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## Eating time of dairy cows: a study focusing on commercial farms

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#### ABSTRACT

The association of daily eating times (ET) of dairy cows, measured by wearable sensors, with diet composition, feeding practices, and performance was investigated. About 800 lactating cows of two breeds (Holstein Friesian - HF and Italian Simmental - IS) reared on 14 commercial farms were considered. Cows were grouped into ET classes (ETC, min/d): ETC-1 < 180; ETC-2 from > 180 to  $\leq$  220; ETC-3 from > 220 to  $\leq$  260; ETC-4 from > 260 to  $\leq$  300; and ETC-5 > 300. Low ETC was positively associated with ether extract, crude protein, nonfibre carbohydrates, and neutral detergent fibre digestibility, and negatively associated with acid detergent lignin and particle size. A higher frequency of feed pushing per day seemed to be able to increase ETC. The relationship between ETC and performance, adjusted for breed, diet composition, days in milk, and parity, showed that cows with the highest ETC were more productive compared to those with shorter ETC (31.9 vs. 27.0 kg/d of milk) without significant changes in milk composition. Moving from short to long ETC, there was a linear reduction in urea, somatic cells, and body condition score. On the contrary, there was a positive association between ETC and time spent ruminating. Considering differences between breeds, HF had a higher milk yield, ruminated 20 min more, and had a higher ET (255 vs. 228 min/d) than IS. The study provides preliminary results for future research to better define the role of ET in the overall feed efficiency and health status of cows.

#### HIGHLIGHTS

- Eating time is linked to the composition of the diet
- Increased frequency of feed pushing lengthens eating time
- The most productive cows have a longer eating time

## Introduction

Maintaining an efficient dairy livestock system is necessary due to the increasing demand for foods of animal origin. Precision dairy farming equipment allows the continuous monitoring and management of individual productivity and health issues (Lovarelli et al. 2020), reducing costs and exploiting the potential of the cows. Changes in behaviours could provide relevant information about the nutrition, reproduction, health, and overall well-being of dairy cows (Benaissa et al. 2019). Nowadays, wearable sensors have been widely tested and validated to automatically assess cow behaviours every day such as feeding time, activity, and other parameters helpful in herd management. Some studies (Borchers et al. 2021; Leso et al. 2021) found substantial correlations between visual observation and sensor-detected feeding behaviour

On the other hand, eating time (ET), which is defined (Beauchemin 2018) as the sole time spent prehending, chewing, and swallowing feed, is occasionally used on commercial farms as a sign of animal conditions. It is strongly affected by feed management, dry matter intake (DMI), diet composition, and environmental conditions and shows a high variability among

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<sup>(</sup>r = 0.85 to 0.93 for eating and r = 0.83 to 0.92 for rumination time, respectively). Rumination time (RT) is widely used in farms as an indicator of animal health (Magrin et al. 2022). As reported in a review by Beauchemin (2018), there is a negative relationship between RT and ruminal acidosis, and other studies have highlighted changes in RT in relation to the oestrous phase or in response to difficult calving (Reith and Hoy 2012; Mammi et al. 2021).

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animals (Grant and Ferraretto 2018; Corazzin et al. 2021).

At an individual level, few studies identified a relationship between performance and feeding behaviour. Johnston and DeVries (2018) found a relationship between ET and milk yield (MY) (+ 1.74 kg/d of MY for every hour of ET increased), as well as RT and MY (+1.26 kg/d of MY for every hour of RT increased). Similarly, in the study of Dado and Allen (1994), higher-producing cows ruminated longer each day with an increase in feed intake. ET and feeding rate have also been studied as factors influencing feed efficiency in cattle (Ben Meir et al. 2019; Romanzin et al. 2021).

Presently, most studies on the feeding behaviour of lactating cows are conducted on research farms with permanent fixed stations, which are very expensive and not suitable for commercial farms. Besides that, these systems allow the gathering of data such as feed intake and feeding time (which differ from eating time and represent meals, which comprise eating bouts interspersed with periods of noneating; Beauchemin 2018). The usage and application of herd monitoring with wearable sensors allow a relatively simple collection of data at the individual level on all sizes and types of livestock farms, both dairy and meat. This study aimed to explore the association of different daily ET of dairy cows, as recorded by wearable sensors, with diet composition, feeding practices, and performance on commercial dairy farms.

## **Materials and methods**

#### Data and samples collection

The study was conducted in May 2022 in 14 dairy farms of the Friuli Venezia Giulia Region (Italy), where dairy cows were equipped with a specific sensor (SenseHub Dairy, Allflex Livestock Intelligence, SCR Engineers Ltd., Netanya, Israel; described by Merenda et al. 2019) able to detect eating, rumination, grazing, resting, and other activity parameters. Dairy cows were kept in a cubicle housing system and milked with an automatic milking system (AMS). Overall, 819 lactating dairy cows were considered, and farms were visited once (within the same month). A questionnaire was created to gather qualitative information about the diet (ingredients and daily amount administered) as well as farm management (e.g. feed administration).

Data on cows' performance (days in milk (DIM), lactation number, MY, milk composition, and somatic cell count (SCC)), and feeding behaviour (ET and RT) were all collected by Lely time-for-cows and Horizon

software (Lely, the management Maassluis, Netherlands) once for each farm, considering the 15 days prior the farm was visited. On the same day the completion of the questionnaire, body condition score (BCS) measurement, and diet sampling were performed. A trained expert evaluated the BCS in all subjects, relying on the 5-point scale method described by Edmonson et al. (1989) with increments of 0.25 points. Partial mixed ration (PMR) samples were collected immediately after feed delivery following the procedure described by Robinson and Meyer (2010). In addition, a sample of the compound feed distributed by the AMS was taken. Samples were labelled and stored frozen at -20 °C until chemical analysis.

#### **Chemical analysis**

The PMR samples were divided into two sub-samples. The first sub-samples were predried at 60°C for 48 h, and then, together with the compound feed samples, were milled through a 1-mm screen (Pulverisette; Fritsch, Idar-Oberstein, Germany). Analysis of residual DM was performed by heating at 105 °C for 3 h (AOAC 2016). Ash was measured by incineration at 550 °C for 2 h (AOAC 2016). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using a fibre analyser (Ankom II Fibre Analyser; Ankom Technology Corporation, Fairport, NY) following the procedure of Van Soest et al. (1991) without correction for residual ash and by applying a pre-treatment of samples with  $\alpha$ -amylase. The N content was determined using the Kjeldahl method (AOAC 2016), and the crude protein (CP) content was calculated as  $N \times 6.25$ . Ether extract (EE) was determined using the Soxhlet method (AOAC 2016). The NDF digestibility (NDFd) was measured by the ANKOM Daisyll Incubator, following the procedure described by the manufacturer (Ankom Technology Corporation, Fairport, NY). Dry feed samples, approx. 0.5 g, were weighed into filter bags (Ankom F57). All samples were contemporarily tested in guadruple in two fermentation runs of 48 h. In each run, the four bags of each sample were distributed in four incubation flasks, which were filled with filtered rumen inoculum (400 mL) and buffer solution (1600 mL), according to Robinson et al. (1999). The rumen liquid of each fermentation run was collected at the slaughterhouse from four cows and pooled. At the end of each incubation, the bags were removed from each bottle, properly rinsed with cold tap water, and then dried for 24 h in a 105°C heater. The bags were weighed and then analysed for their NDF content, as previously reported. Compound feed samples were analysed with the same methods as stated prior for the first sub-sample of PMR.

The second sub-sample of PMR was fractionated according to the procedure of Kononoff et al. (2003) using a particle separator (NASCO®, Pennsylvania State University) composed of three sieves (mesh diameters: 19, 8, and 1.18 mm) and a bottom pan. Samples were spread out on the top 19 mm sieve. The sieve set was shaken five times horizontally in one direction, rotated once, and then shaken five more times. The process was repeated for eight sets of five replications, for a total of forty shakes. Four PMR fractions were then obtained. Each fraction was weighed and combined to form two fractions: a "Short" fraction with particles confined in the bottom pan and a "Long" fraction with particles  $\geq$  1.18 mm in size. The long fraction was then submitted to the NDF analysis to define physical effective NDF (peNDF). The peNDF was calculated by multiplying the NDF content by its physical effectiveness factor, which was defined as the proportion of particles retained by the 1.18 mm sieve according to Cotanch and Grant (2006).

## Data processing and calculations

The questionnaire data was converted to digital form using a spreadsheet (Excel, Microsoft Corp.). Individual data about performance and feeding behaviour taken from farm management software were exported and statistically examined together with questionnaire data. Fat- and protein-corrected milk (FPCM) was calculated using the formula reported by Kok et al. (2016) as follows:

 $\begin{array}{l} \mbox{FPCM } (kg/d) = \mbox{milk } (kg/d) \\ \times \ [0.337 \ + \ 0.116 \ \times \ fat \ content \ (\%) \\ + \ 0.06 \ \times \ protein \ content \ (\%)] \end{array}$ 

The weights of the wet fractions obtained from the particle separator were used to calculate the average particle size using the Particle size spreadsheet made available by the producer (Penn State College of Agricultural Sciences 2022). The nonfibre carbohydrates (NFC) content was calculated as 100 -CP, acid (CP + ash + EE + NDF).The detergent insoluble nitrogen (ADIN) (determined from the protein analysis on the ADF residue), NFC, and NDF contents (in %DM), as well as the NDFd, were used to calculate the truly digestible amounts, according to the equations proposed by NRC (2001). The digestible energy (DE) content at the maintenance level was calculated using Equations 2 - 8a (assuming the fatty acid content equal to EE -1; NRC 2001). This value was then used to determine the DE at production levels by applying a discount factor (Equations 2 -9; NRC 2001). The DE at production levels was used to calculate the net energy for lactation (NE<sub>L</sub>) values for each diet, using the Equations 2-10, 2-11, and 2-12 (NRC 2001).

#### Statistical analysis

Data was analysed using SPSS (ver. 17, SPSS Inc., Illinois) with the exception of Principal Component Analysis (PCA), which was performed with R software (4.1.2 version, R core team). The normality of the data distribution was assessed by the Shapiro-Wilk test. Outlier removal for feeding behaviour traits included daily mean values that were smaller than 5% or larger than 99%, according to Jaeger et al. (2019). In the second step, animals with less than 7 days of available data were deleted. The result was a total of 779 lactating dairy cows, respectively, 540 Italian Simmental (IS) and 239 Holstein Friesian (HF), of whom were considered the last 7 complete days before the date of sampling.

Cows were distributed in five classes of ET length (ETC): ETC-1 < 180 min/d; ETC-2 from > 180 to <220 min/d; ETC-3 from >220 to <260 min/d; ETC-4 from >260 to <300 min/d; and ETC-5 >300 min/d (Grant and Albright 2000). In the statistical models, parity was coded as 1 (primiparous), 2 (multiparous with 2 parities), and 3 (multiparous with more than 2 parities). To explore the relationship between the ETC and the main variables related to diet characteristics, parity, breed, DIM, number of feed distributions, and number of feed pushing per day, PCA was considered. Correlations between variables were assessed using Spearman and Pearson tests when appropriate. In order to avoid redundancy, it was ensured that all variables had a correlation coefficient less than 0.9 (Tabachnick and Fidell 2001). The adequacy of the sample was measured by the Kaiser Meyer Olkin test, and the communalities of the variables were assessed. The multivariate normality was assessed by the Bartlett test of sphericity. After the assumption's assessment, NE<sub>1</sub>, NDF, the number of feed distributions per day, peNDF, and ADF were excluded from PCA analysis. Only the components with eigenvalues greater than 1 were retained in the analysis. PCA was conducted with the PCAmixdata package (Chavent et al. 2014), which allowed for both continuous and categorical variables. In addition, the PCArot function was considered to evaluate the possibility of improving the clarity of the data interpretation (Chavent et al. 2012).

The effect of ETC and breed on MY, milk composition, BCS, and RT was assessed considering ETC and breed as fixed factors, diet and parity as block factors, and DIM as a covariate in a general or generalised linear model when appropriate. A similar model was used to assess the effect of breed on ET. In particular, the breed was considered a fixed factor, diet and parity as block factors, and DIM as a covariate in a general linear model. In all the models considered, multiple testing was conducted using the sequential Bonferroni procedure.

## Results

Descriptive statistics of animal traits, composition of diets, performance, feeding behaviour, and feed management are reported in Table 1. Dairy cows had on

 Table 1. Descriptive characteristics of 779 dairy cows and rations of the farms involved in the study.

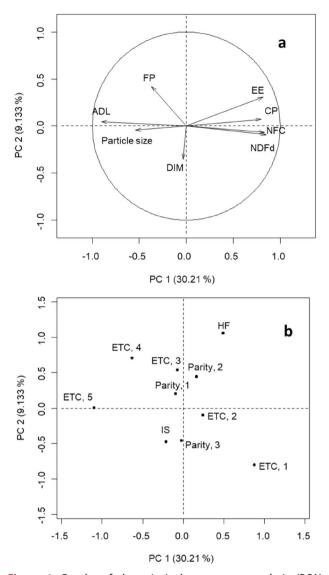
ltem	Total						
item	Mean	SD	Q <sub>25</sub> <sup>1</sup>	Q <sub>75</sub> <sup>1</sup>			
Animal traits							
Parity (n)	2.1	0.9	1	3			
DIM (d)	182	111	102	249			
Diet characteristics							
Particle size (mm)	6.46	2.73	5.14	7.07			
DM (%)	60.48	11.04	56.02	64.25			
Ash (%DM)	7.14	0.76	6.85	7.49			
CP (%DM)	14.98	1.69	13.81	16.84			
EE (%DM)	2.89	0.64	2.39	3.34			
NFC (%DM)	35.40	4.85	32.59	37.90			
NDF (%DM)	39.58	6.16	34.13	43.96			
ADF (%DM)	23.05	4.46	18.51	26.25			
ADL (%DM)	4.18	1.10	3.18	5.26			
NDFd (%NDF)	64.15	6.59	60.27	69.14			
NE <sub>1</sub> (Mcal/kgDM)	1.48	0.17	1.36	1.63			
peNDF (%DM)	40.97	6.71	36.42	43.54			
Performance							
FPCM (kg/d)	29.82	8.55	23.69	35.60			
Milk Yield (kg/d)	30.41	9.11	24.49	36.50			
Milk fat (%)	3.87	0.82	3.35	4.34			
Milk protein (%)	3.42	0.29	3.21	3.61			
Milk lactose (%)	4.80	0.29	4.74	4.97			
Milk urea (mg/dL)	23.09	6.02	18.75	26.80			
SCC (× 1000/mL)	214	515	33	142			
BCS (points)	3.24	0.61	2.75	3.75			
Behaviour							
ET (min/d)	227.6	66.1	179.6	273.4			
RT (min/d)	536.0	58.0	501.1	577.4			
Feed management							
Feed delivery (n/d)	1.50	1.22	1.00	1.00			
Feed pushing (n/d)	11.43	10.01	3.50	24.00			
COf (kg/d)	3.84	1.54	2.70	4.80			

DIM: days in milk; DM: dry matter; CP: crude protein; EE: ether extract; NFC: nonfibre carbohydrates; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; NDFd: neutral detergent fibre degradability; NEL: net energy for lactation; peNDF: physical effective NDF; FPCM: fat– and protein – corrected milk (Kok et al. 2016); SCC: somatic cell count; BCS: body condition score; ET: eating time; RT: rumination time; COf: compound feed offered;  ${}^{1}Q_{25}$ :  $25^{th}$  percentiles.

average  $2.5 \pm 1.5$  lactations,  $183 \pm 111$  DIM. Diets were primarily based on farm-produced forages (corn silage, haylage, hay, and some legumes, and silage accounted for approximately half of the diet (47.57% as fed), with only 14% of farms not using silage in rations. This was also reflected in the particle size data (5.14 to 7.07 mm, Q<sub>25</sub> and Q<sub>75</sub>, respectively), and in DM values (56.02 to 64.25%, Q<sub>25</sub> and Q<sub>75</sub>, respectively). As regards the fibrous component, NDFd ranged from 60.27 to 69.14%NDF (Q<sub>25</sub> and Q<sub>75</sub>, respectively) and peNDF had average values of  $40.97 \pm 6.71\%$ DM. The different composition of the diets led to different energy levels, with 1.36 to 1.63 kcal NE<sub>L</sub>/kg DM (Q<sub>25</sub> and Q<sub>75</sub>, respectively).

The cows had an average production of 30.41 kg of milk per day. Regarding feeding behaviour, the average values of ET and RT were 227.6 and 536.0 min/d, respectively ( $\pm$  66.1 and  $\pm$  58,0 min/d, respectively). Different feeding management practices distinguished the farms. In particular, feed pushing per day showed high variability, with extreme values ranging from 3.5 to 24.0 (Q<sub>25</sub> and Q<sub>75</sub>, respectively), and feed delivery numbers per day (1.50  $\pm$  1.22 on average) showed considerable variability due to various types of conduction using automatic feeding systems or traditional ones.

The PCA of ETC relative to the component map of the numerical variables and of the categorical are shown in Figure 1(a and b), respectively. The first two components explained 39% of the total variance and the ETC was mainly linked to the first principal component (30% of the variance explained) with a squared loading (SL) of 0.44. The ETC-1 and 2 were well discriminated by ETC-3, 4, and 5 on the first principal component, primarily by diet-related characteristics. In particular, the lowest ETC (1 and 2) were positively associated with EE (SL, 0.67), CP (SL, 0.64), NFC (SL, 0.69) and NDFd (SL, 0.72), and negatively associated with ADL (SL, 0.81). Along the second principal component (9% of the variance explained), the lowest ETC (1 and 2) were positively associated with DIM (SL, 0.13) and negatively associated with feed pushing (SL, 0.18). In order to improve the clarity of the relationship between the variables, the simple correlations are shown in Table 3. In agreement with the PCA results, ETC was positively correlated with FP, particle size, and ADL (p < .01) and negatively correlated with parity, CP, EE, NFC, and NDFd (p < .01). In contrast, no significant correlation was found between ETC and DIM (p > .05). Similar results were found with the same variables, but considering the within breed correlations.



**Figure 1.** Results of the principal component analysis (PCA) analysis for the variable related to eating time class. a: component map with factor scores of numerical variables; b: component map with factor scores of levels. (a) FP: number of feed pushing per day; DIM: days in milk; ADL: acid detergent lignin (%DM); EE: ether extract (%DM); CP: crude protein (%DM); NFC: nonfibre carbohydrates (%DM); NDFd: neutral detergent fibre degradability (%NDF). (b) ETC: eating time class. ETC-1  $\leq$  180 min/d; ETC-2 from >180 to  $\leq$ 220 min/d; ETC-3 from >220 to  $\leq$ 260 min/d; ETC-4 from >260 to  $\leq$ 300 min/d; and ETC-5 > 300 min/d. Parity 1: primiparous; Parity 2: multiparous (2 parities); Parity 3: multiparous (>2 parities); HF: Holstein Friesian; IS: Italian Simmental.

Table 2 depicts the results of performance and feeding trait differences among the five ETC. The distribution of cows from the two breeds in different ETC classes revealed a prevalence of IS cows but the frequency of the two breeds was the same across the different ETCs. In fact, the ratio HF/IS cows had a limited range of variation (between 23:77 in ETC-5 to 39:61 in ETC-3), and the statistical analysis (chi-square

test, p > .05, not in table) did not show significant differences in breed ratio across the ET classes. Cows within the highest ETC were the most productive animals compared to those of the shortest ETC (31.91 kg/d vs. 27.04 kg/d of milk, respectively; p < 100 spectral kg/d vs..01). There were no significant differences in milk composition and FPCM increased according to the ET class with a significant linear trend (p < .01). The only difference was given by urea, which was significantly different from ETC-1 with the highest value compared to other ETC (p < .01). Looking at the concentration of SCC in milk, their presence decreases linearly from less to more productive animals (from  $224 \times 1000/mL$  to 118  $\times$  1000/mL; p < .05). Regarding BCS, animals in the lowest ETC had a significantly higher value compared to the highest ETC (3.24 vs. 3.05, respectively; p < .05). On the contrary, animals within the ETC-1 had the lowest RT (520.9 min/d; p < .05).

Considering the differences between the two breeds (Table 2), HF had significantly higher milk production than IS, both in absolute terms (30.60 vs. 28.46 kg/d; p < .01) and as FPCM (29.56 vs. 28.21 kg/d; p < .01) and there were no significant differences in milk composition between the two breeds. The only component that had a significant difference was urea, with half a point in favour of HF (23.17 vs. 22.66 mg/dL, respectively; p < .05). Otherwise, SCC was significantly and strongly higher for HF compared to IS (223 vs.  $157 \times 1000$ /mL, respectively; p < .05). Concerning BCS, significantly higher values were recorded in IS than in HF (3.47 vs. 2.81 points; p <.05). Regarding feeding behaviour, RT was significantly different between the two breeds, with HF ruminating almost 20 min more than IS (p < .01). Finally, the ET differed between the two breeds, with higher values for HF than IS (254.7 ± 5.2 min/d for HF and  $227.9 \pm 2.2 \text{ min/d}$  for IS, p < .01, data not shown in the Table).

#### Discussion

Changes in diet composition and milk production traits are explored in relation to variations in ET of dairy cows on commercial farms, accounting for the contributions of the two breeds considered in the study.

### Eating time and diet composition

The relationship between diet composition and DMI in dairy cows has been extensively studied over the years and dietary NDF has been widely recognised as one of

ltem	Eating Time Class (ETC) <sup>1</sup>					Breed			Breed,	ETC,	Contrasts, ETC <i>p</i> -values	
	1	2	3	4	5	HF	IS	SEM	<i>p</i> -value	<i>p</i> -value	Linear	Quadratic
HF:IS ratio	27:73	29:71	39:61	36:64	23:77	-	-	-	-	-	-	-
Parity (n)	2.3	2.2	2.0	1.9	1.8	-	-	-	_	_	-	_
DIM (n)	199	170	185	183	170	-	-	-	_	_	-	_
Milk yield (kg/d)	27.04 <sup>E</sup>	28.72 <sup>D</sup>	29.89 <sup>C</sup>	30.23 <sup>B</sup>	31.91 <sup>A</sup>	30.60	28.46	0.058	<.01	<.01	<.01	.01
FPCM (kg)	26.71 <sup>e</sup>	27.97 <sup>d</sup>	29.28 <sup>c</sup>	29.54 <sup>b</sup>	31.08 <sup>a</sup>	29.56	28.21	0.059	<.01	<.01	<.01	.13
Milk composition												
Fat (%)	3.87	3.84	3.82	3.87	3.77	3.77	3.90	0.213	.80	1.00	1.00	1.00
Protein (%)	3.45	3.44	3.40	3.39	3.41	3.37	3.47	0.196	.83	1.00	1.00	1.00
Lactose (%)	4.80	4.79	4.80	4.80	4.79	4.80	4.79	0.078	.96	1.00	1.00	1.00
Urea (mg/dL)	23.58 <sup>A</sup>	22.76 <sup>B</sup>	22.95 <sup>B</sup>	22.63 <sup>B</sup>	22.65 <sup>B</sup>	23.17	22.66	0.107	.03	<.01	<.01	<.01
SCC (x 1,000/mL)	224 <sup>a</sup>	255ª	189 <sup>a</sup>	179 <sup>a</sup>	118 <sup>b</sup>	222.5	157.1	11.36	.02	<.01	<.01	.13
BCS (points)	3.24 <sup>a</sup>	3.19 <sup>ab</sup>	3.10 <sup>ab</sup>	3.13 <sup>ab</sup>	3.05 <sup>b</sup>	2.81	3.47	0.024	<.01	.02	<.01	.72
RT (min/d)	520.9 <sup>E</sup>	531.3 <sup>C</sup>	532.3 <sup>B</sup>	528.7 <sup>D</sup>	536.1 <sup>A</sup>	540.1	519.8	0.06	<.01	<.01	<.01	<.01

Table 2. Estimated marginal means of milk yield and composition, body condition score and ruminating time as affected by eating time class and breed.

<sup>1</sup>ETC: eating time class. ETC-1  $\leq$  180 min/d (n = 197); 220 min/d  $\leq$  ETC-2 > 180 min/d (n = 179); 260 min/d  $\leq$  ETC-3 > 220 min/d (n = 174); 300 min/d  $\leq$  ETC-4 > 260 min/d (n = 111); ETC-5 > 300 min/d (n = 118). DIM: days in milk; FPCM: fat- and protein-corrected milk (Kok et al. 2016); SCC: somatic cell count; BCS: body condition score; RT: rumination time; HF: Holstein Friesian; IS: Italian Simmental; <sup>A</sup>, <sup>B</sup>, <sup>C</sup>, <sup>D</sup>, <sup>E</sup> means of ETC in the same row with different superscript are significantly different (p <.01); <sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup> means of ETC in the same row with different superscript are significantly different (p <.05).

Table 3. Correlation coefficient (r<sub>s</sub>) between ETC and variables included in principal component analysis (PCA).

	Parity	Breed	DIM	FP	Particle size	СР	EE	ADL	NFC	NDFd
ETCt	18**	02	06	.30**	.44**	48**	46**	.54**	62**	59**
ETC <sub>HF</sub>	30**	_	.01	.32**	.52**	48**	45**	.51**	52**	52**
ETCt <sub>IS</sub>	13**	-	08	.27**	.42**	54**	58**	.57**	65**	67**

DIM: days in milk; FP: number of feed pushing per day; CP: crude protein (%DM); EE: ether extract (%DM); ADL: acid detergent lignin (%DM); NFC: nonfibre carbohydrates (%DM); NDFd: neutral detergent fibre degradability (%NDF); ETC: eating time class; Parity 1: primiparous; Parity 2: multiparous (2 parities); Parity 3: multiparous (>2 parities); breed: Holstein Friesian (HF), 1; Italian Simmental (IS). \*\*: p < .01.

the dietary components most likely to influence DMI (Mertens 1997; Allen 2000). Instead, much less information is available on the effect of diet on feeding time. Beauchemin (2018), in an extensive review of cow feeding activity, summarises that the main drivers of chewing time are the physical and chemical characteristics of the diet (particle size, moisture, and fibre contents). In particular, the author states that ET and forage NDF intake have an overall positive relationship but with considerable dispersion due to several other factors influencing the feeding behaviour of lactating dairy cows.

This indirectly occurred also in this investigation: the lowest ET classes were associated in the PCA analysis positively with NFC and negatively with ADL, and these latter dietary components were highly correlated with NDF (r = -0.96 and 0.83, respectively). NDFd, on the other hand, had a negative correlation with ETC, as did dietary chemical characteristics that often rise with compound feed consumption (such as NFC, EE, and CP). Several studies have demonstrated the positive impact of fibre digestibility (NDFd) on the performance of dairy cows (Dado and Allen 1994; Oba and Allen 1999), mainly due to a high disappearance and passage rate in the rumen, while there are no studies on its effect on ET. The negative relationship between ETC and NDFd found in our experiment suggests a low necessity for cows to intensively masticate a more degradable fibre. Conversely, high levels of poorly degradable fibre decrease rumen transit time, accelerate rumen filling (Stergiadis et al. 2015), and increase ET.

Beauchemin (2018), in the previously cited review, states that increasing silage particle size augments ET, but this relationship appears to be highly variable across studies. Particle size affects daily DMI, dietary digestibility, and MY not only in maize silage-based diets but also in other forage-based diets (Haselmann et al. 2019). In general, excessively long particle size increases ET because cows require longer mastication to swallow the feed bolus (Grant and Ferraretto 2018). According to this, Kononoff et al. (2003) found an increased daily ET of 36 min/d as silage particle size increased, as well ET was reduced by 43 min/d for cows fed the finer chopped silage (Fernandez and Michalet-Doreau 2002). Particle size also has an effect on RT, with a considerable drop when the particle size is very tiny (<5 mm) (Nasrollahi et al. 2016). The results of our study have confirmed that the increase in particle size was correlated with ETC (r = 0.44; p < .01; Table 3). In the farms involved in this study, we found different dietary strategies (both silage- and hay-based diets), and particle size was related to dietary fibre content expressed as NDF (r = 0.64; p < .01; data not reported in Table). Jiang et al. (2017) observed a 1.8 h/d greater ET when dietary forage content increased from 40 to 70%, but RT only increased by 35 min/d.

In general, eating activity increases throughout the day when there is frequent mobilisation of feed in the bunk (feed delivery or feed pushing). The delivery of fresh feed has been demonstrated to be a strong stimulus to initiate feeding activity, so frequent distribution throughout the day exhibits more desirable feeding patterns to support production and rumen health (Hart et al. 2014). DeVries et al. (2005) tested one, two, and four deliveries of feed per day and found an increase in the daily feeding time with increasing frequency of feed provision (+10 and +14 min/d, respectively for two and four deliveries). It is generally believed that dividing the total daily meal into several portions over 24 h allows ruminal homeostasis, which has advantages in terms of MY and animal welfare. Instead, Benchaar and Hassanat (2020) argue that changing the daily frequency of feed delivery from one to two to four times does not lead to an increase in DMI, milk production, or even in the digestibility of the nutrients. In our study, the frequency of feed pushing contributed only a small part, but highly significant, to ET (r = 0.30; p < .01; Table 3).

## Eating time and milk production

As the ETC decreases, there appears to be a gradual increase in the DIM. Indeed, DIM was weakly related to ETC-1 and 2, along with the second principal component (Figure 1 (a and b)), probably due to the reduction of milk production as lactation progresses and, consequently, to the reduction of nutritional requirements. Cows with more than two lactations were associated with lower ETCs. This is also reflected in Table 3, where a low but significant correlation of parity with ETC is reported for both breeds (r = -0.30for HF and r = -0.13 for IS, p < .01). It is known that multiparous cows spend less time feeding and have a generally higher DMI than primiparous cows (Azizi et al. 2009). This is due to differences in body weight, which, in addition to influencing rumen capacity and meal size, also influence bolus size (Dado and Allen 1994; Aikman et al. 2008).

The positive relationship between DMI and MY is expected and well demonstrated (Dado and Allen

1994; Ben Meir et al. 2019) while more controversial is that with ET. In fact, the same studies report rather weak or absent correlations between milk and ET, although we found a positive relationship (Table 2). In a study comparing two groups with high and low MY (Azizi et al. 2009), no significant difference was found in terms of ET, but the more productive cows had an additional 11% of feeding rate than the other group. However, Dado and Allen (1994) reported a negative correlation of 0.64 between milk production and feeding rate (expressed in min/kg of DM). The aforementioned publications reported experimental data obtained in a controlled environment; our study considered cows on commercial farms with differences between and within herds. Other factors that may have conditioned our results were the PMR feeding technique (high-yielding dairy cows receive more feed from AMS) and the diverse levels of competition at the feed bunk (competitive situations increase the feeding rate).

The group of cows with an ET of less than 3 h/d was essentially made up of low MY cows, where even the little compound feed amount offered by AMS combined with the PMR exceeded their actual nutritional requirements (both energy and protein). This translates into a higher concentration of urea in the milk (an indicator of protein nutrition) and a better BCS (energy nutrition). Another possible interpretation of this result was related to ruminal activity in animals with different feeding behaviours. During the digestion of feeds in the rumen, proteins are partially degraded by bacteria and protozoa into amino acids, which are then deaminated to ammonia. The liver synthesises urea from ammonia absorbed in the rumen, which is subsequently released into the blood, where it is subject to clearance in several ways and, to a minimal extent, excretion in milk. Therefore, a longer RT favours bacteria growth and digestion with increased bacterial protein synthesis, which leads to a reduction in urea production and subsequent elimination through milk (Beauchemin 2018). This was little reflected in our results, in which animals with extreme values of RT had only slightly higher values of milk urea concentration (23.58 mg/dL with 520.9 min/d vs. 22.65 mg/dL with 536.1 min/d, p < .01).

The SCC result is more difficult to interpret. It is clear that in the high ETC, there were cows with a high MY, and among these, there was a lower concentration of SCC in milk, as observed also by Fogsgaard et al. (2012). In fact, the data proposed in this study confirm that the increase in SCC causes a reduction in the synthesis capacity of the mammary gland. However, there does not appear to be a direct effect of mastitis on feeding behaviour. Few studies were performed to probe a relationship between acute or clinical mastitis and changes in animals' behaviour and assess protocols for early detection of disease. In the study by Fogsgaard et al. (2012), the effect of mastitis on behaviour was investigated, and ET and RT decreased from 24 to 48 h after infection. This was probably due to a switch of energy from normal metabolism activities to the immune system to overcome infection. A similar result was found in the study of González et al. (2008), which concluded that feeding time could be a useful early indicator of mastitis if related to other variables such as MY and milk conductivity, which are easily available thanks to the data collected by AMS.

# Breed effects on body conditions, udder health, and feeding behaviour traits

The lower BCS of the HF cows was an expected result, given the higher MY of this breed compared to IS (30.6 vs. 28.4 kg/d, p < .01; Table 2). In fact, the more intensive selection programs favouring only milk production increase the animal's nutritional needs, which are only partly supplied by feed intake increments. This agrees with two studies (Walsh et al. 2008; Ferris et al. 2014) that both compared a dairy breed (HF) with a dual-purpose breed (Norwegian Red) and found that HF cows had a significantly lower BCS during all lactation (p < .001). Another aspect affected by stronger selection for MY in HF is the resilience of the animal, in particular the udder health of dairy cows. Some studies have investigated the different susceptibilities of breeds to the insurgency of mastitis. For example, a study explored potential differences in udder health between breeds on commercial dairy farms and found that HF cows had a higher MY, but udder health was significantly better for dual-purpose cows (Norwegian Red). Furthermore, the proportion of cows that developed mastitis was lower for dual-purpose breeds compared with dairy ones (6% vs. 11.9%, p < .05; Begley et al. 2009). Ferris et al. (2014) discovered that HF and Norwegian Red cows had SCC levels of 282 and  $176 \times 1000$ /mL, respectively, which were fairly similar to our data, indicating that the HF breed has a high susceptibility to mastitis.

As regards differences between the feeding behaviours of HF and IS, we found that the two breeds differed in terms of ETC and RT, with a longer time for HF than IS. In the literature, some studies evaluated feeding behaviour in different breeds and found little or no significant differences in terms of total daily ET but differences in terms of eating rate (Aikman et al. 2008; Olson et al. 2019; Romanzin et al. 2022;). This suggests the possibility that breeds with increased nutrient requirements (like highly productive dairy breeds) may compensate, at least partially, for different ingestion guantities by increasing the amount of feed ingested per unit of time. Other studies found substantial variations in feeding behaviour between HF and other breeds (especially Jersey), with the first spending more time eating than the latter (Munksgaard et al. 2020; Gündel et al. 2022). These results agree with those found in our study, where IS cows ate significantly less than HF (about 27 min/d; Table 2) and are associated with the lowest ETC (Figure 1(b)). This is explained by the higher milk production of HF. Finally, considering RT, Aikman et al. (2008) found that HF animals ruminated longer than Jersey ones (623 vs. 538 min/d; p < .05), despite no significant differences in terms of ET per day.

## Conclusions

Our results revealed that ETC is well related to aspects concerning diet characteristics, the performances of cows, and the health status of the animals. In particular, fibrous and energy content can significantly change the daily duration of the ET, as well as different kinds of ration management. Furthermore, ET and milk performance condition each other, in fact, cows with the highest ETC perform better than others with less ETC. Breed could also define different eating patterns. The study provides preliminary results for future research to better define the role of ET in the causation of the overall feed efficiency and health status of cows. The potential perspective could be using the ET as a useful tool to rank lactating cows within herds for selective and/or management purposes.

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#### **Ethical statement**

All research reported in this study has been conducted in an ethical and responsible manner and is in full compliance with all relevant codes of experimentation and legislation.

### **Disclosure statement**

No potential conflict of interest was reported by the author(s).

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#### Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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