chromatography time-of-flight mass spectrometry (UHPLC-QTOF/MS) analytical platform. To this goal, two different SCG doses were administered to lactating goats, and the results were compared with the control group. Caffeine carryover in milk was also studied. To the best of our knowledge, research data on caffeine and caffeine metabolites carryover in the milk of ruminants are not available in the literature.

2. Materials and Methods

2.1. Animals and Diets

Twelve Saanen dairy goats were divided into three groups that were homogeneous for milk yield, body weight, body condition score, and dry matter intake. All animals were fed a total mixed ration (TMR) as a basal diet composed of haylage (2.3 kg/day), soybean meal (160 g/day), beet pulp (200 g/day), commercial concentrate (1.2 kg/d), and supplemented with 0 (control group, C), 50 g/day (low dose, LD), and 100 g/day (high dose, HD) of SCG. The SCG was randomly collected from different coffee shops and dried at 65 °C to prevent microbial deterioration. The SCG were administered in two aliquots during morning and evening milking. The ingredients and chemical composition of diets offered to dairy goats were reported in the work of Carta et al. [6]. In particular, the caffeine levels in the diet were calculated as 89.4 mg and 44.7 mg per day per animal, for the high dose and low dose groups, respectively. The trial lasted six weeks, with one week of adaptation and five weeks of experimental sampling. The SCG was completely consumed by the goats of both supplemented groups; no adverse effects on goat dry matter intake were observed. Milk compositional parameters were previously reported by Carta et al. [6]. The authors found that SCG supplementation did not influence the milk yield, protein, fat, or lactose contents, while NaCl increased with the SCG supplementation. For the present work, milk samples were collected over the five weeks of treatment at the morning milking after 12 h from the last meal. Samples were collected in sterilized tubes with the preservative bronopol (2-bromo-2-nitro-1,3-propanediol) and immediately frozen at −20 °C.

2.2. Sample Preparation and UHPLC-QTOF/MS Analysis

Individual milk samples collected in the last week of treatment were submitted for lipidomics analysis. Frozen goat's milk samples were thawed at room temperature overnight, vortexed for 1 min, and extracted by a modified Folch extraction method [23]. Briefly, 100 µL of each milk sample was transferred to a microtube containing 10 µL of the internal mixture of standards (Splash, Lipidomics, Sigma Aldrich, Milan, Italy) and then were extracted using 250 µL of methanol and 125 µL of chloroform. Samples were vortexed every 15 min for four times with Wizard IR (VELP Scientifica Srl, Usmate (MB), Italy), then 380 µL of chloroform and 90 µL of aqueous 0.2 M potassium chloride were added. The suspension was centrifuged at 14,000× g for 10 min at 4 °C with a Hermle Z236 (LaborTechnik, GmbH, Wehingen, Germany), and then the lipophilic layer was transferred to a glass vial and dried by a gentle nitrogen stream. The same phase has been resuspended in 30 µL of a methanol/chloroform solution (1:1, v/v), and then 970 µL of an isopropanol/acetonitrile/water solution (2:1:1, $v/v/v$) were added. Each sample was then filtered through a 0.22-µm MS nylon syringe filter, and the lipophilic phase was analyzed with a 6560 Q-TOF/MS (Agilent Technologies, Palo Alto, CA, USA) coupled with an Agilent 1290 Infinity II LC system. An aliquot of 2 µL for positive ionization mode and 5 µL for a negative ionization mode, from each sample was injected in a Kinetex 5 µm EVO C18 100 A, 150 mm × 2.1 µm column (Agilent Technologies, Palo Alto, CA, USA). The column was thermostated at 50 °C at a flow rate of 0.3 mL/min. For the negative ionization mode, the mobile phases consisted of (A) acetonitrile:water (2:3, v/v) with ammonium acetate (10 mM) and (B) acetonitrile:isopropanol (1:9, v/v) with ammonium acetate (10 mM). For the positive ionization mode, the mobile phases consisted of (A) acetonitrile:water (2:3, v/v) with ammonium formate (10 mM) and (B) acetonitrile:isopropanol (1:9, v/v) with ammonium formate (10 mM). The chromatographic separation was obtained with the following gradient: 0 min 70% B kept for 1 min; 1–3.5 min 86% B; 3.5–10 min 86% B; 10.1–17 min 100% B; 17.1–19 min 70% B. Mass spectrometry platform was equipped with an Agilent jet stream technology source, which was operated in both positive and negative ion modes with the following parameters: gas temperature, 200 °C; gas flow (nitrogen), 10 L/min; nebulizer gas (nitrogen), 50 psig; sheath gas temperature, 300 °C; sheath gas flow, 12 L/min; capillary voltage 3500 V for positive and 3000 V for negative; nozzle voltage 0 V; fragmentor 150 V; skimmer 65 V, octupole RF 7550 V; mass range, 40–1700 m/z; capillary voltage, 3.5 kV; collision energy 20 eV in positive and 25 eV in negative mode. An Agilent MassHunter software was used for instrument control [22]. Representative extracted ion chromatograms obtained in the positive and negative ionization modes are reported in Figure S1.

2.3. Analysis of Caffeine by UHPLC-MS/MS

Milk caffeine levels were measured using a UHPLC (Agilent Technologies 1290 LC, Palo alto, CA, USA) coupled to a triple quadrupole (Agilent Technologies Ultivo, Palo Alto, CA, USA) equipped with a Kinetex 5-µm EVO C18 100 A, 150 mm × 2.1 µm column (Agilent Technologies, Palo Alto, CA, USA), and maintained at 25 °C in a thermostated column compartment. Mobile phases consisted of deionized water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The chromatographic separation was obtained by an isocratic elution mode consisting of 55% of A and 45% of B. The mobile phase flow rate was set to 0.7 mL/min. The total chromatographic run time was 9.0 min. The mass spectrometer with a jet stream electrospray source was operated in positive ionization mode with the following parameters: nitrogen drying gas temperature 350 °C, nitrogen sheath gas temperature 325 °C, nitrogen drying gas flow 7.0 L/min, nitrogen sheath gas flow 11.0 L/min, nebulizer pressure 50 psi, capillary voltage 3500 V, and nozzle voltage 1500 V. The following transition, 195.2 > 138.2 (CE = 16V), was used for the quantitation of caffeine levels. Calibration curves were calculated with four points and were considered acceptable when $r^2 \geq 0.998$.

2.4. Caffeine Carry-Over

The carryover ratio (caffeine excreted in milk/caffeine ingested) was calculated. Milk level of caffeine was measured in the morning milk, after 12 h from the last dose administrated to animals. The administered dose of caffeine (44.7 and 22.4 mg in the high and low dose groups, respectively) and the individual morning milk yield were used to calculate the carryover as follows:

$$\text{Caffeine carry} - \text{over} = \frac{(\text{milk caffeine concentration} \times \text{milk yield})}{\text{caffeine administerd}}$$

2.5. Statistical Data Analysis NMR Spectroscopy

ANOVA, Tukey post-hoc comparison test, and linear mixed models were carried out with the software OriginPro, version 2022 (OriginLab Corporation, Northampton, MA, USA). Random effects of weeks and animals on LD and HD mean differences of caffeine concentration and carryover were calculated by a two-way ANOVA. For lipidomics analysis, the QTOF/MS data in positive and negative modes obtained from the KNIME pipeline workflow [24] were submitted to multivariate statistical analysis (MVA) as implemented in SIMCA-P+ software (version 14.1, Umetrics, Umeå, Sweden). The unsupervised principal component analysis (PCA) was performed to investigate sample distributions, deviating features, and prevailing trends (data not shown). The supervised partial least squares discriminant analysis (PLS-DA) was performed for sample classification, while its orthogonal variant (OPLS-DA) was applied to facilitate the identification of the most discriminant variables. The quality of the models was evaluated based on the cumulative parameters R^2Y and Q^2Y, and the latter was estimated by cross-validation. The variable importance in projection (VIP) scores in the predictive component of pairwise OPLS-DA were analyzed, and only those metabolites having VIP values > 1 were considered discriminant and underwent annotation [25].

3. Results and Discussion

3.1. Caffeine Levels in Milk

Milk caffeine levels were measured by LC/MS for eight goats on LD and HD diets during the five weeks of treatment, and the results are reported in Figure 1 and Table 1.

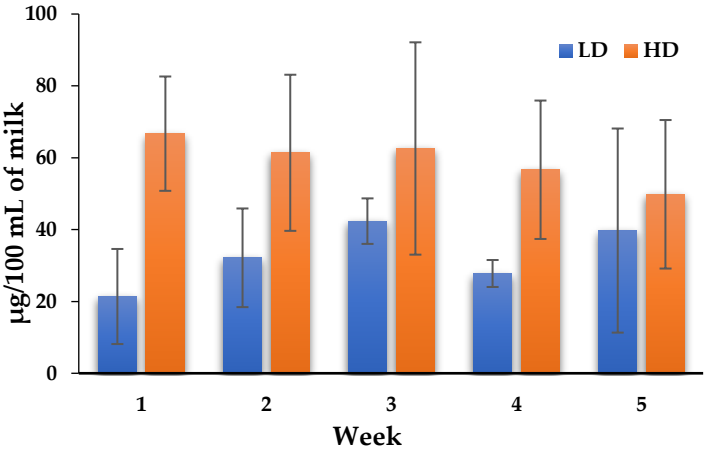


Figure 1. Milk caffeine mean concentrations (SD over four samples are shown as error bars) across the five weeks of experimentation. Within LD and HD groups, week means were not statistically different at $p < 0.001$. Caffeine levels were not detectable in the control samples.

Table 1. Milk caffeine concentrations (mean ± SD) and milk caffeine carryover after 12 h from the last administration.

	HD (n = 20)	LD (n = 20)	Doses	Random Effect	
				Weeks	Animals
	Mean ± SD			p [a]	
Conc (µg/100 mL)	33 ± 16	59 ± 20	***	n.s.	n.s.
Carryover (%)	3.0 ± 1.5	3.1 ± 1.2	n.s.	n.s.	n.s.

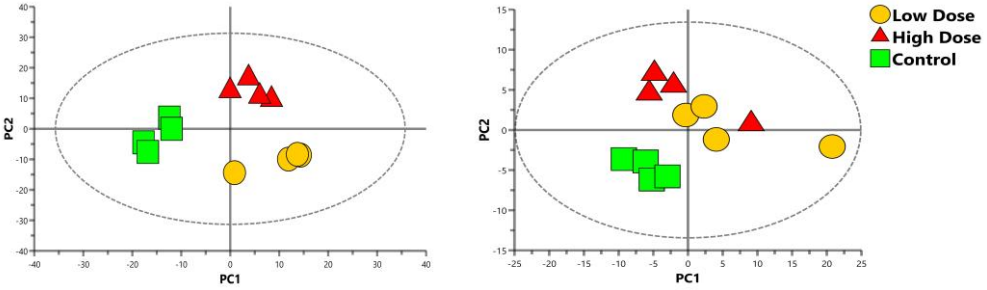
[a] *** $p < 0.001$, n.s. $p > 0.05$.

Figure 1 shows, as a column plot, the mean ± SD concentrations of caffeine (µg/100 mL of milk) for each week of treatment for the HD and LD milk sample groups. As can be seen, the weekly levels of caffeine were very variable (high SD). The overall mean concentration of caffeine in milk was significantly ($p < 0.001$) higher in the high-dose group when compared to the low-dose group; a maximum value of 103 µg/100 mL was detected. Within the same group, the mean levels of each week were not statistically different ($p < 0.001$), suggesting that this alkaloid did not accumulate over time. In this regard, Calvaresi et al. [26] showed that following the consumption of one cup of espresso coffee (containing 80 mg of caffeine), after 24 h the caffeine concentration in breast milk was undetectable.

The means of milk caffeine carryover after 12 h from the last administration for HD and LD groups are reported in Table 1. To calculate the caffeine carryover (caffeine excreted in milk/caffeine in the diet), the milk yield was registered (2050 ± 481, 2047 ± 337, and 2215 ± 301 g for the C, LD, and HD groups, respectively, $p > 0.05$). The carryover, referred to as 12 h after the last SCG dose was offered, was calculated as approximately 3% for both HD and LD groups ($p > 0.05$). Weeks' and animals' random effects on the mean levels were also taken into account, as reported in Table 1, as the results show no influence was deemed significant ($p > 0.05$). To the best of our knowledge, there are no data available regarding the excretion of caffeine or other methylxanthines in ruminant milk. When feeding lactating ruminants with industrial by-products, besides the direct effects on animal wellness and productivity, the indirect effects on the quality of dairy products for human's nutrition should also be taken into consideration. In the last few decades, goat dairy products have gained more attention because of their potential health benefits for the elderly and infants [27]. Therefore, the presence of potential biologically active metabolites, such as caffeine, in caprine dairy products must be studied.

3.2. Multivariate Lipid Signature

To explore changes in the milk lipidome due to SCG supplementation, we analyzed, using the UHPLC-QTOF/MS technique, the three groups of milk samples (C, LD, and HD). Using this analytical platform, we were able to detect, in both the positive and negative ionization modes, hundreds of milk metabolites and in particular lipid species with a total average number of 5000 mass spectrometry features [22]. Only samples collected in the last week of the treatment were studied. In fact, although the caffeine levels in milk did not accumulate during the five weeks of treatment (Figure 1), over time, a daily dose of SCG can induce changes in the overall lipid profile. These changes can be better highlighted in the last stage of SCG supplementation. This choice was also driven by previous observations [22], where we noticed that changes in the lipid profile of sheep's milk due to the diet supplemented with cocoa husks were detectable only after five weeks of treatment. Attempts to differentiate samples based on the diet supplementation were carried out by performing three classes of PLS-DA. In the PLS-DA score plot of data collected in positive ionization mode (Figure 2a), samples clustered into three well-defined groups, while in the negative ionization mode (Figure 2b), HD and LD classes overlapped.



(**a**) (**b**)

Figure 2. Partial least square discriminant analysis (PLS-DA) score plots of the milk lipidome of control, low-dose and high-dose samples. Metabolite data collected in the LC-MS positive ((**a**), n = 2 components, $R^2Y = 0.99$, $Q^2Y = 0.64$) and negative ((**b**), n = 2 components, $R^2Y = 0.61$, $Q^2Y = 0.20$) ionization modes.

Subsequently, with the purpose of finding discriminant metabolites, pairwise OPLS-DA was carried out comparing the control group vs. either the LD or HD groups. The list of discriminant lipid metabolites between the control group and those on diets supplemented with SCG is reported in Tables 2 and 3.

Table 2. Main discriminant metabolites [a] in the milk of goats fed SCG vs. control detected in the positive ionization mode.

Chemical Class	Metabolite	Regulation [b]
Catechins	8,8′-Methylenebiscatechin	Up
Ceramides phosphate	CerP(d18:1/26:1)	Up
	CerP(d18:0/16:0)	Up
Diacylglicerols	DG(18:4/20:5)	Up
	DG(20:5/20:5)	Up
	DG(12:0/22:6)	Up
Flavonoids	Quercetin 3-(6′′′′-ferulylsophorotrioside)	Down
Glucosylceramides	GlcCer(d18:1/24:0)	Up
	GlcCer(d15:1/18:0)	Down
Glycerophosphocholines	PC(24:0/P-18:1)	Up
	PC(O-17:0/20:4)	Up
Glycerophosphoethanolamines	PE(12:0/15:1)	Up
	PE(15:0/18:2)	Up
	PE(13:0/20:0)	Up
	PE(18:0/24:0)	Up
Glycerophosphoethanolamines ceramides	PE-Cer(d14:1/25:0)	Up
	PE-Cer(d15:2/20:0(2OH))	Up
	PE-Cer(d14:2/18:0(2OH))	Down
Glycerophosphoglycerols	PG(20:0/16:1)	Up
Glycerophosphoserines	PS(16:0/16:0)	Down
Monoacylglicerols	MG(22:2)	Up
Monogalactosyldiacylglicerols	MGDG(18:2/18:2)	Up
N-Acylamides	N-docosahexaenoyl glutamine	Up
Polyprenols	Arachisprenol 12	Down
Secosteroids	α,25-dihydroxy-2β-(6-hydroxyhexyl) vitamin D3	Up
Sesquiterpenoids	1,2-Epoxy-1,2,7,7′,8,8′,11,12-octahydro-ψ, ψ -carotene	Up
Sphingomyelins	SM(d16:1/24:1)	Up
	SM(d16:1/22:1)	Up

Table 2. *Cont.*

Chemical Class	Metabolite	Regulation [b]
Sterols	Stigmasteryl ester (16:3)	Up
	CE(20:4)	Up
	CE(22:5)	Up
	CE(22:4)	Up
	Cholesteryl beta-D-glucoside	Up
	11-acetoxy-3β,6α-dihydroxy-24-methyl-27-nor-9,11-seco-5α-cholesta-7,22-dien-9-one	Up
Triacylglicerols	TG(14:1/14:1/17:2)	Up
	TG(12:0/12:0/14:0)	Up
	TG(14:1/14:1/19:1)	Up
	TG(17:2/18:0/18:0)	Down
	TG(16:0/14:0/18:0)	Down
	TG(12:0/18:2/22:0)	Down
	TG(19:0/20:0/20:0)	Up
	TG(17:1/18:0/18:0)	Down
	TG(10:0/10:0/14:0)	Up
	TG(13:0/15:0/22:2)	Down
	TG(10:0/10:0/10:0)	Up
Thioester	Propionyl-CoA	Down
Triterpenoids	Methyl-6′-apo-y-caroten-6′-oate	Down

[a] VIP > 1; [b] up and downregulated metabolites in milk samples of goats fed SCG vs. control.

Table 3. Main discriminant metabolites [a] in the milk of goats fed SCG vs. control, detected in the negative ionization mode.

Chemical Class	Metabolite	Regulation [b]
Caffeine and caffeine metabolites	Caffeine	Up
	Theobromine/Theophylline [c]	Up
	6-Amino-5-(formyl-N-methylamino)-1,3-dimethyluracil (1,3,7-DAU)	Up
Ceramides	Cer(d18:0/24:1)	Up
	Cer(d14:1/28:0)	Up
Glucosylceramides	GlcCer(d18:1/24:0)	Down
	GlcCer(d15:1/18:0)	Down
β-keto acids	Acetoacetic acid	Down
NEFA	Propionic Acid	Up
	Palmitic Acid	Down
	Oleic Acid	Down
	Myristic Acid	Down
	2-Hydroxyadipic acid	Down
	Stearic Acid	Down
Lysophosphatidylethanolamines	LysoPE (18:1/0:0)	Up
Sterols	ST 22:4;O_4	Down
	ST 29:4;O_4	Down
	ST 25:3;O	Up
	ST 23:4;O_2	Down
	ST 23:5;O_3	Down
	ST 27:4;O_4	Down
	ST 27:2;O	Up
	ST 23:5;O_3	Up
	ST 23:4;O_2	Down

[a] VIP > 1; [b] upregulated and downregulated metabolites in milk samples of goats fed SCG vs. control. Nonesterified fatty acids (NEFA); [c] putative annotation for theobromine and/or theophylline.

The analysis of complex lipids in the positive mode (Table 2) allowed us to annotate, among discriminant compounds, cholesteryl esters, sphingomyelins and their metabolites ceramides phosphate, phosphatidylcholines, phosphatidylethanolamines, diacylglycerols, and monoacylglycerols. Several triacylglycerols and sterols were found to be discriminant, although they were differently expressed (upregulated or downregulated).

Cholesteryl esters (CE) were found upregulated in milk samples of goats on the SCG diet. This result agrees with a recent study on human plasma [28] that evidenced an increase in CE in individuals consuming coffee. CE plays a crucial role in lipid metabolism as a transport form for essential fatty acids. Interestingly, the following CE of high-polyunsaturated fatty acids, i.e., CE(20:4), CE(22:4), and CE(22:5), were found upregulated; these fatty acids are present in the milk of ruminants in very low concentrations (<1% of fatty acids) [6].

In milk, the glycerophospholipids (PL) are represented by four main classes: phosphatidylethanolamine (PE, 26.4–72.3% of total PL), phosphatidylcholine (PC, 8.0–45.5%), phosphatidylserine (PS, 2.0–16.1%), and phosphatidylinositol (PI, 1.4–14.1%) [29]. Supplementing the diet with SCG increased the milk contents of PC and PE, indicating a significant effect on phospholipid synthesis in the mammary gland.

Sphingomyelins and ceramide phosphates, minor components of MFGM, were found upregulated in the milk of goats on the SCG diet. Protective properties of milk sphingomyelins against dysfunctional lipid metabolism, gut dysbiosis, and inflammation have been reported [30]. It was also established that sphingomyelins from milk contribute to the central nervous system myelination [31]. Sphingomyelins and their metabolites (ceramide phosphate) have been proposed as candidates for food additives and functional food development for the prevention and treatment of chronic metabolic diseases in humans [32]. A strong positive association between coffee consumption and sphingomyelins in human blood was found by Altmaier et al. [33].

In the negative ionization mode (Table 3), when compared to control samples, we found, as expected, upregulated caffeine and the caffeine metabolites: theobromine, theophylline, and 6-amino-5-(formyl-N-methylamino)-1,3-dimethyluracil (1,3,7-DAU). Caffeine, theobromine, and theophylline are purine alkaloids that share a methyl xanthine structure, which has a significant role in pharmacology and food chemistry. Once caffeine is absorbed, it promptly gets into all the body tissues and crosses the blood-brain, blood-placenta, and blood-testis barriers [11]. In humans, caffeine is subjected to demethylation in the liver, resulting mostly in the release of paraxanthine, theobromine, theophylline, and in urates after oxidation. Theophylline and theobromine share a similar chemical structure; however, in theophylline, the lack of one N-methyl group determines more potent effects than caffeine and theobromine [11]. Theophylline is widely used as an oral treatment for both asthma and pulmonary disease [34]. Furthermore, 1,3,7-DAU is an uracilic metabolite of methylxanthines, which, unlike 5-fluorouracil (5FU), which was markedly cytotoxic, showed mild cytotoxicity [35]. The detected presence of theobromine, theophylline, and 1,3,7-DAU in the milk of lactating goats on the SCG-enriched diet suggests that in these small ruminants, caffeine metabolism follows the same routes as in humans.

Furthermore, we were able to annotate nonesterified fatty acids (NEFA), such as myristic acid, palmitic acid, and oleic acid, ketone bodies (acetoacetic acid), lysophosphatidylethanolamines (LysoPE), ceramides, and their glucosyl derivatives. Lower levels of NEFA (Table 3) were found in the milk of goats on the SCG diet compared to controls. The presence of blood NEFA in dairy cattle has been attributed to the mobilization of adipose tissue in cows with a negative energy balance [34]. Due to the need for energy, NEFA is broken down into ketone bodies, among which acetoacetic acid is downregulated (Table 3) [36]. Lower levels of NEFA and ketone bodies in the milk of goats on the SCG diet suggest a potential role for SCG in reducing the negative energy balance in ruminants; however, further studies are warranted.

We also found propionic acid and propionyl-CoA as discriminants. In ruminants, propionyl-CoA is used as a primer in the lipogenesis of odd-chain fatty acids. The latter is not synthesized by mammalian tissues but originates from the rumen microbial population as a de novo lipogenesis [37]. In agreement, Carta et al. [6] observed that although there was no substantial change in the overall contents of fatty acids, a statistically significant increase in C15:0 level was observed in the milk of goats on the SCG diet, compared to controls. The beneficial effects of C15:0 on human health have been reported [38]. Several sterols were found to be discriminant, although they were expressed differently (upregulated or

downregulated). The 8,8′-methylenebis catechin was found upregulated in goats on the SCG diet. Coffee does not contain catechins that are, however, found in its berries [39].

4. Conclusions

Our results showed that the use of SCG by-products in the diet of lactating goats is associated with potential positive changes in milk lipid metabolite levels, which may have beneficial effects for consumers. However, the inclusion of SCG into the diet of dairy goats caused the carryover of caffeine and caffeine metabolites in milk. Furthermore, the upregulation of complex lipids involved in lipogenesis exerted by SCG suggests a potential beneficial effect of this by-product in the diet of lactating small ruminants with negative energy balance. Although our lipidomics approach covers a broad spectrum of the lipidome, it cannot provide exact quantification. Thus, further investigations using targeted lipidomics are required to provide a better understanding of milk lipid metabolite changes induced by dietary SCG in lactating ruminants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13042477/s1, Figure S1: Representative extracted ion chromatograms of milk sample obtained in the positive (a) and negative (b) ionization modes by UHPLC-QTOF/MS.

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