



**HAL**  
open science

## Effects of forage quantity and access-time restriction on feeding behaviour, feed efficiency, nutritional status, and dairy performance of dairy cows fed indoors

Maëva Cremilleux, M. Coppa, Matthieu Bouchon, Luc Delaby, G. Beure, I. Constant, A. Natalello, Bruno Martin, Audrey A. Michaud

### ► To cite this version:

Maëva Cremilleux, M. Coppa, Matthieu Bouchon, Luc Delaby, G. Beure, et al.. Effects of forage quantity and access-time restriction on feeding behaviour, feed efficiency, nutritional status, and dairy performance of dairy cows fed indoors. *Animal*, 2022, 16 (9), pp.100608. 10.1016/j.animal.2022.100608 . hal-03770112

**HAL Id: hal-03770112**

**<https://hal.inrae.fr/hal-03770112v1>**

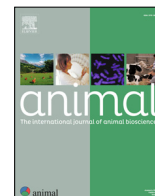
Submitted on 6 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



## Effects of forage quantity and access-time restriction on feeding behaviour, feed efficiency, nutritional status, and dairy performance of dairy cows fed indoors



M. Cremilleux<sup>a,\*</sup>, M. Coppa<sup>b</sup>, M. Bouchon<sup>b</sup>, L. Delaby<sup>c</sup>, G. Beure<sup>d</sup>, I. Constant<sup>a</sup>, A. Natalello<sup>e</sup>, B. Martin<sup>a</sup>, A. Michaud<sup>a</sup>

<sup>a</sup> Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France

<sup>b</sup> HerbiPôle, INRAE, 63122 Saint-Genès-Champanelle, France

<sup>c</sup> INRAE, Institut Agro, Physiologie, Environnement, Génétique pour l'Animal et les Systèmes d'Élevage, 35590 Saint Gilles, France

<sup>d</sup> Facilitator for the Nonprofit 'Association Eleveurs Autrement', 63820 Laqueuille, France

<sup>e</sup> Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy

### ARTICLE INFO

#### Article history:

Received 17 December 2021

Revised 5 July 2022

Accepted 11 July 2022

#### Keywords:

Feed self-sufficiency

Feeding costs

Indoor feeding

Milk production

Time-restricted feeding

### ABSTRACT

Optimising feed is a key challenge for dairy livestock systems, as forage stock shortages are increasingly frequent and feed is the biggest operating cost. The aim of this experiment was to evaluate the effects of reducing forage quantity and access time on dairy performance and animal nutritional status during indoor feeding. Twenty-seven Montbéliarde and Holstein cows were randomly allocated to three groups of nine cows balanced by breed, parity, days in milk, and milk yield. The three groups were given 3.9 kg DM/day of second-cut hay and 4.5 kg/day of concentrate and either i) *ad libitum* access to first-cut hay (*Ad Libitum* group; **AL**), ii) 10.5 kg/day of first-cut hay (Quantity-restricted group; **QR**), or iii) 10.5 kg/day of first-cut hay but with access time restricted to only 2 h in the morning and 2 h in the afternoon (Quantity-and-Time-restricted group; **QTR**). Milk yield, composition and coagulation properties, cow nutritional status (weight, body condition score, blood metabolites) and cow activities were recorded. The AL group ingested 10% more feed than the QR group and 16% more feed than the QTR group. Organic matter digestibility was lower in the AL group than in the QR and QTR groups whereas feed efficiency did not differ. Milk yield was not significantly different among the three groups. Compared to the QR and QTR groups, the AL group had significantly higher milk fat (35.9 vs 32.9 and 32.8 g/kg of milk) and milk protein content (29.5 vs 27.7 and 28.5 g/kg of milk). QR and QTR cows mobilised their body fat, resulting in a lower final body condition score, and tended to have a lower blood non-esterified fatty acid concentration than the AL group. QTR cows showed greater body fat mobilisation, but their final corrected BW was not different from AL cows. Access-time restriction did not impact fat and protein content but led to decreased casein, lactose contents and casein-to-whey protein ratio. The forage savings achieved through this feed management practice could prove economically substantial when forage prices increase. This practice can be of interest in grassland systems to overcome certain climatic hazards without having to resort to purchases or to increase the farm's forage autonomy.

© 2022 The Authors. Published by Elsevier B.V. on behalf of The Animal Consortium. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### Implications

Feed is the biggest operating cost in livestock and can become a critical factor in the event of forage stock shortage. Compared to *ad libitum* feeding, reducing forage quantities given to dairy cows by 2.2 kg/d for 9 weeks has no significant effect on milk yield and BW but decreases milk fat and protein contents as well as

energy-corrected milk. However, the forage savings achieved to compensate for the economic losses due to decreased performance, making it a potentially economically meaningful practice option for farmers. Additional access-time restriction to feed seems to have limited interest in terms of animal performances.

### Introduction

Low-input dairy systems base their economic efficiency on keeping production costs low. Achieving forage self-sufficiency is

\* Corresponding author.

E-mail address: [maeva.cremilleux@vetagro-sup.fr](mailto:maeva.cremilleux@vetagro-sup.fr) (M. Cremilleux).

a major challenge (Lebacqz et al., 2015) to gain resilience in a context of increasingly frequent climatic hazards that put pressure on ruminant feeding systems, especially in low-input and grass-based systems. Optimising feed is key, as feed costs, which account for half of the total dairy-farm operating costs (European Commission, 2018), are significantly increased when forage has to be purchased.

At farm scale, feeding costs can be reduced long term via strategies such as lower age at first calving, increased productive lifespan, and improved genetics (Vandehaar, 1998). In the event of dramatic forage stock shortages, some farmers respond in the short term by selling off some animals or, more likely, by trying to economise some of the forage supplied daily to their cows by limiting refusals and waste and by improving feed efficiency. Farmers who try to improve feed efficiency instead of trying to maximise productivity per animal are looking to find a compromise between average herd output, animal health, reproduction and milk quality. Feeding systems for dairy cows (NRC, 2001; Institut National de la Recherche Agronomique (INRA), 2018) may help farmers limit the amount of forage distributed, as they no longer aim for maximum productivity per animal. The systems describe animal production responses around the recommendations for energy and protein supply by increasing feed-conversion efficiency and addressing other issues like health, environment, milk composition, and so on. Nevertheless, feeding systems are still based on maximisation of forage intake, which will not solve problem of forage shortages.

Beyond feeding optimisation, some authors have also investigated the effects of long-period DM Intake (DMI) restriction on animal responses, but the focus has mainly been on concentrate reduction (Coulon et al., 1996). Forage restriction has been relatively under-researched. Studies have found that 13% and 20% restrictions of DMI decreased daily Energy-Corrected Milk (ECM) output by 2 kg and 3 kg, respectively (Ben Meir et al., 2019; Hervé et al., 2019). However, limiting forage intake often improves digestibility (Zanton and Heinrichs, 2008) and can increase nutrient conversion efficiency for milk yield (Ben Meir et al., 2019). Feed efficiency can also be improved at animal level by targeted feed adaptation for less-efficient cows. According to Fischer et al. (2020), restricting feed quantity (with a diet based on corn silage and concentrate) given to less-efficient cows (a 2.7-kg decrease in DMI) and the most-efficient cows (a 0.8-kg decrease in DMI) had no impact on milk and ECM yields.

Some dairy farmers not only limit the quantity of forage and concentrate but also restrict access to feed to only two rations per day. They argue that restricting both feed quantity and access time ensures all cows are fed the same way, in contrast to *ad libitum* systems where cows can select their preferred feedstuffs, resulting in differences in nutritional intakes and therefore in feed efficiency. Farmers report beneficial effects of feed restriction on milk yield, milk cheesemaking properties and animal nutritional status (Cremilleux et al., 2020). Cavallini et al. (2018) studied a time-limited feeding strategy and found that it increased milk efficiency (ECM kg/DMI kg) without decreasing BW. Manzocchi et al. (2019) compared pasture-based diets without conserved-forage supplements and found that limiting grazing time had no impact on milk yield and milk coagulation properties but decreased milk protein and casein contents. However, as far as we know, there has been no effort to investigate the effects of feed-quantity restriction in tandem with access-time restriction on dairy cow performance and milk cheesemaking ability.

The aim of this experiment was to evaluate the effect of restricting forage quantity versus *ad libitum* feeding and in tandem with restricting access time to feed on dairy performance, milk cheesemaking ability, and animal nutritional status. We posited that limited access time to feed (2 h/meal) could increase feed efficiency compared to a single quantitative restriction (amount of forage

only) due to an increase in digestibility, with the outcome that milk output and animal nutritional status would be only marginally affected under both feeding and access-time restrictions compared to *ad libitum* feeding.

## Material and methods

The experiment was carried out from 6 January to 19 March 2020 at the INRAE's Herbipôle research facility in Marcenat, France (doi.org/10.15454/1.5572318050509348E12; 45°15'N, 2°55').

### Experimental design, animals and diet

The experiment consisted in comparing animal performances and milk quality of three groups of nine cows fed either with i) hay distributed *Ad Libitum* (control group; **AL**), or ii) hay distributed in limited quantities without restricted access time to feed (Quantity-Restricted group; **QR**), or iii) hay distributed in limited quantities and with restricted access time to feed (Quantity-and-Time-Restricted group; **QTR**). The experiment used 16 Holstein and 11 Montbéliarde dairy cows, housed in a pen equipped with freestalls and individual troughs with feeding barriers. During a pre-experimental period of 16 days, all cows were fed the same diet consisting of first-cut hay (7.8% CP, 67.3% NDF, 37.6% ADF, 54.4% Organic Matter (**OM**) digestibility) provided *ad libitum* plus 3.9 kg DM/day of second-cut hay (9.8% CP, 58.5% NDF, 32.1% ADF, 61.8% OM digestibility), 4.5 kg DM/day of a commercial concentrate (17.9% CP, 23.4% NDF, 10.1% ADF, 80.2% OM digestibility) and 200 g of commercial minerals (Galaphos® Axion Repo, Deltavit, Janzé, France). At the end of the pre-experimental period, the cows were randomly allocated into three groups balanced by breed, parity, days in milk and milk yield (Table 1). Then, for a 9-week experimental period, AL cows were fed the first-cut hay provided *ad libitum* whereas QR cows were fed up to 10.5 kg DM/day of the first-cut hay and QTR cows were fed up to 10.5 kg DM/day of first-cut hay but with access to the whole diet restricted to 0800 h–1000 h and 1600 h–1800 h while tied up and without access to the trough between these meals. All cows from all three groups were also provided 3.9 kg DM/d of second-cut hay and 4.5 kg DM/day of commercial concentrate (Table 1). Second-cut hay was distributed 20 min after first-cut hay, and concentrates and minerals were distributed 10 min after the second-cut hay. Forages, concentrates and minerals were distributed half between 0800 and 0830 h and half between 1600 and 1630 h, for AL and QTR cows. QR cows received two-third of both forages between 0800 and 0830 h and the remaining one-third between 1600 h and 1630 h to enable the cows to eat as much as they wanted during the day. For AL cows, quantity of first-cut hay distributed was readjusted each week to maintain 10% refusals of the whole diet (within 5–15% tolerance bounds). All cows had free access to water and were milked at 0630 h and 1530 h in a 2 × 14 herringbone milking parlour where milk yield was recorded automatically at each milking. Individual daily forage intake was measured on two consecutive days per week as the difference between on-day feed offered and the next morning's refusals, giving a total of 18 days of measurement per animal during the experimental period.

### Sampling and analysis

#### Diet

Every week, samples of offered hay (first-cut and second-cut), refused hay and concentrate were collected, dried and ground through a 1-mm screen. Samples from weeks 1 to 3, 4 to 6 and 7 to 9 were pooled for analysis. Samples were analysed for OM

**Table 1**

Characteristics of the cows and the theoretical diet according to treatment. Treatments consisted in dairy cows fed 3.9 kg DM/day of second-cut hay and 4.5 kg/day of concentrate and 1st cut hay provided *Ad Libitum* (AL) or at 10.5 kg DM/day without (Quantitative Restriction – QR) or with (Quantitative and Time Restriction – QTR) restricted access to feed (two meals of 2 h each). Milk production correspond to the average ( $\pm$  SD) observed during the pre-experimental period.

Item	AL		QR		QTR	
Breed	6 Holstein 3 Montbéliardes		5 Holstein 4 Montbéliardes		5 Holstein 4 Montbéliardes	
Parity	6 multiparous 3 primiparous		6 multiparous 3 primiparous		6 multiparous 3 primiparous	
Days in milk (days)	82 $\pm$ 26		100 $\pm$ 42		88 $\pm$ 27	
Milk production (kg/day)	21.1 $\pm$ 3.6		21.2 $\pm$ 6.7		21.0 $\pm$ 4.3	
	Morning	Evening	Morning	Evening	Morning	Evening
1st cut (kg; DM; animal $\times$ day)	<i>Ad libitum</i>		<i>Ad libitum</i>		<i>Ad libitum</i>	
2nd cut (kg; DM; animal $\times$ day)	2.0	1.9	7.0	3.5	5.3	5.2
Concentrate (kg; DM; animal $\times$ day)	2.25	2.25	2.0	1.9	2.0	1.9
Access	Permanent access to feeder		2.25	2.25	2.25	2.25
			Permanent access to feeder		Access to feeder 2 h in the morning and 2 h in the evening, Animals tied up	

determination by calculating the difference between DM and ash obtained by oven-drying at 550 °C for 6 h (EN ISO 5984:2014). Nitrogen was determined using the Dumas Method by combustion (EN ISO 16334-1:2018) with an Elementar Rapid N Cube model 194 (Elementar France, Lyon, France). The resulting nitrogen value was multiplied by 6.25 to calculate CP content. NDF and ADF contents were analysed using the Van Soest method (Van Soest et al., 1991; EN ISO 16472:2006). Nutritive values of the feedstuff and the diet were calculated according to INRA (2018), where Milk Forage Unit (MFU) is net energy requirements for lactation (NEMilk) produced by 1 kg of reference barley and is equivalent to 7.37 MJ of net energy and digestible protein in the small intestine (PDI).

#### Feeding behaviour

Cow activity patterns (eating, ruminating, resting, and other activities) and posture (standing or lying down) were measured throughout the experiment using a commercial Precision Livestock Service device (Axel Medria® sensor; Medria Farm Technologies, Chateaubourg, France) attached to a collar strapped onto the neck of each animal. The device is a 3-axis accelerometer that automatically monitors animal posture and activity at every 5-min interval and sends the data to a radiofrequency receiver in the barn. Cow activity was determined according to neck and muscle movements detected by the accelerometer. The data are made available on a web platform in a table that summarises the different types of activities detected by Medria's algorithms, with a 0 for activity not done or a 1 for activity registered. Only one activity could be different from 0 at any given time, and posture is added to this activity (as a cow can be at rest or ruminate either standing or lying). The data were averaged to have times of feed intake, rumination, and rest per hour (min/h) per week. The data were also averaged by combining activity and posture (rest-lying, rest-standing, eating-lying, eating-standing, ruminating-lying, ruminating-standing). Eating, ruminating, resting and rest-lying are the only data reported. The average pattern of activity during the day was reported graphically per group.

#### Evaluation of feed nutrient utilisation from faecal samples

Faecal grab samples ( $\approx$ 500 g) were collected from the rectum of each animal twice a week (one evening and one morning sample) on weeks 3, 6 and 9. A 250 g aliquot was wet-sieved to evaluate faecal particle size, and the other 250 g aliquot was used to evaluate OM digestibility based on chemical composition of the faeces.

Wet-sieving of faecal samples started immediately after taking the first sample. Each sample was wet-sieved in duplicate using the Nasco Digestion Analyzer kit (Nasco®), which included three stainless-steel screens (top, 4.76 mm; middle, 3.17 mm; bottom,

1.59 mm). The faeces were transferred to the top of the Nasco Digestion Analyzer sieve set that was positioned in the bucket. Wet-sieving was performed until water easily passed through the top sieve, using the same water flow for all samples. After visual inspection to check the absence of small particles, the top sieve was detached from the sieve set. The same procedure was used for middle and bottom. Retained particulates from each sieve were then dried at 80 °C for 48–72 h, and weighed.

DM content was determined by oven-drying at 80 °C for 48–72 h, and each faecal sample was ground through a 1-mm screen. Then, the two samples from each cow in the same week were pooled and analysed for OM determination, following the same method as for forage. OM digestibility was estimated from the nitrogen and ADF content of ground-and-pooled faecal samples and the nitrogen content of hays ingested, according to Peyraud (1998), as follows:

Organic Matter digestibility (g/gOM)

$$= 1.030 - 2.478 / (\text{faeces CP}) - 0.0072 / (\text{faeces ADF}) - 0.0571 * (\text{hay CP}) / (\text{faeces CP}).$$

#### Milk sampling and analysis of chemical and coagulation properties

Milk samples were collected on four consecutive milkings per week during the pre-experimental and experimental periods. All samples were preserved with 2-bromo-2-nitropropane-1,3-diol (Bronopol; D&F Inc., Dublin, CA) at 4 °C until analysis. Fat and protein contents were determined by mid-IR spectroscopy (Agrolab's, Aurillac, France) as per standard ISO 9622:2013. Somatic Cell Count was determined by epifluorescence (Agrolab's) as per standard ISO 13366-2:2006.

On weeks 0 (last week of the pre-experiment period), 3, 6 and 9, urea, lactose and casein contents were determined by mid-IR spectroscopy (Agrolab's) as per standard ISO 9622:2013, and a 30-ml aliquot was taken and stored at –20 °C for the analysis of milk coagulation properties. This sample was thawed overnight at 4 °C and mixed for 15 min in a rotary shaker. Milk coagulation properties were determined in duplicate using a milk coagulation meter (Maspres, Florence, Italy and Foss Italia, Padua, Italy) according to Menci et al. (2021). The milk coagulation meter measures coagulation based on the movement of small loop pendulums that are immersed in linearly oscillating samples of coagulating milk (Bittante, 2011). The following rennet parameters were determined: clotting time, i.e. time (in min) from rennet addition to the beginning of coagulation; firming time, i.e. time (in min) from coagulation to reach the amplitude of 20 mm; curd firmness; amplitude of the graphical trace at 30 min, 60 min and two times

clotting time, respectively, after rennet addition. Milk samples that did not coagulate within 60 min were classified as non-coagulating.

A last aliquot of 20 ml was taken to perform a lactofermentation test (Bérodier et al., 2001). All the stoppered tubes, each containing 20 ml of fresh milk, were placed in a water bath at 37 °C for 24 h, and the appearance of the coagulum thus obtained was visually evaluated as either 'gelled', 'flaky', 'digested', or 'caseous'. Milk pH was measured before and after being placed in water bath using an electrode (InLab Expert, Mettler Toledo, Switzerland) placed in the milk.

#### Body condition score and body weight

All cows were scored for body condition at weeks 0, 3, 6 and 9 by two skilled assessors using the scale developed by Bazin (1984), which ranges from 0 for an emaciated cow up to 5 for a fat cow, in 0.25-unit increments. At weeks 0, 3, 6 and 9, all cows were weighed on a balance (Delaval France, Elancourt, France) at 0200 h the weight was corrected based on the average DMI of the same week, using the formula  $\text{WeighCor (kg)} = \text{Weight (kg)} - 4 \times \text{DMI (kg/d)}$  (Chilliard et al., 1987), in order to correct the weight from the variation in digestive content.

#### Blood metabolites

On weeks 0, 3, 6 and 9, just after morning milking, individual blood samples were taken from the tail vein into vacuum EDTA-containing tubes (Terumo France, Guyancourt, France) and then immediately centrifuged at 1 200g for 20 min at 4 °C. Blood plasma was then stored at -20 °C until analysis on a chemistry analyser (Arena 20 XT Chemistry System, Thermo Scientific, Waltham, MA) to determine the concentration of non-esterified fatty acids (NEFA-HR2 kit, Fujifilm WAKO; Arena 20 XT Chemistry System; Thermo Scientific, Waltham, MA), beta-hydroxybutyrate (product code 984325; Thermo Scientific), glucose (product code 981379; Thermo Scientific), and urea (product code 981818; Thermo Scientific).

#### Data analysis

##### Estimation of feed efficiency

The following parameters were calculated to evaluate feed efficiency: Milk (kg)/DMI (kg); Milk (kg)/MFU ingested; ECM (kg)/DMI (kg); ECM (kg)/MFU (NEMilk).

ECM was calculated according to the following equation:

$$\text{ECM (kg/d)} = [\text{MilkProd} \times [0.42 + 0.0053 \times (\text{MFC} - 40) + 0.0033 \times (\text{MPC} - 31)]] / 0.42 \text{ (INRA, 2018)}.$$

where MilkProd is milk production (output) in kg per day, MFC is Milk Fat Content in grams per kg, and MPC is Milk Protein Concentration in grams per kg.

##### Evaluation of energy balance

Energy balance of each group was calculated according to INRA (2018) based on individual needs (maintenance, lactation, reproduction, growth) and energy intake.

##### Economic evaluation for field application

To analyse the economic impact of the implementation of QR and QTR practices, we determined the forage price threshold at which the savings on forage compensate for the losses due to altered milk composition. For this purpose, we used the *Sodiaal year-2019* milk payment grid (Sodiaal is one of the biggest French dairy cooperatives), in which milk price decreases by €5.0 and €2.7 per 1 000 l of milk for each g/kg of protein and fat lost, respectively.

The price scale for fodder corresponds to the prices commonly encountered in France based on market-price records in a year of standard climate (€80–110/T) or in a year of forage shortage (€110–160/T). The economic gain due to the decrease in forage distributed during 60 days and the economic loss due to the decrease in fat and protein levels in milk were calculated for one QR and one QTR cow for a 60-day period. The difference between this economic gain of forage and economic loss of milk then served to quantify total gain or loss according to forage price.

#### Statistical analyses

In order to analyse the effect of restricted forage quantity and restricted access time, all data were averaged by week. The first two pre-experiment weeks, used for progressive adjustment to the diet, were not included in the statistical analysis. The Somatic Cell Count data were log-transformed to achieve a normal distribution. Per-treatment averages for coagulation properties were computed with non-coagulating samples treated as missing values according to Koczura et al., 2019, and concerned 12 %, 8 % and 10 % of AL, QR and QTR data, respectively.

All data (feeding activity, OM digestibility, sieved faecal particle size, milk yield and composition, BW, body condition score, blood metabolites, feed efficiency) except for data from the lactofermentation test were analysed with a mixed model in SAS (SAS version 3.8, SAS Institute Inc., Cary, NC). The model used included parity (primiparous, multiparous), treatment (AL, QR, TR), experimental week (3–9 or 3, 6 and 9 according to the traits considered), and the treatment  $\times$  parity, treatment  $\times$  week, and treatment  $\times$  parity  $\times$  week interactions were considered as fixed effects. The repeated factor was the week, and the subject (random factor) was the individual cow. To obtain the best-fit models for each variable, fixed factors (other than treatment) and interactions with  $P > 0.15$  were discarded using manual backward stepwise selection. Days in milk and the response variable during the pre-experimental period, centred to parity, were used as covariates. The covariance structure was first-order autoregressive for heterogeneous variances. The threshold for statistical significance was set at  $P < 0.05$ . Normality of the data and residuals were checked using the Shapiro–Wilk Test. For all data, in order to compare the AL cows against the two restricted groups (QR and QTR) and compare QR cows against QTR cows, we established the orthogonal contrasts 2; -1; -1 and 0; -1; 1, respectively. Curd features following the lactofermentation test were analysed with a  $\kappa^2$  test of independence in R (R Core Team, 2018) using the `chisq.test` function.

## Results

### Intake, feed behaviour, and digestibility

As expected, QR and QTR cows ingested significantly less first-cut hay than AL cows and their DMI decreased by 13 % (2.5 kg of DM/d) and 17.0 % (3.2 kg of DM/d), respectively (Table 2). This resulted in lower intakes of Net Energy (NEMilk) and PDI for QR and QTR cows compared to AL cows and for QTR cows compared to QR cows. Energy density of ingested diets were 0.65, 0.69, 0.68 MFU/kgDMI, for AL, QR and QTR cows, respectively, and forage:concentrate ratios were 77:23, 74:26, 73:27 for AL, QR and QTR cows, respectively.

AL cows spent more time ingesting and ruminating than QR cows (+33 min/d, and +17 min/d, respectively) and QTR cows (+78 min/d and +59 min/d, respectively), to the detriment of time spent resting and lying down which was lower for AL cows than for QR and QTR cows (-52 and -86 min/d, respectively). On a DMI basis, QR and QTR cows also showed increased rumination time

**Table 2**

Dairy cow intake, daily activity, digestibility of organic matter and faeces sieving according to treatment, parity (primiparous and multiparous) and experimental week (3–9). Treatments consisted in dairy cows fed 3.9 kg DM/day of second-cut hay and 4.5 kg/day of concentrate and 1st cut hay provided *Ad Libitum* (AL) or at 10.5 kg DM/day without (Quantitative Restriction – QR) or with (Quantitative and Time Restriction – QTR) restricted access to feed (two meals of 2 h each).

Item	Treatment, LSM			Parity		SEM	P-values				
	AL	QR	QTR	Primi.	Multi.		AL vs (QR&QTR)	QR vs QTR	Parity (P)	Week (W)	T × W
<b>Intake</b>											
1st cut ingested (kg/d)	11.4	9.0	8.3	8.5	10.6	0.397	<0.001	0.169	<0.001	0.017	
DMI (kg/d)	19.2	16.7	16.0	16.2	18.4	0.401	<0.001	0.169	<0.001	0.017	
NEMilk <sup>1</sup> (MFU/d)	12.5	11.3	10.8	11.0	12.0	0.178	<0.001	0.036	<0.001	0.613	0.063
PDI <sup>2</sup> (g/d)	1 928	1 699	1 616	1 667	1 828	28.83	<0.001	0.040	<0.001	0.566	0.054
<b>Animal activity</b>											
Rumination (min/d)	554	537	495	522	535	8.78	0.001	0.001	0.219	0.052	
Ingestion (min/d)	288	255	210	282	220	21.2	0.035	0.110	0.021	0.009	
Rest-Lying (min/d)	203	255	289	240	258	10.7	<0.001	0.017	0.120	0.403	0.033
Rumination/DMI (min/kg)	1.23	1.34	1.30	1.35	1.23	0.030	0.011	0.265	0.003	<0.001	
Ingestion/DMI (min/kg)	15.6	15.4	13.3	17.3	12.1	1.186	0.375	0.170	0.001	<0.001	
Digestibility of OM	0.668	0.683	0.680	0.678	0.676	0.004	0.008	0.621	0.760	<0.001	
<b>Sieving</b>											
Coarse (%)	19.3	22.1	22.4	21.6	21.0	2.485	0.337	0.929	0.836	0.016	
Medium (%)	30.7	28.8	29.2	27.6	31.6	2.122	0.521	0.889	0.124	0.009	
Fine (%)	50.0	49.0	48.5	50.9	47.4	2.51	0.672	0.877	0.245	0.072	

Abbreviations: LSM = least square means; Primi. = primiparous; Multi. = Multiparous; T = Time; DMI = DM Intake.

<sup>1</sup> NEMilk = net energy for milk production. One MFU (Milk forage Unit) is the net energy for milk production brought by 1 kg of reference barley and is equivalent to 7.37 MJ of net energy (INRA, 2018).

<sup>2</sup> PDI = digestible protein in the small intestine.

compared to AL cows, but the time spent ingesting was not significantly different. Compared to QR cows, QTR cows spent significantly less time ruminating and more time resting.

Quantity-and-Time-Restricted group cows spent on average more time ingesting during the two daily meals (from 0800 h to 1000 h and from 1600 h to 1800 h) than the other groups, but AL cows spent on average more time ingesting outside of the meal times, especially in the evening from 2100 h to midnight (Fig. 1a). AL cows ruminated on average more after the second meal of the day (from 1600 h to 1800 h) and during the night (from 0100 h to 0400 h) than QR and QTR cows, whereas QTR cows had the lowest average rumination time, especially during the night (Fig. 1b). QTR cows rested on average more than AL and QR cows, particularly after the first daily meal (from 1000 h to 1300 h) and during the night (from 2100 h to 0400 h) (Fig. 1c).

Organic matter digestibility estimated from faecal samples was significantly lower for AL cows than QR cows (–2.2%) and QTR cows (–1.8%; Table 3), and there were no significant differences in the proportion of coarse, medium and fine fibres contained in the faeces (Table 2).

#### Milk yield, composition, and coagulation properties

AL cows tended to have a higher daily milk yield than QR cows (+0.5 kg) and QTR (+1.2 kg) cows ( $P = 0.081$ ), whereas AL cows also had significantly higher ECM, protein and fat yields than QR cows (+1.3 kg/d, +31 g/d and +81 g/d, respectively) and QTR cows (+1.0 kg/d, +53 g/d and +80 g/d, respectively) (Table 4). AL cows had significantly higher milk fat, protein, casein and lactose contents and a significantly higher casein-to-whey protein ratio than QR cows (+3.7 g/kg, +2.4 g/kg, +0.8 g/kg, +0.0 g/kg and +0.012, respectively) and QTR cows (+3.2 g/kg, +1.4 g/kg, +1.7 g/kg, +2.6 g/kg and +0.013, respectively) (Table 3).

Energy-corrected milk, protein and fat yield were similar between QR and QTR cows, but QTR cows had lower lactose and casein contents than QR cows. There were no significant differences in Somatic Cell Count and milk urea between AL vs QR and QTR cows and between QR vs QTR cows.

Concerning significant interactions between treatments and the other effects included in the model, the difference in milk lactose

content between QTR cows and the other groups was higher in week 6 than in weeks 3 and 9. Primiparous cows had similar casein contents whatever the treatments, except in weeks 6 and 8 where AL cows had a higher casein content than QTR and QR cows. Casein-to-whey protein ratio was significantly different between the three groups in weeks 3 and 6, whereas there was no significant difference in week 9 (Table 3).

There were no significant between-treatment differences in curd features ( $P = 0.125$ ; Table 4), variables describing milk coagulation, and initial pH and pH change during the lactofermentation test. Curd firmness at 60 min after rennet addition tended to be higher for AL cows compared to QTR and QR cows.

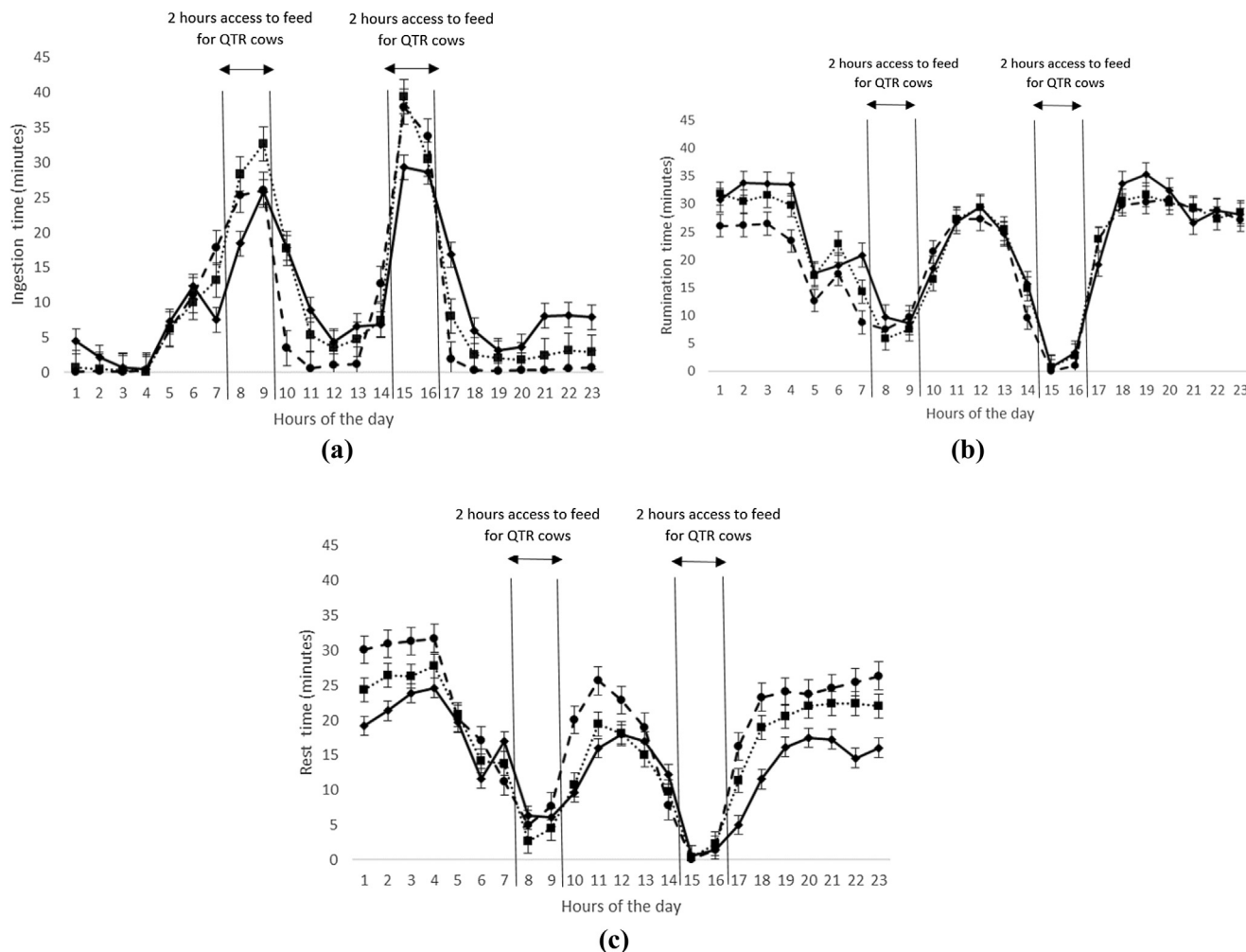
#### Body weight, body condition score, blood metabolites, and energy balance

*Ad Libitum* cows had a significantly higher final BW (636 kg) and body condition score (1.73 points) than QR cows (+20 kg and +0.07 points, respectively) and QTR cows (+18 kg and +0.29 points, respectively), whereas the final corrected BW was similar between AL vs QR and QTR cows and between QR vs QTR cows (Table 5). QTR cows had a lower final body condition score than QR cows and were the only cows that lost body condition from week 0 to week 9, but without impact on corrected BW (Table 5).

Energy balance was more negative for QR and QTR cows than for AL cows. Blood non-esterified fatty acid concentration tended ( $P = 0.052$ ) to be lower for AL cows (0.121 mmol/l) compared to QR and QTR cows (0.156 and 0.211 mmol/l, respectively; Table 5). QTR cows had a higher blood beta-hydroxybutyrate concentration than QR cows (+0.086 mmol/l). There were no significant between-treatment differences in blood glucose and urea concentration.

#### Feed efficiency

*Ad Libitum* cows had a lower milk yield by net energy intake (MFU/d) and tended to have a lower milk/DMI ratio than QR cows (+0.068 kg/DMI) and QTR cows (+0.078 kg/DMI) ( $P = 0.074$ ). ECM by kg of DMI or by net energy intake did not differ significantly according to treatments (Table 5).



**Fig. 1.** Daily ingestion time (a), rumination time (b), rest time (c) per hour in the *Ad libitum* group (—●—), Quantity-restricted group (···■···, 10.5 kg DM/day), Quantity-and time-restricted group (—●—, 10.5 kg DM/day with restricted access to feed – two meals of 2 h each). Data presented correspond to the average per hour (±SE) from week 2 to week 9 of the activities of each group of cows. Abbreviations: AL = *ad libitum* group; QR = quantity-restricted group; QTR = quantity-and time-restricted group.

*Economic aspects for field applications*

During a period of 2 months, QR and QTR practices saved 234 kg DM of forage by cow (on an offered quantity basis) compared to AL cows. However, the milk price was reduced by 19.1 and 13.9 €/cow for QR and QTR practices, respectively, due to the decrease in fat and protein content (Table 6). Therefore, QR would become economically profitable from a forage price of €85/T whereas QTR would be economically profitable whatever the hay price within its range of variation in France (Table 6).

**Discussion**

*Effect of hay supply restriction on milk performance and nutritional status*

*Milk yield*

Feed restriction (QR and QTR cows) tended to decrease milk yield, but not significantly, thus confirming our hypothesis. Recent studies reported that similar (–13%) or slightly higher (–20%) decreases in DMI (corn silage, soybean meal, and concentrates) significantly reduced milk yield (by 8% and 9%, respectively; Ben Meir et al., 2019; Hervé et al., 2019). Here, the absence of impact of feed restriction can be explained by several factors. First, OM

digestibility of the diet, which is the main component of net energy, was improved when feed intake was reduced. This is in line with INRA feed recommendations (2018), which state that each percentage-point decrease in DMI (per kg of BW) increases OM digestibility by 2.74%. Similarly, Santana et al. (2019) found that a 51% restriction of DMI increased DM digestibility by 13.76%. However, as other studies (Cavallini et al., 2018; Ben Meir et al., 2019) did not find any effect of feed restriction on digestibility, the evidence suggests that DMI would have to be decreased by at least 20% to have an effect on digestibility, which was not the case in our study. Furthermore, even if diet-restricted cows ruminated less than AL cows, their rumination time increased when adjusted on a DMI basis. This higher rumination per kg DMI was observed even when the forage-to-concentrate ratio was decreased in the feed-restricted diets, as forage intake was decreased while concentrate offered remained constant. This may partly explain the higher OM digestibility observed for feed-restricted cows. Indeed, when the fractional passage rate out of the rumen decreases, it leads to an increased mean retention time of feed in the rumen (INRA, 2018).

Second, feed-restricted groups spent less time ingesting. They did not increase the intake rate but spent more time at rest than AL cows. As each additional hour of rest lying down could result in an increase of 1 kg of milk per cow per day (Grant, 2003), the increasing resting time of feed-restricted cows could also explain

**Table 3**

Milk yield and milk composition, coagulation properties and lactofermentation according to treatment, parity (primiparous and multiparous) and experimental week (3–9). Treatments consisted in dairy cows fed 1st cut hay provided *Ad Libitum* (AL) or at 10.5 kg DM/day without (Quantitative Restriction – QR) or with (Quantitative and Time Restriction – QTR) restricted access to feed (two meals of 2 h each).

Item	Treatment, LSM			Parity		SEM	P-values					
	AL	QR	QTR	Primi.	Multi.		AL vs (QR&QTR)	QR vs QTR	Parity (P)	Week (W)	T × W	T × P × W
Milk production												
Milk yield (kg/d)	17.3	16.8	16.1	15.6	17.9	0.372	0.081	0.202	<0.001	<0.001	0.139	
ECM <sup>1</sup> (kg/d)	15.8	14.5	14.8	14.4	15.7	0.385	0.017	0.673	0.007	<0.001		
Fat (g/kg)	36.0	32.3	32.8	34.7	32.7	0.864	0.002	0.663	0.058	<0.001		
Fat yield (g/d)	618	538	537	545	583	16.5	<0.001	0.969	0.050	<0.001		
Protein (g/kg)	29.9	27.5	28.5	29.5	27.8	0.498	0.003	0.169	0.004	<0.001		
Protein yield (g/d)	507	466	454	456	495	11.7	0.002	0.480	0.007	<0.001	0.071	
Urea (mg/kg)	237	230	220	248	209	8.22	0.228	0.432	<0.001	<0.001		
Lactose (g/kg)	48.2	48.2	45.6	47.8	46.8	0.364	0.005	<0.001	0.028	<0.001	0.036	
Casein (g/kg)	27.2	26.4	25.5	27.2	25.4	0.227	0.001	0.033	<0.001	<0.001	0.055	0.024
Casein: protein ratio	0.919	0.907	0.906	0.914	0.907	0.006	0.034	0.867	0.204	<0.001	0.004	
SCC (log <sub>10</sub> /ml)	4.88	5.00	5.00	4.90	5.02	0.094	0.282	0.984	0.233	0.004		
Coagulation properties												
Rennet coagulation time, min	15.1	15.2	14.6	13.2	16.8	0.870	0.861	0.622	0.001	0.141		
Firming time, min	10.4	11.7	8.23	6.98	13.2	1.66	0.829	0.161	0.007	0.426		
A30 <sup>2</sup> (mm)	27.2	24.6	26.4	30.9	21.2	2.00	0.482	0.529	<0.001	0.666		
A2R <sup>3</sup> (mm)	28.2	25.6	25.9	29.3	23.7	1.32	0.140	0.894	0.001	0.578		
A60 <sup>4</sup> (mm)	32.5	28.8	28.5	33.5	26.4	1.58	0.059	0.862	<0.001	0.495		
Lactofermentation												
pH initial	6.65	6.65	6.64	6.62	6.68	0.010	0.593	0.306	<0.001	0.421	0.502	0.083
Δ pH (after – before lactofermentation)	2.08	2.07	2.15	2.10	2.10	0.052	0.623	0.344	0.979	<0.001		

Abbreviations: LSM = least square means; Primi. = primiparous; Multi. = Multiparous; T = Time; SCC = Somatic Cell Count.

<sup>1</sup> ECM = Energy-Corrected Milk (kg/d); ECM = NEMilk/0.44 with NEMilk (MFU/d) = MilkProd × [0.42 + 0.0053 × (Milk Fat Concentration – 40) + 0.0033 × (Milk Protein Concentration – 31)].

<sup>2</sup> Curd firmness A30 = amplitude of the trace at 30 min, after rennet addition.

<sup>3</sup> Curd firmness A2R = amplitude of the trace at two times R, after rennet addition.

<sup>4</sup> Curd firmness A60 = amplitude of the trace at 60 min, after rennet addition.

**Table 4**

Contingency table presenting the appearance of the curds after the lactofermentation test of 24 h for the three treatments. Treatments consisted in dairy cows fed 1st cut hay provided *Ad Libitum* (AL) or at 10.5 kg DM/day without (Quantitative Restriction – QR) or with (Quantitative and Time Restriction – QTR) restricted access to feed (two meals of 2 h each).

Item	Gelled	Flaky	Digested	Caseous	Total number of observation	P-value
AL	30	7	16	1	54	0.125
QR	23	19	12	0	54	
QTR	22	15	15	2	54	

the non-significant difference in milk production. Indeed, resting lying down has benefits for welfare and health (less stress on the hoof and less lameness, less fatigue stress) and potentially affords greater milk synthesis due to greater blood flow through the udder (Grant, 2003).

Surprisingly, even though milk yield did not decrease significantly under DMI restriction, we found only a trend on feed efficiency. Milk yield by net energy intake (MFU/d) was the only factor that was improved under DMI restriction. This runs contrary to our hypothesis and to reports from other studies (Cavallini et al., 2018; Ben Meir et al., 2019) where a DMI restriction of between 7.6 % and 12.8 % improved ECM/DMI as ECM was only marginally affected (–3.4 % and –5.4 %, respectively). Here, even though feed restriction did not significantly decrease milk yield, it decreased milk fat and protein synthesis, which led to a higher reduction of ECM for QR and QTR cows compared to AL cows (–8% and –7%, respectively). These discrepancies could be explained by the law of diminishing returns: the more the cows are overfed (in relation to their needs), the less the feed restriction affects their milk production (Zanton and Heinrichs, 2008). In this experiment, cows were not fed to the same level as in Cavallini et al. and Ben Meir et al., where cows ingested between 23 and 30 kg/cow/d of an energy-dense diet. Here, intakes were much lower, and the

negative calculated energy balance and low body condition score (2.1 at the beginning of the pre-experimental period) both indicate that the herd was not overfed. Therefore, the feed restriction practised in this study was likely to have a higher impact on milk output, which could explain why feed efficiency was not improved. This practice may therefore have greater value on farms where cows are frequently overfed.

In this experiment, feed-restricted cows also mobilised their body fat reserves, as evidenced by their lower final body condition score and BW, but without impacting corrected BW, which was in line with the blood non-esterified fatty acid concentration that tended to be lower for fed-restricted cows. There appeared to be less mobilisation of body fat reserves here than in Hervé et al. (2019) where a 20 % DMI restriction resulted in a 5 % BW loss. Nevertheless, the consequence of this variation in body condition score and blood non-esterified fatty acid concentration should be studied over a longer time window to verify a possible long-term effect on reproduction and health that could not be evaluated in this study. Moreover, even if the proportion of forage was still high in the QR and QTR diets (74 and 73 % respectively), reducing the dietary proportion of fodder can lead to health problems, as shown in studies including a higher proportion of concentrates (75 % after forage restriction) (Cavallini et al., 2021). The reduction of physically



**Table 5**

Body weight and Body Condition Score, energetic balance, blood metabolites and feed efficiency according to treatment, parity (primiparous and multiparous) and experimental week (3–9). Treatments consisted in dairy cows fed 1st cut hay provided *Ad Libitum* (AL) or at 10.5 kg DM/day without (Quantitative Restriction – QR) or with (Quantitative and Time Restriction – QTR) restricted access to feed (two meals of 2 h each).

Item	Treatment, LSM			Parity		SEM	P-values					
	AL	QR	QTR	Primi.	Multi.		AL vs (QR&QTR)	QR vs QTR	Parity (P)	Week (W)	T × W	T × P × W
<b>BW and BCS</b>												
Final BW	636	616	618	596	650	7.49	0.044	0.810	<0.001			
Final corrected BW	601	584	588	565	616	7.9	0.124	0.721	<0.001			
Final BCS	1.73	1.66	1.44	1.68	1.54	0.004	0.044	0.034	0.128			
Δ BCS (Week 9 – Week 0)	0.09	0.02	–0.194	0.042	–0.094	0.070	0.044	0.034	0.128			
Energy balance, MFU	–0.91	–2.06	–1.78	–1.61	–1.56	0.203	<0.001	0.310	0.820	<0.001	0.085	
<b>Blood metabolites</b>												
NEFA (mmol/l)	0.121	0.156	0.211	0.169	0.156	0.027	0.052	0.156	0.644	<0.001		
BHB (mmol/l)	0.500	0.514	0.428	0.454	0.507	0.019	0.206	0.004	0.014	0.023	0.829	0.114
Glucose (g/l)	0.555	0.540	0.540	0.485	0.604	0.017	0.449	0.941	<0.001	0.542		
Urea (g/l)	0.161	0.148	0.138	0.160	0.139	0.010	0.182	0.459	0.097	<0.001		
<b>Feed efficiency</b>												
Milk (kg / kg DMI)	0.922	0.990	1.00	0.968	0.978	0.034	0.074	0.742	0.801	<0.001	0.535	0.087
Milk (kg / MFU ingested)	1.39	1.48	1.49	1.41	1.49	0.040	0.046	0.811	0.085	<0.001	0.003	
ECM <sup>1</sup> (kg/ kg DMI)	0.804	0.826	0.859	0.824	0.817	0.028	0.268	0.408	0.399	<0.001	0.867	0.029
ECM (kg/UF) (NEMilk)	1.20	1.23	1.27	1.24	1.24	0.033	0.148	0.374	0.957	<0.001		

Abbreviations: LSM = least square means; Primi. = primiparous; Multi. = Multiparous; T = Time; BCS = Body Condition Score; MFU = Milk Forage Unit; NEFAs = Non-Esterified Fatty Acids; BHB = Beta-hydroxybutyrate.

<sup>1</sup> ECM = Energy-Corrected Milk (kg/d); ECM = NEMilk/0.44 with NEMilk (MFU/d) = MilkProd × [0.42 + 0.0053 × (Milk Fat Concentration – 40) + 0.0033 × (Milk Protein Concentration – 31)].

**Table 6**

Economic impact of the Quantitative Restriction (QR, 10.5 kg DM/day) and the Quantitative Time Restriction (QTR, 10.5 kg DM/day with restricted access to feed – two meals of 2 h each) for one average cow, calculated as a difference with cows fed *Ad Libitum*. Calculations take into account fat and protein content of milk observed during a period of 60 days – according to forage price.

Item	Forage price (€/T of DM)	80€	90€	100€	110€	120€	140€	160€
QR	Economic loss (€) due to lower fat and protein contents in milk – QR practice	19.1	19.1	19.1	19.1	19.1	19.1	19.1
	Economic gain (€) due to the reduction in the quantities of fodder distributed – QR practice	18.7	21.1	23.4	25.7	28.1	32.8	37.4
QTR	Gain/loss compared to the loss on milk rates – QR practice	–0.4€	+2€	+4.3€	+6.6€	+9.0€	+13.7€	+18.3€
	Economic loss (€) due to lower fat and protein contents in milk – QTR practice	13.9	13.9	13.9	13.9	13.9	13.9	13.9
	Economic gain (€) due to the reduction in the quantities of fodder distributed – QTR practice	18.7	21.1	23.4	25.7	28.1	32.8	37.4
	Gain/loss compared to the loss on milk rates – QTR practice	+4.8€	+7.2€	+9.5€	+11.8€	+14.2€	+18.9€	+23.5€

effective fibre can impair rumen health and lead to subacute ruminal acidosis (Humer et al., 2018) but also homeostasis and inflammatory response (Cavallini et al., 2021).

*Milk composition and coagulation*

Feed-restricted cows decreased their in-milk protein and fat content, in line with other studies (Abdelatty et al., 2017; Hervé et al., 2019). Here, the observed reduction in milk fat content can be explained by the increased proportion of concentrate in the diet (Aguerre et al., 2011) of feed-restricted cows, as all three groups had the same concentrate intake whereas forage intake was reduced in feed-restricted groups. Forage-to-concentrate ratio is an important factor of variation in milk fat content: when forage-to-concentrate decreases, milk fat content is reduced due to the higher starch content of the diet that depresses the synthesis of FA precursors in the rumen (acetate and butyrate) to the benefit of propionate (Vazirigohar et al., 2014). The decreased protein content in milk from feed-restricted cows could be a consequence of their reduced energy intake. The average reduction observed here for feed-restricted cows (–1.6 g/kg of protein) was slightly higher than expected based on the reference provided by Coulon and Remond (1991), who reported that milk protein content is reduced by an average of 0.6 g/kg for each unit decrease in net energy intake. However, in this trial, the milk protein content of the three groups remained low compared to the French average (29.5 g/kg for AL cows here versus a Holstein-breed average of 31.8 g/kg;

Institut de l'élevage, 2021). This is partly explained by a low nutritional value and energy density of the diet, which is common in upland-area livestock systems (Baumont et al., 2012). As expected, casein content was decreased in the milk of feed-restricted cows, but surprisingly the casein-to-whey protein ratio was also decreased. Casein-to-whey protein ratio is known to vary much more according to animal-related factors (mainly genetic variants of β-lactoglobulin and κ-casein and udder health) than dietary factors (Coulon et al., 1998). Nevertheless, the decrease observed here was only modest and needs to be confirmed in further trials with reference-standard analysis of milk casein content instead of infrared predictions.

Surprisingly, despite milk compositional differences, its cheesemaking ability remained similar in the three groups. Concerning milk coagulation ability, curd firmness, which is well known to be positively correlated to milk casein content (Martin and Coulon, 1995), was not reduced in the milk of the feed-restricted cows despite their lower milk casein content. This absence of effect cannot be related to milk Somatic Cell Count, which was similar in the three groups, nor to animal characteristics (lactoprotein genetic variants), as we used pre-experimental data as covariates in the statistical model. This absence of effect could be due to the fact that the differences in milk composition remained minor. Further studies considering the cheese yield and coagulation properties of milk should be undertaken to explore this interesting finding in greater depth. The absence of effect of

feed restriction on rennet coagulation time was less surprising given that the initial milk pH was similar in the three groups. Concerning lactofermentation, this process is partly related to milk microbiota: a milk that produces a gelled curd translates a strong development of lactic bacteria, for example (Bérodier et al., 2001). Milk microbiota is partly influenced by hygiene during milking, environment, and milking equipment (Montel et al., 2014). Considering that all these factors of variation were standardised for all three groups here, it was not really surprising to find no differences in the lactofermentation tests. An in-depth characterisation of the milk microbiota from the three groups would be usefully informative, as some authors recently reported diet-related differences even when the animals were reared and milked in the same conditions (Fréтин et al., 2018).

#### Effect of additional access-time restriction on milk performances

We hypothesised that a limited access time to feed (2 h per meal) could further increase feed efficiency compared to a single quantitative restriction, due to a decrease in transit rate enabling an increase in digestibility, with the outcome that milk production and animal nutritional status would only be marginally affected. Compared to the single quantitative restriction of forage offered, the limitation of access time to feed modified the behaviour of the animals but only marginally reduced their intakes and had no further effect on milk yield (whether expressed as kg/d or ECM/d). This is in line with our hypothesis.

Interestingly, the QTR cows managed to ingest a similar quantity of forage to QR cows but within just 4 h per day, which resulted only in a marginal decreased NEMilk and PDI intakes.

Animals performed the same activity at the same time, whatever the group. For every group, there were two large meals of about 2 h each, and the intake during the other time periods was ultimately relatively limited. Resting and ruminating periods were also unaffected by the time restriction on feeding. Few studies have investigated the restriction of access time to feed, and only 'mild' time restrictions have been tested (19 h vs 24 h access in Cavallini et al., 2018), which is therefore not comparable to the time restriction applied here. This time restriction, with cows fed a diet based on grass hay, steam-flaked corn, and mixed grain, reduced DMI by 10% (-2.49 kg) but did not impact milk yield. Manzocchi et al. (2019) studied part-time grazing with similar time reductions to those implemented here (access to pasture for 4 h after the morning milking and 2 h after the evening milking) and found that the cows had lower milk protein and casein contents but similar milk yield and milk coagulation properties to full-time-grazing cows. However, pasture intakes were not measured.

Here, the fact that access-time restriction had no impact on milk yield can be explained by similar DMI and similar OM digestibility between groups. Even though QR and QTR cows had similar DMI, QR cows spent more time ruminating and less time resting than QTR cows, but these behavioural differences did not impact rumination time by DMI and ingestion time by DMI, which could explain why digestibility was not improved.

Access-time restriction had no significant impact on milk composition other than on lactose and casein content. Even though NEMilk level decreased in QTR cows, protein content surprisingly remained unaffected. Nevertheless, contrary to our hypothesis, this seems to have been achieved through a further mobilisation of body fat reserves instead of increased ration digestibility and feed efficiency. To maintain the same level of production as QR cows, QTR cows had a lower final body condition score and a higher blood beta-hydroxybutyrate concentration in blood. It could thus be suggested that access-time restriction led to a higher mobilisation of cow body reserves in order to produce milk, in line with

Manzocchi et al. (2019) who found that time-restricted cows up-mobilised their lipid reserves. Access-time restriction had no significant impact on feed efficiency and digestibility. The time restriction did not add to the impact of feed restriction under the conditions tested here, i.e. with cows that were not overfed.

Finally, the possible animal welfare issues raised by this practice (animals not fed *ad libitum* and tied up during meals) and the allied work management constraints (having to return to release the animals) may be obstacles to the implementation of this practice that, contrary to our hypothesis, seems to have limited value in terms of animal performances.

#### Economic implications for field application

Farms are increasingly being forced to make forage purchases to cope with climate-event hazards, but it is often difficult to find affordable good-quality forage. Farmers therefore prefer to work with what they have on their farm rather than buying off-farm. This study finds evidence that QTR and QR feed management practices can have a net-positive economic outcome for farms that buy-in forage at prices higher than €60/T (for QTR) and €80/T (for QR) on a DM-basis, which is a very common situation in France as the mean price of hay varied between €80 and €160 per tonne according to the period of the year and prevailing climatic conditions. In a context where climate hazards are recurring increasingly frequently, and with an increase in demand for forage due to these hazards, the forage savings made possible with these management practices offer potentially valuable perspectives for coping with these hazards (in both the short term or long term) instead of buying fodder or readjusting feed. In the event of forage stock shortage, one solution open to farmers would be to compensate for the decrease in forage by using more concentrate. However, as concentrates are the most expensive feed component of the ration, this option would nevertheless penalise the farm's economic results, unless the concentrates can be self-produced.

While quantity-only forage restriction seems economically interesting, it is equally also necessary to account for previous nutritional (body condition score and blood metabolites) aspects and farm organisation factors. If the difference in milk yield is not significant over 2 months but becomes significant over the longer term, then economic implications will need to be reassessed. A longer trial period would also allow a better understanding of the effects of these practices on the nutritional status, health and performance of the animals and on farm system organisation in the longer term.

#### Conclusion

Restricted access time to feed reduced DMI by 16% without significant effect on milk yield and tended to increase feed efficiency due to better forage utilisation (better digestibility). Cows adapted to a restriction of feed quantity by significantly decreasing milk solids synthesis—i.e. ECM, fat, protein, casein and lactose—and by mobilising their body reserves (body condition score, blood non-esterified fatty acids), but they did not lose corrected weight and their milk cheesemaking ability was not impacted. Time restriction did not impact milk composition and coagulation properties other than casein, casein-to-whey protein ratio and lactose which were decreased. Time restriction also increased the mobilisation of body fat reserves. The saving in forage induced by quantity and time restriction appears to be economically interesting when the forage price climbs higher than €60/tonne. Future research should therefore focus on longer-term experiments to better understand the impact of restricted feeding practices on nutritional status, health and reproduction. Moreover, these practices are expected to be

more viable with animals that are usually overfed, as is often the case in dairy farms.

### Ethics approval

The local institutional animal care and use committee and the French Ministry for Higher Education, Research and Innovation (approval number #2019102916176881).

### Data and model availability statement

None of the data was deposited in an official repository. Data supporting the findings of this study can be made available on request.

### Author ORCIDs

**Maeva Cremilleux:** 0000-0002-2380-4314; **Mauro Coppa:** 0000-0003-2073-0599; **Matthieu Bouchon:** 0000-0002-7175-7233; **Luc Delaby:** 0000-0002-9805-4108; **Antonio Natalello:** 0000-0002-9802-2501; **Bruno Martin:** 0000-0003-2501-8306; **Audrey Michaud:** 0000-0002-3666-6678

### Author contributions

**M. Cremilleux:** Formal analysis, Investigation, Data Curation, Visualization, Writing – Original draft, Writing – Review & editing; **M. Coppa:** Methodology, Investigation, Validation, Supervision, Writing – Review & editing; **M. Bouchon:** Resources, Data Curation, Writing – Review & editing; **L. Delaby:** Validation, Writing – Review & editing; **G. Beure:** Conceptualization, Writing – Review & editing; **I. Constant:** Resources, Writing – Review & editing; **A. Natalello:** Resources, Writing – Review & editing; **B. Martin:** Conceptualization, Methodology, Validation, Supervision, Writing – Review & editing; **A. Michaud:** Conceptualization, Methodology, Supervision, Writing – Review & editing, Funding acquisition, Project Administration.

### Declaration of interest

None

### Acknowledgements

The authors thank the staff at the INRAe–Marcevat experimental farm for the technical support during the experiment. We also thank Sandrine Faure and Aline Le Morvan (Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France) for handling laboratory analysis, and the farmer-members of the association ‘Elevateurs Autrement’ for sharing their knowledge, which helped set up of this experiment.

### Financial support statement

This work was supported by the French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25, grant number PRHI-3.2-CRI190627-MICHAUD) and FRE (Federation Recherche Environnement)

### References

Abdelatty, A.M., Iwaniuk, M.E., Garcia, M., Moyes, K.M., Teter, B.B., Delmonte, P., Kadegowda, A.K.G., Tony, M.A., Moha-mad, F.F., Erdman, R.A., 2017. Effect of short-term feed restriction on temporal changes in milk components and

mammary lipogenic gene expression in mid-lactation Holstein dairy cows. *Journal of Dairy Science* 100, 4000–4013. <https://doi.org/10.3168/jds.2016-11130>.

Aguerre, M.J., Wattiaux, M.A., Powell, J.M., Broderick, G.A., Arndt, C., 2011. Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide, and ammonia, lactation performance, and manure excretion. *Journal of Dairy Science* 94, 3081–3093. <https://doi.org/10.3168/jds.2010-4011>.

Baumont, R., Michaud, A., Delaby, L., 2012. Services fourragers des prairies permanentes: production d’herbe et valeur alimentaire pour les ruminants. *Fourrages* 211, 219–228.

Bazin, S., 1984. Grille de notation de l’état d’engraissement des vaches Pie Noires. Institut Technique de l’élevage Bovin, Paris, France.

Ben Meir, Y.A., Nikbachat, M., Portnik, Y., Jacoby, S., Levit, H., Bikel, D., Adin, G., Moallem, U., Miron, J., Mabeesh, S.J., Halachmi, I., 2019. Dietary restriction improved feed efficiency of inefficient lactating cows. *Journal of Dairy Science* 102, 8898–8906. <https://doi.org/10.3168/jds.2019-16321>.

Bérodier, A., Parguel, P., Dasen, A., Duboz, G., Renaud J.P., Billot, M., Berodier, F., Ducret, J.B., Marguet, B., 2001. Validation du test de lactofermentation en filière Comté (1999–2000). Comité Technique du Comté, Poligny, France.

Bittante, G., 2011. Modeling rennet coagulation time and curd firmness of milk. *Journal of Dairy Science* 94, 5821–5832. <https://doi.org/10.3168/jds.2011-4514>.

Cavallini, D., Mammi, L.M.E., Fustini, M., Palmonari, A., Heinrichs, A.J., Formigoni, A., 2018. Effects of ad libitum or restricted access to total mixed ration with supplemental long hay on production, intake, and rumination. *Journal of Dairy Science* 101, 10922–10928. <https://doi.org/10.3168/jds.2018-14770>.

Cavallini, D., Mammi, L.M.E., Buonaiuto, G., Palmonari, A., Valle, E., Formigoni, A., 2021. Immune-metabolic-inflammatory markers in Holstein cows exposed to a nutritional and environmental stressing challenge. *Journal of Animal Physiology and Animal Nutrition* 105, 42–55. <https://doi.org/10.1111/jpn.13607>.

Chilliard, Y., Rémond, B., Agabriel, J., Robelin, J., Vérité, R., 1987. Variations du contenu digestif et des réserves corporelles au cours du cycle gestation-lactation. *Bulletin Technique CRZV Theix* 70, 117–131.

Coulon, J.B., Remond, R., 1991. Variations in milk output and milk protein content in response to the level of energy supply to the dairy cow: a review. *Livestock Production Science* 29, 31–47. [https://doi.org/10.1016/0301-6226\(91\)90118-A](https://doi.org/10.1016/0301-6226(91)90118-A).

Coulon, J.B., d’Hour, P., Garel, J.P., Petit, M., 1996. Long-term effect of level and pattern of winter concentrate allocation in dairy cows. *Annales de Zootechnie* 45, 233–251. <https://hal.archives-ouvertes.fr/hal-00889556/>.

Coulon, J.B., Hurtaud, C., Remond, B., Verite, R., 1998. Factors contributing to variation in the proportion of casein in cows’ milk true protein: A review of recent INRA experiments. *Journal of Dairy Research* 65, 375–387. <https://doi.org/10.1017/S0022029998002866>.

Cremilleux, M., Cayre, P., Rigolot, C., Martin, B., Michaud, A., 2020. Les systèmes d’élevage agroécologiques: une caractérisation pragmatique croisant la zootechnie avec les sciences humaines. *Rencontres Recherches Ruminants* 25, 570–574.

European Commission, 2018. EU Dairy farms report based on 2016 FADN data. Page 185 in Agriculture and Rural Development. E. Commission, ed. European Commission Directorate-General for Agriculture and Rural Development. Retrieved on 19 September 2019 from [https://ec.europa.eu/agriculture/rica/pdf/Dairy\\_report\\_2016.pdf](https://ec.europa.eu/agriculture/rica/pdf/Dairy_report_2016.pdf).

Fischer, A., Edouard, N., Faverdin, P., 2020. Precision feed restriction improves feed and milk efficiencies and reduces methane emissions of less efficient lactating Holstein cows without impairing their performance. *Journal of Dairy Science* 103, 4408–4422. <https://doi.org/10.3168/jds.2019-17654>.

Fréte, M., Martin, B., Rifa, E., Verdier-Metz, I., Pomiès, D., Ferlay, A., Montel, M.C., Delbès, C., 2018. Bacterial community assembly from cow teat skin to ripened cheeses is influenced by grazing system. *Scientific Reports* 8. <https://doi.org/10.1038/s41598-017-18447-y>.

Grant, R.J., 2003. Taking advantage of dairy cow behavior: Cost of ignoring time budgets. Paper presented at the 65th conference for feed manufacturing, 21–23 October 2003, Syracuse, USA.

Hervé, L., Quesnel, H., Véron, M., Portanguen, J., Gross, J.J., Bruckmaier, R.M., Boutinaud, M., 2019. Milk yield loss in response to feed restriction is associated with mammary epithelial cell exfoliation in dairy cows. *Journal of Dairy Science* 102, 2670–2685. <https://doi.org/10.3168/jds.2018-15398>.

Humer, E., Petri, R.M., Aschenbach, J.R., Bradford, B.J., Penner, G.B., Tafaj, M., Südekum, K.H., Zebeli, Q., 2018. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. *Journal of Dairy Science* 101, 872–888. <https://doi.org/10.3168/jds.2017-13191>.

INRA, 2018. *Alimentation des ruminants*. Edition QUAE, Versailles, France. Institut de l’élevage, 2021. *Résultats de contrôle laitier, espèce bovine, France 2020. Collection résultats*, Paris, France.

Koczura, M., Martin, B., Turille, G., De Marchi, M., Kreuzer, M., Berard, J., 2019. Milk composition, but not cheese properties, are impaired the day after transhumance to alpine pastures. *International Dairy Journal* 99. <https://doi.org/10.1016/j.idairyj.2019.104540> 105540.

Lebacqz, T., Baret, P.V., Stilmant, D., 2015. Role of input self-sufficiency in the economic and environmental sustainability of specialised dairy farms. *Animal* 9, 544–552. <https://doi.org/10.1017/S1751731114002845>.

Manzocchi, E., Koczura, M., Coppa, M., Turille, G., Kreuzer, M., Berard, J., 2019. Grazing on upland pastures part-time instead of full-time affects the feeding behavior of dairy cows and has consequences on milk fatty acid profiles. *Animals* 9, 908. <https://doi.org/10.3390/ani9110908>.

- Martin, B., Coulon, J.B., 1995. Milk production and cheese characteristics. 1. Influence of milk production conditions on herd milk clotting ability. *Lait* 75, 61–80.
- Menci, R., Natalello, A., Luciano, G., Priolo, A., Valenti, B., Difalco, A., Rapisarda, T., Caccamo, M., Constant, I., Niderkorn, V., Coppa, M., 2021. Cheese quality from cows given a tannin extract in 2 different grazing seasons. *Journal of Dairy Science* 104, 9543–9555. <https://doi.org/10.3168/jds.2021-20292>.
- Montel, M.C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmasures, N., Berthier, F., 2014. Traditional cheeses: rich and diverse microbiota with associated benefits. *International Journal Food Microbiology* 177, 136–154. <https://doi.org/10.1016/j.ijfoodmicro.2014.02.019>.
- Nrc, 2001. *Nutrient Requirements of Dairy Cattle*. National Academies Press, Washington, DC, USA.
- Peyraud, J.L., 1998. Techniques for measuring fecal flow, digestibility and intake of herbage in grazing ruminants. In: North Wyke, U.K. (Ed.), *Proceedings of the IXth European Intake Workshop*, pp. 39–43.
- R Core Team, 2018. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Sodiaal, 2019. Grille mensuelle de paiement du lait cru de vache-Bassin grand Sud SODIAAL – en vigueur au premier janvier 2019. Retrieved on 4 April 2021 from <https://www.coordinationrurale.fr/wp-content/uploads/2019/01/num%C3%A9risation0005.pdf>
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Vandehaar, M.J., 1998. Efficiency of nutrient use and relationship to profitability on dairy farms. *Journal of Dairy Science* 81, 272–282. [https://doi.org/10.3168/jds.S0022-0302\(98\)75576-6](https://doi.org/10.3168/jds.S0022-0302(98)75576-6).
- Vazirigohar, M., Dehghan-Banadaky, M., Rezayazdi, K., Krizsan, S., Nejati-Javaremi, A., Shingfield, K., 2014. Fat source and dietary forage-to-concentrate ratio influences milk fatty-acid composition in lactating cows. *Animal* 8, 163–174. <https://doi.org/10.1017/S175173111300181X>.
- Zanton, G.L., Heinrichs, A.J., 2008. Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. *Journal of Dairy Science* 91, 3579–3588. <https://doi.org/10.3168/jds.2008-1210>.