



Feed restriction has no effect on milk lipolysis, although it results in a marked reduction in milk lipoprotein lipase activity in dairy goats

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ABSTRACT

Climate change pressures livestock feeding systems by reducing the availability and nutritional quality of forages and pasture. Previous studies have shown that dietary restriction impacts milk lipolysis (i.e. the hydrolysis of milk fat by lipoprotein lipase (LPL)) in cows and ewes. However, data regarding the effects of feed restriction on milk lipolysis in goats are scarce. Therefore, the present study aims to investigate the impact of feed restriction on goat milk composition, with a specific focus on lipolysis, LPL activity and milk fat characteristics. For that purpose, two groups of 12 dairy goats (121 ± 7 days in milk) balanced with regard to the α_{s1} -casein (*CSN1S1*) genotype (7 A/A goats, corresponding to strong *CSN1S1* genotypes and 5 O/O goats, corresponding to *null CSN1S1* genotypes) received either a control diet (100 % of the dry matter intake (DMI) ad libitum: non-restricted; NR) or the experimental diet (65 % of the DMI ad libitum: restricted; R) according to a 2×2 cross-over design. Dietary restriction did not significantly affect either lipolysis levels in milk or milk fat globule size, whereas a sharp decrease was observed in milk LPL activity within the short-term restriction window tested. A genotype effect was evidenced for milk lipolysis (copper soap method), milk fat content and milk fat yield. No genotype $\times$ diet effect was observed with regard to lipolysis or LPL activity under feed restriction. This work provides a solid framework for follow-up lipidomic and proteomic studies to gain a comprehensive understanding of the regulation of the lipolytic system in goats.

1. Introduction

Goat milk and caprine dairy products are perceived by consumers as natural and authentic. In Europe, goat milk production is mainly concentrated in southern European countries with Greece, Spain, and

France accounting for nearly 90 % of total output (Pulina et al., 2018). Among European countries, production systems vary, ranging from extensive breeding farms to more specialized dairy farms with an increased level of industrialization, as seen in France and Spain compared to other countries. Climate change affects both the quantity

Abbreviations: BDI, Bureau of Dairy Industry; FA, fatty acids; FFA, free fatty acids; LPL, lipoprotein lipase; MFG, milk fat globule; MUFA, Monounsaturated Fatty Acids; NEFA, non-esterified Fatty Acids; NR, non-restricted diet; PUFA, Polyunsaturated Fatty Acids; R, restricted diet; SCC, somatic cell count; SFA, Saturated Fatty Acids.

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and quality of milk by reducing the availability and composition of pasture and harvested forage for animals, which in the future is expected to represent a major challenge for the dairy sector, particularly in Mediterranean countries. This context highlights the need to study the impact of feeding practices on milk quality, as changes in forage availability and composition can significantly influence the nutritional and technological properties of milk.

Spontaneous lipolysis results from milk fat breakdown by lipoprotein lipase (LPL enzyme; EC: 3.1.1.34) releasing free fatty acids (FFA) and mono and di-glycerides. This phenomenon alters the quality of milk as well as its capacity to be further processed. Indeed, FFA and their oxidation by-products can negatively impact the organoleptic properties of milk, resulting in a strong rancidity, which is not appreciated by consumers (Deeth, 2006).

Previous studies have shown that cows under feed restriction or a low-energy diet produce milk with higher levels of spontaneous lipolysis (Hurtaud et al., 2023; Vanbergue et al., 2018). While the effect of feed restriction is well characterized in cows, it has only been recently documented in dairy ewes (Bernard et al., 2024). Only few studies have reported the effects of either fasting or feed restriction on the milk FFA content in the goat species (Dønnem et al., 2011; Eknæs and Skeie, 2006; Chilliard et al., 1984). Therefore, the objective of the present study was to thoroughly investigate the effect of feed restriction on milk composition in dairy goats, with a special focus on the lipolytic system in the milk and mammary gland. Because LPL is regulated at multiple levels, we evaluated its enzymatic activity using a combination of biochemical and molecular approaches. In addition, since the *CSN1S1* gene polymorphism modulates milk fat characteristics in goats, we hypothesize that the response to feed restriction may vary according to the goat's genotype at the α s1-casein locus, which influences milk fat globule size and composition (Cebo et al., 2012).

2. Material and methods

2.1. Animals and experimental design

The experiment was conducted at the experimental INRAE research unit at Bourges (INRAE P3R Unit Expérimentale Pôle de Phénotypage des Petits Ruminants Bourges, Domaine de la Sapinière 18390 Osmoy – France; Unit approval C18–174–01 <https://doi.org/10.15454/1.5483259352597417E12>). Goats were housed in straw-bedded paddocks, with 12 animals per unit. The study used 24 Alpine goats in mid-lactation with different genotype at the *CSN1S1* locus (14 AA (strong) and 10 OO (null)) according to a crossover design experiment with feeding level as the main factor. At the beginning of the experimental period, on average, goats were 121 ± 7.0 DIM (mean $\pm$ SD) and produced 3.0 ± 0.3 L (mean $\pm$ SD) of milk/d. Their body weight (BW) was 58.7 ± 9.9 kg (mean $\pm$ SD), and the lactation rank was 3 ± 1 (mean $\pm$ SD). Goats were allocated to two groups of 12 animals according to their genotype at the *CSN1S1* locus (AA vs OO), lactation stage, lactation rank, milk yield, and BW. Goats had been previously genotyped at Labogena (Palaiseau, France) for their *CSN1S1* locus allowing to discriminate AA (strong) and OO (null) genotypes, as previously described (Chilliard et al., 2006). Two dietary treatments were applied differing by feeding level: “non-restricted” (NR) with goats fed 100 % of ad libitum dry matter intake (DMI) and “restricted” (R) with goats fed at 65 % of ad libitum DMI. The trial was conducted in May 2021 during 2 consecutive weeks split into two one-week periods. During period 1, each group of goats received either an orchardgrass hay-based diet 100 % of ad libitum DMI, or at 65 % of ad libitum DMI. During period 2, feeding levels were reversed.

Before the experiment, the goats were group-fed. Orchardgrass hay was offered ad libitum and the quantity of concentrate distributed was calculated based on the predicted forage consumption (INRA, 1989) to cover 115 % of the metabolizable energy requirements for maintenance and milk yield. The hay was distributed once per day after morning

milking in the paddock. The concentrate fraction was offered in two equal meals of lactation concentrate of 400 g each distributed at 8.00 am and 4.30 pm during milking, with the animals blocked into the feeding rack to support similar individual consumption. The rest of the concentrates distributed mid-day consisted of 100 g of lactation concentrate with 300 g of barley.

2.2. Treatments and feeding

Ingredients, chemical composition and nutritional value of the diets are given in Table 1. All the goats received a diet consisting of 65 % forage (orchardgrass hay) and 35 % concentrate (26 % Lactation Concentrate and 9 % barley), and 14 g of minerals. All goats were fed with this diet ad libitum during four weeks prior to a one-week pre-experimental period. During the ad libitum period, the feed distribution was adjusted for each goat to an allowance rate of 115 % of the previous day's voluntary intake. Goats allocated to the R treatment were adapted during a 3-days transition period (gradual reduction of feed) to switch to the restricted diet for 4-days. Similarly, for the second experimental period, the level of feeding was reversed with a 3-days transition period (gradual increase of feed) followed by 4-days of treatment. Goats allocated to the NR treatment were fed at 100 % of their ad libitum DMI.

2.3. Measures, sample collection and laboratory analysis

2.3.1. Feeds and refusals

Goats were housed within the barn in 2 paddocks of 12 on straw-bedding and had permanent access to fresh water. Goats had access to a group-feeding delivered by a conveyor belt. Milking took place in the morning at 8.00 a.m., during which time 1/3 part of the concentrates (lactation concentrate) was distributed and the goats had access to group-feeding (hay) when they returned from milking between 8:30 and 9:00 a.m. and another 1/3 part of concentrates at mid-day (lactation concentrate and barley). Evening milking took place at 4:30 p.m., with the last 1/3 part distribution of concentrates (lactation concentrate). Refusals were collected and weighed daily to evaluate DMI for each group of goats. Throughout the experiment, the methods to calculate DMI and diet chemical and nutritional composition (dry matter, mineral matter, crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, organic matter digestibility, phosphorus, calcium, fat) of grass silage, alfalfa hay, and energy concentrate were the same as in Vanbergue et al. (2018).

Table 1

Diet components, dry matter intake (DMI) and refusals based on the non-restricted (NR) and restricted (R) feeding treatments for dairy goats. ADF: Acid detergent fiber; ADL: Acid detergent lignin; NDF: Neutral detergent fiber. Values represent means per treatment (R = restricted, NR = non-restricted) $\pm$ standard error of the mean (SEM). The indicated p-values from a Type I ANOVA (linear model) are considered significant at $p < 0.05$ (indicated in bold).

Feed	Dry matter, %	Crude protein, %	ADF	NDF	ADL	Energy (kcal)
Hay	88.8	8.1	34.5	59.2	4.2	1156
Concentrate	90.0	19.1				1598
Barley	87.2	112.0	65.0	215	12.0	1853
			NR	R	SEM	p-value
Concentrate intake (kg DM/d)			0.88	0.58	0.087	0.002
Barley intake (kg DM/d)			0.29	0.19	0.029	0.002
Hay intake (kg DM/day)			2.0	1.5	0.15	0.040
Total intake of concentrates (kg DM/day)			1.2	0.8	0.11	0.002
Total dry matter intake (DMI, kg DM/day)			3.2	2.3	0.27	0.016
% of concentrates in the diet (% of DMI)			36.7	33.9	0.99	0.212
% of forage in the diet (% of DMI)			63.3	66.1	0.99	0.212

2.4. Milk yield and traits

Milk yield was recorded individually at each milking. Milk fat, protein contents and somatic cell counts (SCC) were determined from two consecutive milkings every week. These analyses were performed by mid-infrared spectrometry (MilkoScan™ FT+ spectrometer, Foss, Hillerød, Denmark) for fat, and protein contents and by flow cytometry for SCC, all at the LILCO dairy laboratory (Surgères, France).

Lipolysis, fatty acid (FA) profile, MFG size and milk protein and mineral composition were determined from milk from the same 2 milkings. Milk samples were individually collected on the total milking recovered from evening and morning milkings at the end of each experimental period (one day) and then stored at -20°C for analysis of FA composition. Milk LPL (EC 3.1.1.34) activity was measured on morning milking as described in Bernard et al. (2005). The mRNA abundance of lipogenic genes was measured in morning milk following a total mRNA extraction from milk fat (Brenaut et al., 2012).

2.4.1. Milk lipolysis

Two vials with colorless bronopol (Merck, Darmstadt, Germany) per goat were collected for the measure of FFA contents, indicator of milk lipolysis, and kept at 4°C . FFA analyses were performed on both samples by the copper-soap method (Shipe et al., 1980) (LILCO, 17700 Surgères, France) and by ISO/TS 22113 standard (BDI method; Actalia, Poligny, France).

2.4.2. Milk fat globule and casein size

A milk vial per goat was collected and kept at room temperature with bronopol (Merck, Darmstadt, Germany) for further evaluation of MFG size distribution by laser light scattering (Mastersizer 3000, Malvern, UK) (Hurtaud et al., 2023).

The milk was skimmed by two successive centrifugations which removed the fat. The mean diameter $d_{4,3} = \sum (N_i x d_i^4) / \sum (N_i x d_i^3)$ (with N_i the number of particles in diameter class d_i) of the casein micelles was measured with the Mastersizer 3000.

2.4.3. Milk fatty acid composition

Two milk vials per goat were collected and stored at -20°C to perform the milk FA analysis. Samples of morning and evening milk samples were freeze-dried and pooled according to the calculated mean milk fat yield at each milking (60/40) to create a representative 100-mg sample. Fatty acid composition was measured as previously described (Fougère et al., 2018).

2.4.4. Milk nitrogen and minerals

Milk samples (250 mL) were collected per goat and stored at -20°C for total nitrogen, non-protein nitrogen (NPN), non-casein nitrogen (NCN), casein and urea analysis determined according to the Kjeldahl methods described by Pien, 1969. Total and soluble calcium were analysed analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES 5110 Agilent Technology, Les Ulis, France) on milk and milk ultrafiltrate, respectively as previously described (Hurtaud et al., 2023). Total and soluble phosphorus contents were determined using a KONE PRO multiparameter analyzer (ThermoFisher Scientific, Illkirch, France) by the Allen method (Pien, 1969).

2.4.5. Plasma metabolites

Jugular blood samples were collected using 5 mL heparinized tubes (VT-050SHL, Venoject, Terumo Europe, Leuven, Belgium) at the end of each experimental period. Blood sampling was performed in the morning before milking and before feeding. After centrifugation of blood and storage of plasma at -20°C until analysis (Hurtaud et al., 2023), plasma glucose, urea, acetate, NEFA, triglycerides, cholesterol, lactose and β -hydroxybutyrate contents were assayed using colorimetric enzymatic reactions on 2 replicates as reported in Delamaire and Guinard-Flament, 2006. Plasma insulin and IGF1 concentrations were determined by

RadioImmuno Assays (RIA) as in Hurtaud et al. (2023). Plasma concentrations of prolactin was assessed using the method described by Herve et al., 2019.

2.4.6. Candidate gene expression analysis

2.4.6.1. Milk fat sampling and RNA isolation. RNA was isolated from milk fat globule as a non-invasive alternative to mammary gland tissue for studying lactation traits in lactating goats (Brenaut et al., 2012). Samples of 50 mL of milk from morning milking were individually collected from each animal in sterilized, RNase free Falcon tubes and immediately centrifuged at $2000 \times g$ 10 min at 4°C . One gram of milk fat layer was used for RNA extraction performed following TRIzol™ LS Reagent protocol as described in Cebo et al. (2012). The RNA concentrations were determined by measuring absorbance at 230, 260, and 280 nm using a NanoDrop (ND-1000 spectrophotometer; NanoDrop, Labtech, Palaiseau, France). RNA integrity was assessed prior to RT-qPCR using an Agilent 2100 Bioanalyzer (Agilent Technologies) to ensure the reliability of subsequent analyses.

2.4.7. Reverse transcription and RT-qPCR

Reverse transcription (RT) was performed from 1 μg of total RNA by using High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems) in a total volume of 20 μL , composed by 6 μL of ARN, 3 μL (2 ng/ μL) of Luciferase RNA Control (Promega) and 11 μL of Master Mix, according to the manufacturer's instructions. The complementary DNA (cDNA) obtained was then stored at -20°C for RT-qPCR.

The cDNA samples were subjected to RT-qPCR in duplicate with MicroAmp™ Fast Optical 96-Well Reaction Plates (Applied Biosystems) using Power SYBR™ Green PCR Master Mix (Applied Biosystems) and TaqMan™ Fast Advanced Master Mix for qPCR (Applied Biosystems) for primers with probes. The mRNA abundance of the following 11 candidate genes related to lipid metabolism was measured via quantitative real-time reverse transcription PCR: Lipoprotein lipase (LPL) involved in the uptake of FA from circulating triacylglycerols and in lipolysis; Stearoyl-CoA Desaturase 1 (SCD1) involved in FA desaturation; FA synthase (FASN) involved in de novo FA synthesis; Hormone Sensitive Type (LIPE) involved in lipolysis; Lipin1 (LPIN1) involved in lipid synthesis; Toll-like receptor 4 (TLR4) involved in inflammation pathway; Lactadherin (MFGES8), Glycosylation-dependent cell adhesion molecule 1 (GLYCAM1) which encodes two major proteins in the milk fat globule membrane; Glycerol-3-phosphate acyltransferase 1, mitochondrial (GPAT) involved in triglyceride synthesis; α S1-casein (CSN1S1) which encodes a major protein in the milk; FA translocase (CD36) involved in FA uptake. Primers were specifically designed on goat reference mRNA sequences and were purchased by Merck (Sigma-Aldrich). Gene primers are reported in Supplementary Table S1. The mRNA abundance was normalized by adopting *Luciferase* as the reference gene. Each well was filled with 5 μL of cDNA (previously diluted 1:50 with nuclease free water) and 15 μL of Master Mix assembled by 10 μL of Mix SYBR or TaqMan, 2 μL of specific primers (1 μL Forward and 1 μL Reverse), 0.5 μL of specific probes (in TaqMan Mix) and adjusted to volume of 15 μL with nuclease free water.

RT-qPCR reaction was performed using StepOnePlus real-time PCR system (Applied Biosystems) set up with the following program: denaturation at 95°C for 10 min; 40 cycles of PCR reactions at 95°C for 15 s and 58 or 60 or 62°C for 45 s according hybridization temperature.

Samples were run in duplicate and compared to reference gene to obtain ΔCt where cycle threshold (CT) is the endpoint of real-time PCR analysis, and the delta CT corresponds to "CT gene – CT of the luciferase reference genes". Three samples were discarded for extremely high ΔCt leaving 32 samples balanced for diet and genotype which were used for the subsequent statistical analyses. Validation of reference gene stability was obtained with GeNorm software (<https://genorm.cmgg.be/>).

2.5. Statistical analyses

The experimental design was a crossover design over two successive 1-week periods with feeding level as the main factor. Daily values were considered for DMI, BW, plasma parameters and milk traits; for the latter, daily values of milk composition were obtained by calculating the average of the morning and evening values, weighted by the milk yields of each milking. Twice-daily values (morning, evening) for lipolysis, MFG size, protein and mineral composition were considered. The effects of feed treatment, genotype and their interaction were evaluated with a linear mixed model including as fixed effects the group of goats (as described in the paragraph “*Animals and experimental design*”), genotype (AA, OO), period (first, second), feeding level (R, NR), milking time (morning, evening; only included for lipolysis, MFG size, protein and mineral composition), and the interaction between genotype and feeding level, as well as a random effect for the goats within group: $Y_{ijklmn} = \mu + \text{Group}_i + \text{Genotype}_j + \text{Period}_k + \text{Feeding level}_l + \text{Milking Time}_m + \text{Genotype}_j \times \text{Feeding level}_l + \text{Goat}_n(\text{Group}_i) + \varepsilon_{ijklmn}$, where $\text{Goat}_n(\text{Group}_i)$ correspond to the random effect of animal n nested within group i and $\varepsilon_{ijklmn(i)}$ corresponds to standard Gaussian noise. Gene expression ΔCt values were analyzed using a repeated measures ANOVA with group, genotype, period, feeding level, and the interaction between genotype and feeding level as fixed factors. The statistical significance threshold was set at $P < 0.05$. Trend was set at $0.05 < P < 0.10$. We verified modeling assumptions of the linear mixed model for all variables studies by visual inspection of plots of the homoscedasticity and normality of residuals as well as the normality of random effects. All statistical analyses were performed using R software (version 4.4.2).

3. Results

3.1. Intake, milk composition and metabolic profile of dairy goats

The detailed composition of the experimental diets is given in [Table 1](#). Due to the experimental design, feed restriction resulted in a

roughly 30 % drop in total DMI, which is therefore close to the expected 35 % reduction for the experimental feed restriction ([Table 1](#)). The applied feed restriction did not result in any significant changes in the BW of the goats ([Table 2](#)). However, the R treatment caused a significant decrease in milk yield (-11 % and -9 % for AA and OO goats, respectively), in milk fat yield (-11 %, observed in both AA and OO goats) and in milk protein yield (-15 % and -9 % for AA and OO goats, respectively) ($P < 0.001$; [Table 2](#)). Feed restriction did not affect significantly milk somatic cell counts ([Table 2](#)). The observed decrease in milk production under feed restriction is consistent with the reduction in prolactin secretion (-27 % and -33 % for AA and OO goats, respectively, $P < 0.001$). The R treatment changed the metabolism of dairy goats, as indicated by the sharp increase in levels of plasma NEFA (Non-Esterified Fatty Acids), most probably as a result of negative energy balance, especially in AA goats (+57 % and +9 % in AA and OO goats, respectively; $P = 0.019$; [Table 2](#)). Similarly, under feed restriction, there was a slight decrease in milk FAs from de novo mammary synthesis ($\text{FA} < \text{C16}$; -4 % and -5 % for AA and OO goats, respectively, $P = 0.005$), whereas long-chain FA taken up by the udder ($\text{FA} \geq \text{C18}$) increased (+3 %, observed in both AA and OO goats; $P = 0.013$; [Table 3](#)). No effect of feed restriction was observed with regard to the mineral composition of milk, except for the soluble P content of milk ([Supplementary Table S2](#)). Taken together, these results show that, although moderate, feed restriction significantly affects the metabolism and milk composition of goats, regardless of their AA or OO genotype.

3.2. Milk fat characteristics, lipolysis and milk lipoprotein lipase enzyme activity

The main finding of this study is that under a moderate feed restriction regime, lipolysis in goat milk remains unchanged, whereas the enzymatic activity of LPL is drastically reduced ([Table 4](#) and [Fig. 1](#)). Indeed, the R treatment markedly reduced LPL activity in milk (-33 % and -34 % for AA and OO goats, respectively; $P < 0.001$; [Table 4](#)). In contrast, lipolysis estimated in milk by two analytical methods (both the copper soap and the BDI methods) remained stable under feed

Table 2

Effects of diet (R = restricted, NR = non-restricted), *CSN1S1* genotype (AA, OO) and their interaction on milk gross composition and plasma metabolic and hormonal profiles of goats. Results are expressed as mean $\pm$ SD ($n = 14$ AA and $n = 10$ OO for each diet). Results are expressed as mean $\pm$ SD ($n = 14$ for AA and $n = 10$ for OO). Significant p-values (from Type III ANOVA tests) are shown in bold.

	Diet						
	NR		R		Significance		
	AA	OO	AA	OO	genotype	diet	genotype x diet
<i>Milk composition</i>							
Milk yield (kg)	2.48 $\pm$ 0.50	2.36 $\pm$ 0.53	2.20 $\pm$ 0.41	2.14 $\pm$ 0.57	0.639	< 0.001	0.565
Milk fat content (g/kg)	31.3 $\pm$ 3.9	26.5 $\pm$ 1.8	31.3 $\pm$ 3.5	26.0 $\pm$ 2.0	0.001	0.536	0.629
Milk fat yield (g/d)	77.1 $\pm$ 15.0	62.7 $\pm$ 16.2	68.4 $\pm$ 12.3	55.6 $\pm$ 14.6	0.029	< 0.001	0.626
Milk protein content (g/kg)	30.8 $\pm$ 2.9	23.5 $\pm$ 2.5	29.6 $\pm$ 3.0	23.7 $\pm$ 2.6	< 0.001	0.014	0.001
Milk protein yield (g/d)	75.5 $\pm$ 11.3	54.7 $\pm$ 11.4	64.5 $\pm$ 9.2	49.8 $\pm$ 10.2	< 0.001	< 0.001	0.066
Caseins, g/kg	25.3 $\pm$ 1.9	19.3 $\pm$ 2.3	24.5 $\pm$ 2.1	19.5 $\pm$ 1.9	< 0.001	0.190	0.016
Soluble proteins, g/kg	5.82 $\pm$ 1.44	4.49 $\pm$ 0.65	5.55 $\pm$ 1.31	4.47 $\pm$ 0.91	0.021	0.188	0.237
Somatic cell score ^a	5.72 $\pm$ 1.41	5.12 $\pm$ 0.72	5.86 $\pm$ 1.46	4.97 $\pm$ 0.74	0.144	0.487	0.337
<i>Metabolic profile</i>							
BW, kg	55.6 $\pm$ 6.4	58.0 $\pm$ 12.9	55.4 $\pm$ 6.6	58.1 $\pm$ 13.2	0.539	0.943	0.510
Acetate, mmol/L	0.71 $\pm$ 0.24	0.57 $\pm$ 0.13	0.63 $\pm$ 0.18	0.58 $\pm$ 0.13	0.154	0.240	0.161
Non-esterified fatty acids, $\mu\text{mol/L}$	221 $\pm$ 114	310 $\pm$ 110	347 $\pm$ 165	338 $\pm$ 168	0.386	0.019	0.122
β -hydroxybutyrate, $\mu\text{mol/L}$	298 $\pm$ 75	285 $\pm$ 144	308 $\pm$ 82	297 $\pm$ 112	0.754	0.498	0.943
Glucose, mg/L	553 $\pm$ 48	558 $\pm$ 54	542 $\pm$ 43	566 $\pm$ 39	0.410	0.834	0.294
Lactose, mg/L	34.4 $\pm$ 14.6	23.7 $\pm$ 8.1	33.9 $\pm$ 17.2	25.1 $\pm$ 13.9	0.094	0.830	0.657
Triglycerides, mg/L	170 $\pm$ 30	235 $\pm$ 82	155 $\pm$ 43	216 $\pm$ 67	0.008	0.057	0.785
Cholesterol, g/L	1.45 $\pm$ 0.26	1.45 $\pm$ 0.35	1.50 $\pm$ 0.26	1.46 $\pm$ 0.32	0.861	0.209	0.350
Urea, mg/L	308 $\pm$ 61	411 $\pm$ 93	262 $\pm$ 55	298 $\pm$ 52	0.004	< 0.001	0.036
<i>Hormonal profile</i>							
Insulin, $\mu\text{UI/mL}$	11.4 $\pm$ 6.6	12.3 $\pm$ 6.4	8.6 $\pm$ 5.3	12.0 $\pm$ 4.0	0.370	0.027	0.068
Insulin-like growth factor 1, ng/mL	107 $\pm$ 31	125 $\pm$ 23	105 $\pm$ 31	112 $\pm$ 17	0.370	0.027	0.068
Prolactin (ng/mL)	183 $\pm$ 74	191 $\pm$ 89	133 $\pm$ 43	129 $\pm$ 56	0.946	< 0.001	0.494

^a Somatic cell score = $\log_2$ (somatic cell counts/100) + 3

Table 3

Effects of diet (R = restricted, NR = non-restricted), *CSN1S1* genotype (AA, OO) and their interaction on milk fatty acid composition. Results are expressed as mean $\pm$ SD (n = 14 AA and n = 10 OO for each diet). ($\Sigma <C16:0$ = sum of FA from C4:0 to C15:0; $\Sigma C16$ = sum of C16:0, isoC16:0, cis-9-C16:1, trans-9-C16:1 and cis-7-C16:1; $\Sigma \geq C18:0$ = sum of FA from C18:0 to C20:4 n-6).

Fatty acids (g/100 g total FA)	Diet		Diet		Significance		
	NR		R		genotype	diet	genotype x diet
	AA	OO	AA	OO			
$\Sigma <C16:0$	30.1 $\pm$ 3.0	28.4 $\pm$ 3.1	28.9 $\pm$ 3.4	26.8 $\pm$ 1.7	0.095	0.005	0.729
C16:0	22.9 $\pm$ 2.3	25.1 $\pm$ 1.5	22.4 $\pm$ 1.9	24.7 $\pm$ 1.7	0.009	0.046	0.647
$\Sigma C16$	24.2 $\pm$ 2.2	26.4 $\pm$ 1.4	23.8 $\pm$ 1.9	26.0 $\pm$ 1.6	0.008	0.184	0.714
$\Sigma \geq C18$	43.5 $\pm$ 3.6	43.1 $\pm$ 3.8	44.8 $\pm$ 3.6	44.6 $\pm$ 2.9	0.837	0.013	0.687
cis-9-C18:1	21.8 $\pm$ 2.2	23.1 $\pm$ 2.7	22.6 $\pm$ 2.3	24.4 $\pm$ 2.2	0.110	0.001	0.393
Delta-9 desaturation ratio							
cis-9-C14:1/C14:0	0.016 $\pm$ 0.004	0.017 $\pm$ 0.003	0.017 $\pm$ 0.005	0.018 $\pm$ 0.002	0.557	0.079	0.682
cis-9-C16:1/C16:0	0.025 $\pm$ 0.005	0.025 $\pm$ 0.003	0.028 $\pm$ 0.006	0.027 $\pm$ 0.004	0.640	< 0.001	0.881
cis-9-C18:1/C18:0	2.02 $\pm$ 0.38	2.45 $\pm$ 0.21	2.08 $\pm$ 0.40	2.68 $\pm$ 0.29	0.001	0.001	0.051
cis-9, trans-11-C18:2/ cis-9,trans-11-C18:1	1.27 $\pm$ 0.21	1.44 $\pm$ 0.21	1.21 $\pm$ 0.26	1.37 $\pm$ 0.28	0.091	0.172	0.874

Table 4

Effects of milking time (morning, evening), diet (R = restricted, NR = non-restricted), *CSN1S1* genotype (AA, OO), their interaction and on milk fat characteristics, lipolysis and lipoprotein lipase activity from milk samples. Results are expressed as mean $\pm$ SD (n = 14 AA and n = 10 OO for each diet). Significant p-values (from Type III ANOVA tests) are shown in bold.

		Diet		Diet		Significance			
		NR		R		genotype	milking	diet	genotype x diet
		AA	OO	AA	OO				
Lipolysis, mEq/100 g fat (copper soap method)	Morning	2.50 $\pm$ 2.65	5.53 $\pm$ 5.07	1.87 $\pm$ 2.02	6.80 $\pm$ 5.30	0.018	< 0.001	0.855	0.358
	Evening	3.62 $\pm$ 3.84	8.45 $\pm$ 5.86	3.69 $\pm$ 3.42	8.03 $\pm$ 6.56				
Lipolysis, mEq/100 g fat (BDI method)	Morning	1.25 $\pm$ 1.49	1.81 $\pm$ 1.46	1.10 $\pm$ 1.19	2.72 $\pm$ 2.92	0.116	< 0.001	0.766	0.692
	Evening	2.16 $\pm$ 2.86	4.30 $\pm$ 4.54	1.97 $\pm$ 2.15	4.62 $\pm$ 5.41				
Lipoprotein lipase activity, nmol/mn/mL	Morning	514 $\pm$ 15	517 $\pm$ 8	346 $\pm$ 24	343 $\pm$ 16	0.928	n/a	< 0.001	0.414
	Evening	n/a	n/a	n/a	n/a				
Milk fat content, g/kg	Morning	37.1 $\pm$ 4.6	30.0 $\pm$ 2.3	35.6 $\pm$ 4.5	29.5 $\pm$ 3.0	< 0.001	< 0.001	0.474	0.891
	Evening	27.6 $\pm$ 3.8	24.1 $\pm$ 2.0	28.6 $\pm$ 3.4	23.8 $\pm$ 1.6				
Milk fat yield (g/d)	Morning	35.8 $\pm$ 9.5	28.6 $\pm$ 7.6	29.8 $\pm$ 6.2	24.2 $\pm$ 6.2	0.029	0.580	< 0.001	0.661
	Evening	41.3 $\pm$ 6.7	34.1 $\pm$ 9.5	38.7 $\pm$ 7.7	31.3 $\pm$ 9.2				
Milk fat globule diameter ($d_{4,3}$ ^a , μ m)	Morning	3.74 $\pm$ 0.95	3.06 $\pm$ 0.40	3.74 $\pm$ 0.94	3.16 $\pm$ 0.45	0.074	0.438	0.872	0.867
	Evening	3.73 $\pm$ 0.90	3.21 $\pm$ 0.48	3.71 $\pm$ 0.92	3.11 $\pm$ 0.37				
Milk fat globule surface area (m^b /g of fat) ^b	Morning	2.18 $\pm$ 0.37	2.62 $\pm$ 0.27	2.21 $\pm$ 0.36	2.61 $\pm$ 0.33	0.072	0.653	0.263	0.986
	Evening	2.19 $\pm$ 0.37	2.59 $\pm$ 0.33	2.23 $\pm$ 0.37	2.66 $\pm$ 0.30				

^a $d_{4,3} = \Sigma(N_i \times d_i^4) / \Sigma(N_i \times d_i^3)$, with N_i = the number of MFG in diameter class d_i

^b Milk fat globule area = $6 / (\rho \times d_{3,2})$, with ρ = the density of the particle considered (0.92 for fat) and $d_{3,2} = \Sigma(N_i \times d_i^3) / \Sigma(N_i \times d_i^2)$, with N_i = the number of MFG in diameter class d_i

restriction in both AA and OO genotype goats. Interestingly, we did not observe any variation in the LPL mRNA abundance, thus suggesting that the regulation of LPL activity in milk under feed restriction in the goat species is post-transcriptional (Table 5).

The time of milking (morning or evening) had a pronounced effect on milk lipolysis, regardless of the method used to measure lipolysis in milk. On the whole, lipolysis was higher in evening milk than in morning milk. No effect was observed under feed restriction with regard to milk fat globule characteristics (Table 4).

With regard to goat genotypes, milk fat content was lower in OO goats than in AA goats (37.09 $\pm$ 4.64 g/kg and 29.97 $\pm$ 2.32 g/kg in morning milk for AA and OO goats, respectively; $P < 0.001$). A similar finding was observed for milk fat yield (35.85 $\pm$ 9.53 and 28.57 $\pm$ 7.65 g/kg in morning milk for AA and OO goats, respectively; $P = 0.029$; Table 4). A genotype effect was observed on milk lipolysis only when using the copper soap method (+121 % and +133 %) in morning and evening milk from OO goats fed a NR diet compared with AA goats; similarly, we observed +264 % and +118 % in morning and evening milks from OO goats fed a R diet compared with AA goats; $P = 0.018$; Table 4). Using the BDI method, despite higher values for OO milks compared with AA milks, the difference was not statistically significant. A trend was observed regarding the effect of goat genotype on

fat globule diameter and surface area. Overall, fat globules were smaller in milk from OO genotype goats, although this difference was not statistically significant at $P < 0.05$ ($P = 0.074$).

3.3. Candidate gene expression from milk fat

Gene expression was reported as Δ CT values; note that higher Δ CT value correspond to lower expression, and genes that are more highly expressed than the reference gene will tend to have negative Δ CT values. Among the 11 mRNAs encoding for genes involved in mammary metabolism, none was found to be affected by the feed restriction regime. However, as expected *CSN1S1* expression was significantly affected by genotype. *FASN* expression was similarly affected by genotype ($P = 0.036$), and a trend was observed for the interaction between genotype and treatment for *CSN1S1* ($P = 0.068$).

4. Discussion

Feed costs account for approximately 50 % of total production costs in dairy goat farms (Pulina et al., 2018). Therefore, it may be tempting to reduce concentrate intake or adopt alternative feeding strategies to lower overall production costs (Romero-Huelva et al., 2017). A

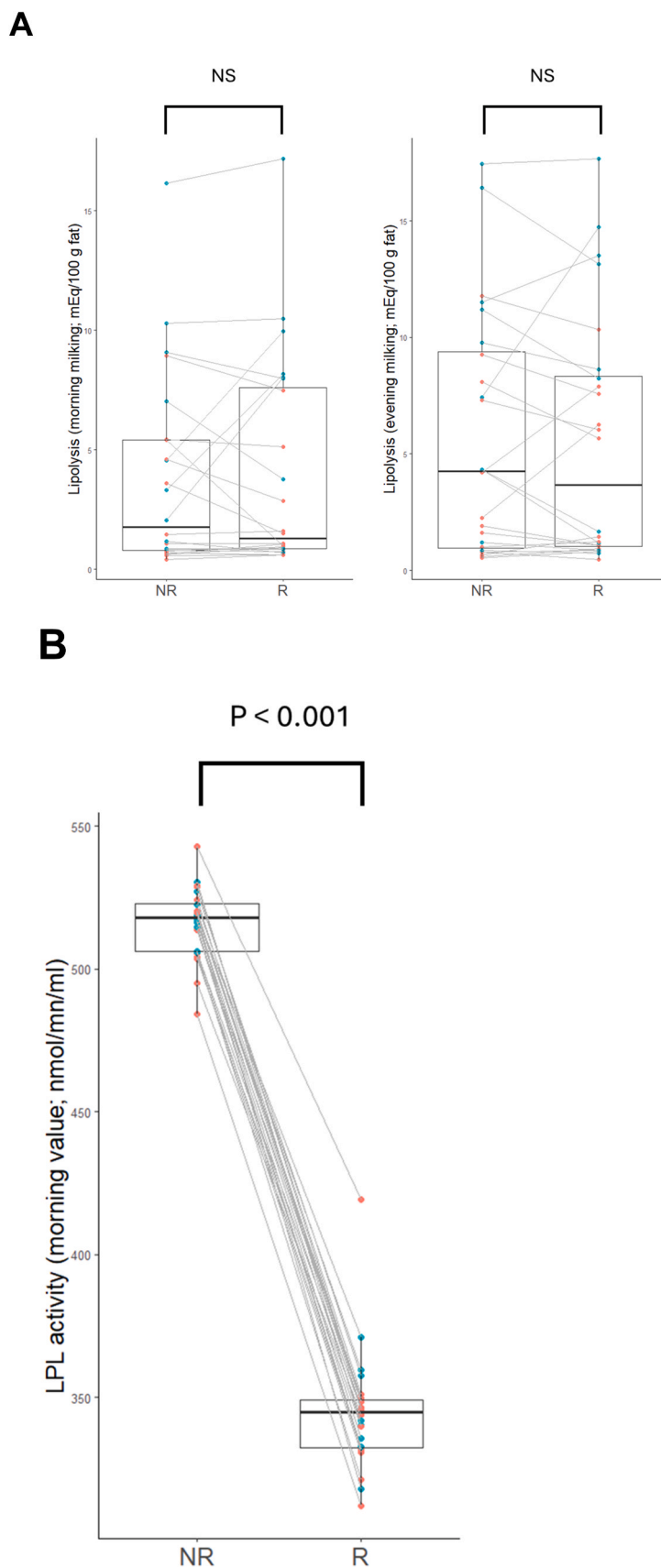


Fig. 1. Paired boxplot showing the distribution of milk lipolysis (morning and evening milks; panel A) and lipoprotein lipase (LPL) activity (panel B) according to *CSN1S1* genotype of goats and diet. Paired data between the non-restricted (NR) treatment and the restricted (R) treatment are connected by solid lines for each goat. Data derived from A/A and O/O goats are featured by red and blue circles, respectively. NS: non significant.

Table 5

Effects of diet (R = restricted, NR = non-restricted), *CSN1S1* genotype (AA, OO), and their interaction on mean mRNA abundance of candidate genes expressed as Δ Ct values. Results are expressed as mean $\pm$ SD (n = 9 AA and n = 7 OO for each diet). Significant p-values (from Type III ANOVA tests) are shown in bold.

	Diet				genotype	Significance	
	NR		R			diet	genotype x diet
	AA	OO	AA	OO			
<i>LPL</i>	10.92 ± 2.22	9.06 ± 3.55	10.35 ± 3.25	10.31 ± 2.62	0.376	0.755	0.267
<i>SCD1</i>	12.31 ± 1.48	11.42 ± 1.58	11.94 ± 2.70	11.66 ± 2.01	0.529	0.949	0.653
<i>FASN</i>	13.22 ± 1.19	14.27 ± 2.30	12.77 ± 1.46	14.02 ± 1.03	0.036	0.421	0.884
<i>LIPE</i>	18.35 ± 1.47	18.48 ± 1.16	18.37 ± 0.89	18.32 ± 1.67	0.965	0.893	0.869
<i>LPIN1</i>	12.95 ± 0.92	13.11 ± 1.01	12.65 ± 0.76	12.52 ± 1.04	0.870	0.196	0.575
<i>TLR4</i>	15.84 ± 2.31	14.17 ± 1.26	14.96 ± 2.29	15.06 ± 1.55	0.397	0.985	0.121
<i>MFGE8</i>	11.30 ± 1.93	10.76 ± 2.61	10.72 ± 2.04	10.63 ± 1.95	0.505	0.576	0.875
<i>GLYCAM1</i>	4.11 ± 2.35	2.92 ± 2.46	3.39 ± 2.60	3.42 ± 2.61	0.508	0.871	0.381
<i>GPAT</i>	13.79 ± 1.24	13.89 ± 1.19	13.67 ± 1.27	13.26 ± 1.10	0.659	0.433	0.473
<i>CSN1S1</i>	−1.04 ± 2.57	3.31 ± 4.37	−1.45 ± 2.52	5.81 ± 3.36	< 0.001	0.354	0.068
<i>CD36</i>	8.05 ± 1.78	6.91 ± 2.04	7.60 ± 2.19	6.71 ± 1.62	0.117	0.643	0.681

semi-intensive system that integrates natural grazing with supplementary feeding can lower feed costs, although seasonal variations in forage availability or prolonged droughts due to climate change may pose challenges. As a result, it is essential to conduct studies to evaluate the impact of feed restriction on milk quality in dairy species.

Lipolysis plays a significant role in determining milk quality. Indeed, FFA and partial glycerides released during the lipolytic process can alter both the organoleptic and the technological properties of milk (Deeth, 2006). Nutrition is a key factor that influences fat breakdown in milk. Previous research has demonstrated that cows subjected to feed restriction or a low-energy diet produce milk with increased levels of spontaneous lipolysis (Hurtaud et al., 2023; Vanbergue et al., 2018). Underfeeding dairy ewes results in milk with lower lipolysis (Bernard et al., 2024). In the present study, we demonstrated that milk produced by underfed goats (65 % of DMI as in our previous cow and ewe studies) exhibited no change in lipolysis rate compared to milk produced by goats receiving 100 % of their nutritional requirements. So far, data in the literature on the effect of feed restriction in dairy goats are not clear. Indeed, a 2-days fasting period was shown to reduce lipolysis and milk LPL activity (Chilliard et al., 2003) and others reported that decreased energy balance or energy intake increased lipolysis (Dønnem et al., 2011; Eknæs and Skeie, 2006). Our results indicate that, unlike what happens in cows (Hurtaud et al., 2023), reducing feed intake in goats does not significantly affect body weight or milk lipolysis, although it reduces milk yield by approximately 10 %. This suggests that the goat is relatively resilient to short-term nutritional changes, at least with respect to milk lipolysis. Nonetheless, these results must be interpreted carefully, since feed restriction could potentially impact other parameters that were not measured in this study. In addition, our results need to be confirmed with other goat breeds as only Alpine goats were included here. Indeed, large differences in the levels of LPL activity and spontaneous lipolysis between the Norwegian, Alpine, and Saanen breeds have been reported (Chilliard et al., 2003). However, taken together, our results highlight a species-specific response to feed restriction with regard to FFA levels and milk LPL activity in ruminant species.

Some authors have also suggested that LPL activity and milk lipolysis are well correlated in goat milk, in contrast to what has been observed in cows; notably, the lipase enzyme is primarily associated with the fat fraction in goats, whereas in cows, it is mainly linked to the skim milk fraction (Chilliard et al., 1984). However, the more striking result of our study is that under feed restriction, milk lipolysis remains stable despite a significant decrease in LPL activity.

The regulation of LPL activity is highly complex and occurs at multiple levels (Wu et al., 2021; Deeth, 2006). First, as a protein, LPL may be regulated through both transcriptional and post-transcriptional mechanisms. Our results showed that LPL activity was reduced, although LPL mRNA expression was not affected by the feed restriction regime. This could suggest post-transcriptional regulation of this gene by microRNAs,

small non-coding RNAs that are highly conserved across species. Direct evidence for the post-transcriptional regulation of LPL by microRNAs has recently been reported in ewes (Hao et al., 2021). Additionally, studies in goats have shown correlations between microRNA over-expression and the expression of genes involved in lipid metabolism (Lin et al., 2013).

Second, as an enzyme, LPL is also regulated at the post-translational level. Our results strongly suggest that under dietary restriction, a release of LPL activators into milk could occur and may compensate for the decreased enzymatic activity to maintain the level of lipolysis in goat milk. In this regard, we have recently identified putative regulators of lipolysis in the cow and ewe species (Bernard et al., 2025; Delosi  re et al., 2023, 2025). We are currently conducting similar work in the context of this study in the goat species to better understand the mechanisms regulating lipolysis in goats. Regarding the milk FA composition during the feed restriction, cis-9 C18:1 increased as an indicator of fat mobilization in adipose tissue, as evidenced by the increase in plasma NEFA, which classically occurs in dairy ruminants when the energy balance is negative (Vanbergue et al., 2017). As a consequence, we observed an increase in $\sum \geq C18$ which is concomitant to a decrease in $\sum < C16$. Goats exhibit a notable polymorphism at the α_{s1} -casein (*CSN1S1*) locus, leading to considerable variation in both the concentration and properties of milk proteins (Marletta et al., 2007; Grosclaude et al., 1987). The main alleles at this locus are classified based on their level of gene expression: the high-expression group includes most notably alleles A, B, and C, while the low-expression group comprises alleles D, F, G, with alleles E and I considered to have intermediate expression of the α_{s1} -casein in milk. In goats homozygous for O alleles (null variants), α_{s1} -casein is absent from the milk, and this is linked to marked impairments in milk fat secretion (Chanat et al., 1999). Allelic frequencies vary across breeds, with high-expression genotypes reported in goat populations from Southern European countries, whereas low or null alleles are predominant in Scandinavia (Devold et al., 2011; Sacchi et al., 2005). A relationship between the genetic polymorphism at the α_{s1} -casein locus and milk lipolysis (as indicated by FFA levels) has already been demonstrated (Valenti et al., 2010; Chilliard et al., 2006). As a consequence, the “goat flavor” is more pronounced in cheeses made from goats displaying E or F alleles for α_{s1} -casein than in cheeses made from goats displaying strong (A) alleles (Chilliard et al., 2006; Lamberet et al., 1996). Similarly, we show here that lipolysis is lower in the milk from goats displaying strong alleles for the α_{s1} -casein gene compared with the milk from goats displaying null alleles at the *CSN1S1* locus. In contrast, *in vitro* LPL activity levels are comparable in milk from AA and OO goats, thus suggesting that some *in vivo* regulating factors may intervene in AA goats’ milk to reduce lipolysis. However, during a 4-days period of feed restriction, we show here that LPL activity is significantly reduced in both AA and OO genotypes, whereas the milk lipolysis rate remains unchanged, regardless of the genotype. Besides

milk regulators, the milk fat globule itself could represent a key factor in regulating lipolytic activity in milk. The size of fat globules, by modulating the surface and hence, the accessibility of the triglyceride core, can impact the lipolysis in milk. We have demonstrated that the size of fat globules at mid-lactation was linked to the *CSN1S1* genotype (Cebo et al., 2012). We do not confirm this result despite a tendency for an effect of the genotype ($P = 0.074$), with goats at 121 ± 7 DIM in our study vs 149 ± 17 d in the previous study. Moreover, the composition of the milk fat globule membrane (MFGM) may affect lipolysis. In this regard, we have recently shown that, in cows, lipolysis is associated with a specific profile of MFGM phospholipids and proteins, suggesting an effect on membrane fluidity and lipid raft composition, which play a role in LPL anchoring and activation (Bernard et al., 2025). Consistent with this hypothesis, we have demonstrated that MFGM composition is dependent upon the genetic polymorphism at the *CSN1S1* locus (Cebo et al., 2012). Taken together, our results suggest that LPL regulation in goat milk is not solely determined by its distribution among the different milk fractions, but also involves other regulatory factors within the milk, highlighting a more complex regulatory mechanism than initially assumed.

With respect to FA, the C16:0 percentage was higher in OO vs AA genotype, which agrees with earlier studies comparing low (FF) and high genotype (AA) goats (Chilliard et al., 2003). This is also in line with the positive correlation between C16:0 percentage and the goat flavor that has been previously observed (Lamberet et al., 1996; Bakke et al., 1977).

Moreover, we showed lower expression of the lipogenic gene encoding FA synthase in agreement with the low fat concentration associated with OO genotype which is in line with a previous study comparing *CSN1S1* genotype AA and AB vs EF goats (Ollier et al., 2007). The milk delta-9 desaturation ratio cis-9-C18:1/C18:0 was higher in OO vs AA genotype in agreement with previous data reporting an increased delta-9 desaturation ratio and higher spontaneous lipolysis in goats displaying low *CSN1S1* genotypes (Chilliard et al., 2014). However, in our study, the SCD gene expression was unchanged, which is suggestive of a post-transcriptional level of regulation for this gene (Bernard et al., 2008).

5. Conclusion

The present study demonstrates that feed restriction dramatically decreases the activity of milk lipoprotein lipase enzyme without affecting lipolysis in the goat species at least within the short-term restriction window tested. This is in sharp contrast to what has been observed in cows and ewes with similar dietary conditions. This means that lipolysis-related mechanisms under feed restriction differ markedly among ruminant species, a point that needs to be confirmed by a comprehensive interspecies comparison. In addition, we anticipate that complementing our results in the future with lipidomic and proteomic data in blood and milk samples from goats with highly contrasted lipolysis levels will lead to an improved understanding of lipolytic system regulation in goats.

CRedit authorship contribution statement

L. Bernard: Conceptualization and management of the Lipomec project, Resources, Supervision, Data curation, Visualization, Writing – original draft. **C. Hurtaud:** Conceptualization and management of the Lipomec project, Resources, Data curation, Writing – review & editing. **H. Larroque:** Conceptualization of the experiment, Resources, Data curation, Writing – review & editing. **P. Iommelli:** Monitoring of Experimentation, Resources, Data Curation, Writing – review & editing. **T. Fossier:** Conceptualization and Monitoring of Experimentation, Resources, Data Curation. **A. Rau:** Resources, Data Curation, Writing – review & editing. **C. Cebo:** Conceptualization and management of the Lipomec project, Funding acquisition, Supervision, Data curation,

Visualization, Writing – original draft. All authors agree to be accountable for the content of the work.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence assisted technologies in the writing process.

Ethics approval

The experiment was conducted in accordance with the French legislation on animal experimentation and was approved by the French National Committee for Consideration of Ethics in Animal Experimentation (Authorization: APAFIS n° #30012–2021022308572260 v2 delivered on 4 May 2021).

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Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.smallrumres.2025.107649](https://doi.org/10.1016/j.smallrumres.2025.107649).

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