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Assessing short-term feed efficiency and its association with biological markers in herbage-fed dairy cows

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ABSTRACT

Feed efficiency is an important trait of dairy production. However, assessing feed efficiency is constrained by the associated cost and difficulty in measuring individual feed intake, especially on pastures. The objective of this study was to investigate short-term feed efficiency traits of herbage-fed dairy cows and screening of potential biomarkers (n = 238). Derived feed efficiency traits were ratio-based (i.e., feed conversion ratio (**FCR**) and N use efficiency (**NUE**)) or residual-based (i.e., residual feed intake (**RFI**), residual energy intake (**REI**), and residual N intake (**RNI**)). Thirty-eight Holstein and 16 Swiss Fleckvieh dairy cows underwent a 7-d measurement period during mid- and/or late-lactation. The experimental data (n = 100 measurement points) covered different lactational and herbage-fed system situations: mid-lactation grazing (n = 56), late-lactation grazing (n = 28), and late-lactation barn feeding (n = 16). During each measuring period, the individual herbage intake of each cow was estimated using the n-alkane marker technique. For each cow, biomarkers representing milk constituents (n = 109), animal characteristics (n = 13), behaviour, and activity (n = 46), breath emissions (n = 3), blood constituents (n = 35), surface, and rectal temperature (n = 29), hair cortisol (n = 1), and near-infrared (**NIR**) spectra of faeces and milk (n = 2) were obtained. The relationships between biomarkers and efficiency traits were statistically analysed with univariate linear regression and for NIR spectra using partial least squares regression with feed efficiency traits. The feed efficiency traits were interrelated with each other (r: −0.57 to −0.86 and 0.49–0.81). The biomarkers showed varying R^2 values in explaining the variability of feed efficiency traits (FCR: 0.00–0.66, NUE: 0.00–0.74, RFI: 0.00–0.56, REI: 0.00–0.69, RNI: 0.00–0.89). Overall, the feed efficiency traits were best explained by NIR spectral characteristics of milk and faeces (R^2: 0.25–0.89). Biomarkers show potential for predicting feed efficiency in herbage-fed dairy cows. NIR spectra data analysis of milk and faeces presents a promising method for estimating individual feed efficiency upon further validation of prediction models. Future applications will depend on the ability to improve the robustness of biomarkers to predict feed efficiency in a greater variety of environments (locations), managing conditions, feeding systems, production intensities, and other aspects.

Implications

Achieving greater efficiency in livestock production is paramount to improve profitability and reduce its environmental footprint. The dairy industry has been slow to integrate feed efficiency into its breeding goals. This is primarily attributed to a lack of accurate feed intake data, especially in pasture-based feeding systems and a lack of consistency in defining feed efficiency. Therefore, considerable interest remains in finding proxies for predicting feed efficiency on a routine basis. Multiple biomarkers, especially spectra analysis of milk and faeces, showed potential for predicting feed efficiency. Therefore, biomarkers present a promising method for estimating individual feed efficiency in grazing dairy cows.

Introduction

To achieve greater sustainability in dairy production, it is paramount to improve productivity, profitability and reduce its environmental footprint. Due to limited resource availability,

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strategies must be developed to optimise the efficiency of animal-based food production. Feed efficiency is a key factor to improve profitability and sustainability of the sector (Cantalapiedra-Hijar et al., 2018). However, feed efficiency is a multitrait phenotype, as feed intake and nutrient utilisation are determined by multiple biological and physical mechanisms. For example, variability in feed efficiency can be due to differences in feed intake capacity and rates, digestion of feed and the associated energy costs, absorption of nutrients, metabolism, physiological stage, health status, rumen microbial metabolism, activity, and thermoregulation (Li et al., 2016). In cattle, feed efficiency is moderately heritable and appears to be low compared to other livestock (Taussat et al., 2020). However, it is highly variable between animals raised under similar conditions (Arthur et al., 2001), which gives rise to the possibility of improving this animal trait through genetic selection. Overall, two different metrics reflecting different underlying mechanisms are typically used as measures of feed efficiency in cattle. It is either commonly implemented as ratio traits between an animal's input and output (e.g., feed conversion ratio (**FCR**) and N use efficiency (**NUE**)) or as residual intake (i.e., residual feed intake (**RFI**), residual energy intake (**REI**), and residual N intake (**RNI**)), which is the difference between observed and predicted intake after accounting for energy or protein sinks. In terms of residual intake, more efficient cows have lower values compared to less efficient cows or even negative values, as they consume less in relation to standard nutrient requirements.

Regardless of the definition, the greatest challenge in assessing feed efficiency is the measurement of individual animal DM intake (**DMI**). While measurements may be accurate, their use in dairy cattle, especially on pasture has generally been limited to smaller research herds due to workload, feasibility and high cost. Thus, it is applicable to find proxies that are phenotypically accurate and genetically related to feed efficiency traits, especially for animals in high-forage or pasture-based feeding systems (Toral et al., 2021). The objectives of this study were (1) to screen a multitude of biomarkers with the goal to identify valid and widely applicable biomarkers for short-term feed efficiency of grazing lactating dairy cows, and (2) to investigate the relationship between short-term feed efficiency traits.

Material and methods

Experimental design, animals, and housing

Three grazing trials were conducted at the organic farm Ferme École in Sorens, Switzerland, and one at the Agroscope experimental farm in Posieux, Switzerland from 2018 to 2019. Details about the trials are presented in Table 1. Before selecting the experimental cows, all cows passed a medical check before each trial, which encompassed vital parameters and udder and claw health. In Sorens, for the flow of work and equipment reasons, the cows were equally divided into two consecutive data collection periods, with a 21-d adaptation period followed by a 7-d measuring period. Twenty-eight grazing dairy cows, comprising 14 Swiss Holstein and 14 Swiss Fleckvieh, were formed in matched pairs of Swiss Holstein and Swiss Fleckvieh cows according to parity, days in milk, and BW. In trials one, two and three at Sorens, the experimental cows were in milk for an average of 128, 222 and 122 days, respectively. All experimental cows grazed in a single herd in a rotational grazing system. The pastures were long established, composed predominantly of grasses (86.4%; mainly *Lolium perenne* and *Poa pratensis*), herbs (4.7%; mainly *Taraxacum officinale* and *Plantago lanceolata*) and clover (8.9%; mainly *Trifolium repens* and *T. pratense*). The two daily grazing periods together lasted between 16 and 19 h. The cows were housed in a free-stall barn and milked at 0530 and 1630 h in a parlour in the barn. Around 2 h after milking, the cows were released to pasture. In the second trial at Sorens, the cows were brought into the barn about 3 h earlier in the afternoon than in trials 1 and 3 to reduce heat exposure. Concentrate (No. 8311, Mühle Rytz AG, Biberen, Switzerland) was allocated to the animals, according to days in milk, and provided through an automatic concentrate feeder in the barn. In Posieux, the trial consisted of a 21-d adaptation period, followed by a 7-d measuring period. Sixteen Swiss Holstein cows, with an average of 233 days in milk and accustomed to herbage-based ration, were fed with fresh cut herbage for *ad libitum* intake using weighing troughs (Insentec B.V., Marknesse, Netherlands). The mown herbage was composed predominantly of grasses (57.4%; mainly *L. perenne* and *P. pratensis*) and clover (42.6%, mainly *T. repens* and *T. pratense).* The cows were housed in a free-stall barn and milked at 0530 and 1630 h in a parlour. In Sorens and Posieux, the cows had access to mineral salt and water. For sampling reasons and dotriacontane administration, at both sites, the animals were briefly tethered in cubicles after milking.

Data recording, measurements and sample collection

Milk yield was measured twice daily in the milking parlour (Sorens: MidiLine, DeLaval AG, Sursee, Switzerland; Posieux: Fullwood, Arnold Bertschy AG, Guschelmuth, Switzerland; with an MM15 (Sorens: DeLaval AG) and Pulsameter 2 (Posieux: SAC, Kolding, Denmark). Milk composition was determined for all trials on days 1, 4, and 7 during the measuring week. Aliquots of subsamples from morning and evening milking were pooled and preserved in one sample tube per cow containing a Broad-Spectrum Microtab II (Gerber Instruments AG, Effretikon, Switzerland) and stored at 8 °C for subsequent analysis of milk fat, protein, lactose, and casein content. Further subsamples were pooled per week per cow and frozen at −20 °C without preserving agents. These pooled subsamples of frozen milk were lyophilised (Delta 1–24 LSC; Christ, Osterode, Germany) and subsequently milled with a grinder (Vertec, JNJ Automation SA, Romont, Switzerland). After each milking, BW was measured with a constrained walk-over animal weighing system (Sorens: W-2000, DeLaval; Posieux: Ga5010, Insentec B.V., Marknesse, Netherlands). The body condition score was assessed according to a five-point system (1 = thin, 5 = fat; Edmonson et al., 1989) by the same, experienced person before the first measurement period of each trial.

Individual herbage intake was estimated using the n-alkane double-indicator method used in a similar way as described by Rombach et al. (2019): From 6 days before until the next-to-last day of each measuring week, cows were dosed twice daily with one gelatine capsule (HGK-17-60 sl; Capsula GmbH, Ratingen, Germany) containing 0.5 g of dotriacontane (C32H66, HC32; Minakem Beuvry Production S.A.S., Beuvry la Forêt, France) as the external alkane marker on a carrier of 4.5 g of dried fruit pomace. During the 7 d, once per day after the morning milking, the faeces of each cow were spot-sampled indoors to determine the concentration of alkanes. Samples were taken from spontaneous defecations or with mild stimuli, pooled for each cow and measuring week, and stored at −20 °C until lyophilisation. To determine the concentration of alkanes in the herbage eaten by the cows, herbage collection was carried out for 7 d in the morning and afternoon. For Sorens, samples were taken from herbage cut with a battery grass shearer (Gardena, Husqvarna Schweiz AG, Maegenwil, Switzerland) by following cows and mimicking their selections. In Posieux, herbage samples were obtained from the weighing troughs by random sampling with an auger. Herbage sampling started 24 h before faeces sampling and ended 24 h earlier. These samples were chopped and stored at −20 °C until lyophilisation. The frozen faeces and herbage samples were lyophilised (Delta 1–24 LSC; Christ, Oster-

Table 1
General information about the trials: location, dairy cow key figures and efficiency criteria (n = 100; numbers are means with their SD in parentheses).

Item	Trials			
	1	2	3	4
Farm	Sorens	Sorens	Sorens	Posieux
Location	Pasture	Pasture	Pasture	Barn
Number of Cows	28	28	28	16
Breed	HO, FV	HO, FV	HO, FV	HO
Parity (% prim.)	64.3	64.3	14.3	25.0
Days in milk (d)	128 (±29)	222 (±35)	122 (±35)	233 (±18)
Milk (kg/d)	22.5 (±3.6)	16.0 (±3.6)	21.9 (±4.7)	22.6 (±3.0)
BW (kg)	577 (±43)	617 (±53)	618 (±43)	670 (±48)
DMI (kg DM/d)	14.0 (±2.1)	14.5 (±1.6)	15.6 (±1.8)	20.9 (±1.8)
Herbage	13.3 (±1.6)	14.2 (±1.6)	14.6 (±1.7)	19.9 (±1.8)
Concentrate	0.7 (±1.1)	0.3 (±0.3)	1.0 (±1.0)	0.7 (±0.1)
FCR	0.66 (±0.08)	0.89 (±0.20)	0.79 (±0.13)	0.88 (±0.08)
NUE	0.29 (±0.03)	0.21 (±0.03)	0.30 (±0.06)	0.19 (±0.02)
RFI (kg DM/d)	−2.17 (±1.87)	−0.88 (±1.84)	−0.59 (±1.85)	0.17 (±1.48)
REI (MJ NEL/d)	−20.5 (±10.32)	−3.68 (±11.06)	−17.68 (±11.67)	2.68 (±7.16)
RNI (g N /d)	3.01 (±36.58)	91.5 (±45.61)	−8.36 (±49.25)	272.8 (±38.6)

Abbreviations: DMI = DM intake; FCR = feed conversion ratio; FV = Swiss Fleckvieh; HO = Swiss Holstein; NEL = net energy of lactation; NUE = N use efficiency; prim. = primiparous; REI = residual energy intake; RFI = residual feed intake; RNI = residual N intake.

ode, Germany). Subsequently, all samples, including concentrate supplements, were milled through a 1.0-mm screen (Brabender mill with titanium blades; Brabender GmbH & Co. KG, Duisburg, Germany).

Each cow's eating and rumination behaviour were recorded during the entire respective measurement periods using an automatic jaw movement recorder (RumiWatch System; Itin and Hoch GmbH, Liestal, Switzerland; validated by Rombach et al., 2018). A similar approach was taken as in (Rombach et al., 2019): To accustom the cows to the RumiWatch halter, they were attached to the cows 4 d before the start and left on until the end of each measurement period. The data were read through the interface software RumiWatch Manager (version 2.2.0.0; Itin and Hoch GmbH) and processed using the evaluation software RumiWatch Converter (version 0.7.3.36; Itin and Hoch GmbH). The activity of each cow (time spent standing, lying, and walking, and the number of steps) and the motion index (a summed indication of motion in all three dimensions) were determined during the measurement period using a pedometer (RumiWatch Pedometer; Itin and Hoch GmbH). The device was attached to the left-hind leg at the metatarsus level. The cows were accustomed to the pedometer for at least 4 d. The data were read through the interface software RumiWatch Manager (version 2.2.0.0; Itin and Hoch GmbH) and compiled over 24 h intervals using the evaluation software RumiWatch Converter Version 0.7.3.36.

Venous blood was collected once at 0700 h in the middle of each measurement period by puncture of the jugular vein using the Vacuette® System (Greiner Bio-One GmbH, Kremsmünster, Austria). Plasma was obtained using Vacuette® EDTA tubes, and serum was acquired with Vacuette® serum tubes. After sampling, the tubes were cooled in ice water until further processing. Vacuette® EDTA and Vacuette® serum tubes were stored upside down for at least 1 h at room temperature. The Vacuette® serum tubes were inversed 10–15 times before analysis. The Vacuette® serum tubes were centrifuged at 3 000 × *g* for 15 min and then at 4 000 × *g* for an additional 2 min and stored in Eppendorf vials at −20 °C (Thanner et al., 2014). Whole blood was obtained with Vacuette® serum tubes, emptied into small, round aluminum trays, and stored at −20 °C until being lyophilised (Delta 1–24 LSC; Christ, Osterode, Germany). The hair from the rump above the hip was clipped once with electric hair clippers (Delta 3, Heiniger, Herzogenbuchsee, Switzerland) at the start of each trial and for sampling at the end of the measurement period (28 d regrowth). This procedure was chosen mainly to account for cortisol deposition during the experiment. The hair was cut as close to the skin as possible and stored at room temperature in the dark until processed.

Individual spot measurements of methane (CH_4), carbon dioxide (CO_2) emission, and oxygen (O_2) consumption were made using the GreenFeed® system (C-Lock Technology Inc., Rapid City, SD, USA). The system consists of a mobile feeding station with integrated gas flow measurement equipment. The cows were accustomed to the Greenfeed® during the last week of the adaptation period, and data recording took place during the entire measurement period. Cows were allowed a maximum of four visits to the station over a day, one visit per 4-h time slot, and encouraged to stay by releasing, per visit, up to eight portions of 32 g of bait feed at 20-s intervals. The calculation of methane production per day from the data obtained per visit and day was made according to Huhtanen et al. (2015). During the measurement period in Sorens, the dairy cows had access to one GreenFeed® unit in the barn and one on pasture. In Posieux, dairy cows had access to two GreenFeed® units located in the barn, except during milking.

Infrared thermography was performed once at the end of each trial for all cows, according to Montanholi et al. (2010), using an IR camera (FLIR T620 Thermal Imager, FLIR Systems-Boston, North Billerica, MA, USA) with an external lens (45°). An emissivity value of 0.98 was used following the manufacturer's recommendation for biological tissue. IR images of multiple (n = 28) body locations were taken at the end of each measurement periods between 0600 and 0800 h, while being held in a self-locking yoke. The IR images of the left and right sides of the rump, hip, back and front legs, claws, flank, ribs, and ears of the animal were taken, as well as one image of the backside, backside of udder, neck, head, and nose. All IR images were taken at a distance of 1.50 m from each of the body locations. The IR was analysed using FLIR Tools software (FLIR Systems, Inc., Wilsonville, OR, USA). Five variables were defined for each body location, representing the average of maximum and minimum, the average of analysed area, and the minimum and the maximum surface temperatures of the sub-area. Before thermal images were taken, during trials 2 and 3 in Sorens and during trial 4 in Posieux, the rectal temperature was measured using a digital thermometer (SC 12, SCALA Electronic GmbH, Stahnsdorf, Germany).

Laboratory analysis

The three aliquot milk samples per measurement period were analysed to determine fat, protein, casein, and lactose content, using Fourier-transform mid-IR spectrometry (Combi-Foss FT +;

Foss, Hillerød, Denmark). The number of somatic cells in milk was counted with fluorescence flow cytometry (Fossomatic FC200; Foss). Additionally, one aliquot sample was produced out of the three milk samples per measurement period. Pooled (according to individual milk yield) urea in milk was analysed with a differential pH analyser (Eurochem, Ardea, Italy) before and after hydrolysis with urease (International Dairy Federation, 2004). The milk fatty acid (**FA**) composition was determined using high-resolution gas chromatography with flame ionisation detection (Collomb and Bühler, 2000). The results were expressed in g FA per 100 g fat.

The samples of concentrates and lyophilised herbage were dried for 3 h at 105 °C to analysed DM, and subsequently incinerated at 550 °C until a stable mass was obtained to determine the ash content. The contents of the HC32 and tritriacontane (C33H68) were determined as described by Thanner et al. (2014). The herbage and supplement N contents were analysed using the Dumas method (ISO 16634-1: 2008) on a C/N analyser (Trumac CNS; Leco Instruments, St. Joseph, MI). The N content of the samples was multiplied by 6.25 to get the CP content. The contents of acid detergent fibre (ADF, ISO 13906:2008) and neutral detergent fibre (NDF, ISO 16472:2006) for the herbage and supplement samples were analysed with Gerhardt Fibretherm (Gerhardt GmbH & Co. KG, Königswinter, Germany). For the NDF analysis, heat-stable amylase and sodium sulphite were used. Prior to the ADF determination, an NDF procedure was performed. A correction for the residual ash (2 h of incineration at 550 °C) was made for the ADF and NDF values. Organic matter digestibility of lyophilised herbage samples was determined according to Tilley and Terry (1963). The organic matter digestibility of concentrate was based on a regression for wheat-containing concentrate (Agroscope, 2021).

Near-infrared (**NIR**) spectra were recorded from freeze-dried faeces and milk (week-pooled) with a NIRFlex N-500 (Büchi, Flawil, Switzerland) equipped with a rotary cup. For each sample, three replicates were taken, with 21 scans per replicate, in the range of 4 000–10 000 cm^{-1}. Repeated measurements were averaged to one measurement per cow per trial.

Metabolite concentrations, hormones, and enzyme activity in plasma and serum were determined using the following methods: albumin (No. 103016; Greiner, Pleidelsheim, Germany), alkaline phosphatase (No. 12117; Phosphatase Alkaline, Human, Wiesbaden, Germany), asparate-aminotransferase (No. 12011, ASAT/GOT, Human), b-hydroxybutyrate (No. RB 1007; Randox Laboratories, Crumlin, UK), cholesterol (No. TA-135L; Cliniline SA, Vionnaz, Switzerland), creatine kinase (No. 120.016 CK NAC, Cliniline SA) creatinine (No. T-146 L, Creatinine JK, Cliniline SA), gamma-glutamylglucose (No. 1447513,; Roche diagnostics; Basel, Switzerland), non-esterified fatty acids (**NEFA**: FA115; Randox laboratories), total protein (TP: No. 1553836; Roche diagnostics), triacylglycerides (TGL: No. 61236; bioMerieux; Marcy l'Etoile, France), urea (No. 61974, UV 250; bioMerieux), alanine aminotransferase (ALAT: No. 63312; bioMerieux), aspartate aminotransferase (ASAT: No. 63212; bioMerieux), creatine kinase (CK: No. 61141; bioMerieux), cholecystokinin octapeptide (CCK-8) (Bovine CCK8 Elisa kit, Mybiosource, San Diego, CA, USA), and leptin (Multi-Species Leptin kit XL-85K, EMD Milipore Corp., Darmstadt, Germany). Plasma insulin (Porcine Insulin radioimmunoassay (RIA) kit PI-12K, EMD Milipore Corp.) and insulin-like growth factor-1 (IGF-1) concentrations were measured using kit no. A15729 from Beckman Coulter (Fullerton, CA, USA). 3,5,3′trijodthyronine (T3) and thyroxin (T4) were measured by RIA using a Coat-A-Count® Total T3 kit (Siemens Schweiz AG, Zurich, Switzerland) and Coat-A-Count® Total T4 kit (Siemens Schweiz AG), respectively.

Hair samples were weighed, washed, and ground, as suggested by Davenport et al. (2006). Hair cortisol was extracted following the procedure of Koren et al. (2002). Hair cortisol was analysed using a commercially available assay kit designed for salivary cortisol (Salimetrics Expanded Range, High Sensitivity 1-E3002, State College, PA, USA). For the nitrogen isotope analysis, the lyophilised samples of herbage, faeces, milk, blood, and concentrates were ground using a ball mill (Retsch MM 400, Retsch GmbH, Haan, Germany). A 4 mg subsample was used to determine the N content and the ^{15}N enrichment in the samples as the ratio of ^{15}N:^{14}N compared to a standard (air-N_2). The measurement of the N and $\delta^{15}N$ values was performed using a Flash EA 1112 Series elemental analyser (Thermo Italy, Rhodano, Italy) coupled to a Finnigan MAT DeltaplusXP isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) via a 6-port valve and a ConFlo III (Brooks et al., 2003).

Calculations and statistical analysis

All calculations were performed using the mean values for each measurement week. The energy-corrected milk yield (**ECM**) was calculated based on a 4% fat, 3.2% protein, and 4.8% lactose standard (Agroscope, 2021). Herbage DMI (**HDMI**) estimation was based on the equations proposed by Mayes et al. (1986). The following equation was used to calculate the daily HDMI of every single experimental cow in the four experiments:

$$HDMI = \frac{\frac{F33}{F32} \times \{[A32 + (C \times C32)] - (C \times C33)\}}{H33 - \frac{F33}{F32} \times H32}$$

where HDMI represents the daily herbage DMI (kg); *F*33, *H*33, and *C*33 are the concentrations of tritriacontane (mg/kg of DM) in faeces, herbage, and concentrate, respectively; *F*32, *H*32, and *C*32 are the concentrations of HC32 (mg/kg DM) in faeces, herbage, and concentrate, respectively; *C* is the concentrate intake (kg of DM/d); *A*32 is the daily dose of HC32 (mg/d) administered via the gelatine capsules.

Efficiency traits

The FCR was calculated as follows:

$$FCR = \frac{DMI\ (kg/d)}{ECM\ (kg/d)}$$

The daily N intake was calculated from DMI and the CP content of the diets and then divided by 6.25; milk N content was calculated from the concentration of milk true protein analysed divided by 6.38. NUE was calculated as follows:

$$NUE = \frac{milk\ N\ yield\ (kg/d)}{N\ intake\ (kg/d)}$$

The predicted daily total DMI for individual cows was calculated based on ECM, lactation week and number as well as corrections for ration composition (Agroscope, 2021; chapter 7.6). Finally, the individual RFI (kg DM) of dairy cows results from the difference of the actual minus the predicted total DMI. The daily predicted net energy of lactation and nitrogen (CP requirements divided by 6.25) requirements for individual dairy cows were determined based on the respective requirements for maintenance and ECM production (Agroscope, 2021; table 7.1 in chapter 7.9). Finally, to obtain REI or RNI, the predicted net energy of lactation or N requirement was subtracted from the actual net energy of lactation or N intake, respectively.

Statistical analysis

The linear relationships within and between FCR, NUE RFI, REI, and RNI in mid- and late-lactation were assessed using Pearson correlations. Data from potential markers were collected over several days and averaged per cow for each measurement period. The

"Hmisc" package (version 5.1–1) of R statistical software (R Core Team, 2021) was used to determine the simple linear regression of feed efficiencies with markers and traits. The NIR models were developed with R (version 4.3.3; R Core Team, 2021) with "caret" package (version 6.0–94; Kuhn et al., 2023). Calculating the leave-one-out cross-validated partial least squares regression and data pretreatment: with signal package (v0.7–7; Ligges et al., 2015), with normalisation standard normal variate and 1st derivative Savitzky-Golay 9 points Gap 2.

Results

Feed efficiency traits – values and correlations

Feed efficiency trait values from a total of 100 measurements (n = 100) over one week based on 54 different cows ranged from 0.44 to 1.59 kg DMI/kg ECM for FCR, 0.12–0.53 g milk N/g N intake for NUE, −5.82–4.65 kg DM/d for RFI, −57.5–23.5 MJ net energy of lactation/d for REI, and −131.6–332.7 g N/d for RNI (Table 1). The correlation coefficients shown in Table 2 indicated moderate to very strong relationships (r = -0.57 to −0.86 and r = 0.49–0.81) between the feed efficiency traits FCR, NUE, RFI, REI, and RNI.

Relationship between feed efficiencies and markers

The coefficient of determination of the linear relationship of individual markers used to explain the variance of feed efficiency traits ranged from none to very strong, at 0.00–0.89 (Tables 3–7). The largest proportion of variance in short-term feed efficiency was explained by RNI (R^2 = 0.89), followed by NUE (R^2 = 0.74), FCR (R^2 = 0.66), REI (R^2 = 0.69), and RFI (R^2 = 0.56). Furthermore, Tables 3–7 contain the three best markers for each marker group for FCR, NUE, RFI, REI and RNI, respectively. The complete information for markers (n = 238) can be found in Supplementary Tables S1–S7.

Discussion

Relationships among feed efficiency traits

Feed efficiency traits, especially residual intakes, seem to have high repeatability and moderate heritability in dairy cows (Connor et al., 2013). However, limited knowledge exists in terms of relationships between different feed efficiency traits in grazing, lactating dairy cows. Our results indicate that the investigated feed efficiency traits were moderately to strongly correlated with each other at the same time point. These results are consistent with those of Liu and VandeHaar (2020) who concluded that energy−efficient cows appear to be also protein efficient. Similarly, Xie et al. (2021) found that the utilisation of metabolisable protein for milk protein, and mammary amino acid utilisation was more efficient in cows with a lower RFI. Variation in dairy cattle maintenance requirements (at the same feed intake and milk production) can also account for up to 37.6% of the variation in milk energy efficiency (Onken et al., 2011). This variation is mainly driven by protein turnover, ion pumping and proton leakage which is a major function of maintenance requirements and accounts for 30–40% of basal energy expenditure (Baldwin, 1968). Thus, the phenotypic selection of cows for improved energy efficiency (i.e., FCR, RFI, or REI) will also result in improved N efficiency (NUE or RNI).

Assessing potential biomarker relationships with feed efficiency

The present study investigated a plethora of biomarkers (n = 238) and their relations to feed efficiency traits of lactating herbage-fed dairy cows. Therefore, in the subsequent discussion, promising biomarkers are grouped according to the following marker groups: milk constituents, animal characteristics, behaviour and activity, breath emissions, blood constituents, surface and rectal temperature, hair cortisol, and NIR spectra.

Table 2
Relationship between short-term feed efficiency traits of dairy cows on herbage-based diets.

Feed efficiency	r	R^2	RMSE	*P*-value	α	β
FCR vs						
NUE	−0.78	0.60	0.10	< 0.001	1.33	−2.09
RFI	0.78	0.61	0.10	< 0.001	0.86	0.07
REI	0.80	0.65	0.10	< 0.001	0.90	0.01
RNI	0.54	0.29	0.14	< 0.001	0.74	0.00
NUE vs						
FCR	−0.78	0.60	0.04	< 0.001	0.48	−0.29
RFI	−0.57	0.32	0.05	< 0.001	0.24	−0.02
REI	−0.86	0.74	0.03	< 0.001	0.21	0.00
RNI	−0.79	0.62	0.04	< 0.001	0.28	0.00
RFI vs						
FCR	0.78	0.61	1.21	< 0.001	−8.41	9.32
NUE	−0.57	0.32	1.60	< 0.001	3.62	−18.19
REI	0.74	0.55	1.29	< 0.001	0.19	0.10
RNI	0.49	0.24	1.69	< 0.001	−1.59	0.01
REI vs						
FCR	0.80	0.65	8.17	< 0.001	−65.65	68.28
NUE	−0.86	0.74	7.01	< 0.001	38.58	−196.72
RFI	0.74	0.55	9.19	< 0.001	−6.02	5.29
RNI	0.81	0.65	8.13	< 0.001	−18.28	0.10
RNI vs						
FCR	0.54	0.29	90.10	< 0.001	−217.03	357.48
NUE	−0.79	0.62	65.71	< 0.001	423.82	−1405.42
RFI	0.49	0.24	93.45	< 0.001	94.57	27.04
REI	0.81	0.65	63.32	< 0.001	138.46	6.28

Abbreviations: α = intercept ; β = slope (coefficient); FCR = feed conversion ratio; NEL = net energy of lactation; NUE = N use efficiency; REI = residual energy intake (MJ NEL/d); RFI = residual feed intake (kg DM/d); RNI = residual N intake (g N/d).

Table 3
Relationship between markers and short-term feed conversion ratio in herbage-fed dairy cows, including the three best markers per marker group (milk constituents, animal characteristics, behaviour and activity, breath emissions, blood constituents, surface temperature, hair cortisol, and NIR spectra of milk and faeces).

Item	Biomarker	Milk constituents	Animal characteristics	Behaviour & activity	Breath emissions	Blood constituents	Temperature	Hair	NIR spectra
Range	r	−0.40–0.62	−0.61–0.35	−0.33–0.29	−0.21–0.75	−0.49–0.57	−0.38–0.23	−0.20	0.66–0.81
	R^2	0.00–0.39	0.01–0.38	0.00–0.11	0.03–0.56	0.00–0.33	0.01–0.14	0.04	0.43–0.66
	RMSE	0.13–0.18	0.13–0.16	0.15–0.16	0.11–0.16	0.13–0.16	0.15–0.16	0.16	0.09–0.12
	n	109	13	46	3	35	29	1	2
Rank									
1	Biomarker	Δ15N (‰)	Milk production (kg)	Strides (n/d)	CO_2 (g/kg ECM)	Δ15N (‰)	Leg back r. max. (°C)	Cortisol (ng/g)	Milk (nm)
	r	0.62	−0.61	−0.33	0.75	0.57	−0.38	−0.20	0.81
	R^2	0.39	0.38	0.11	0.56	0.33	0.14	0.04	0.66
	RMSE	0.13	0.13	0.15	0.11	0.13	0.15	0.16	0.09
	P-value	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001	0.043	< 0.001
	α	0.20	1.21	0.95	0.31	0.31	1.11	0.85	
	β	0.17	−0.02	0.00	0.00	0.14	−0.01	−0.03	
2	Biomarker	β-casein (%)	Milk flow (kg/min)	Walking (min/d)	O_2 (g/kg ECM)	IGF-1 (ng/ml)	Cheeks mean min. (°C)		Faeces (nm)
	r	0.60	−0.54	−0.30	0.70	0.51	−0.38		0.66
	R^2	0.35	0.29	0.09	0.49	0.26	0.14		0.43
	RMSE	0.15	0.14	0.15	0.11	0.14	0.15		0.12
	P-value	< 0.001	< 0.001	0.004	< 0.001	< 0.001	< 0.001		< 0.001
	α	−0.22	1.14	0.96	0.32	0.58	1.09		
	β	0.36	−0.16	0.00	0.00	0.00	−0.01		
3	Biomarker	C12:1 (g/100 g FA)	BW (kg)	Stride duration (s/stride)	CH_4 (g/kg ECM)	NEFA (mmol/l)	Flank & Rib l. min. (°C)		
	r	0.59	0.35	0.29	0.68	−0.49	−0.38		
	R^2	0.35	0.12	0.09	0.46	0.24	0.13		
	RMSE	0.13	0.15	0.15	0.12	0.14	0.15		
	P-value	< 0.001	< 0.001	0.005	< 0.001	< 0.001	< 0.001		
	α	0.33	0.17	0.26	0.41	0.87	1.06		
	β	5.24	0.00	0.00	0.02	−0.49	−0,01		

Abbreviations: α = intercept; β = slope (coefficient); C12:1 = lauroleic acid; Cheeks mean min. = cheeks mean minimum; ECM = energy-corrected milk; FA = fatty acids; Flank & Rib l. min. = flank and ribs of left side minimum; IGF-1 = insulin-like growth factor 1; n = number of biomarkers; NEFA = non-esterified fatty acids; NIR = near-infrared spectroscopy; Leg back r. max. = leg back right maximum, Δ15N = 15 N animal-diet.

Table 4
Relationship between markers and short-term N use efficiency (milk N yield / N intake) in herbage-fed dairy cows, including the three best markers per marker group (milk constituents, animal characteristics, behaviour and activity, breath emissions, blood constituents, surface temperature, hair cortisol, and NIR spectra of milk and faeces).

Item	Biomarker	Milk constituents	Animal characteristics	Behaviour & activity	Breath emissions	Blood constituents	Temperature	Hair	NIR spectra
Range	r	−0.68–0.53	−0.34–0.57	−0.50–0.60	−0.44–0.53	−0.65–0.47	−0.29–0.70	0.37	0.80 – 0.86
	R^2	0.00–0.47	0.01–0.33	0.00–0.36	0.12–0.28	0.00–0.43	0.04–0.49	0.14	0.64 – 0.74
	RMSE	0.04–0.06	0.04–0.06	0.05–0.06	0.05–0.06	0.05–0.06	0.04–0.06	0.06	0.03 – 0.04
	n	109	13	46	3	35	29	1	2
Rank									
1	Biomarker	Urea (mg/kg)	Milk production (kg)	Strides (n/d)	O_2 (g/d)	$\Delta^{15}N^b$ (‰)	Foot back r. min. (°C)	Cortisol (ng/g)	Milk (nm)
	r	−0.68	0.57	0.60	0.53	−0.65	0.70	0.37	0.86
	R^2	0.47	0.33	0.36	0.28	0.43	0.49	0.14	0.74
	RMSE	0.04	0.05	0.05	0.05	0.05	0.04	0.06	0.03
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	α	0.40	0.11	0.14	0.90	0.46	0.12	0.22	
	β	0.00	0.01	0.00	0.00	−0.06	0.01	0.02	
2	Biomarker	$\Delta^{15}N$ (‰)	Milk flow (kg/min)	Walking (min/d)	CH_4 (g/d)	Urea (mmol/l)	Leg back r. surf. avg. (°C)		Faeces (nm)
	r	−0.68	0.43	0.57	0.49	−0.60	0.69		0.80
	R^2	0.46	0.19	0.32	0.24	0.36	0.48		0.64
	RMSE	0.04	0.05	0.05	0.06	0.05	0.04		0.04
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001
	α	0.20	0.17	0.14	0.88	0.37	0.07		
	β	0.17	0.02	0.00	0.00	−0.02	0.01		
3	Biomarker	β-casein (%)	BW (kg)	Stride duration (s/stride)	CO_2 (g/d)	IGF-1 (ng/ml)	Lower hip mean min. (°C)		
	r	−0.66	−0.34	−0.50	0.47	−0.55	0.68		
	R^2	0.44	0.11	0.25	0.23	0.30	0.46		
	RMSE	0.04	0.06	0.05	0.06	0.05	0.04		
	P-value	< 0.001	0.001	0.001	< 0.001	< 0.001	< 0.001		
	α	0.55	0.48	0.26	0.88	0.34	0.07		
	β	−0.11	0.00	0.00	0.00	0.00	0.01		

Abbreviations: α = intercept; β = slope (coefficient); Foot back r. min. = foot back right minimum; IGF-1 = insulin-like growth factor-1; Leg back. r. surf. avg. = leg back right surface area average; Lower hip mean min. = lower hip mean minimum; n = number of biomarkers; NIR = near-infrared spectroscopy; $\Delta^{15}N = {}^{15}N_{animal\text{-}diet}$.

Table 5
Relationship between markers and short-term residual feed intake in herbage-fed dairy cows, including the three best markers per marker group (milk constituents, animal characteristics, behaviour and activity, breath emissions, blood constituents, surface temperature, hair cortisol, and NIR spectra of milk and faeces).

Item	Biomarker	Milk constituents	Animal characteristics	Behaviour & activity	Breath emissions	Blood constituents	Temperature	Hair	NIR spectra
Range	r	−0.37–0.45	−0.48–0.30	−0.25–0.36	−0.21–0.46	−0.45–0.28	−0.22–0.12	0.05	0.50–0.74
	R^2	0.00–0.20	0.01–0.23	0.00–0.13	0.03–0.21	0.00–0.20	0.00–0.05	0.00	0.25–0.56
	RMSE	1.73–1.94	1.71–1.93	1.71–1.93	1.58–1.76	1.73–1.94	1.77–1.96	1.94	1.28– 1.68
	n	109	13	46	3	35	29	1	2
Rank									
1	Biomarker	C16:1 (g/100 g FA)	Milk flow (kg/min)	Grazing bouts (n/d)	O_2 (g/kg ECM)	NEFA (mmol/l)	Foot back r. max. (°C)	Cortisol (ng/g)	Milk (nm)
	r	0.45	−0.48	0.35	0.46	−0.45	−0.22	−0.05	0.74
	R^2	0.20	0.23	0.13	0.21	0.20	0.05	0.00	0.56
	RMSE	1.73	1.71	1.80	1.58	1.73	1.89	1.94	1.28
	P-value	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.028	0.595	< 0.001
	α	−3.57	2.49	−3.50	−4.43	−0.16	−0.10	−0.83	
	β	12.80	−1.65	0.38	0.01	−5.43	−0.06	−0.08	
2	Biomarker	C13:0 + C12:1 (g/100 g FA).	Milk production (kg)	Stride duration (s/stride)	CO_2 (g/kg ECM)	Hemoglobin (g/dl)	Leg back r. max. (°C)		Faeces (nm)
	r	0.38	−0.30	0.32	0.46	−0.34	−0.21		0.50
	R^2	0.15	0.09	0.10	0.21	0.11	0.04		0.25
	RMSE	1.79	1.85	1.71	1.59	1.83	1.90		1.68
	P-value	< 0.001	0.002	0.002	< 0.001	0.001	0.038		< 0.001
	α	−4.66	1.44	−7.78	−4.24	7.61	1.08		
	β	22.63	−0.12	0.00	0.01	−0.82	−0.09		
3	Biomarker	β-casein (%)	BW (kg)	Strides (n/d)	CH_4 (g/kg ECM)	Triglyceride (mmol/l)	Backside min. (°C)		
	r	0.37	0.30	−0.26	0.43	−0.33	−0.20		
	R^2	0.14	0.09	0.07	0.19	0.11	0.04		
	RMSE	1.79	1.85	1.75	1.61	1.83	1.91		
	P-value	0.005	0.003	0.014	< 0.001	0.001	0.046		
	α	−7.95	−7.40	0.47	−4.43	1.54	1.36		
	β	2.33	0.01	0.00	0.01	−15.95	−0.08		

Abbreviations: α = intercept; Backside min. = backside minimum; β = slope (coefficient); C13:0 + C12:1 = tridecanoic acid and fatty acids & lauroleic acid; C16:1 = hexadecenoic acid; ECM = energy-corrected milk; FA = fatty acids; Foot back r. max. = foot back right maximum; Leg back r. max. = leg back right maximum; n = number of biomarkers; NEFA = non-esterified fatty acids; NIR = near-infrared spectroscopy.

Table 6
Relationship between markers and short-term residual energy intake (MJ NEL/d) in herbage-fed dairy cows, including the three best markers per marker group (milk constituents, animal characteristics, behaviour and activity, breath emissions, blood constituents, surface temperature, hair cortisol, and NIR spectra of milk and faeces).

Item	Biomarker	Milk constituents	Animal characteristics	Behaviour & activity	Breath emissions	Blood constituents	Temperature	Hair	NIR spectra
Range	r	−0.52–0.61	−0.28–0.43	−0.57–0.51	−0.48–0.34	−0.55–0.54	−0.58–0.21	−0.28	0.73–0.83
	R^2	0.00–0.37	0.05–0.15	0.00–0.33	0.07–0.23	0.00–0.30	0.01–0.34	0.08	0.53–0.69
	RMSE	10.9–13.8	11.5–13.4	10.7–13.6	11.8–13.0	11.5–13.8	11.2–13.8	13.3	7.4– 7.7
	n	109	13	46	3	35	29	1	2
Rank									
1	Biomarker	C10:0 (g/100 g FA)	Milk flow (kg/min)	Strides (n/d)	O_2 (g/d)	NEFA (mmol/l)	Foot back r. min. (°C)	Cortisol (ng/g)	Milk (nm)
	r	0.61	−0.39	−0.57	−0.48	−0.55	−0.58	−0.28	0.83
	R^2	0.37	0.15	0.33	0.23	0.30	0.34	0.08	0.69
	RMSE	10.9	12.1	10.7	11.8	11.5	11.2	13.2	7.7
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.004	< 0.001
	α	−52.64	2.87	10.96	3.97	−4.07	13.00	−5.24	
	β	460.17	−4.42	−0.01	0.00	−47.00	−1.55	−2.92	
2	Biomarker	C10:1 (g/100 g FA)	Milk production (kg)	Walking (min/d)	CH_4 (g/d)	$\Delta^{15}N$ (‰)	Leg back r. avg. (°C)		Faeces (nm)
	r	0.57	−0.34	−0.57	−0.46	0.54	−0.34		0.73
	R^2	0.33	0.12	0.29	0.21	0.29	0.31		0.53
	RMSE	11.3	12.9	11.0	12.0	11.6	11.5		7.4
	P-value	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001
	α	−53.38	8.30	11.43	3.61	−50.38	21.22		
	β	122.72	−0.95	−0.23	−0.04	11.52	−1.59		
3	Biomarker	Urea (mg/kg)	BW (kg)	Stride duration (s/stride)	CO_2 (g/d)	Urea (mmol/l)	Udder back avg.(°C)		
	r	0.56	0.28	0.51	−0.44	0.46	−0.53		
	R^2	0.31	0.08	0.26	0.19	0.21	0.28		
	RMSE	11.4	13.2	11.2	12.1	12.2	11.6		
	P-value	< 0.001	0.005	< 0.001	< 0.001	< 0.001	< 0.001		
	α	−38.37	−54.30	−90.22	2.62	−32.10	56.08		
	β	0.09	0.07	0.05	0.00	3.56	−2.30		

Abbreviations: α = intercept; β = slope (coefficient); C10 = decanoic acid; C10:1 = decenoic acids; FA = fatty acids; Foot back r. min. = foot back right minimum; Leg back r. Avg. = leg back right average; n = number of biomarkers; NEFA = non-esterified fatty acids; NEL = net energy of lactation; NIR = near-infrared spectroscopy; Udder back avg. = udder back average; $\Delta^{15}N$ = ^{15}N isotopic discrimination.

Table 7
Relationship between markers and short-term residual N intake (g N/d) in herbage-fed dairy cows, including the three best markers per marker group (milk constituents, animal characteristics, behaviour and activity, breath emissions, blood constituents, surface temperature, hair cortisol, and NIR spectra of milk and faeces).

Item	Biomarker	Milk constituents	Animal characteristics	Behaviour & activity	Breath emissions	Blood constituents	Temperature	Hair	NIR spectra
Range	r	−0.67–0.84	−0.35–0.46	−0.86–0.81	−0.85–0.03	−0.40–0.69	−0.73–0.20	−0.35	0.91– 0.94
	R^2	0.00–0.71	0.00–0.21	0.00–0.74	0.00–0.73	0.00–0.48	0.00–0.54	0.13	0.84– 0.89
	RMSE	49.8–107.2	58.3–107.0	56.0–109.4	59.4–113.5	77.6–107.2	71.6–112.5	100.5	36.3– 43.4
	N	109	13	46	3	35	29	1	2
Rank									
1	Biomarker	Urea (mg/kg)	BW (kg)	Strides (n/d)	O_2 (g/d)	Urea (mmol/l)	Udder back min. (°C)	Cortisol (ng/g)	Milk (nm)
	r	0.84	0.46	−0.86	−0.85	0.69	−0.73	−0.35	0.94
	R^2	0.71	0.21	0.74	0.73	0.48	0.54	0.13	0.89
	RMSE	56.7	95.3	56.0	59.4	77.6	71.6	100.5	36.3
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	α	−248.34	−480.45	345.42	272.67	−175.04	385.31	125.19	
	β	1.03	0.89	−0.11	−0.03	41.45	−13.54	−27.90	
2	Biomarker	C18:1 (g/100 g FA)	Milk flow (kg/min)	Walking (min/d)	CH_4 (g/d)	$\Delta^{15}N$ (‰)	Foot back r. avg. area (°C)		Feces (nm)
	r	−0.67	−0.35	−0.82	−0.83	0.51	−0.70		0.91
	R^2	0.45	0.12	0.67	0.69	0.26	0.49		0.84
	RMSE	56.7	58.2	62.7	63.6	92.5	76.6		43.4
	P-value	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001
	α	382.48	116.21	358.17	273.98	−218.06	402.54		
	β	−697.65	−38.96	−3.02	−0.68	84.14	−15.62		
3	Biomarker	C18:2 (g/100 g FA)	BCS (score)	Activity index (n/d)	CO_2 (g/d)	NEFA (mmol/l)	Head min. (°C)		
	r	0.66	−0.28	−0.82	−0.82	−0.40	−0.69		
	R^2	0.44	0.08	0.67	0.68	0.16	0.48		
	RMSE	49.8	103.0	63.3	64.6	98.4	77.3		
	P-value	< 0.001	0.005	< 0.001	< 0.001	< 0.001	< 0.001		
	α	−188.47	343.53	342.20	263.19	108.18	200.43		
	β	636.26	−102.41	−2.09	−0.02	−264.09	−7.35		

Abbreviations: α = intercept; β = slope (coefficient); BCS = body condition score; C18:1 = oleic acid; C18:2 = octadecadienoic acids; FA = fatty acids; Foot back r. avg. area = foot back right average area; Head min. = head minimum; n = number of biomarkers; NEFA = non-esterified fatty acids; NIR = near-infrared spectroscopy; Udder B. Min. = udder back minimum; Δ15N = 15 N isotopic discrimination.

Milk constituents

Milk protein and milk urea nitrogen are important indicators of protein metabolism. Milk urea represents 2.5–3.0% of the total N compounds in milk and strongly correlates with blood urea (Roy et al., 2011). The excretion of excess urea is an energy-requiring process; therefore, milk urea could be used as an indicator of feed efficiency (e.g., r for RNI: 0.84, NUE: − 0.68, and REI: 0.57) and efficient N metabolism in animals. However, the evidence regarding the potential of this biomarker to reflect the between-animal variation in feed efficiency (i.e., NUE) and its association with N partitioning at the individual animal level is inconclusive (Beatson et al., 2019). Nousiainen et al. (2004) reported a strong relationship between milk urea and N utilisation; however, this was mainly driven by diet. As β-casein constitutes about 30% of milk protein, a positive correlation between milk β-casein content and feed efficiency of cows was observed (e.g., r for FCR: 0.60, NUE: −0.66, and RFI: 0.37). This could have been due to dilution and increased feed intake (Arndt et al., 2015). The N isotopic discrimination (Δ^{15}-N = $\delta^{15}N_{animal} - \delta^{15}N_{diet}$) in animal protein is a promising biomarker for the prediction of feed efficiency because of its direct link with ruminal microbial N metabolism and with the catabolism of AA in the liver. In short, N isotopic discrimination is negatively correlated with feed efficiency (e.g., R^2 for FCR: 0.39, NUE: 0.47) and this finding is consistent with FCR (R^2 = 0.29 (Correa-Luna et al., 2022)) and NUE (R^2 = 0.50 (Cantalapiedra-Hijar et al., 2016b)).

Milk FA can be derived from four major pathways: diet, modified or produced within the gastrointestinal tract (biohydrogenation, bacterial degradation, and synthesis), body fat mobilisation, and de novo synthesis in the mammary gland. Some of these milk FAs may indicate the feed efficiency of cows, as lower carbon chain FA (≤ C14:0) and to some extent also C16:0 in milk originate from mammary de novo synthesis of FA (Gross et al., 2011), whereas C18:0 and C18:1 cis-9 are predominantly released from adipose tissue (Rukkwamsuk et al., 2000). The milk FA, such as C18:1 and C18:2, as well as C12:1, C10:1, and C16:1, have been identified as moderate to strong indicators of feed efficiency. According to Van Haelst et al. (2008), elevated proportions of C18:1 and C18:2 in milk fat are suitable markers for energy balance not only in the early lactation period but also in mid-and late-lactation. Reports indicate increased concentrations of C16:0 (Patel et al., 2013) and C18:1 cis-9 (Neveu et al., 2013) in milk with an increasing proportion of forage in the diet. Thus, because diet composition influences the efficiency of feed utilisation, their effects on traits and biomarkers of feed efficiency should be further evaluated. The increase in C18:1 cis-9 concentration is explained by the additional dietary supply of monounsaturated FA and the greater mammary availability of its precursor, C18:0, due to the improved digestion processes of feed-efficient cows, rather than a greater mobilisation of adipose tissue (rich in C18:1 cis-9) in animals (Khiaosa-ard et al., 2020).

Animal characteristics

Selection for a higher milk yield and lower BW will increase the short-term FCR and NUE. Negative correlations were found between body condition score and ratio traits and improving ratio traits at the expense of body reserves is expected to compromise reproduction and health status. Thus, because FCR and NUE do not differentiate for the energy and nitrogen accreted or released by changes in BW and body condition score, it would be misleading to use such a metric to assess feed efficiency in dairy cows for selection purposes (Liu and VandeHaar, 2020). Feed−efficient cows with a low residual intake showed lower DMI without a concomitant response in ECM yield. The result agrees with most previous studies indicating that low residual is a consequence of lower intake while ECM yield is maintained (VandeHaar et al., 2016; Ben Meir et al., 2019). The process of digestion and reduced maintenance requirements may explain parts of the variance in residual intake (Richardson and Herd, 2004; VandeHaar et al., 2016; Potts et al., 2017).

Eating behaviour characteristics and activity

Our findings were consistent with Green et al. (2013); the more efficient animals had less consumption time and were consequently less active (longer time interval between strides) and had fewer grazing bouts. The daily number of strides correlated best with feed efficiency. The exception was RFI, which was best correlated (r = 0.35) with the daily number of grazing bouts. In addition, the activity results of the Posieux trial are consistent with the studies by Ben Meir et al. (2018), as feed efficiency was not related to the activity estimated by a pedometer. For NUE and RNI, the higher protein content of the fresh herbage fed in barn in Posieux, with reduced animal activity for cows kept indoors could explain the good relationship between activity markers and the nitrogen-based feed efficiencies.

Breath emissions

The CH_4 emissions (g/d) were positively related to feed−efficient dairy cows. According to Mertens et al. (2002), more efficient animals showed higher CH_4 production (g/d) and intensity (g/kg ECM), presumably due to the generation of gases during the ruminal fermentation, waste excretion, and heat production, which arise from improved digestion of feed efficient animals. However, the relationship between feed efficiency (RFI in most cases) and CH_4 yield (g/kg DMI) has been inconsistent (Lovendahl et al., 2018). Data from dairy cattle have shown that reduced CH_4 yield is associated with reduced DM and fibre digestibility, such that those animals that are inefficient at digesting fibre revealed lower CH_4 yields (Cabezas-Garcia et al., 2017). Eructed methane, measured with the Greenfeed system, is a product of fermentation in the rumen, while CO_2 comes from both fermentation and tissue metabolism. However, CO_2 and O_2 could be used as a marker of animal efficiency, as they are more closely related to whole-animal heat production (Huhtanen et al., 2021). The O_2 uptake (g/d) was best correlated with feed efficiency, except for FCR, which was best correlated with CO_2 intensity and RFI best correlated with O_2 consumption per kg ECM.

Blood constituents

Depending on the feed efficiency criteria, urea, IGF-1, NEFA, haemoglobin, triglyceride, and Δ^{15}N showed the most explanatory regressions (Tables 3–7). A positive correlation exists between efficiency and the blood constituents NEFA, haemoglobin, and triglyceride levels, and a negative correlation with urea, IGF-1, and Δ^{15}N. The higher haemoglobin shown in lactating cows compared to heifer calves may be due to a more significant oxygen requirement related to the metabolic demands of lactation and pregnancy (Bauman and Currie, 1980). Moreover, the blood urea concentrations correlated well with feed efficiency traits. The excretion of excess urea is an energy-consuming process, and the proper reduction of blood urea can increase milk production. The potential for IGF-1 as a biomarker appears to be due to its role as part of the growth hormone-IGF axis in regulating growth and cellular metabolism (Bishop et al., 1989). The IGF-1 concentrations correlated well with FCR and NUE, which agrees with Brown et al. (2004), with positive correlations between IGF-1 and feed efficiency in high-forage diets (Moore et al., 2005). The NEFA (FCR: R^2 = 0.24, RFI: R^2 = 0.20, REI: R^2 = 0.30, and RNI: R^2 = 0.16) are an important energy supply *in vivo* and a critical contributing factor to the incidence of glucose metabolic disorders and insulin resistance. More efficient dairy cows had higher concentrations of NEFA and triglycerides (RFI: R^2 = 0.11), which may be related to the increased carcass fat of more efficient cattle and their mobilisation of body

reserves throughout lactation (Xi et al., 2016). A negative relationship between blood $\Delta^{15}N$ and feed efficiency was observed, corresponding to $\Delta^{15}N$ of plasma for FCR and NUE of Cantalapiedra-Hijar et al. (2016a). Feed efficiency could be weakly to moderately explained by blood $\Delta^{15}N$, which corresponds to the relation found in literature about feed efficiency traits in beef heifers (FCR, R^2 = 0.35) and lactating dairy cows (NUE; R^2 = 0.45) (Wheadon, 2014).

Temperature

In our study, higher surface temperatures of different body parts were generally associated with more short-term efficient dairy cows in terms of FCR, NUE, REI and RNI. For short-term RFI, the relationships were less clear and very little or none of the variability could be explained by the surface temperatures of different body parts. The results found in the literature on the relationship between body surface temperature and efficiency are inconsistent and very sparse for dairy cows. For lactating cows, DiGiacomo et al. (2014) found results opposite to ours for some body parts, but similarity with our data for dry cows. They hypothesised that inefficient cows may waste energy on inefficient processes, resulting in increased heat production, which may be reflected in differences in surface temperatures. Martello et al. (2016) found concurrent results to ours for beef cattle, namely that more efficient animals had higher surface temperatures for at least some body parts. In contrast, Montanholi et al (2010) found lower cheek and snout surface temperatures in more efficient beef cattle. Probable reasons for these conflicting results could be that the conditions for measuring body surface temperature are not sufficiently standardised (ambient temperature – thermoregulation, roofing, animal restraint – stress level, measurement accuracy – number of thermal images per animal) and that different parts of the body are considered. Interestingly, in our study, rectal temperature in most cases behaved in the opposite way to surface temperature, and explaining less of the variability in the efficiency traits analysed. In DiGiacomo et al. (2014), the efficient and inefficient cows had similar rectal temperatures.

Hair cortisol

Hair cortisol concentration is an indicator of chronic stress and long-term activity of the hypothalamic–pituitary–adrenal axis (Heimbürge et al., 2019). In our study, the hair cortisol concentration (n = 1) explains at best up to 14% of the variability of different short-term feed efficiency traits, reflecting no to weak relationships. No relationship was found between hair cortisol concentration and RFI, but more efficient dairy cows in terms of FCR, NUE, REI and RNI had higher hair cortisol concentrations. In Montanholi et al. (2010), faecal cortisol metabolites in beef cattle, a medium-term indicator of circulating cortisol concentrations, were negatively correlated with RFI, meaning that more efficient beef cattle also had higher faecal cortisol metabolite concentrations. These authors hypothesised that more efficient cattle have a shy coping style, which is associated with higher baseline concentrations of faecal cortisol metabolites and superior feed efficiency.

Near-infrared spectroscopy spectra of milk and faeces

The NIR spectra of milk and faeces (n = 2) explain, at best, 29–89% of the variability of the different investigated feed efficiency traits, reflecting moderate to very strong relationships. The freeze-dried milk NIR spectra showed the best overall R^2 of feed efficiency traits. Many milk recording schemes globally already use mid-IR spectroscopy to estimate protein, fat, casein, lactose, and urea contents. Therefore, spectra data of milk can be a readily available and useful source of information on large-scale operations. However, McParland et al. (2014) reported a weaker R^2 of 0.28 for RFI. This could be due to differences in terms of breed, lactation stage, parity, and diets (Toral et al., 2021). The freeze-dried faeces NIR spectra showed a moderate to strong R^2 across feed efficiency traits. This is probably based on the principle that faeces contain good spectral information that allows for a description of the diet composition and digestive process (Coleman and Murray, 1993). The NIR spectra in faeces and milk seem to be strong candidates for identifying feed efficiency in lactating dairy cows. However, the present NIR spectra models may lead to biased predictions when applied to conditions different from those of this study. Indeed, they would gain in robustness, in predictive potential and thereby in wider applicability, by extending the reference database with the inclusion of additional variabilities such as different diets, breeds, feeding systems, managing conditions and environmental setups.

Conclusion

The main factors hindering the widespread use of the animal's feed efficiency information, especially in breeding strategies, are the limited availability of individual animal feed intake records and the plethora of definitions of the animal's efficiency. We identified a moderate to strong relationship, between energy and nitrogen efficiency in herbage-fed, lactating dairy cows. Various biomarkers, especially NIR spectra of milk and faeces, are associated with short-term energy and protein efficiency traits such as FCR, NUE, RFI, REI and RNI.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101211.

Ethics approval

All experimental procedures were in accordance with Swiss guidelines for animal welfare and were approved by the Animal Care Committee of the Canton Fribourg, Switzerland (No. 2017_35_FR, 2018_07_FR, 2018_37_FR, and 2019_04_FR).

Data and model availability statement

The data/models were not deposited in an official repository. Data supporting the findings of this study can be made available upon reasonable request.

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

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare no conflicts of interest.

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