



Article Relationships between Dairy Cows' Chewing Behavior with Forage Quality, Progress of Lactation and Efficiency Estimates under Zero-Concentrate Feeding Systems

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Abstract: Adaptivity of eating and rumination behavior are assumed to play a particular role for cows in low-input systems, because they are more frequently challenged by altering forage quality. The present study examined relations between forage quality, chewing behavior and efficiency estimates in dairy cows from Swiss zero-concentrate organic farming systems. A total of 102 Swiss Fleckvieh cows on two organic dairy farms were observed during one full production year. Each farm was visited eight times. At each visit, up to 45 cows were equipped with RumiWatch $^{\oplus}$ (Itin and Hoch GmbH, Liestal, Switzerland) sensor head collars, from which eating and rumination time and the frequency of activity changes were obtained for 48 h. Milk from one complete day was analyzed individually. All offered roughages (pasture herbage, grass silages and hay) were sampled at each visit and analyzed for crude fiber, crude protein and net energy, and a feed quality score was calculated. Metabolic production efficiency was estimated based on entire lactation data, and feed efficiency was estimated based on the individual farm visits. Lactation stage and forage quality significantly affected the chewing sensor variables. Eating time increased and rumination time decreased with the improved nutritive quality of feed. Coefficients of variance of the factor animal in the sensor variables showed a contribution of the individual cow to chewing behavior. Significant correlations between chewing sensor variables and efficiency estimates were not found. In conclusion, chewing behavior under on-farm conditions in low-input dairy farms alters during lactation and during changing forage quality, with significant animal effects, indicating potential for new phenotypes, albeit with no indications for efficiency.

Keywords: grazing; chewing sensor; feeding behavior; milk yield; on-farm research; organic dairy; roughage

1. Introduction

Low-input dairy production systems, which aim at reducing the use of concentrate feeds, are expanding in Europe [1] and in the US [2], particularly in the organic sector. These farms need cow types well adapted to roughages and pastures with varying nutrient density and digestibility [3,4]. Responses to varying nutritive quality may consist in adapting milk yields, mobilizing and restoring body adipose tissues, altering intake and digestion or avoiding pregnancy. From a biological perspective, the animal's main goals are to stay healthy and to reproduce [5], while, from the farmers' economic perspective, the main goal is the efficient conversion of the feed offered to dairy products. These goals are antagonistic, to a certain degree, which is evident from the antagonism between high milk yields and robustness traits across breeds [6]. There is a general trade-off between advanced high-yielding breeds, which are often not flexibly adaptive to changing pasture conditions [7], and well-adapted traditional or dual-purpose breeds, which cope well with the challenges of low-input systems but have lower milk yields [6,8]. Therefore, resilience,



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). adaptability and roughage conversion efficiency are important aspects to characterize suitable breeds and phenotypes for low-input systems [9]. For such characterizations, behavioral traits such as eating and rumination may play a helpful role.

The assessment of feed efficiency on cow level under grazing conditions is challenging. Individual determination of dry matter and nutrient intake is hardly practicable in dairy systems with loose housing and grazing [10]. Thus, calculation of efficiency sensu strictu [11] is hardly possible on such farms. Intake and rumination activity measured with chewing sensors might provide meaningful proxies to estimate intake and responses to feed quality in practice [12]. Several such sensors were brought to market in recent years [13,14], and reasonable correlations of the achieved data with intake could be shown, e.g., for noseband-situated pressure sensors [15,16].

Chewing activity curves, translated into eating and rumination patterns [17], can reflect changes in feed availability and quality [18,19], but they may also express individual responses to feed properties in terms of intake and rumination phenotypes [10,20]. However, such approaches will work only if they provide consistent responses under practice conditions. In order to evaluate the suitability of chewing sensor data for senseful phenotyping, it appears necessary to evaluate (a) the strength of chewing behavior in response to different forage qualities and (b) the correlation of intake and rumination traits with milk production efficiency estimates at the cow level.

Therefore, we conducted a study on two commercial Swiss organic dairy farms with zero-concentrate feeding strategies. Covering a full production season, each of the herds was assessed eight times with chewing behavior sensors at the cow level over 48 h. The sensor data were analyzed to investigate the relationships between foraging behavior and production parameters such as feed quality, lactation stage, performance and efficiency values. Three levels of analysis were (i) the general development of the assessed variables across lactation, (ii) the effects of feed quality on chewing sensor and production variables and (iii) the corrected correlation of chewing sensor data with two different efficiency estimates. The target of the study was to evaluate the potential of chewing sensor data for phenotyping cows under practice conditions for selection on low-input dairy farms.

2. Animals, Materials and Methods

2.1. Farms and Animals

We conducted on-farm assessments on two Swiss organic dairy farms. The animal experiment was controlled and permitted under the Swiss permit number 29374, issued by the veterinary authority in Aarau, Switzerland. The commercial farms were chosen in order to have sites well representative for a low-input but nonetheless dedicated dairy production agriculture in grassland-rich sub-alpine regions. Both farms are economically fully profitable under current conditions and thus they represent a reasonable model for future low-input dairy production in the region. Both farms were located in Central Switzerland (farm 1: close to the city of Aarau; farm 2: close to the city of Luzern; both were in lowland sites with a high proportion of semi-natural grassland).

Both farms followed a zero-concentrate feeding regime with grazing whenever possible. The animals had always ad libitum access to water and forage, the latter either as pasture or as cut grass, grass silage or hay in the barn (Table 1). Salt and mineral mixtures were accessible daily in the barn for each cow. The cows were milked daily in the morning (6.00) and in the evening (17.00). Each farm herd consisted of 40–50 lactating dairy cows of the Swiss Fleckvieh breed [21]. All lactating animals were kept in one group, respectively.

Run	Starting Date	Ration	Botanical Characterization ¹	THI ²	N _{cows}
Farm1_a	29 August 2017	Pasture	K2-3	63.8	39
Farm1_b	3 September 2017	Pasture	A3	62.1	39
Farm1_c	6 June 2018	Mixed	G2 + G4	54.6	34
Farm1_d	11 April 2018	Pasture	A4	56.7	34
Farm1_e	23 August 2018	Mixed	A2	65.0	39
Farm1_f	28 August 2018	Mixed	K3 + L4	59.2	39
Farm1_g	10 October 2018	Pasture	L3 + G3	NA	35
Farm1_h	15 October 2018	Mixed	G3	NA	35
Farm2_a	5 November 2017	Mixed	A2-3	43.4	42
Farm2_b	10 November 2017	Mixed	L2-3	40.9	42
Farm2_c	21 March 2018	Hay	G3-4	46.3	27
Farm2_d	26 March 2018	Mixed	G1 + G3-4	42.8	27
Farm2_e	16 November 2018	Mixed	G2 + G3-4	35.2	30
Farm2_f	21 November 2018	Hay	G3-4	39.0	30
Farm2_g	16 April 2019	Mixed	G3 + G3-4	59.0	21
Farm2_h	21 April 2019	Pasture	G3	56.1	21

Table 1. Experimental setup showing the eight runs per farm, starting date, ration including botanicalcharacterization, Temperature–Humidity Index (THI) and number of cows involved.

¹ botanical composition, assessed following the recommendations of AGFF, 2007 (https://www.eagff.ch/files/ images/bilder/Raufutter_produzieren/Futterqualitaet/agff-mb3_1707_D_21_bewertung_von_wiesenfutter_ ohne_06.05.pdf (accessed on 15 July 2022)): G =rich in grasses, K =rich in herbs, L =rich in legumes, A = balanced; development stages: 1 = start of shooting, 2 = panicle rises in 50% of the fermenters, 3 = start of panicle pushing, 4 = full panicle pushing. ² Temperature–Humidity Index.

The investigation schedule comprised eight visits per farm, which were intended to cover the whole grazing season (March–November) and as many as possible of different feed quality situations. For reasons of feasibility, we organized the visits always pairwise around a foreseen change in pasture or in feeding schedule. Each farm was visited eight times between August 2017 and April 2019, to collect chewing behavior data in association with feed nutritional quality and lactation status, and to assess the body condition and body weight of the cows. Whereas, initially, all lactating cows were studied at each farm visit, technical errors of sensors or mismatch with validation criteria caused a posteriori exclusion of individual animals. The effective sample size and basic descriptors of the different farm visits are shown in Table 1, and the farm characteristics are displayed in Table 2.

Table 2. Descriptive summary of chewing sensor variables and cow characteristics by farm.

Data Origin	Variable	(Ob	Farm 1 servations $n = 29$	4)	(Ob	240)	
	-	$\mathbf{Mean} \pm \mathbf{SD}$	Median	Min-Max	$\mathbf{Mean} \pm \mathbf{SD}$	Median	Min–Max
	Eating time (min/d) Ruminate time (min/d) Other activity time (min/d)	$\begin{array}{c} 609.9 \pm 88.8 \\ 413.1 \pm 79.4 \\ 412.8 \pm 102.4 \end{array}$	628.6 399.5 394.6	341.3–768.3 240.5–606.7 233.3–692.8	$\begin{array}{c} 538.3 \pm 71.7 \\ 490.4 \pm 71.9 \\ 401.0 \pm 80.8 \end{array}$	542.3 486.9 410.0	325.6–714.2 324.5–666.5 219.6–603.9
	Ruminate chews per minute (avg. of 24 h)	43.2 ± 6.8	42.5	26.6-67.5	50.3 ± 7.0	49.8	31.2–71.4
	Ruminate chews per bolus (avg. of 24 h)	34.5 ± 6.2	34.2	18.7–55.9	43.7 ± 6.8	43.8	28.3-60.7
Trial ¹	Activity changes (n/d)	121.9 ± 20.0	120.0	77.3-183.0	133.1 ± 26.5	128.7	79.3-220.3
	Body weight (kg)	581.8 ± 61.1	583	456-787	648.4 ± 75.4	658	441-810
	Body condition score	2.90 ± 0.29	2.75	2.25-3.75	3.21 ± 0.34	3.25	2.50 - 4.00
	ECM (kg/d) ³	16.6 ± 5.7	15.1	6.7-37.0	21.3 ± 5.7	21.4	5.2-33.0
	Milk protein (%)	3.37 ± 0.30	3.34	2.76-5.12	3.70 ± 0.50	3.72	2.77 - 5.40
	Milk fat (%)	4.34 ± 0.59	4.29	2.96-6.35	4.44 ± 0.79	4.49	1.88-6.98
	Milk lactose (%)	4.73 ± 0.15	4.73	4.27-5.48	4.67 ± 0.17	4.69	3.78-5.13
	Days in milk	182.5 ± 76.1	210.5	3–300	148.7 ± 108.6	161	3–308
	Lactation number	3.5 ± 2.8	2	1–12	3.9 ± 2.4	3.5	1–10
	Lactation yield (kg)	6362 ± 1815	6406	2295-10,621	5857 ± 1226	5863	2135-10,208
	Lactation length (d)	336 ± 101	307	90-602	297 ± 70	306	80-584
HB ²	$DECM_{TL}^{4}$ (kg/d)	19.6 ± 3.2	19.7	12.6-26.3	20.8 ± 2.9	20.9	14.4-27.7
	Milk protein _{TL} (%)	3.31 ± 0.24	3.32	2.72-3.96	3.42 ± 0.18	3.43	3.00-3.86
	Milk fat _{TL} (%)	4.22 ± 0.43	4.15	3.48-5.52	4.28 ± 0.35	4.29	3.37-5.20
	Milk lactose _{TL} (%)	4.77 ± 0.10	4.77	4.46-5.02	4.70 ± 0.11	4.70	4.43-4.93

¹ data generated during the trial, ² data extracted from herd book data (Qualitas AG, Zug, Switzerland), ³ energy-corrected milk yield measured during the trial, ⁴ $_{TL}$ = averages of total lactation.

2.2. Data Collection

We equipped up to 45 cows of each herd with RumiWatch[®] (RW) head collars for at least four days per farm visit, on eight runs in total. RumiWatch[®] head collars are noseband pressure sensors detecting chewing movements; the signals are translated into temporal eating and ruminating patterns (Itin and Hoch GmbH, Liestal, Switzerland, [17]). After the cows were holstered, they followed their daily routines on the farm.

Individual milk samples from one complete milking (evening and morning) were taken on day 2/day 3 of each farm visit and analyzed for fat, protein, lactose and urea through infrared spectroscopy (Milko-Scan-FT TM, FOSS Hilleroed, Denmark) by Suisselab (Zollikofen, Switzerland). At the end of each visit, cows were weighed with a digital balance with a riffle sheet that cows could walk on and two Trutest weighing bars (M800; Grüter Waagen GmbH, Eschenbach). Further, body condition score was assessed using the method described as "dBCS" by [22]. Additionally, herd book data on total milk yield (kg), average milk composition (i.e., percentage of milk fat, milk protein and milk lactose content) and length of the respective lactation of each cow in the trial were obtained from the competence center for informatics and genetics of Swiss breeding organizations, Qualitas AG (Zug, Switzerland), and included in the analysis.

We used TinyTag loggers located on a pasture close to each barn to measure relative humidity and temperature in °C and calculated the Temperature–Humidity Index (THI), defined in Equation (1) as

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$$
(1)

where T is the air temperature in $^{\circ}$ C and RH is the relative humidity in % (Table 1).

2.3. Feed Sampling and Analysis

At each farm visit, we collected representative samples of the actual feedstuffs, including indoor fed hay, grass silage and freshly cut green fodder, as well as pasture. On pasture, samples were collected by cutting, respectively, five plots of 0.25 m² at 3–5 cm above ground. The plots were randomly chosen spots, distributed across the respective pasture actually grazed. From hay, grass silage or cut grass, we collected five samples filling a 1 L bag from different places of the feedstocks in the barn, respectively. We dried the samples for 24 h at 60 °C to determine dry matter. Dried samples were milled (Cutting Mill SM100, Retsch GmbH, 42781 Haan, Germany) and analyzed with near-infrared spectroscopy (NIRFlex N-500 equipped with a NIRflex Solids measuring cell, Büchi Labortechnik AG, 9230 Flawil, Switzerland) for crude protein (CP), crude ash and crude fiber. The NIRFlex device had been calibrated based on a sample of >100 roughage samples of a wide quality range. Net energy for lactation (NEL) and absorbable protein at the duodenum (APD) were calculated according to the Swiss feed grading system [23].

2.4. Processing and Validation of RumiWatch Data

The first day of each farm visit was considered an adaptation day to the head collars, and data were not used. From each visit, RumiWatch data from day two, 2:00 p.m., to day four, 1:59 p.m., were used for analysis. Raw data recorded by the RumiWatch head collars were converted using the RumiWatch Converter V.0.7.3.36 [24] to calculate eating and rumination patterns, aggregated at 1-h time intervals (minutes per hour). If a cow had spent less than 30 min within two consecutive hours with either ruminating or eating, the data of the corresponding 24 h (defined as time slot from 2:00 p.m. to 1:59 p.m.) were excluded for this cow. If we had to exclude more than 24 h of data from a cow, we excluded that cow for the current visit.

We used the variables rumination time (min/h), eating time (min/h) and number of activity changes, i.e., the frequency of switches between eating, ruminating and idling (n/h; [19]), as descriptors of chewing behavior, which we validated against the following threshold values (aggregated to 24 h): eating, 240–795 min/d; ruminating, 220–720 min/d; and activity changes, 55–300 n/d. The validation ranges for eating and ruminating are larger than the averages given in the review of Beauchemin (2018). This reflected the longer activity time for grazing cows [25]. A descriptive summary of chewing sensor variables by farm in the validated data set is shown in Table 2.

2.5. Definition of Efficiency Parameters

Energy-corrected milk yield (ECM in kg/d) was calculated with a standard of 4% milk fat, 3.2% milk protein and 4.8% milk lactose, applying Formula (2) [23]. We estimated dry matter intake (DMI) in kg per day using Formula (3) proposed by [26].

ECM [kg] = ((MilkFat [%] × 0.38 + MilkProtein [%] × 0.24 + MilkLactose [%] × 0.17) × DMY [kg])/3.14 (2)

 $DMI_{DeSouza} = [(3.7 + Parity \times 5.7) + 0.305 \times (0.0929 \times MilkFat [\%] \times DMY [kg] + 0.0563 \times MilkProtein [\%] \times DMY [kg] + 0.0395 \times MilkLactose \times DMY [kg]) + 0.022 \times Body weight [kg] + (-0.689 + Parity \times -1.87) \times BCS]$ (3) $\times [1 - (0.212 + Parity \times 0.136) \times exp^{(-0.053 \times DIM)}]$

where DMY = daily milk yield in kg, MilkFat = milk fat content in %, MilkProtein = milk protein content in %, MilkLactose = milk lactose content in %, Parity = a two-level categorical variable indicating whether a cow is primiparous (0) or multiparous (1), BCS = body condition score of the cow on a scale of 1 to 5 with 0.25 intervals [22] and DIM = days in milk.

Data were limited to cows with an actual lactation status of maximal 305 days in milk. We had to discard the attempt to apply the formula proposed by [16], which uses RumiWatch variables to estimate DMI, as we partly obtained unrealistically low DMI estimates.

We used herd book data on total lactation milk yield, milk fat, milk protein and milk lactose content and lactation length (Qualitas, Zug, Switzerland) for each cow involved in the trial. Herd book data on lactation milk yield were energy-corrected by applying Formula (2). The resulting energy-corrected lactation milk yield (in kg) was divided by the lactation length (in days) to obtain values for energy-corrected average daily milk yield of total lactation (DECM_{TL}).

We calculated two different efficiency estimates. First, the metabolic milk production efficiency at total lactation level (MPE_{TL}) [27] was calculated as

$$MPE_{TL} = DECM_{TL} / (average body weight^{0.75})$$
(4)

where DECM_{TL} is the average energy-corrected daily milk yield of the total lactation divided by the average metabolic body weight of the respective lactation based on the weighings during our farm visits. Second, we calculated a day-based feed efficiency value

$$FE_{day} = ECM_{day} / DMI_{DeSouza}$$
(5)

where ECM_{day} is the energy-corrected daily milk yield measured during the respective farm visit, divided by the actual dry matter intake estimated according to Formula (3).

2.6. Statistical Analysis

All statistical analyses were performed in R (v 3.6.2; [28]).

Because the nutritional values analyzed in the feed were highly correlated, and in order to reduce the dimensions of the data, we performed a Principal Component Analysis (PCA) using the "prcomp" function in R (v 3.6.2, [28]). The broken stick approach was applied to identify the relevant principal components (Table 3). The first principal component (PC1) explained 88% of the variance in feed quality across the different farm visits. Therefore, we used the coefficients from PC1 to calculate a general "feed quality score" using Formula (6).

Feed quality score = DM
$$[g/kg] \times 0.51 + CF [g/kg] \times 0.50 + NEL [MJ/kg] \times -0.50 + CP [g/kg] \times -0.49$$
 (6)

	PC1	PC2	PC3	PC4
Eigenvalue	3.54	0.27	0.12	0.08
% variance	88.38	6.70	2.88	2.03
% variance cum.	88.38	95.08	97.97	100.00
Variables' % contribution				
Dry matter	25.70	9.65	38.69	25.97
Crude fiber	25.47	13.99	37.83	22.71
NEL	24.65	33.90	11.29	30.15
Crude protein	24.18	42.46	12.19	21.16
Variables' coordinate contribut	ion			
Dry matter	0.95	-0.16	0.21	0.15
Crude fiber	0.95	0.19	-0.21	0.14
NEL	-0.93	-0.30	-0.11	0.16
Crude protein	-0.92	0.34	0.12	0.13

Table 3. Results of the Principal Component Analysis for the feed composition.

This feed quality score has only a dimension in the sense of distances on the x-axis. It distinguishes fiber- and dry-matter-rich feed (positive values) from protein- and energy-rich feedstuff (negative values). The score was subsequently used in the models calculating the feed effects of chewing sensor variables.

Furthermore, we categorized three main types of rations according to the respective feeding schedules at each farm visit (Table 1): (1) a pure hay diet indoors, during winter ("hay"), (2) a pastureonly regime in summer ("pasture") and (3) a pasture-based diet with additional supply of hay, freshly cut grass or grass silage in the barns during spring and autumn or drought periods ("mix"). This classification was used to provide a summarized overview of the feeding regimes (Tables 1 and 4).

Table 4. Chemical feed composition and nutritive values aggregated to three ration types (hay, mixed and pasture) across visits and farms.

Ration		DM (%)	CP (g/kg DM)	Ash (g/kg DM)	CF (g/kg DM)	NEL (MJ/kg DM)	APD (g/kg DM)
Hay (<i>n</i> = 57)	$\text{Mean} \pm \text{SD}$	$89.7\ ^{a}\pm1.0$	124.8 $^{\rm c}$ \pm 7.9	91.8 $^{\rm b}\pm3.5$	279.8 $^{a}\pm2.7$	$5.2\ ^{\rm c}\pm 0.0$	79.2 $^{\rm c}$ \pm 5.5
	Min–Max	88.8–90.7	117.4-133.0	88.5-95.4	277.1-282.2	5.2-5.2	74.0-85.0
Mixed (<i>n</i> = 274)	$Mean \pm SD$	$32.4 \ ^{\mathrm{b}} \pm 17.7$	178.0 $^{ m b} \pm 19.6$	110.4 $^{\mathrm{b}}$ \pm 24.8	236.6 $^{\rm b} \pm 18.0$	5.8 $^{\mathrm{b}}\pm0.2$	$100.2^{\text{ b}} \pm 5.1$
	Min–Max	11.9-67.3	149.0-204.8	72.2-141.8	206.9-263.8	5.5-6.2	92.5-107.8
Pasture	Mean \pm SD	15.4 $^{ m c}$ \pm 3.4	207.5 a \pm 15.8	113.6 a \pm 10.2	215.0 c \pm 11.9	$6.2~^{a}\pm0.2$	108.9 $^{\mathrm{a}}\pm2.8$
(n = 203)	Min–Max	9.3-20.4	181.0-228.3	96.1-130.8	195.3-226.6	5.8-6.5	104.0-111.8

DM = dry matter, CP = crude protein, CF = crude fiber, NEL = net energy lactation, APD = absorbable protein at the duodenum. ^{a,b,c} Different superscript letters within columns represent significant differences at*p*< 0.05 between rations based on mean comparisons by Tukey contrasts.

In order to test differences in chemical composition (Table 4) between the rations "hay", "mix" and "pasture", respectively, we performed pairwise comparisons as Tukey contrasts on the linear models for the respective chemical component (dependent variable) and ration (independent variable) using the "emmeans" package (version 1.4.5, [29]).

We investigated the effect of days in milk (DIM) and feed quality score on chewing sensor variables, applying linear mixed effects models in the "lme4" package in R (version 1.1-21, [30]). DIM, feed quality score and lactation class (levels: 1st, 2nd or 3rd lactation onwards) were fixed effects:

Model 1: $Y_{ijklm} = \mu + DIM_i + FQS_j + LC_k + herd_l + cow_m (herd_l) + e_{ijklm}$

where Y_{ijklm} = response variable (ruminate time (min/d), eating time (min/d) or activity changes (n/d), μ = overall mean, DIM_i = covariate of days in milk i, FQS_j = covariate of feed quality score j, LC_k = fixed effect of lactation class k (k = 1st, 2nd or 3rd lactation onwards), herd_l = random effect of herd l, N = 2, cow_m (herd_l) = random effect of cow m nested within herd l and e_{ijklm} = random error. Intake estimates, body weight and BCS had been excluded from the model to avoid confounding with the efficiency estimates in the subsequent step (Model 2).

To account for repeated measurements, cows nested within farms were used as random effects. We extracted pseudo marginal and conditional R squared estimates with the "MuMIn" package (version 1.43.15, [31]) to assess the variance explained by the models.

In order to display the non-linear relationships of relevant variables with DIM, we calculated smooth splines to show the empirical development of chewing sensor data, performance, body condition score and body weight across lactation. For this, we used an expanded version of model 1, which included DMI_{DeSouza}, BCS and body weight.

The smooth spline calculations were subsequently used to correct chewing sensor data for days in milk with a smoothing parameter (spar) of 0.95. For the last step, we used the estimates from the models for feed effects, which are displayed in Table 5, to correct the chewing sensor variables for effects of the feed quality score. Thereafter, the chewing sensor variables are labeled with "corrected".

Finally, we applied mixed models in "lme4" with efficiency traits as dependent variables, corrected chewing sensor variables as fixed effects and cow nested within farm as a random effect.

Model 2: $Y_{ijklmn} = \mu + Eat_i + Rum_j + AC_k + LC_l + herd_m + cow_n (herd_m) + e_{ijklmn}$

where Y_{ijklmn} = response variable (MPE_{TL}: total lactation average daily metabolic milk production efficiency [kg ECM/kg BW^{0.75}], FE_{day} = day-based feed efficiency value [kg ECM/kg DM intake]), μ = overall mean, Eat_i = eating time corrected for days in milk and feed quality score i, Rum_j = ruminate time corrected for days in milk and feed quality score j, AC_k = number of activity changes corrected for days in milk and feed quality score, LC₁ = fixed effect of lactation class (l = 1st, 2nd or 3rd lactation onwards; applied only in model with MPE_{TL} as dependent variable), herd_m = random effect of herd l, N = 2, cow_n (herd_m) = random effect of cow nested within herd and e_{ijklmn} = random error.

The significance of random effects was tested by comparing models with and without random effects through likelihood ratio tests in the "lmerTest" package [32]. This was done for animal within farm and also separately for farm only. Statistical significance was determined at p < 0.05, with tendency at p > 0.05 and <0.1 in all analyses.

3. Results

3.1. Feed Quality

The three ration types differed significantly regarding their nutritional properties (Table 4). The PCA on the feed analyses shown in Table 3 and Figure 1 revealed that most of the variation (88.4%) was explained by one single component (PC1, Eigenvalue =3.53). PC1 was composed of dry matter content (%), fiber content (g/kg DM), net energy used for lactation (NEL in MJ/kg DM) and protein content (g/kg DM), with a contribution to PC1 of 25.7, 25.5, 24.6 and 24.2, respectively. The broken stick approach revealed that PC1 was the only component explaining more of the variation than expected by chance. Based on this result, the feed quality score was calculated from PC1 (coefficients provided in Materials and Methods section). The farm visits were well distributed along the feed quality score (Figure 1), with farm 2 tending towards more fiber-rich and farm 1 tending towards more protein-rich score values.



Figure 1. Result of the Principal Component Analysis (PCA) on feed quality parameters. The PCA revealed that PC1 explains more than 88% of the variance in the data, reflecting a gradient from energy- and protein-rich feed (PC1 < 0) to fiber-rich feed (PC1 > 0). The arrows in the lower right indicate the loadings of the different parameters on the first two components (DM = dry matter, NEL = net energy lactation), and the shape of the symbols represents the two different farms. The letters in the symbols reflect the eight different trials (a–h) on each farm to enable the link to Table 1.

3.2. Temporal Development of Intake, Rumination, Body Condition and Yields

Across both farms, all variables of interest for this study were significantly altered with the days in milk (Figure 2, Table 5). While rumination time showed a smoothed U shape, where the early and the late days in milk showed the highest rumination time, eating time had an inverse U shape, with peak eating times in mid-lactation (Figure 2A). The variation in activity changes across DIM was a small but statistically significant decrease over time. While ruminate time did not differ between cows of different lactation classes, eat time was lower in cows from third lactation onwards. Activity changes were less frequent in cows from second lactation onwards.

Table 5. Influence of days in milk (DIM), feed quality score (FQS) and lactation class on chewing behavior traits ($N_{observations} = 534$, $N_{cows} = 102$, $N_{farms} = 2$).

	Intercept Slope by DIM			Slope by FQ	QS							
						_	1	2	3			
Response Variable	Est. \pm SE	p	Est. \pm SE	р	Est. \pm SE	р	Est. \pm SE	Est. \pm SE	Est. \pm SE	р	$\mathbb{R}^2 \mathbf{m}$	R ² c
Rumination time (min/d)	466 ± 19	*	-0.13 ± 0.03	***	29.1 ± 1.5	***	440 ± 19	445 ± 19	455 ± 18	0.121	0.443	0.590
Eating time (min/d)	651 ± 31	*	-0.33 ± 0.03	***	-14.9 ± 1.9	***	$597\pm31~^{a}$	$581\pm31~^{a}$	563 ± 30 $^{\rm b}$	**	0.191	0.471
Activity changes (n/d)	147 ± 7	*	-0.05 ± 0.01	***	-0.4 ± 0.5	0.434	$138\pm7~^a$	124 ± 7 b	$125\pm7~^{b}$	***	0.079	0.514

Est. = estimate, *p* values show *t*-test results using Satterthwaite's method in linear mixed models fit by REML for the intercept, days in milk, feed quality score and the *p* values of the F-Test with Kenward Roger degrees of freedom for lactation class (1st, 2nd and 3rd lactation onwards) as fixed and cow nested within farm as random effect. ^{a,b} Estimates for levels of lactation class are least square means, where different superscript letters in the same row indicate pairwise differences at *p* < 0.05 in the post hoc analysis. R² m = marginal R squared, i.e., proportion of variance explained by the fixed effects, R² c = conditional R squared, i.e., proportion of variance explained by the fixed and random effects. * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001.



Figure 2. Influence of days in milk (DIM) on (**A**) the chewing behavior parameters rumination time (\min/d) , eating time (\min/d) and number of activity changes (n/d), and on (**B**) the efficiency-related parameters body weight, body condition score (BCS), dry matter intake (DMI) based on the formula of de Souza et al. (2019) and daily energy-corrected milk yield (ECM). Lines represent the smooth splines between DIM and the corresponding variable. Variables have been multiplied or divided by the values added to the labels. Vertical lines represent 120-day intervals of DIM.

As shown in Figure 2B, body weight and BCS decreased in early lactation, but started to increase again at around 200 DIM. While dry matter intake (DMI), after de Souza et al. (2019), showed an increase at the beginning of the lactation, which leveled out after approximately 50 DIM, ECM clearly showed a negative relationship with DIM.

3.3. Impact of Feed Quality Score and Rations on Chewing Sensor Data and Milk Yield

Rumination and eating time were significantly affected by feed quality score, while the number of activity changes was not (Table 5). Rumination time increased with fiber content in feed (positive feed quality score values), while eating time showed the inverse pattern, namely an increase in eating time with more protein-rich feed (negative feed quality score values). Eating time and number of activity changes decreased with increasing lactation class.

3.4. Relation of Chewing Behavior Traits and Lactation Class with Efficiency Parameters

The three chewing sensor traits rumination time, eating time and number of activity changes, all corrected for days in milk and feed quality score, had no significant relation with the variation in the day-based efficiency parameter, FE_{day} . By contrast, corrected rumination time significantly influenced the total-lactation based MPE_{TL}, albeit with a small effect size (Table 6). Moreover, lactation class significantly influenced MPE_{TL}, with animals of second lactation and third lactation onwards showing higher MPE_{TL} estimates than primiparous animals (i.e., least square means of 0.147 (SE: 0.003), 0.159 (SE: 0.002), and 0.175 (SE: 0.002) for first, second and \geq third lactation, respectively, all pairwise comparisons with *p* < 0.001). The marginal R squared values representing the variation explained by the fixed factors were 0.281 and 0.814, representing the variation explained by the whole model (Table 6).

	Intercept Rumination Time Co			tion Time Corrected	Eating Time Corrected				Activity Changes Corrected					
Response Variable	Estimate	SE	p	Estimate	SE	р	Estimate	SE	p	Estimate	SE	p	R ² m	R ² c
MPE _{TL} ¹ FE _{day} ²	0.1602 0.9938	0.0086 0.2000	*** ***	$\begin{array}{c} -2.17\times 10^{-5} \\ -6.42\times 10^{-6} \end{array}$	$6.32 imes 10^{-6} \ 1.40 imes 10^{-4}$	*** 0.963	$-5.25 imes 10^{-6}\ 1.15 imes 10^{-4}$	$8.59 imes 10^{-6}\ 1.83 imes 10^{-4}$	0.542 0.532	$\begin{array}{c} -3.32\times 10^{-6} \\ -5.06\times 10^{-4} \end{array}$	$\begin{array}{c} 2.89 \times 10^{-5} \\ 5.66 \times 10^{-4} \end{array}$	0.908 0.37	0.232 0.002	0.814 0.280

Table 6. Influence of chewing sensor variables corrected for days in milk and feed quality score on metabolic milk production efficiency calculated on the basis of total lactation data (MPE_{TL}) and feed efficiency at daily basis (FE_{day}) in Swiss organic dairy cows (N_{observations} = 534, N_{cows} = 102, N_{farms} = 2).

¹ MPE_{TL}: total lactation average daily metabolic milk production efficiency [kg ECM/kg BW^{0.75}]. ² FE_{day}: day-based feed efficiency value [kg ECM/kg DM intake]. *p* values show *t*-test results using Satterthwaite's method in linear mixed models fit by REML with the corrected chewing traits ruminate time, eating time and number of activity changes as fixed effects, for both response variables, plus lactation class (3 levels: 1st, 2nd and 3rd lactation onwards) in the MPE_{TL} model, and cow nested within farm as random effect in both models. R² m = marginal R squared, i.e., proportion of variance explained by the fixed effects, R² c = conditional R squared, i.e., proportion of variance explained by the fixed and random effects. *** p < 0.001.

3.5. Animal and Farm Effects

The variability between animals and between farms was observed in all chewing sensor variables (Figure 3). The respective coefficients of animal variability for eating time, rumination time and activity changes were 14.6%, 19.2% and 16.4%.



Figure 3. Plots of the random effects farm and animal from models on chewing sensor variables. The plots show the mean effect (dot) and the confidence interval (horizontal lines) of farm or animal on the respective mean model estimate, represented by the 0 value.

For all investigated traits, models with animal and farm as random effects differed significantly from models without a random effect at p < 0.001, showing that the inclusion of the random effects was reasonable in order to explain the variation in the data. The only random effect without a significant effect was farm for the variable MPE_TL.

4. Discussion

Grassland-based low-concentrate dairy cattle systems can claim high sustainability in environmental scales, if human-edible feed conversion efficiency [3] and land-surface-related effects such as eutrophication potential are considered [33,34]. However, even if differentiated land use (arable land versus permanent grassland) is one of the core arguments pro low-input dairy production [34], also, in such systems, feed conversion efficiency must not be neglected. The detection of cow types that are resilient in low-input grassland systems and show fair milk yields at the same time is a challenge [6]. For selection in such systems, it is important to have more phenotypic traits besides milk yields, fitness and fertility, because feed intake is almost impossible to control. An increasing number of sensors are currently appearing on the market that detect the chewing movements of the individual animals. Such chewing behavior data may be used for the detection of estrus [35], diseases [36] or the start of parturition [12], and there appears to be potential for estimating intake [15]or even efficiency by such approaches. In particular, the head-collar-based devices appear to have high accuracy, because they directly measure jaw movements [13,17]. For such devices, equations for absolute [16] or relative [15] intake estimates have been developed. Therefore, they might be helpful as indirect proxies for the efficiency of individual cows. Whether or not this is possible under practice conditions on-farm is, however, not proven.

Instead of standardized station experiments, which may be better controlled, but are also always specific cases [37], the immanent diversity of low-input production systems requires repeated data collections and evaluation under varying on-farm practices [1,38]. The current study provides such on-farm-generated data showing the feasibility of sensor-based phenotypic relations between chewing behavior, milk yield development and efficiency estimates in dairy cows with altering forage quality on low-input farms.

We used the system Rumiwatch[®], which has high precision in detecting eating and rumination patterns [17,39]. The initial goal had been a sound estimate for intake on pasture with the sensor head collars. However, during the current study, it became clear that none of the existing equations [15,16] would lead to plausible intake data for the herds investigated. In this light, we decided to use intake [26] and efficiency [27] estimates based on cow performance data and evaluate whether or not the sensor data would relate to them.

In a first step, the general sensitivity of chewing sensor variables towards different nutritional quality of forages (pasture, hay and mixed) was assessed. The finding that eating time was strongly affected by feed quality was well in line with earlier studies, which, however, did not report clear feed quality effects on rumination time [40–42]. Nonetheless, there are other studies reporting such effects on rumination [18,43,44] and the results of the present study appear plausible in terms of the direction and strength of these effects.

The development of eating time across lactation (Figure 2) reflected common intake curves, and the curve of rumination time along DIM was similar to the one published by Zetouni et al. [20]. The development of rumination time during lactation appeared reciprocal to that of eating time, decreasing when eating time increased and vice versa (Figure 2A). In addition, in the literature [18–20], there seems to be a slight partial antagonism between intake (eat time) and rumination time. This points towards a potential time conflict between these activities. Since the eating time development with lactation in our data followed the commonly known intake curve during lactation, we assume that, given an existing time conflict between eating and rumination, the former dominates the curve of the latter. Thus, when a high feed intake is required (as, for instance, during high performance in the first months of lactation), less intensive rumination and, eventually, less efficient feed degradation might happen. This context will, however, need further investigation.

Additionally, our study showed the effects of animal on chewing sensor variables, with considerable variation in the individual effects. The coefficients of variance of the animal effects on eating and rumination time were well in line with the animal variability reported by [20,38]. We could show that the variation among animals is significant, even if corrected for feed effects and days in milk, indicating a potential to phenotype individuals based on sensor variables for foraging behavior. This underlines that sensor-based phenotypes can be related to chewing activity patterns as such, but also in response to feed quality changes and in development during lactation. The practical applicability of these phenotypes with respect to the resilience or productivity of the cow still needs to be further elaborated.

While we were able to link behavioral changes directly to feed quality and stage of lactation, we did not find clear relations between the applied efficiency estimates and the behavioral data. One of the main issues in studies in pasture-based systems is the difficulty to gain an appropriate estimate for feed intake on pasture [45], which was a relevant obstacle for FE_{day} estimates in the current study. None of the efficiency estimates applied was related to chewing sensor variables. Generally, the fact that effect estimates were very small shows that there was no functional relation between chewing behavior and both chosen efficiency parameters. Even though a significant effect of corrected rumination time on MPE_{TL} was detected, it had such a small effect size (a longer rumination time of 100 min/day would reduce MPE_{TL} by roughly 1%) that its relevance is negligible.

Considering that sensor data were obtained only a few times during the lactation of the individual animals, it appears plausible that the regression on an efficiency parameter, which builds on whole lactation data (MPE_{TL}), was weak. However, the rather low frequency of data collection is a real constraint in the practice of pasture-based production systems, and it makes rather limited sense to test sensors at unrealistically high usage frequencies, which are subsequently not affordable. Moreover, the absence of any effects in the FE_{day} model indicates that there was no correlation between chewing behavior and estimated feed efficiency in the studied herds. This makes it rather unlikely that chewing sensors could be successfully used to approximate the efficiency of dairy cows in low-concentrate pasture-based dairy systems. There may be potential if chewing sensor data are used as auxiliary traits in models when intake is known [46], but under barely controllable practice conditions with pasture, as in the current study, the predictive use for efficiency appears limited. The lack of intake data will remain difficult to overcome in pasture-based systems. We have used two efficiency estimates in order to see trends at least, but it appears that the road to efficiency proxies in these contexts is still long.

The curves for body weight and BCS development, as well as intake estimates along the days in milk, are plausibly in accordance with expectations of physiological development in the lactating cow. The ECM curve, which was estimated based on the Swiss herd book test-day data, did not show the expected early-lactation increase, because the first test day is usually one month after parturition.

5. Conclusions

The present study assessed the potential of chewing sensor data as traits for contributing to the estimation of the individual production efficiency of dairy cows in low-input pasture-based systems. Clear animal-related variance in eating and rumination time was found, and effects of nutritional feed quality and days in milk on these variables were detected, which may be useful information in low-input breed phenotyping. After correction for these effects, no notable relation of eating and rumination time with efficiency approximations was found. This lack of effect was found regardless of whether efficiency had been calculated as milk yield over metabolic body weight on lactation level or as milk yield over estimated intake on day level. Thus, in pasture-based systems, the difficulty of quantifying intake remains an obstacle, which cannot be overcome by the use of chewing sensors only. However, the sensor data may serve as interesting phenotypes, in response to feed quality and stage of lactation. To what degree the individual variation of the sensor traits may be indicative of the adaptability and thus resilience of low-input dairy cows is subject to further investigation.

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