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Research article

Feed restriction as a tool for further studies describing the mechanisms underlying lipolysis in milk in dairy cows

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ABSTRACT

Milk lipolysis is defined as the hydrolysis of triglycerides, which are the main component of milk fat. Short-chain fatty acids (**FAs**) released in milk are responsible for rancid flavour. In addition, the presence of partial glycerides impairs the functional properties of milk, such as foaming and creaming abilities. Milk lipolysis, a key criterion used to assess milk quality, depends on animal parameters and breeding factors. Low-energy diets are associated with higher levels of spontaneous lipolysis, particularly in late lactation. In this study, dairy cows were fed a restricted diet (i.e. 65% of their ad libitum DM intake (**DMI**)) to induce spontaneous lipolysis in milk and to study milk composition associated with lipolysis. Two groups of 22 cows each received a control diet (100% of ad libitum DMI) or the restricted diet according to a 2 × 2 crossover design. The restricted diet was fed for five days. As expected, feed restriction increased milk spontaneous lipolysis which was associated with an increase in lipoprotein lipase activity. At the same time, milk yield and protein content decreased and no effect was observed on milk fat content. The increase in spontaneous lipolysis was associated with an increase in milk fat globules diameter, without influencing casein micelles diameter. Feed restriction altered the parameters of dairy cow metabolism, with increases in plasma non-esterified FAs, triglycerides and urea, indicating body fat mobilisation and protein catabolism associated with feed restriction. Feed restriction also altered hormonal parameters, with decreases in plasma insulin, insulin-like growth factor 1 and prolactin. As expected, lipolysis was higher in evening milk and was associated with a larger diameter of milk fat globules. This zootechnical approach will be completed with proteomic, lipidomic and transcriptomic studies of milk and/or mammary gland of animals selected for their extreme lipolysis.

Implications

In most French milk-producing regions, the lipolysis rate, i.e. the content of milk fat degradation products, is one of the milk quality criterions used for its payment. Indeed, short-chain fatty acids released during lipolysis cause the development of rancid flavours in milk, which is undesirable for consumers. Moreover, the presence of partial triglycerides in milk impairs its technological properties, like foaming and creaming abilities. In this study, feeding restriction induced milk lipolysis, which was more important in evening milks than in morning milks. Economic losses can be avoided by farmers by taking care to cover the energy and nitrogen needs of their dairy cows.

Specification table

Subject	*Quality of Animal Products*
Related research article	Milk lipolysis
Type of data	Table
How data were acquired	Fibertec, Agilent ICP OES, Foss Milkoscan, Büchi analyzer, MasterSizer 3000, gas chromatograph Agilent 7890A, RIA Wizard 2 gamma counter 2470 Perkin Elmer, KONE PRO multiparameter

(*continued on next page*)

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	analyzer ThermoFisher Scientific, Wizard2 gamma counter 2470 Perkin-Elmer
Data format	Raw and pretreated data
Parameters for data collection	44 cows were milked twice a day with a Delaval ROTO milking parlour, and individual milk was collected in milking cans
Description of data collection	Milk samples were collected individually from individual samplers (6 times a week) or from milk cans from morning and evening milkings at the end of each period. Blood was collected from the tail vein using 5 mL heparinised and EDTA tubes after morning milking during the last week of each experimental period.
Data source location	Institution: INRAE City/Town/Region: St-Gilles/Brittany Country: France Latitude and longitude: 48°8037.33200N, 1°49056.59500; 48.14370346069336, –1.8323876857757568
Data accessibility	https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/VJ145K

Introduction

Milk lipolysis is defined as the hydrolysis of triglycerides, which are the main component of milk fat. Short-chain fatty acids (**FAs**) subsequently released in milk severely impair the organoleptic and functional properties of milk and dairy products (Deeth, 2006). Therefore, milk lipolysis, which is routinely measured by accredited inter-professional dairy laboratories, is one of several criteria used to assess milk quality in France.

Lipolysis can be induced during milking processes or when manufacturing dairy products; however, spontaneous lipolysis (**SL**) of milk results from complex interactions among farming practices, animal physiology and animal genetics (Vanbergue et al., 2020). Dairy farmers currently implement practices to minimise lipolysis in milk. However, the growing development of automatic milking systems could reduce in future the quality of milk due to lipolysis (De Marchi et al., 2017). The mechanisms underlying SL are not well known and thus require future study. The LIPOMEC project, funded by APIS-GENE, by the French National Research Agency (ANR-19-CE21-0010) and by dairy stakeholders, is the first large-scale integrated project to study the lipolytic system in milk and the mammary glands of three dairy species (dairy cow, ewe and goat).

The objective of the present study was to characterise milk of dairy cows fed a restricted diet at 65% of ad libitum DM intake (**DMI**) and the metabolism and hormonal status of these cows. We hypothesised that this feeding practice would induce SL in milk as in Vanbergue et al. (2018a, and 2018b). The final objective of this study was to then characterise the mechanisms of SL to better control the degradation of triglycerides. Thus, the generated samples (i.e., milk and blood samples and mammary biopsies) were then used for further in-depth analysis. This article, which describes the experimental study, the milk composition of all samples and the blood metabolite and hormonal composition, will be used as a reference article for all future studies, including lipidomics, proteomics and transcriptomics. Preliminary results have been published in EAAP congress and in Rencontres Recherches Ruminants in 2020.

Material and methods

Animals and experimental design

The experiment was conducted at IE PL, INRAE, Dairy nutrition and physiology (IE PL, 35650 Le Rheu, France; https://doi.org/10.15454/yk9q-pf68) with the agreement for animal housing number C-35–275-23 and in accordance with French legislation on animal experimentation and was approved by the French National Committee for Consideration of Ethics in Animal Experimentation (Authorisation: APAFiS #17944-2018120416536243 v2 delivered on 19 February 2019). The study used 44 primiparous and multiparous Holstein cows in mid-lactation. At the beginning of the experiment, on average (±1 standard deviation), days in milk were 165 ± 16 d, cows produced 34.3 ± 4.8 kg of milk/d with 3.91% ± 0.32% fat content and 3.07% ± 0.21% protein content, and 0.60 ± 0.26 mEq/100 g of fat for evening milk SL. Cows' body weight (BW) was 653 ± 58.1 kg, and parity was 1.8 ± 1.24. All cows were kept indoors, with a mean area of 8.75 m^2 per cow. Two diet treatments used differed in the feeding level. Cows were allocated to two groups of 22 animals each according to the following criteria and in this order: milk, SL, lactation stage, lactation rank (primiparous vs multiparous), milk yield, milk fat and protein contents, somatic cell count (**SCC**) and BW. Each group was divided into two batches that were staggered by 1 week due to experimental constraints and to ease laboratory analyses (Fig. 1). Diets were based on maize silage. The two diet treatments were "non-restricted" (**NON RESTR**), with cows fed 100% of ad libitum DMI, and "restricted" (**RESTR**), with cows fed at 65% of ad libitum DMI. Begun in March 2019, the experiment was conducted using a 2 × 2 crossover design with feeding level as the main factor for 4 weeks, divided into two 2-week periods. During period 1, each group of cows received NON RESTR or RESTR, and during period 2, the feeding level was reversed.

Treatments and feeding

The ingredients, chemical composition and nutritional value of the diets differed (Supplementary Tables S1 and S2). All cows received a diet consisting of 65% maize silage, 12.5% energy concentrate (20% wheat, 20% maize, 20% barley, 20% beet pulp, 15% wheat bran, 3% cane molasses, 1% vegetable oil and 1% salt), 12.5% soybean meal, 10% dehydrated alfalfa and 300 g of minerals. All cows were fed this diet ad libitum for 4 weeks before the experiment (i.e. the pre-experiment period). Cows allocated to the RESTR treatment were given a 4-day transition period to switch to this treatment before beginning the first 2-week experimental period. For the second experimental period, the feeding level was reversed. As before, cows allocated to the RESTR treatment were given a 4-day transition period to switch to this treatment before beginning the second 2-week experimental period. The restricted diet was fed for five days. Cows allocated to the NON RESTR treatment were fed 100% of ad libitum DMI (Fig. 1). Diets were formulated to meet energy and protein requirements when distributed at 100% of ad libitum DMI (INRA, 2018).

Measures, sample collection and laboratory analysis

Feeds and feed refusal

Throughout the experiment, cows had free access to water and were fed via individual electronic gating twice daily, at 0800 h and

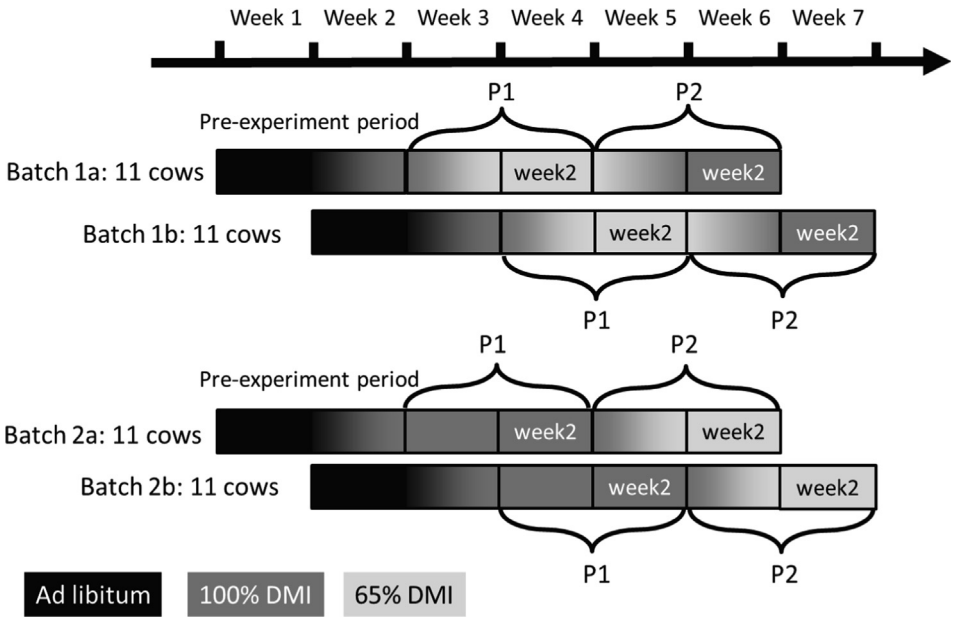

Fig. 1. Description of the experimental design. P: period. DMI: DM intake.

1800 h. All feed refusals were collected and weighed daily to determine each cow's net DMI. To calculate DMI, refusals were assumed to have the same composition as the offered diet. To determine the chemical and nutritional compositions of the diets, throughout the experiment, fresh maize silage was sampled five times per week, while energy concentrate, soybean meal and dried alfalfa were sampled once per week, and all samples were stored at −20 °C and pooled to provide one sample per type of feed per period. Samples were lyophilised and analysed for DM, mineral matter (NF V18-101, 1977), CP (NF EN ISO 16634, 2008), crude fibre (NF V03-040, 1993), neutral detergent fibre (**NDF**), acid detergent fibre (**ADF**) (van Soest et al., 1991), starch (Thivend et al., 1965), organic matter, organic matter digestibility (Aufrère et al.,1989), phosphorus (NF ISO 6491, 2011) and calcium (NF EN 6869, 2002). Fat content of the samples was extracted with a mixture of chloroform/methanol (v/v 2:1) according to the method of Folch et al. (1957).

Milk yield and traits

Cows were milked twice daily, at 0630 h and 1630 h, in the milking parlour, and milk yield was recorded individually at each milking. Milk fat, protein and lactose contents, and somatic cell count (**SCC**) were determined from six consecutive milkings each week. These analyses were performed by mid-infrared spectrometry for fat, protein and lactose contents and by flow cytometry for SCC at the MyLab dairy laboratory (Châteaugiron, France).

Milk used to determine SL, the FA profile, milk fat globule (**MFG**) diameter and milk protein and mineral composition was collected from the same milking. Milk samples were collected individually from milk cans from morning and evening milkings at the end of the pre-experiment period and the end of each experimental period (one day), and then stored at 4 °C or at −20 °C according to the analyses.

Spontaneous lipolysis of milk

Two vials of milk per cow were collected to calculate SL as free FA (**FFA**) contents after 24 h of storage at 4 °C minus initial FFA contents. Immediately after milking, a 50 mL sample was heated for 2.5 min in a water bath at 100 °C to stop lipase activity and then kept at 4 °C. A second sample was first stored at 4 °C for 24 h before being heated to 100 °C and then kept at 4 °C, as for the first sample. FFAs in both samples were analysed using the copper-soap method (Shipe et al., 1980).

Milk fat globule and casein size

Vials of milk, with bronopol (Merck, Darmstadt, Germany) added, were kept at room temperature to assess MFG diameter distribution using laser-light scattering (Mastersizer 3000, Malvern, UK). Mean diameters $d_{4,3} = \Sigma(N_i \times d_i^4)/\Sigma(N_i \times d_i^3)$ and $d_{3,2} = \Sigma(N_i \times d_i^3)/\Sigma(N_i \times d_i^2)$, and MFG area $S = 6/(\rho \times d_{3,2})$ (with N_i the number of MFG in diameter class d_i and ρ the density of the particle considered (0.92 for fat)) were calculated using Malvern software. The milk was skimmed in two successive centrifugations (3157 g, 4 °C) to remove the fat. The mean diameter $d_{4,3}$ of casein micelles was measured using the Mastersizer 3000.

Milk fatty acid composition and lipoprotein lipase activity

To analyse milk FAs, freeze-dried morning and evening milk samples were pooled according to the mean milk fat yield at each milking (60/40) to create a representative 100 mg sample. FA methyl esters of the milk samples were then prepared and analysed after injection into a gas chromatograph (Agilent 7890A GC System, Massy, France) equipped with a flame ionisation detector and a CP-Sil 88 capillary column (100 m × 0.25 mm, 0.2 µm thickness; Agilent Technologies, Inc., Santa Clara, California, USA) as previously described (Fougere et al., 2018). Milk lipoprotein lipase (EC 3.1.1.34) activity was measured from morning milk, as described by Bernard et al. (2005).

Nitrogen and mineral contents of milk

Total nitrogen, non-protein nitrogen, non-casein nitrogen, and casein were determined according to the Kjeldahl methods described by Alais (1984). Urea was analysed on milk ultrafiltrate in two replicates of colorimetric enzymatic reactions. Total and soluble calcium, sodium, potassium and magnesium were analysed via inductively coupled plasma optical emission spectroscopy (ICP-OES 5110 Agilent Technology, Les Ulis, France) of milk and milk ultrafiltrate (only for calcium), respectively. A 500 µL sample of milk was first diluted 40 times in water. Then, 2.5 mL of 0.01% Triton X100 (Sigma Aldrich, Saint- Quentin Fallavier, France) and 7.5 mL of 65% nitric acid were added. Water was then added to

the samples to fill them to 50 mL. The analyses were performed in duplicate according to manufacturer instructions using calibration standards for ICP-OES Certipur calcium, potassium, magnesium and sodium 1000 µg/mL (Agilent Technology, Les Ulis, France) and standard milk sample ERM-BD151 (European Reference Materials, milk sample with mineral guaranteed contents, European Commission Joint Research Centre, Brussels, Belgium). Total and soluble phosphorus and chlorine contents were determined using a KONE PRO multiparameter analyzer (ThermoFisher Scientific, Illkirch, France) according to the Allen method for phosphorus (Pien, 1969) and as described by Henry et al. (1974) for chlorine.

Plasma metabolites and hormones

Blood was sampled from the tail using 5 mL heparinised and EDTA tubes (VT-050SHL, Venoject, Terumo Europe, Leuven, Belgium) after morning milking during the last week of each experimental period. Blood was centrifuged at 2,264 g for 15 min, and plasma was removed and stored at −20 °C until analysis. Plasma glucose, urea, acetate, non-esterified FA, triglyceride, cholesterol, lactose and β-hydroxybutyrate contents were analysed in two replicates of colorimetric enzymatic reactions, as reported by Delamaire and Guinard-Flament (2006). Plasma insulin and insulin-like growth factor 1 (**IGF1**) concentrations were determined by radioimmunoassays (**RIAs**) using the Wizard2 gamma counter 2470 (Perkin-Elmer) with commercial kits (Insulin RIA kit, PI-12K, Millipore, Billerica, Massachusetts, USA; IGF-1 RIA-CT, Diasorin, Antony, France). Plasma concentrations of prolactin were assessed using the method described by Herve et al. (2019).

Calculations and statistical analyses

All statistical analyses were performed using SAS software (SAS 9.2 Institute Inc., Cary, North Carolina, USA). Effects of feeding level were evaluated using daily values for DMI, energy requirements and balance, the supply and efficiency of protein digested in the small intestine (**PDI**), BW, milk traits and plasma parameters. For milk traits, daily values were obtained by calculating the average of morning and evening values weighted by milk yield. Effects of milking time (morning vs evening), feeding level and their interactions were evaluated using twice-daily values for SL, MFG diameter and protein and mineral compositions. The statistical model was a mixed model (MIXED procedure of SAS) that included the group of cows, feeding level, milking time and the interaction of milking time and feeding level as fixed effects, a cow within a group as a random effect, and a covariable that corresponded to the value of variable Y_{ijkl} during the pre-experiment period ($CovY_{ijkl}$):

$$Y_{ijkl} = \mu + Group_i + Milking\ Time_j + Period_k + Feeding\ level_l + Milking\ Time_j \times Feeding\ level_l + CovY_{ijkl} + \varepsilon_{ijkl}.$$

The threshold for statistical significance was set at $P < 0.05$, while that for a trend was set at $P < 0.10$.

Results

Effects of feed restriction

By design, feed restriction decreased DMI (−5.7 kg/d), which resulted in a decrease in PDI intakes (−498 g/d), in a negative energy balance (−16.9 MJ/d) and in an increase in PDI efficiency (+0.02) (Table 1). As expected, the RESTR treatment increased milk SL (+0.25 mEq/100 g of fat) (Table 2), while lipolysis values ranged from 0.09 to 3.48 mEq/100 g of fat (individual results in Results-Morning-Evening-Milking-2.tab published in https://doi.org/10.57745/VJ145K). The increase in SL was associated with an increase in milk lipoprotein lipase activity and with an increase in MFG diameter ($d_{4,3}$) only in evening milk (+0.23 µm) (Table 2), without no effect on the diameter of casein micelles (Supplementary Table S3). Under feed restriction, as observed in previous studies, milk FAs from de novo mammary synthesis (FAs < C16) decreased, while long-chain FA uptake by the udder (FAs > C16) increased, which resulted mainly in a specific increase in milk cis-9 C18:1 (Table 3). At the same time, milk production and protein content decreased greatly (−5.1 kg/d and −1.3 g/kg, respectively) (Table 4). Feed restriction increased milk urea content (+21.5 mg/L) (Supplementary Table S4). Milk fat content did not change (Table 4). The RESTR treatment increased SCC. The RESTR treatment decreased colloidal calcium (−19 mg/kg), total, soluble and colloidal phosphorus contents (−53.5, −24.0 and −29.0 mg/kg, respectively), and increased soluble calcium (+26 mg/kg) and chlorine content (+1.2 mmol/L) (Supplementary Table S5). The RESTR treatment also changed the metabolism of dairy cows (Table 5), as indicated by the increase in levels of plasma non-esterified FAs (+302 µmol/L), triglycerides (+21.5 mg/L), β-hydroxybutyrate (+38 µmol/L), cholesterol (+141.7 mg/L) and urea (+26 mg/L) and the decrease in plasma glucose levels (−29 mg/L). Plasma acetate and lactose tended to decrease (−0.12 mmol/L, $P = 0.077$ and −6.3 mg/L, $P = 0.066$). Similarly, plasma insulin and IGF1 concentrations decreased under feed restriction (−1.33 µUI/mL and −22.24 ηg/mL, respectively) (Table 5). Feed restriction also decreased plasma prolactin concentrations (−3.2 ηg/mL).

Effects of the time of milking

SL was higher in evening milk (+0.56 mEq/100 g of fat) and associated with an increase in fat content (+7.7 g/kg), in MFG diameter ($d_{4,3}$) (+0.20 µm), the latter especially with the RESTR treatment and a decrease in MFG-specific area (−0.09 m^2) (Table 2). Compared with milk from the morning milking, milk from the eve-

Table 1
Weight, DM, protein and energy intake, energy balance and efficiency of protein digestible in the intestine (PDI) based on the non-restricted (NON RESTR) and restricted (RESTR) feeding treatments for dairy cows.

Characteristic	ATOL[1] id	Feeding		SEM	*P*-value	
		NON RESTR	RESTR		Group	Treatment
Body weight, kg	ATOL_0000351	662	625	1.7	0.347	<0.001
DM intake, kg/d	ATOL_0005395	21.8	16.1	0.15	0.025	<0.001
PDI[2], g/d	/	1 815	1 317	13.5	0.022	<0.001
NE_L[3] requirements, MJ/d	ATOL_0002559	151.5	132.4	0.96	0.932	<0.001
NE_L balance, MJ/d	/	−7.4	−24.3	1.03	0.088	<0.001
PDI efficiency[4]	ATOL_0001584	0.83	0.85	0.003	0.005	<0.001

[1] Traits in reference to ATOL: Animal Trait Ontology for Livestock, https://www.atol-ontology.com/en/erter-2/.
[2] PDI: protein digested in the small intestine.
[3] Net energy for lactation.
[4] PDI efficiency = (67.5–0.52 × (PDI-100) + 0.014 × (MilkProteins-1 000))/100 (INRA, 2018).

Table 2
Milk fat characteristics (free fatty acid contents, spontaneous lipolysis, milk fat globule diameter and lipoprotein lipase activity only measured for morning milk) based on non-restricted (NON RESTR) and restricted (RESTR) feeding treatments for dairy cows on the sampling day.

Characteristic	ATOL[1] id	Milking	Feeding			*P*-value			
			NON RESTR	RESTR	SEM	Group	Feeding	Milking	F[2] * M[3]
Free fatty acids just after milking (T0), mEq/100 g fat	ATOL_0001753	Morning	0.19	0.21	0.018	0.527	0.978	0.135	0.275
		Evening	0.23	0.21					
Free fatty acids after 24 h at 4 °C (T24), mEq/100 g fat	ATOL_0001753	Morning	0.49	0.72	0.076	0.081	<0.001	<0.001	0.753
		Evening	1.06	1.32					
Spontaneous lipolysis, mEq/100 g fat	/	Morning	0.30	0.51	0.077	0.005	<0.001	<0.001	0.516
		Evening	0.82	1.11					
Lipoprotein lipase activity, ηmol/min per mL	ATOL_0000188	Morning	501.7	708.8	12.75	0.833	<0.001	nd[4]	nd[4]
		Evening	nd[4]	nd[4]					
Milk fat content, g/kg	ATOL_0001520	Morning	39.4	37.4	0.72	0.754	0.033	<0.001	<0.001
		Evening	44.0	48.2					
Milk fat globule diameter ($d_{4,3}$[5]), μm	ATOL_0000729	Morning	3.83	3.76	0.046	0.104	0.014	<0.001	0.001
		Evening	3.88	4.11					
Milk fat globule diameter ($d_{3,2}$[6]), μm	ATOL_0000729	Morning	3.35	3.22	0.034	0.036	0.745	<0.001	<0.001
		Evening	3.38	3.50					
Milk fat globule area (s[7]), m^2	ATOL_0000730	Morning	1.96	2.05	0.020	0.038	0.320	<0.001	<0.001
		Evening	1.94	1.88					

[1] Traits in reference to ATOL: Animal Trait Ontology for Livestock, https://www.atol-ontology.com/en/erter-2/.
[2] F: Feeding.
[3] M: Milking.
[4] nd: not determined.
[5] $d_{4,3} = \Sigma(N_i \times d_i^4)/\Sigma(N_i \times d_i^3)$.
[6] $d_{3,2} = \Sigma(N_i \times d_i^3)/\Sigma(N_i \times d_i^2)$.
[7] $s = 6/(\rho \times d_{3,2})$ (with N_i the number of milk fat globules in diameter class d_i and ρ the density of the particle considered (0.92 for fat)).

ning milking had a higher protein content (+0.5 g/kg) due to an increase in casein content (+0.7 g/kg) at the expense of soluble protein content (−0.3 g/kg), without influencing the milk lactose content; however, the casein micelle diameter was smaller (−5.5 ηm) (Supplementary Table S3). The casein:protein ratio was therefore higher in evening milk (+1.1 point) (Supplementary Table S3). Evening milk contained a higher SCC (+21.4 × 10^3/mL) (Supplementary Table S5). Evening milk contained more total calcium (+43 mg/kg) as soluble calcium (+9 mg/kg) and colloidal calcium (+35 mg/kg) (Supplementary Table S4). Evening milk contained less total and soluble phosphorus (−24 mg/kg and −18 mg/kg, respectively), potassium (−56.5 mg/kg), magnesium (–3.5 mg/kg), sodium (−11 mg/kg) and chlorine (−1.25 mmol/L). Evening milk had a higher freezing point (+0.001 °C) (Supplementary Table S4).

Author's point of view

Increase of lipolysis and of lipoprotein lipase is consistent with the results of Vanbergue et al. (2018a and 2018b), in cows under similar feed restriction (75–80% of ad libitum DMI) regardless of the lactation stage. The concomitant increase in SL and MFG diameter suggests that changes in MFG composition could explain greater sensitivity of milk fat to lipolysis. Further studies to explore the composition of MFG membranes (protein and lipids) are necessary and will be conducted with the milk sampled during this experiment. During feed restriction, cis-9 C18:1 increased as an indicator of fat mobilisation in adipose tissue (Chilliard, 1987) that occurs when the energy balance is negative. Therefore, a positive relationship between the cis-9 C18:1 content in milk and SL in cows under feed restriction was previously observed (Chilliard, 1987; Vanbergue et al., 2017). Milk production and the protein content decreased as observed by Gabbi et al. (2016) and Vanbergue et al. (2018a and 2018b). The decrease in protein content resulted from the decrease in casein and soluble protein contents due to a lack of energy for protein synthesis (Coulon and Rémond, 1991). Milk urea increased as observed by Gross et al. (2021), likely due to lower growth of rumen microorganisms caused by decreased intake of protein and energy (Gross and Bruckmaier, 2019). The increase in urea may also have been related to amino acid catabolism, as observed in dairy cows under intense feed restriction (Leduc et al., 2021). Milk fat content did not change unlike the results of Gross et al. (2021) (increase of milk fat after concentrate withdrawal) and Gabbi et al. (2016) (increase of milk fat with 40–50% feed restriction according to NRC). Differences in milk fat content due to feed restriction have already been observed (Leduc et al., 2021). The RESTR treatment increased SCC as observed by Herve et al. (2019). Since SCC contains mammary epithelial cells, their increase could partly result from an increase in the exfoliation rate of these cells, as observed in a previous study during feed restriction (Herve et al., 2019). Increase of plasma non-esterified FAs, triglycerides, β-hydroxybutyrate, cholesterol and urea and the decrease in plasma glucose levels indicated mobilisation of adipose tissue, catabolism of body protein and a decrease in growth of rumen microorganisms associated with feed restriction (Leduc et al., 2021; Vanbergue et al., 2018a; 2018b), which decreased BW (−37 kg). Plasma acetate tended to decrease unlike observations of Vanbergue et al. (2018a and 2018b) (Table 5). A tendency towards a decrease is also observed for plasma lactose (Table 5) and can be related to the decrease in milk lactose synthesis (Guinard-Flament et al., 2011). Plasma insulin and IGF1 concentrations were consistent with endocrine adaptations due to a negative energy balance (Leduc et al., 2021; Vanbergue et al., 2018a; 2018b). Feed restriction also decreased plasma prolactin concentrations as previously observed under intense feed restriction (Ollier et al., 2015), in contrast with the lack of change in plasma prolactin observed under a moderate feed restriction of 80% of ad libitum DMI (Herve et al., 2019).

SL higher in the evening and associated with an increase in fat content as in Vanbergue et al. (2017, 2018a; 2018b) was mainly due to the interval between the two milkings (14 and 10 h, respectively) and to the smaller quantity of milk produced during evening milking. Conversely, Ferlay et al. (2010) observed that the percentages of milk protein and lactose did not change as a function of time of milking. According to Manoni et al. (2021), the calcium content of milk is correlated with MFG diameter; smaller MFGs indicate a higher calcium content. In this experiment, however, opposite results were found.

Table 3
Milk fatty acid composition based on non-restricted (NON RESTR) and restricted (RESTR) feeding treatments for dairy cows.

Fatty acids (% total fatty acids)	ATOL[1] id	Feeding		SEM	*P*-value	
		NON RESTR	RESTR		Group	Feeding
C4:0	ATOL_0000638	2.89	2.91	0.054	0.165	0.779
C6:0	ATOL_0000640	1.94	1.70	0.026	0.724	<0.001
C8:0	ATOL_0000642	1.12	0.88	0.016	0.656	<0.001
C10:0	ATOL_0000644	2.79	2.02	0.050	0.494	<0.001
cis-9-C10:1	ATOL_0005629	0.32	0.23	0.007	0.468	<0.001
C12-0	ATOL_0000646	3.43	2.40	0.064	0.461	<0.001
iso C13:0	ATOL_0005626	0.031	0.028	0.0005	1.000	<0.001
cis-9-C12:1	ATOL_0005628	0.090	0.056	0.0027	0.207	<0.001
iso C14:0	ATOL_0000254	0.090	0.090	0.0031	0.291	0.942
C14:0	ATOL_0000647	11.9	10.0	0.14	0.721	<0.001
iso C15:0	ATOL_0000256	0.21	0.20	0.003	0.439	0.294
anteiso C15:0	ATOL_0000257	0.41	0.37	0.006	0.229	<0.001
cis-9-C14:1	ATOL_0005627	1.22	0.99	0.031	0.149	<0.001
C15:0	ATOL_0000255	1.04	0.81	0.019	0.418	<0.001
Σ <C16	/	27.9	23.0	0.319	0.849	<0.001
ΣC16	/	36.9	33.5	0.41	0.740	<0.001
iso C16:0	ATOL_0000258	0.24	0.25	0.006	0.837	0.095
C16:0	ATOL_0000648	34.7	31.2	0.39	0.858	<0.001
iso C17:0 + trans-9-C16:1	ATOL_0000259	0.34	0.40	0.006	0.925	<0.001
anteiso C17:0	ATOL_0000260	0.36	0.40	0.006	0.693	<0.001
cis-9-C16:1	ATOL_0000702	1.70	1.86	0.059	0.161	0.012
C17:0	ATOL_0000649	0.64	0.62	0.007	0.317	0.060
cis-9-C17:1	ATOL_0000660	0.20	0.28	0.007	0.965	<0.001
Σ>C18:0	/	33.5	41.6	0.54	0.688	<0.001
ΣC18	/	31.9	39.7	0.53	0.586	<0.001
C18:0 + cis-9-C18:1	/	25.3	31.6	0.46	0.391	<0.001
ΣC18:1	/	21.6	28.3	0.44	0.872	<0.001
ΣC18:2		2.23	2.50	0.036	0.766	<0.001
C18:0	ATOL_0000650	7.69	8.49	0.139	0.019	<0.001
trans-10-C18:1	ATOL_0000666	0.44	0.50	0.016	0.162	0.004
trans-11-C18:1	ATOL_0000661	1.07	1.70	0.048	0.154	<0.001
trans-12 + cis-6-C18:1	/	0.40	0.44	0.007	0.914	<0.001
Σ trans C18:1	/	2.75	3.55	0.069	0.227	<0.001
cis-9 + cis-10 + trans-15-C18:1	/	17.6	23.1	0.387	0.957	<0.001
cis-11-C18:1	ATOL_0000668	0.56	0.79	0.019	0.562	<0.001
cis-12-C18:1	ATOL_0000669	0.43	0.47	0.009	0.572	<0.001
cis-13-C18:1	ATOL_0000670	0.092	0.124	0.0025	0.953	<0.001
cis-15-C18:1 + C19:0	ATOL_0000671	0.16	0.17	0.002	0.804	<0.001
cis-9,trans-13-C18:2 + cis-10,trans-14-C18:2	/	0.22	0.23	0.005	0.484	0.056
cis-9,trans-14-C18:2	ATOL_0005630	0.10	0.11	0.003	0.489	0.069
cis-9,cis-12-C18:2	ATOL_0000657	1.74	1.98	0.031	0.946	<0.001
C18:3 (n-3)	ATOL_0000699	0.30	0.34	0.007	0.969	0.0005
cis-9,trans-11 CLA (+trans-7,cis-9 + trans-8,cis-10 CLA)	ATOL_0000657	0.5189	0.7761	0.02291	0.0134	<0.001
Σ≥C20:0	/	0.58	0.63	0.011	0.923	<0.001
cis-9 C14:1/C14:0	/	0.103	0.099	0.0030	0.145	0.077
cis-9 C16:1/C16:0	/	0.049	0.060	0.0018	0.179	<0.001
cis-9 C18:1/C18:0	/	2.31	2.76	0.052	0.020	<0.001
cis-9 trans-11 CLA/trans-11 C18:1	/	0.49	0.46	0.009	0.034	0.003

[1] Traits in reference to ATOL: Animal Trait Ontology for Livestock, https://www.atol-ontology.com/en/erter-2/.

Table 4
Milk yield and composition based on non-restricted (NON RESTR) and restricted (RESTR) feeding treatments for dairy cows.

Characteristic	ATOL[1] id	Feeding		SEM	*P*-value	
		NON RESTR[1]	RESTR[2]		Group	Feeding
Milk yield, kg/d	ATOL_0001518	30.6	25.5	0.30	0.327	<0.001
Milk fat content, g/kg	ATOL_0001520	40.0	40.1	0.32	0.043	0.786
Milk fat yield, g/d	ATOL_0000549	1 221	1 021	13.3	0.391	<0.001
Milk protein content, g/kg	ATOL_0001521	31.4	30.1	0.14	0.411	<0.001
Milk protein yield, g/d	ATOL_0000550	961	766	9.0	0.668	<0.001
Milk lactose content, g/kg	ATOL_0000619	49.3	49.5	0.18	0.122	0.127
Milk lactose yield, g/d	ATOL_0000618	1 520	1 301	29.9	0.703	<0.001
SCC[2], $\times 10^3$/mL	ATOL_0000991	29.1	37.5	4.27	0.153	0.097

[1] Traits in reference to ATOL: Animal Trait Ontology for Livestock, https://www.atol-ontology.com/en/erter-2/.
[2] SCC: somatic cell count.

On dairy farms, high lipolysis can be avoided by feeding the requirements of the cows, and an adapted milking interval (not too short) (Rémond et al., 2009). Once-day milking can also be a way to reduce lipolysis (Rémond and Pomiès, 2005). The nature of the diet can also have an impact on lipolysis, in particular diets rich in lipids (Vanbergue et al., 2018a; 2018b).

Table 5
Plasma metabolites and hormone concentrations based on non-restricted (NON RESTR) and restricted (RESTR) feeding treatments for dairy cows.

Characteristic	Ontology[1] id	Feeding		SEM	*P*-value	
		NON RESTR	RESTR		Group	Feeding
Acetate, mmol/L	/	1.16	1.04	0.044	0.138	0.077
Non-esterified fatty acids, µmol/L	VT:0001553	147	449	34.1	0.610	<0.001
β-hydroxybutyrate, µmol/L	VT:0010996	609	647	34.0	0.066	0.227
Glucose, mg/L	ATOL_0000097	734	705	5.4	0.220	<0.001
Lactose, mg/L	/	51.2	44.9	2.49	0.408	0.066
Triglycerides, mg/L	VT:0002644	71.2	92.7	2.98	0.895	<0.001
Cholesterol, mg/L	VT:0000180	1 724.8	1 866.5	57.06	0.751	0.001
Urea, mg/L	VT:0005265	198	224	6.5	0.347	<0.001
Insulin, µUI/mL	VT:0001560	8.045	6.719	0.497	0.103	0.042
Insulin-like growth factor 1, ηg/mL	ATOL_0000990	180.49	158.25	4.966	0.054	<0.001
Prolactin, ηg/mL	ATOL_0001699	12.58	9.38	6.27	0.046	<0.001

[1] Traits in reference to ontologies: ATOL (Animal Trait Ontology for Livestock, https://www.atol-ontology.com/en/erter-2/) and VT (Vertebrate Trait ontology, https://bioportal.bioontology.org/ontologies/VT/?p=summary).

Conclusion

As expected, feed restriction in dairy cows induced lipolysis in milk, with highly variable individual responses depending on individual cow's susceptibility to lipolysis. The increase in lipolysis was associated with an increase in MFG diameter and a decrease in MFG area in evening milk. A positive relationship was observed between the cis-9 C18:1 content of milk and SL due to fat mobilisation in adipose tissue, as illustrated by the increase in plasma non-esterified FAs. Lipolysis was also higher in evening milk and associated with higher fat content, larger MFG diameter and smaller casein micelle diameter than those in morning milk. This study confirms that feed restriction is a useful tool for studying milk lipolysis in cattle. We thoroughly described an experimental design that induced lipolysis in dairy cows and provided in-depth biophysical and biochemical analyses of milk samples under lipolysis. The data will be enriched with future detailed studies of lipids, proteins and gene expression under lipolysis in the milk and mammary glands of cows.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.anopes.2022.100035.

Ethics approval

The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The experiment was approved by the French National Committee for Consideration of Ethics in Animal Experimentation (Authorisation: APAFiS #17944-2018120416536243 v2 delivered on 19 February 2019).

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Declaration of interest

The authors declare that they have no conflict of interest.

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