



The energy expenditure of 2 Holstein cow strains in an organic grazing system

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ABSTRACT

Until recently, measurements of energy expenditure (EE; herein defined as heat production) in respiration chambers did not account for the extra energy requirements of grazing dairy cows on pasture. As energy is first limiting in most pasture-based milk production systems, its efficient use is important. Therefore, the aim of the present study was to compare EE, which can be affected by differences in body weight (BW), body composition, grazing behavior, physical activity, and milk production level, in 2 Holstein cow strains. Twelve Swiss Holstein-Friesian (H_{CH} ; 616 kg of BW) and 12 New Zealand Holstein-Friesian (H_{NZ} ; 570 kg of BW) cows in the third stage of lactation were paired according to their stage of lactation and kept in a rotational, full-time grazing system without concentrate supplementation. After adaption, the daily milk yield, grass intake using the alkane double-indicator technique, nutrient digestibility, physical activity, and grazing behavior recorded by an automatic jaw movement recorder were investigated over 7 d. Using the ^{13}C bicarbonate dilution technique in combination with an automatic blood sampling system, EE based on measured carbon dioxide production was determined in 1 cow pair per day between 0800 to 1400 h. The H_{CH} were heavier and had a lower body condition score compared with H_{NZ} , but the difference in BW was smaller compared with former studies. Milk production, grass intake, and nutrient digestibility did not differ between the 2 cow strains, but H_{CH} grazed for a longer time during the 6-h measurement period and performed more grazing mastication compared with the H_{NZ} . No difference was found between the 2 cow strains with regard to EE (291 ± 15.6 kJ) per kilogram of metabolic BW, mainly due to a high between-animal variation in EE. As efficiency and energy use are important in sustainable, pasture-based, organic milk production systems, the determining factors for EE, such as methodology, ge-

netics, physical activity, grazing behavior, and pasture quality, should be investigated and quantified in more detail in future studies.

Key words: energy expenditure, dairy cow, Holstein-Friesian, pasture

INTRODUCTION

Pasture-based milk production systems have recently gained international interest due to economic, environmental, animal welfare, and product quality issues. The economic benefit of such systems is based on the efficient use of pasture herbage and linked with reasonable milk production per cow (Dillon et al., 2005). If pasture herbage is used efficiently per area, so that milk production per hectare is optimized, then the herbage intake per cow, which is the main determinant for individual milk production, is limited by the reduced herbage allowance (Delagarde et al., 2001). Consequently, high-genetic merit cows for milk production in pasture-based systems suffer from a negative energy balance accompanied by lower BCS and impaired fertility. It has been previously shown that cows fed on pasture alone benefit from supplemental feeding to express their high-milk production potential in an efficiently managed grass-based system and to reduce the need to mobilize excessive amounts of body reserves in early lactation (Kennedy et al., 2002; Pedernera et al., 2008). Therefore, it is advisable to use dairy cows that are able to meet their energy requirements for production and maintenance in a pasture-based system. Bruinenberg et al. (2002) found that grass-fed dairy cows have a 10% higher metabolizable energy requirement for maintenance (ME_m) in indirect calorimetry experiments. However, ME_m is not only influenced by diet, but also by physical activity. For example, grazing cows had 21% higher energy expenditure (EE) compared with grass-fed cows kept indoors (Kaufmann et al., 2011). The energy requirements relative to maintenance may increase up to 50% depending on grazing conditions, including herbage availability (allowance and mass) and digestibility, distances walked (distance to the milking parlor and watering points), weather, topography, and

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interaction between these factors (CSIRO, 2007). More generally, according to Gruber et al. (2007), current energy systems established in Europe and the United States underestimate the ME_m for dairy cows. Those authors concluded that an increase in internal organ mass and feed intake, as well as a decrease in BCS, can be reasons for the increased ME_m in high-yielding dairy cows. In fact, it has been reported that recommended ME requirements for zero energy balance in cows fed fresh pasture were too low (Mandok et al., 2013). Currently, precise information about additional energy costs under pasture-based conditions is not available.

In New Zealand, Holstein cows are bred for the specific needs of pasture-based, low-input dairy production, including selection for milk solids, lower BW, fertility, and longevity (Miglior et al., 2005). This is in contrast to most other countries, where selection in the past was done primarily for enhanced productivity without taking body size or feed conversion efficiency into account (Pryce et al., 2007). In the future, this may change as new breeding goals could be defined and less intensive production environments may gain in importance (Boichard and Brochard, 2012). McCarthy et al. (2007) showed that New Zealand (NZ) Holstein cows were able to achieve high DMI and milk production in a pasture-based feeding system by grazing for a longer period of time in comparison to other high-yielding Holstein strains. Compared with Swiss Holstein cows, the NZ Holstein cows had a lower BW, showed a different body condition around calving, ruminated for a longer period of time, and tended to take more steps on the pasture (Schori and Münger, 2010; Piccand et al., 2013). As a moderate positive correlation exists between EE and walking and eating time (Kaufmann et al., 2011), the higher energy requirements of dairy cow strains on pasture may be partly caused by differences in grazing behavior, and physical activity. Brosh et al. (2006) and, more recently, Aharoni et al. (2013) allocated specific energy costs to foraging activities and locomotion of beef cows during grazing. The objective of the current study was to determine EE based on CO_2 production using the ^{13}C bicarbonate dilution technique with New Zealand Holstein-Friesian cows and heavier, high-producing Swiss Holstein-Friesian cows in a full-time grazing system without concentrate supplementation. To explain possible differences in EE between strains with differences in grazing behavior or physical activity, these variables were recorded simultaneously.

MATERIALS AND METHODS

Animals and Experimental Design

All experimental procedures were in accordance with the Swiss guidelines for animal welfare and were

approved (No. 2011_10_FR) by the Animal Care Committee of the Canton of Fribourg, Switzerland. Before selecting the cows for the experiment, a medical check-up including vital parameters, as well as udder and claw health, was performed. The 12 selected Swiss Holstein-Friesian (H_{CH}) cows were from a strain of North American origin (52% of the third generation of their ancestors originated from the United States or Canada) and selected for high milk yield. Their average economic breeding value (ISEL; Swiss Holstein Breeding Association, Posieux, Switzerland), which includes productivity, quality of milk, conformation, udder health, longevity, and fertility, was 981 ± 25.9 . The average ISEL was similar to that of the Swiss Holstein cow population in 2011 (1,023 ISEL; September 2012, E. Barras, Holstein Association of Switzerland, Posieux, Switzerland, personal communication). The 12 chosen New Zealand Holstein-Friesian (H_{NZ}) cows were from a strain selected within a seasonal calving, pasture-based dairy system with a high emphasis on the production of milk solids, fertility, and longevity. At least 2 generations of male ancestors were Holstein-Friesians with genetics from New Zealand. The ISEL of the H_{NZ} was 801 ± 31.0 . The experiment was set up as a balanced complete block design and consisted of 2 consecutive experimental weeks. Twelve matched pairs of H_{CH} and H_{NZ} cows were formed according to the following criteria: number of lactations, DIM, and age for primiparous cows. The cow pairs were equally divided between the 2 consecutive experimental weeks so that each cow passed a period of 7 d where data was collected. At the start of the first experimental week, H_{CH} cows were on average in the 2.6 (SD 1.8) lactation, had been 173 (SD 19) DIM, had an average BW of 616 (SD 30.9 kg), a BCS of 2.6 (SD 0.26), an average height at the withers of 147 (SD 3.6) cm, a chest circumference of 197 (SD 6.0) cm, and were producing 21.1 (SD 2.26) kg of milk/d. The H_{NZ} cows were on average in the 2.6 (SD 1.7) lactation, had been 179 (SD 16) DIM, had on average a BW of 570 (SD 55.9) kg, a BCS of 2.9 (SD 0.27), an average height at the withers of 135 (SD 4.7) cm, a chest circumference of 188 (SD 8.5) cm, and produced 17.6 (SD 3.96) kg of milk/d. On March 30, the grazing period started. The cows received hay and were supplemented with 264 (SD 16.0) kg of cereal mixture and 48 (SD 17.1) kg of a protein concentrate per cow during the first 80 d of lactation. The supplementation was terminated in mid-May so that the cows received solely herbage until the start of the experiment in mid-September. During the experiment, the cows grazed from 0800 to 1400 h and from 1800 to 0430 h on the pasture. Between the daily grazing periods, the cows were kept in a freestall barn and milked at 0530 and 1630 h in a milking par-

lor. Fresh water, a mineral mix, and common salt were available at all times.

Grazing Management, Pasture, and Climate

The experiment was carried out in a rotational grazing system from September 12 to 25. All 24 experimental dairy cows were managed as a single group separated from the rest of the lactating herd (about 44 cows). The average walking distance from the barn to grazed paddocks was 460 (SD 310) m. Paddocks used with the experimental herd were rotationally grazed for 1 to 3 d based on decision rules considering sward height, the pregrazing herbage availability measured with an electronic rising plate meter [Jenquip, Feilding, New Zealand; 1 click unit (CU) = 0.5 cm] and a postgrazing sward surface height of 4 cm (8 CU) from ground level. The maximum pregrazing sward height was 8 cm, equivalent to a maximum herbage mass of 1,300 kg of DM/ha above 4 cm. The average pregrazing sward height was 6.4 (SD 0.7) cm, corresponding to 810 (SD 197) kg of DM/ha above 4 cm, and the average postgrazing sward surface height was 4.4 (SD 0.6) cm. Herbage mass in kilograms of DM per hectare above 4 cm or 8 CU, respectively, was calculated according to $995 + 141 \times \text{sward height (CU)}$. This regression was calibrated for the pastures of the organic farm "L'Abbaye" ($n = 281$, $R^2 = 0.86$) and is valid from September to October. The pastures of "L'Abbaye" are

long-established pastures composed predominantly of grasses (mainly *Lolium perenne*, *Dactylis glomerata*, and *Phleum pratense*) but also of clover (mainly *Trifolium repens*) and other herbs (mainly *Taraxacum officinale*), and are situated in Sorens (Switzerland, 824 m above sea level). The pastures were fertilized once per year with 25 m³ of farm-produced manure per hectare (corresponding to 80 kg of N, 51 kg of P₂O₅, and 130 kg of K₂O per hectare). The chemical composition of the herbage during the experimental period is shown in Table 1. The ambient outdoor temperature and rainfall were recorded daily by the meteorological station in Grangeneuve (Meteo-Schweiz, Station Grangeneuve, Switzerland), located about 15 km north of the experimental pastures. During the experiment, the average temperature was 15.4 (minimum 9.3, maximum 18.1) °C. On 4 of the 14 d, scattered rain showers occurred, with an average daily precipitation of 9.1 (SD 9.4) mm.

Sample Collection and Data Recording

Milk yield (Flo Master Pro, DeLaval AG, Sursee, Switzerland) was recorded twice daily in the milking parlor. Milk composition was analyzed for each cow on d 1, 4, and 7 during each experimental week, respectively. The aliquot amount from a subsample of morning milk and evening milk was pooled together and preserved in sample tubes containing Broad-Spectrum Microtabs II (Gerber Instruments AG, Effretikon,

Table 1. Chemical composition of hand-plucked pasture samples¹

Item	First week		Second week	
	Mean	SD	Mean	SD
DM (g/kg of wet weight)	162	9.5	135	12.1
Analyzed nutrients and mineral composition (g/kg of DM)				
OM	821	45.8	841	27.5
CP	181	40.2	205	22.7
Ether extract	43	6.6	45	6.2
ADF	277	18.8	273	22.3
NDF	393	29.7	381	36.4
Crude fiber	184	12.3	177	18.1
Water-soluble carbohydrates	108	13.7	119	22.7
Ca	8.6	1.31	8.1	1.15
P	5.1	0.56	5.0	0.67
Mg	3.0	0.35	2.8	0.25
Na	0.4	0.18	0.3	0.09
K	35	4.2	36	5.1
Calculated energy and protein supply ² per kg of DM				
NE _L (MJ)	5.9	0.47	6.2	0.27
APDE ³ (g)	100	10.7	107	5.7
Analyzed <i>n</i> -alkane contents (mg/kg of DM)				
C32	5.4	1.80	5.3	1.05
C33	57	14.1	58	9.4

¹Means of 14 samples per week.

²According to Agroscope (2012).

³APDE = absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen.

Switzerland) at 5°C. The BW was recorded twice daily after milking, and BCS (1 = thin, 5 = fat) was assessed before the start and after the end of the experiment. As an experimental period of 1 wk is too short to estimate BW changes accurately, these were modeled with linear regressions over a period of 4 wk (beginning 2 wk before the 2 experimental wk).

Using the *n*-alkane double-indicator technique described by Mayes et al. (1986), individual feed intake and digestibility were estimated. Six days before the experimental week, the cows received gelatin capsule twice per day (Capsula GmbH, Ratingen, Germany, HGK 17–60 sl) containing a 0.5-g (weighing accuracy 0.001) alkane marker C32 (dotriacontane, C₃₂H₆₆, Argenta Ltd., Auckland, New Zealand) on a carrier of dried fruit pomace. During the experimental weeks, a daily spot sample of feces was collected indoors from each cow's defecation, with or without stimulus, between 0700 and 0730 h. Samples were pooled by cow and experimental week and stored at –20°C for later analysis. Herbage sample collection was carried out as described by Graf et al. (2005). Briefly, hand-plucked herbage samples were collected daily between 0800 and 0900 h by following and mimicking cow selection and cutting with a battery grass shearer (Gardena, Husqvarna Schweiz AG, Mägenwil, Switzerland). Daily samples were pooled by cow strain and stored at –20°C until further analysis.

Grazing and ruminating behavior were recorded on 3 consecutive days using an automatic jaw movement recorder with a pressure sensor (Datenlogger MSR145, MSR Electronics GmbH, Hengart, Switzerland), as described by Nydegger et al. (2011), which recorded digitally the cows' jaw movement frequency and amplitude. Data was compiled and analyzed using the software programs MSR-Reader, MSR-Viewer (V 1.64, MSR Electronics GmbH, Hengart, Switzerland), and R (R Development Core Team, 2012), as described by Nydegger et al. (2011) and in the MSR145 User Manual (MSR Electronics GmbH, Hengart, Switzerland).

Physical activity, including time spent standing, lying, and walking, and numbers of steps were determined using the IceTag pedometer (IceRobotics Ltd., Edinburgh, Scotland UK). The pedometer was attached to the right hind leg of the cow at the metatarsus level and recorded acceleration in 3 dimensions at 0.1-s intervals for 72 h. Using the software program IceTag-Analyzer (V 4.005, IceRobotics Ltd.), the data were downloaded and compiled over 60-s intervals. Walking was defined as >3 steps per minute, as suggested by Kaufmann et al. (2011).

Using the ¹³C bicarbonate dilution technique (Jungmans et al., 2007), the CO₂ production of 1 cow of each strain per day was determined from 0745 to 1345

h. The detailed application of the technique and the calculation procedure of EE have been described by Kaufmann et al. (2011). Briefly, 1 d before the start of measurements, cows were fitted with a catheter (Tygon S-54-HL, Saint-Gobain Performance plastics, Akron, OH) in the left external jugularis vein and a backpack to later hold the IceSampler. On the sampling day at 0700 h, blood samples for determination of plasma enzymes, metabolites, and hormones were taken manually from the catheter using the Vacuette System (Greiner Bio-One GmbH, Kremsmünster, Austria). Vacuette lithium heparin tubes were used to retrieve plasma. After sampling, these tubes were cooled in wet ice until they were centrifuged at 1,500 × *g* for 15 min at ambient room temperature (20°C). To obtain serum, the Vacuette serum tubes were stored upside down for at least 1 h at room temperature after sampling. They were then centrifuged at 1,500 × *g* for 15 min and then at 2,000 × *g* for an additional 5 min at ambient room temperature (20°C). The retrieved serum and plasma were stored at –20°C until analysis. Additionally, basal samples were taken manually 10 and 5 min before tracer administration using lithium heparin Monovettes (Sarstedt, Nümbrecht, Germany). The NaH¹³CO₃ tracer (0.7 mg of NaH¹³CO₃/kg of BW; Cambridge Isotope Laboratories, Tewksbury, MA) was administered as an intravenous bolus at 0745 h. Then blood was sampled automatically for 6 h at 2, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min after the tracer administration by an automatic blood sampling system (IceSampler) carried by the cow in a backpack. After sampling was completed and the cows had returned from the pasture (1400 h), all blood samples were frozen at –20°C until further analysis. Prior to the beginning of the experiment, the cows were accustomed to the jaw movement recorder, the pedometer, and the backpack.

Laboratory Analysis

Milk samples were analyzed by Combi-Foss FT 6000 (Foss, Hillerød, Denmark), using Fourier transformed infrared spectrometry (Milkoscan FT 6000), for contents of fat, protein, lactose, and urea. Herbage and feces samples were lyophilized (Christ, Mod. Delta, 1–24 LSC, Osterode, Germany). Samples were milled through a 1.0-mm screen (Brabender mill with titanium blades, Brabender, Duisburg, Germany). Afterward, herbage and feces subsamples were dried for 3 h at 105°C to determine DM and subsequently incinerated at 550°C until they reached a stable mass to assess the ash contents. Mineral residues in the ash were dissolved with nitric acid and analyzed for Ca, Na, P, Mg, and K with inductively coupled plasma optical emis-

sion spectrometry (ICP-OES Optima 2000 DV, Perkin Elmer, Shelton, CT; with system ICP-OES Optima 7300) based on European Standard EN 155510:2008. The contents of *n*-alkanes C32 and C33 (triotriacontane, C₃₃H₆₈) were determined as described by Peiretti et al. (2006). One microliter of alkane extract containing an internal standard C34 (tetratriacontane, C₃₄H₇₀) was injected by a split injector (ratio 1:45) on a 30-m × 0.32-mm capillary column (film = 0.25 μm, Agilent 19091Z-413E, Agilent Technologies, Santa Clara, CA) in a gas chromatograph fitted with flame ionization detection (GC-FID, Agilent 6850, Agilent Technologies). The carrier gas (H₂) flow rate was 1.6 mL/min. The injector and detector temperatures were both set to 320°C. The gradient of temperature for the column was 270°C for 2 min, then 5°C/min to 320°C, and finally 3 min at 320°C. The N content was determined using the Dumas method (AOAC International, 1995) on a C/N analyzer (type FP-2000, Leco Instruments, St. Joseph, MI) and then multiplied by 6.25 to get the CP content. The ether extract was determined using the Soxtec Avanti 2050 apparatus for extraction following the guidelines of ISO 6492:1999 and VDLUFA 5.1.1. Acid detergent fiber (procedure 973.18; AOAC International, 1995) and crude fiber (procedure 978.10; AOAC International, 1995; analyzed only in herbage) were determined with correction for residual ash obtained after incineration at 500°C for 1 h, as well as NDF (procedure 2002.04; AOAC International, 1995), which was assessed with the addition of heat-stable amylase and sodium sulfite. Water-soluble carbohydrates were determined as described by Hall et al. (1999).

The extraction of the blood CO₂ fraction and the subsequent determination of the ¹³C:¹²C ratio by mass spectrometry was done as described in detail by Kaufmann et al. (2011). Briefly, 0.5 mL of thawed blood was placed in a 10-mL syringe that had previously been filled with argon. Two milliliters of 10% lactic acid were added and the syringe was closed by means of a multi-directional stop-cock (Discofix-3, B. Braun Melsungen AG, Melsungen, Germany). After 2 h, the released CO₂ in the headspace of the syringe was transferred into an evacuated 13-mL screw cap Exetainer (Labco Ltd., Buckinghamshire, UK) for dispatch. The ¹³C:¹²C was determined using an isotope ratio mass spectrometer (IRMS DELTA plus XL, Finnigan MAT GmbH, Bremen, Germany) at the Leibniz Institute for Farm Animal Biology in Germany. Metabolite concentrations and enzyme activities were determined enzymatically using commercial test kits for albumin (No. 1970 569; Roche Diagnostics, Rotkreuz, Switzerland), urea (No. 61974, UV 250; bioMérieux, Marcy l'Etoile, France), creatinine (No. 11489291216; Roche Diagnostics), glucose (No. 1447516; Roche Diagnostics), BHBA (No.

RB1007; Radox Laboratories, Crumlin, UK), cholesterol (No. 61218; bioMérieux), NEFA (FA115; Radox Laboratories), aspartate aminotransferase (No. 63212; bioMérieux), creatine kinase (No. 61141; bioMérieux), and glutamate dehydrogenase (No. 1929992; Roche Diagnostics). Plasma insulin and IGF-1 concentrations were quantified using RIA as described by Vicari et al. (2008). The 3,5,3'-trijodthyronine and thyroxin were measured by RIA using the Coat-A-Count Total T4 kit and Coat-A-Count Total T3 kit, respectively, from Siemens (Siemens Schweiz AG, Zurich, Switzerland).

Calculations and Statistical Analysis

The NE_L was calculated for fresh herbage according to Swiss nutrient recommendations for ruminants (Bickel and Landis, 1978). For the NE_L estimation, the OM digestibility based on the regression for fresh herbage with unknown botanical composition (Agroscope, 2012) was used:

$$\begin{aligned} \text{OM digestibility (\%)} = & 56.7 + (0.1262 \times \text{CP}_{\text{OM}}) \\ & + (0.0939 \times \text{CF}_{\text{OM}}) - (0.000231 \times \text{CP}_{\text{OM}}^2) \\ & - (0.000312 \times \text{CF}_{\text{OM}}^2), \end{aligned} \quad [1]$$

where CP_{OM} = CP (g/kg of OM); and CF_{OM} = crude fiber (g/kg of OM).

The absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen (APDE) was calculated for fresh herbage according to Swiss nutrient recommendations for ruminants (Agroscope, 2012) as

$$\begin{aligned} \text{APDE} = & (0.093 \times \text{FOM}) + \{\text{CP} \times [1.11 \\ & \times (1 - \text{deCP}/100)]\} \times (\text{vASF}/100), \end{aligned} \quad [2]$$

where FOM = fermentable OM (g/kg of DM); deCP = degradability of CP (%); and vASF = amino acids digestibility of the feed (%). The values for FOM, deCP, and vASF were calculated for each herbage sample according to Agroscope (2012).

The ECM was calculated based on a 4.0% fat, 3.2% protein, and 4.8% lactose basis (Agroscope, 2012). Feed intake and digestibility were calculated using the ratio of the *n*-alkanes C32 and C33 on the basis of the equation proposed by Mayes et al. (1986), which, as no concentrate was fed, was adapted.

Energy expenditure (kJ/6 h) was calculated according to Kaufmann et al. (2011) as

$$\text{EE} = (4.96 + 16.07/\text{RQ}) \times \text{RCO}_2, \quad [3]$$

where RQ is the respiration quotient and a value of 1 was used based on data from the respiration measurements of cows in advanced lactation (Derno et al., 2009). The RCO_2 term was calculated according to Junghans et al. (2007) as described by Kaufmann et al. (2011):

$$\text{RCO}_2 = (\text{D}/\text{AUC}) \times \text{RF} \times 6, \quad [4]$$

where $\text{RCO}_2 = \text{CO}_2$ production rate (L/6 h); D = administered ^{13}C dose (mol); and AUC = calculated area under the ^{13}C enrichment (atom percent excess, APE)-time curve (APE·h). To correct for the incomplete ^{13}C recovery in breath from the rate of CO_2 appearance in the blood, the recovery factor (RF) 0.7 was used (Junghans et al., 2007).

Data for BW, BCS, milk yield and composition, ruminating and grazing behavior, and physical activity were collected over several days and averaged per cow. The aforementioned averages, as well as BW losses, intake, efficiency criteria, nutrient digestibility, EE, and blood metabolites were analyzed using SYSTAT-12 (SYSTAT Software Inc., Chicago, IL) according to the linear mixed model

$$Y_{ijk} = \mu + \tau_i + \pi_j + (\tau\pi)_{ij} + P_k + \varepsilon_{ijk}, \quad [5]$$

where Y_{ijk} = response (respectively its logarithm); μ = LSM; τ_i = fixed effect of cow strain i ($i = \text{H}_{\text{CH}}, \text{H}_{\text{NZ}}$); π_j = fixed effect of week j ($j = 1, 2$); $(\tau\pi)_{ij}$ = effect of the interaction between cow strain i and week j ; P_k = random effect of cow pair k ; and ε_{ijk} = random error.

The fixed effect of week is a blocking factor with 2 levels, as it combines the biological effects that could not be modulated in the fixed effect of cow strain and the random effect of cow pair (e.g., having different cows in the 2 periods and changing grass quality in the pasture). As the blocking factor period combines known and unknown nuisance factors, it is not included in the tables. Before using the statistical model described herein, data not normally distributed were transformed to fit a normal distribution using log- or reciprocal-transformation. If a normal distribution could not be achieved by transformation, the data were analyzed using the Kruskal-Wallis test. Data presented in the tables were back-transformed. The effects were considered significant at $P < 0.05$. A value of $P < 0.10$ was considered a trend.

RESULTS

The H_{CH} cows had a greater BW ($P = 0.01$) but a lower BCS ($P = 0.01$) and showed the same BW

changes ($P = 0.91$) compared with the H_{NZ} cows (Table 2). Neither milk yield ($P = 0.31$) nor ECM ($P = 0.96$) differed between the cow strains. The milk fat ($P = 0.03$) and protein ($P = 0.001$) content were lower for H_{CH} than for H_{NZ} cows, but these differences became negligible when milk fat ($P = 0.73$) and protein ($P = 0.61$) were expressed as yield per day. No influence of cow strain was found for the content of urea ($P = 0.21$) and for the content ($P = 0.49$) and daily yield ($P = 0.36$) of lactose. The 2 cow strains consumed the same amount of grass DM per day ($P = 0.89$; Table 2). Consequently, the intake of OM ($P = 0.92$), CP ($P = 0.87$) and NDF ($P = 0.87$) did not differ between the 2 cow strains. Greater digestibility of NDF ($P = 0.04$) was observed for H_{CH} compared with H_{NZ} cows, but no differences with regard to the digestibility of OM ($P = 0.17$) and CP ($P = 0.56$), respectively, were found. The production efficiency measures, namely ECM produced per 100 kg of $\text{BW}^{0.75}$ ($P = 0.25$), ECM produced per kilogram of grass DMI ($P = 0.85$), and grass DMI per 100 kg of $\text{BW}^{0.75}$ ($P = 0.41$), were not affected by cow strain.

During the 6 h of blood sampling for EE determination, H_{CH} spent more time grazing ($P < 0.001$) and performed more grazing mastication ($P = 0.001$) than the H_{NZ} (Table 3). Ruminating behavior did not differ between the 2 cow strains during the 6 h. Daily time spent grazing ($P = 0.59$), as well as the number of grazing mastications ($P = 0.23$), the time spent ruminating ($P = 0.43$), the number of ruminating mastications ($P = 0.59$), and the number of ruminating mastications per boli ($P = 0.17$), did not differ between cow strains. A tendency toward a greater number of boli chewed per day by the H_{NZ} ($P = 0.06$) was observed. The grazing time and rumination time per kilogram of DMI ($P = 0.87$ and 0.40 , respectively) and per kilogram of NDF intake ($P = 0.87$ and 0.60 , respectively) did not differ between cow strains.

Cow strain had no effect on the physical activities of standing ($P = 0.92$) and lying ($P = 0.92$) during the 6 h of EE measurements, and no difference was seen between the 2 cow strains in regard to the time spent walking ($P = 0.34$) and the number of steps ($P = 0.43$; Table 4). Similar to the 6-h measurement period, no difference between the 2 cow strains was observed in regard to the time spent standing ($P = 0.60$), lying ($P = 0.60$), and walking ($P = 0.43$) or the number of steps ($P = 0.74$) when measured over 24 h.

Cow strain did not affect EE per kilogram of $\text{BW}^{0.75}$ ($P = 0.27$) and EE per cow ($P = 0.13$, Table 5) during the 6-h measurement period, although H_{CH} were heavier. Apart from greater concentrations of BHBA ($P = 0.05$) and a higher activity level of glutamate dehydrogenase ($P = 0.03$), as well as a tendency toward

Table 2. Effect of cow strain¹ on milk production performance, BW, BCS, nutrient intake, digestibility, and production efficiency measures

Item	H _{CH}	H _{NZ}	SEM	Effect of cow strain (<i>P</i> -value)
Day in lactation	197	203	3.9	
BW (kg)	615	567	12.6	0.01
BW loss (g/d)	96	112	96	0.91
BCS	2.54	2.84	0.07	0.01
Milk yield (kg/d)	18.8	17.5	1.04	0.31
ECM (kg/d)	18.3	18.3	0.82	0.96
Fat (%)	4.01	4.49	0.15	0.03
Protein (%)	3.26	3.65	0.06	0.001
Lactose (%)	4.51	4.46	0.05	0.49
Fat (kg/d)	0.76	0.77	0.03	0.73
Protein (kg/d)	0.62	0.64	0.03	0.61
Lactose (kg/d)	0.86	0.79	0.05	0.36
Urea (mg/kg)	344	328	8.03	0.21
Grass intake per cow (kg of DM/d)	16.5	16.3	0.74	0.89
Nutrient intake (kg of DM/d)				
OM	13.6	13.6	0.62	0.92
CP	3.17	3.14	0.14	0.87
NDF	6.30	6.37	0.29	0.87
Digestibility (%)				
OM	72.8	71.5	0.61	0.17
CP	70.2	69.4	0.85	0.56
NDF ²	79.8	77.7	0.69	0.04
ECM (kg/100 kg of BW ^{0.75})	14.8	15.7	0.61	0.25
ECM (kg/kg of grass DMI)	1.12	1.13	0.04	0.85
Grass DMI (kg/100 kg of BW ^{0.75})	13.3	14.0	0.52	0.41

¹H_{CH} = Swiss Holstein-Friesian; H_{NZ} = New Zealand Holstein-Friesian.²Nonparametric test (Kruskal-Wallis) for statistical analysis.

a lower activity level of aspartate aminotransferase ($P = 0.07$) and higher accumulations of creatinine ($P = 0.09$) and urea ($P = 0.07$) for H_{CH} cows compared with H_{NZ} cows, no differences were noted in measured blood metabolites, enzymes, or hormones between the 2 cow strains.

Table 3. Effect of cow strain¹ on grazing and ruminating behavior

Item	H _{CH}	H _{NZ}	SEM	Effect of cow strain (<i>P</i> -value)
6-h measurement				
Time ruminating (min)	37.2	40.7	4.31	0.56
No. of boli	51	55	5.6	0.68
No. of ruminating mastication	2,523	2,818	302	0.49
No. of ruminating mastication/boli ²	50.9	51.8	2.10	0.59
Time grazing (min)	235	213	7.82	<0.001
No. of grazing mastication	17,514	15,634	736	0.001
24-h measurement				
Time ruminating (min)	368	381	10.9	0.43
No. of boli ³	479	513	11.8	0.06
No. of ruminating mastications ²	26,049	26,654	944	0.59
No. of ruminating mastication/boli	55	52	1.5	0.17
Time grazing (min)	548	540	17.5	0.59
No. of grazing mastication	41,136	39,900	1,594	0.23
Per intake				
Time grazing/DMI (min/kg)	33.8	34.1	1.79	0.87
Time grazing/NDF intake (min/kg)	88.4	87.5	4.64	0.87
Time ruminating/DMI (min/kg)	22.7	24.0	1.03	0.40
Time ruminating/NDF intake (min/kg)	59.4	61.4	2.65	0.60

¹H_{CH} = Swiss Holstein-Friesian; H_{NZ} = New Zealand Holstein-Friesian.²Log₁₀-transformed for statistical analyses.³Nonparametric test (Kruskal-Wallis) for statistical analysis.

Table 4. Effect of cow strain¹ on physical activity

Item	H _{CH}	H _{NZ}	SEM	Effect of cow strain (<i>P</i> -value)
6-h measurement				
Standing (min)	280	281	14.0	0.92
Lying (min)	80	79	14.0	0.92
Walking ² (min)	109	95	11.0	0.34
Steps	1,186	1,106	73.3	0.43
24-h measurement				
Standing (min)	913	927	18.2	0.60
Lying (min)	528	513	18.2	0.60
Walking (min)	352	330	18.9	0.43
Steps	4,086	4,019	181.9	0.74

¹H_{CH} = Swiss Holstein-Friesian; H_{NZ} = New Zealand Holstein-Friesian.

²If the number of steps per minute was >3, this minute was counted as walking.

DISCUSSION

Differences in EE Between and Within Cow Strains in Grazing Conditions

Indirect calorimetry under controlled conditions in respiration chambers is considered the gold standard to measure EE, but this method cannot be used to measure EE (heat production) in free-ranging farm animals. Thus, additional energy expenses occurring in relation to pasture-based production systems, due, for example, to physical activity, grazing behavior, herbage composition, and so on, cannot be estimated, although these expenses may increase up to 50% relative to maintenance (CSIRO, 2007). Kaufmann et al. (2011) combined the ¹³C bicarbonate dilution technique (Junghans et al., 2007) with an automatic blood sampling system (Fønss and Munksgaard, 2008) to assess the EE of grazing

dairy cows. In bulls, the ¹³C bicarbonate dilution technique allow EE determination with an accuracy of 10% when compared with EE measurements obtained with indirect calorimetry in respiration chambers (Junghans et al., 2007). In addition, according to Kaufmann et al. (2011), it is a suitable method to determine the EE of lactating dairy cows on pasture. Another indication for the reliability of this method is the fact that similar estimates of EE per kilogram of BW^{0.75} were obtained in the present study compared with earlier results of Kaufmann et al. (2011).

Energy expenditure measured with the ¹³C bicarbonate dilution method includes the ME_m (includes energy for fasting metabolism and activity allowance, measured under thermoneutral conditions), as well as heat increment changes (heat losses, differences between ME, and net energy for maintenance, production, and gestation), but it does not include heat from rumen

Table 5. Effect of cow strain¹ on energy expenditure, blood metabolites, enzymes, and hormones

Item	H _{CH}	H _{NZ}	SEM	Effect of cow strain (<i>P</i> -value)
EE ² (kJ/6 h per kilogram of BW ^{0.75})	309	273	22.0	0.27
EE (MJ/6 h per cow)	37.9	31.8	2.70	0.13
BSA (g/L)	41.0	39.0	0.80	0.11
BHBA (mmol/L) ³	1.05	0.85	0.10	0.05
Cholesterol (mmol/L)	6.10	6.42	0.38	0.49
Creatinine (μmol/L)	68.0	64.1	1.76	0.09
Glucose (mmol/L)	3.45	3.40	0.07	0.59
NEFA (mmol/L) ⁴	0.07	0.08	0.01	0.20
Urea (mmol/L)	6.93	6.45	0.47	0.07
Aspartate aminotransferase (U/L)	71.9	75.5	2.40	0.07
Creatine kinase (U/L) ³	195	158	25.6	0.35
Glutamate dehydrogenase (U/L)	17.7	14.6	0.99	0.03
IGF-1 (ng/mL) ³	128	140	10.7	0.34
Insulin (μU/mL)	9.45	10.55	1.87	0.49
3,5,3'-Triiodothyronine (nmol/L)	1.44	1.60	0.08	0.11
Thyroxin (nmol/L)	42.5	48.1	2.41	0.14

¹H_{CH} = Swiss Holstein-Friesian; H_{NZ} = New Zealand Holstein-Friesian.

²EE = energy expenditure.

³Log₁₀-transformed for statistical analyses.

⁴Reciprocal transformation for statistical analyses.

methane production. To our knowledge, no comparison of EE between differing dairy cow strains under pasture-based conditions is available.

By considering BW in the breeding worth, H_{NZ} , in contrast to H_{CH} , were indirectly selected for feed conversion efficiency. Although this may limit the intake capacity or the maintenance requirements per milk solids in H_{NZ} , equal ECM per kilogram of DMI and EE per kilogram of $BW^{0.75}$ were found for both cow strains. Possible reasons could be that, compared with earlier studies (Schori and Munger, 2010; Piccand et al., 2011), an increasing similarity in the BW of the 2 cow strains was found, which can contribute to the similarity in EE per animal. Additionally, the BCS had apparently no substantial effect on the EE, although cows with higher BCS, such as H_{NZ} , consequently have a lower amount of body protein per kilogram of BW, which would suggest a lower fasting heat production per kilogram of BW (Agnew and Yan, 2000). Concerning the efficiency of utilization of ME for milk or the energy requirement for maintenance per kilogram of $BW^{0.75}$, Munger et al. (1996) found no differences between Holstein, Simmental, and Jersey dairy cows. This is in agreement with recent studies by Xue et al. (2011) comparing Holstein-Friesian and Jersey-Holstein crossbred cows, and by Dong et al. (2013), who investigated Holstein and a group of non-Holstein-Friesian cows consisting of Norwegian Red, Norwegian Red \times Holstein-Friesian and Jersey \times Holstein-Friesian. However, Aharoni et al. (2006) were able to indicate lower efficiency of ME utilization by Montbeliarde \times Holstein compared with Holstein cows.

Interestingly, in the present study, H_{CH} spent more time grazing during the EE measurement period of 6 h, which did not lead to significant differences in EE per kilogram of $BW^{0.75}$. However, the correlation between EE and eating time was moderate ($R^2 = 0.18$) (Kaufmann et al., 2011). Nevertheless, equal EE per kilogram of $BW^{0.75}$ between strains can be partly caused by a lack of differences in DMI per day, in the digestibility of the eaten herbage, in ECM, and in physical activity during the 6-h EE-measurement period. Furthermore, the variability of EE between animals was high ($CV = 26\%$), and clearly higher compared with a CV of 17%, as observed by Kaufmann et al. (2011). The larger variation may be explained by the use of different cow strains, the larger variation of the length of the path to the paddocks, and the topography of the foothills pastures. Former studies have reported a considerable between-animal variation in ME_m . For example, Van Es (1961) found that the ME_m of cows of the same breed and similar size may vary by as much as 8 to 10% under controlled activity conditions. Likewise, Xue et al. (2011) reported a CV of 16 to 17% of ME_m due to

sire breed, maturation stage, plane of nutrition, and season. Hotovy et al. (1991) identified a ratio of variance components of 3:1 (between twin pair and within twin pair of beef cattle) for fasting EE when excluding the effect of sex and breed. The authors assumed that a major contributor to the variation in fasting EE is the genetically inherited amount of organ mass. Van Es (1961) discussed not only the between-animal variation but also analytical and physiological variation, including daily variation in the animal's production of feces, urine, CO_2 , CH_4 , and heat, as well as variation in the composition of rations as additional effects that can contribute to the variation in ME_m . Not to be neglected are the variability generated by corrections for energy gains and losses as well as by BW measurements. Moreover, under practical grazing conditions, variability is even greater compared with respiration chambers as, among others, herbage availability and quality, climate, and the physical activity of dairy cows varies.

The analysis of plasma hormones and metabolite concentrations, which were considered relevant for interpreting the cows' energy metabolism (Reist et al., 2002), did not indicate differences between the 2 cow strains except for higher BHBA concentrations and glutamate dehydrogenase activity in H_{CH} . The higher activity of glutamate dehydrogenase for H_{CH} is in the normal range for healthy cows (Kaneko et al., 2008) and therefore does not indicate increased liver cell damage. Also, the concentrations of plasma NEFA and glucose were in the normal range (Cozzi et al., 2011). Therefore, the moderate elevation of BHBA, as seen for H_{CH} , cannot be associated with a negative energy balance, but might be indicative of better use of ketone bodies as an energy source in H_{NZ} .

Estimation of EE over 24 h Under Grazing Conditions

Beside the 6-h measurements, where cow types and other factors can be investigated, the EE over 24 h would be worth knowing. This would allow comparisons with the daily energy recommendations for grazing dairy cows under different grazing conditions. Actually, a consequence of the specific validated methodology used in the present study, the chosen ^{13}C bicarbonate dose, causes the plasma $^{13}CO_2$ enrichment return to baseline approximately 6 h after tracer application (Junghans et al., 2007). As it is known (Kilgour, 2012) and can also be seen in the present study, that intake behavior and physical activity are subject to diurnal patterns. Thus, an extrapolation to the EE per day could be biased, and a comparison with daily energy requirements misleading. However, a measurement over 24 h would be preferable. It is generally possible to use

higher ^{13}C bicarbonate doses to enable EE estimates over time periods >6 h, but this requires further validation of EE. Additionally, it makes it necessary to take more blood samples in free-ranging cows over a period of >6 h.

One alternative technique to measure EE in free-ranging farm animals is the use of continuous heart rate monitoring coupled with the short-term measurements of the oxygen pulse per heartbeat (Brosh, 2007). This technique has been used to derive diurnal estimations of EE in grazing cattle, assess the relationship between EE and physical activity (Aharoni et al., 2013), and to compare EE and efficiency of energy use between dairy cattle breeds (Aharoni et al., 2006). Despite the recent wide adoption, the accuracy of this technique is also questionable because of the use of extrapolations based on short-term measurements of oxygen pulse per heartbeat, the use of arbitrary respiration quotients, or the error-free recording of heartbeats over time.

Concerning efficiency, the daily EE or ME_m plays a major role in low-input milk production systems, such as pasture-based organic systems, because the ratio between the energy for production to EE or ME_m , respectively, is usually lower compared with that of high-input systems. Thus, it is important for low-input systems to quantify the EE, ME_m , and their determining factors.

Several studies (Agnew and Yan, 2000; Gruber et al., 2007; Mandok et al., 2013) suggest that energy requirements for maintenance are underestimated, and not merely in pasture-based production systems. A higher proportion of body protein mass, a higher metabolic rate due to greater production in the modern high genetic dairy cow, rations with increased fiber concentration, and the greater physical efforts of grazing cattle were discussed as possible sources of increased EE or ME_m . Further, dairy cows outside thermo-neutral zone, depending on environmental factors and animal characteristics, have an increased heat production (Kadzere et al., 2002). As energy intake recommendations assume a well-balanced dietary nutrient composition, increased heat production for ingestion, absorption, and metabolism of nutrients from fibrous, protein-rich herbage (Bruinenberg et al., 2002) can also contribute to increased ME_m requirements for grazing cows. In general, grazing cows are exposed to several factors that influence their EE or ME_m , many of which are hard to quantify under real grazing.

CONCLUSIONS

Similar production levels in late lactation, small differences in BW, and physical activity of the 2 examined grazing Holstein cow strains led to similar values of EE

per kilograms of $\text{BW}^{0.75}$ in both strains. The high variability suggests that potential to improve the efficient use of consumed energy exists. As efficiency and energy use are important in sustainable, pasture-based organic milk production systems, the determining factors for EE should be investigated and quantified in more detail in future studies.

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