Energy expenditure of grazing cows and cows fed grass indoors as determined by the ^{13}C bicarbonate dilution technique using an automatic blood sampling system

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ABSTRACT

The objectives of the study were to assess the ^{13}C bicarbonate dilution technique using an automatic blood sampling system and to use this technique to estimate energy expenditure (EE) based on the CO_2 production of 14 lactating Holstein cows on pasture or in a freestall barn. The effects of physical activity and eating behavior on EE were also assessed. Cows were exposed to each feeding system in a crossover design with two 14-d experimental periods, each consisting of an adaptation period and a 7-d data collection period. Cows either grazed on pasture or had ad libitum access, in the freestall barn, to grass cut daily from the same paddock. All cows were supplemented with a cereal-based concentrate. The EE of each cow was determined from 0700 to 1300 h on 1 d of each collection period. Blood samples for the ^{13}C bicarbonate dilution technique were taken either manually in the barn or using an automatic blood sampling system on pasture. Eating pattern and physical activity were recorded from 0700 to 1300 h using a behavior recorder and an activity meter, respectively. Milk yield was recorded daily. Individual feed intake was estimated using the alkane double-indicator technique. Two preceding experiments confirmed that the sampling technique (manual or automatic) and the following storage of the blood samples (frozen directly after withdrawal or first cooled on ice and then frozen 6 h later) had no effect on $^{13}\text{CO}_2$ enrichment in the extracted blood CO_2 or on the subsequent calculation of CO_2 production. During the 6-h measurement period, the EE of cows on pasture was higher than that of cows in the freestall barn. Daily feed intake and milk production were not affected by the feeding treatment. Grazing cows spent more time walking and less time standing and lying than did cows fed indoors. Time spent eating was greater and time spent ruminating was lower for cows on pasture compared with grass-fed

cows in the barn. In conclusion, the ^{13}C bicarbonate dilution technique, combined with an automatic blood sampling system, is a suitable method to determine the EE of lactating dairy cows on pasture. Positive correlations between EE and walking and eating time indicate that the higher energy requirements of dairy cows on pasture may be at least partly caused by a higher level of physical activity. However, before specific recommendations about additional energy supply can be given, it must be determined whether EE measured over 6 h can be extrapolated to 24 h. Furthermore, the apparent inconsistency between EE, feed intake, and milk production needs to be resolved.

Key words: ^{13}C bicarbonate dilution technique, energy expenditure, dairy cow, pasture

INTRODUCTION

When applying standard factors for digestive and metabolic use, the milk production of cows fed a grass-based diet is often lower than expected based on the calculated energy supply. The reason for this unsatisfactory performance is not entirely clear. Bruinenberg et al. (2002) suggested that a dietary imbalance or the effect of the diet on endocrine control, that in turn affects the repartitioning of nutrients, might be responsible. Cattle on grass-based diets may suffer from an energy deficiency as a result of small amounts of fermentable organic matter in the rumen. This could lead to inefficient use of CP and higher urea production (Bruinenberg et al., 2002). Kolver and Muller (1998) concluded that dietary energy, rather than the supply of metabolizable protein or amino acids, is limiting milk production of cows consuming high-quality pasture. Thus, the lower milk production of grass-fed cows might also be due to less efficient use of metabolizable energy for milk than is commonly specified, or higher requirements for NE_m (Bruinenberg et al., 2002). Although the discrepancy between energy output and intake in grazing cattle can partly be explained by energy costs for metabolism and excretion of excess N (discussed by Bruinenberg et al., 2002; Tyrrell et al., 1970) grazing activity and locomotion

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tion are believed to have great effect (Agnew and Yan, 2000). Thus, higher energy expenditure (**EE**) during grazing may result from the greater physical effort required to forage.

The EE of grass-fed cows has been determined mostly under controlled conditions that do not reflect a grazing behavior. Based on regression analysis of data from Dutch and Irish experiments using indirect calorimetry, Bruinenberg et al. (2002) concluded that the NE_m of grass-fed dairy cows is 10% higher than has previously been assumed. However, this value may vary considerably for grazing cattle (Di Marco and Aello, 1998) and increase up to 50%, depending on grazing conditions (NRC, 2001).

Junghans et al. (2007) validated the ^{13}C bicarbonate dilution technique to estimate the EE of young bulls using indirect calorimetry. To our knowledge, this method has never been applied in experiments with grazing dairy cows, possibly due to the necessity of taking blood samples at timed intervals. Combining this method with an automatic blood sampling system, which has been used before to determine cortisol concentration in dairy cow blood over a period of 2.5 h (Fønss and Munksgaard, 2008), might facilitate the estimation of EE for dairy cows on pasture.

The objectives of the current study were to 1) validate the procedure of automatic blood sampling, 2) determine CO_2 production and EE of dairy cows grazing full-time or fed identical grass indoors using the ^{13}C bicarbonate dilution technique, and 3) evaluate the effect of physical activity and feeding behavior on EE.

MATERIALS AND METHODS

Preliminary Experiments to Validate the Procedure of Automatic Blood Sampling

Test of Temperature Sensitivity of Blood CO_2

Four lactating Holstein cows (BW 654 kg), fed hay ad libitum, were fitted with a catheter (Tygon S-54-HL, Saint-Gobain Performance Plastics, Akron, OH) used to infuse $NaH^{13}CO_3$ tracer (99 atom % ^{13}C , Chemotrade Ltd., Leipzig, Germany) solution and to collect blood samples. Using sterile techniques, the catheter was inserted into the right external jugular vein under local anesthesia by subcutaneous injection of 2 mL of lidocaine (lidocaine 2%, Vétoquinol AG, Ittigen, Switzerland) 1 d before the start of measurements. To ensure patency, the catheter was filled with 2 mL of saline solution in which heparin was added to a final concentration of 100 IU/mL (Bichsel Heparin 1,000 IU/mL, ref 1400020, Laboratorium Dr. G. Bichsel AG, Interlaken, Switzerland). On the sampling day, blood was collected 10 and 5 min before the intravenous bolus administration of

$NaH^{13}CO_3$ (0.7 mg/kg of BW, dissolved in 0.9% saline, sterile filtered through 0.2 μm) at 0700 h and 2, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min after the administration. Blood samples were collected in Li-heparinate monovettes (Sarstedt, Nümbrecht, Germany). Two blood samples were taken from each cow at each sampling time. One blood sample was kept on ice bricks and frozen at $-20^{\circ}C$ after the sampling series was completed. The second blood sample was frozen at $-20^{\circ}C$ immediately after withdrawal.

Comparison of Automatic and Manual Blood Sampling. Five lactating Holstein cows (BW: 666 ± 29 kg), fed hay ad libitum, were fitted with 2 catheters, which were inserted into the right and left external jugular vein 1 d before the start of the measurements. On the sampling day, blood was sampled, using Li-heparinate monovettes (Sarstedt), at the same time points as described above. The first 2 samples, before tracer administration, were taken simultaneously by hand from both catheters of each cow. The next samples were drawn manually from one catheter and automatically from the other catheter. For this purpose, cows were equipped with an automatic blood sampling system (IceSampler, IceRobotics Ltd., Roslin, UK), as described in detail by Fønss and Munksgaard (2008). The sampler was mounted in a backpack, which was positioned on the left side of the cows' thoracic vertebrae and kept in place by 2 elastic straps. To prevent the backpacks from sliding off, a counterweight containing 3 kg of sand was added on the right side. The sampler was surrounded by 4 ice bricks and polyurethane foam sheets to keep the temperature of the blood samples low. Blood samples taken manually were cooled on ice bricks. All samples were frozen at $-20^{\circ}C$ after the sampling was completed.

Main Experiment

Experimental Design and Animals. Fourteen lactating, multiparous Holstein cows were randomly assigned to 2 treatments (feeding systems: barn or pasture; 7 cows per treatment) in a crossover design. The experiment started in late April, 2008 when cows were, on average, 38 (SD 10.8) DIM, had a BW of 660 (SD 65.0) kg, and produced 44 (SD 2.7) kg/d of milk. In the treatment pasture, cows grazed full-time from 0730 to 1430 h and from 1700 to 0430 h. The grass for the cows subjected to the treatment barn was cut once daily from the same paddock. The barn cows were kept in a partially open freestall barn and had ad libitum access to the grass at the same time as the cows on pasture. In addition, all cows were supplemented with a cereal-based concentrate that included a mineral mix (Table 1) to meet their predicted nutrient requirements

(ALP, 2008). The concentrate was offered after milking in 2 equal meals, at 0630 and 1630 h, in the freestall barn using automatic weighing troughs (Insentec B.V., Marknesse, the Netherlands). Fresh tap water was available at all times. Each cow completed 2 consecutive 14-d experimental periods. Each period consisted of a 7-d adaptation period and a 7-d data and sample collection period.

Grazing Management and Climatic Conditions. The experimental pastures were long-established pastures with mixed swards. The average botanical composition, as determined once weekly, was characterized by a high proportion of grasses (66% by fresh weight, dominated by 43% *Lolium perenne*), 20% herbs (dominated by 18% *Taraxacum officinale*), and 14% white clover (*Trifolium repens*). Pasture paddocks were divided into 2 plots. One plot was used for grazing, whereas the other was used to provide grass for the cows in the barn. To have the best possible homogeneity of grass, paddocks were cut once 20 d before the start of the experiment. Grass for cows in the barn was cut daily between 0700 and 0800 h, at a height of 5 cm. The harvested quantity was weighed to determine pasture allowance, which was, on average, 25.5 kg of DM/cow per day. To offer the same amount of grass to the grazing cows, grazing plots were subdivided, allowing 3.5 meals per plot. The walking distance from pasture to the milking parlor averaged 474 m (range: 343 to 649 m). Ambient outdoor temperature and rainfall were recorded daily using a stationary meteorological station (Meteo-Schweiz, Station Grangeneuve, Switzerland) located less than 1 km away from the experimental pastures. Temperatures at 0700, 1300, and 1900 h were 7.9 (SD 2.7), 17.2 (SD 3.6), and 15.9 (SD 3.6) °C, respectively, and average daily precipitation was 2.9 (SD 5.7) mm.

Sample Collection and Data Recording

During each data collection period, milk yield and BW after milking were determined twice daily in the milking parlor. Milk samples were taken from each cow during each milking. Samples were pooled per day, preserved with Broad-Spectrum Microtabs II (Gerber Instruments AG, Effretikon, Switzerland), and stored at 5°C for later analysis of milk components. A second milk sample per cow was taken on the first and last day of each collection period and stored at -20°C for urea and acetone analysis. The health status of all cows was monitored daily. Rectal body temperature was, on average, 38.2 (SD 0.37) °C, indicating no clinical illnesses.

Individual grass intake and nutrient digestibility were estimated using the alkane double-indicator technique, as described by Mayes et al. (1986). Two days before

Table 1. Ingredients of the concentrate

Ingredient	Amount (%)
Barley	53.50
Wheat	31.70
Fat	4.95
Molasses	3.96
CaCO ₃	1.98
NaCl	1.49
CaHPO ₄	1.49
MgO	0.79
Trace elements and vitamin mix ¹	0.20

¹Containing per 100 g of DM: 7.0 g of Zn, 3.3 g of Mn, 0.9 g of Cu, 0.1 g of I, 0.04 g of Se, 0.02 g of Co, 7.0 g of vitamin E, 1,075,268 IU of vitamin A, 86,021 IU of vitamin D₃.

the start of the collection periods, 347 ± 7.63 mg of alkane marker (dotriacontane, C₃₂H₆₆) in a gelatin capsule (HGK 17-60 sl, Capsula GmbH, Ratingen, Germany), filled with dried fruit pomace, was administered to each cow twice daily. During the collection periods, one daily spot sample of feces was taken indoors between 0500 and 0600 h from cows defecating with or without stimulus. Samples were frozen at -20°C and later pooled by cow and collection period for analysis. Samples of cut herbage offered to the cows in the barn and samples of concentrate were taken daily between 0800 and 0900 h; collection lasted from 2 d before the start of the actual collection period to 2 d before the end of that week. Collection of herbage samples on pasture was carried out during the same time, following the procedure described by Graf et al. (2005). Daily herbage samples were immediately frozen at -20°C and later pooled by treatment over 2 and 3 d, respectively, for alkane analysis and over the collection period for the analysis of nutrients and minerals. Daily concentrate samples were pooled across the collection periods. To recheck the estimated grass intake [16.1 (SD 2.4) kg of DM], the intake of cows in the barn was measured simultaneously using weighing troughs [15.9 (SD 1.8) kg DM]. These values proved that, in our hands and under our experimental conditions, the alkane double-indicator technique was a reliable method to accurately estimate grass intake.

On each day of the collection periods, CO₂ production of one cow in the barn and one cow on pasture was determined from 0700 to 1300 h using the ¹³C bicarbonate dilution technique (Junghans et al., 2007). One day before the start of the measurements, cows were catheterized and fitted with the backpacks, as described above. On the sampling day, NaH¹³CO₃ tracer (0.7 mg of NaH¹³CO₃/kg of BW) was administered at 0700 h. Sampling intervals were the same as described in the preceding experiments. Blank samples (10 and 5 min before tracer administration) were taken by hand.

After the tracer administration, blood was sampled either manually in the barn or automatically on pasture using an automatic blood sampling system (IceSampler, IceRobotics Ltd.), as described previously. The sampler was put in the backpack of the pasture cow just before tracer administration. The backpack of the barn cow was equipped with ballast, corresponding to the weight of the sampler. The manually-taken samples were cooled on ice bricks. After sampling was completed and pasture cows were back in the barn (1430 h), all of the blood samples were frozen at -20°C until further analysis.

During the day when CO_2 production was measured, and for a further 3 d during the data collection period, cow physical activity and position status were monitored from 0700 to 1300 h using IceTag activity meters (IceRobotics Ltd.). The activity meter was attached by a Velcro belt to the right hind leg of the cow and recorded acceleration in all 3 dimensions at 0.1-s intervals. Data were downloaded from the activity meter using the software program IceTag-Analyzer version 3.005, 2008 (IceRobotics Ltd.) and summarized over 60-s intervals. Activities were interpreted as time spent standing, lying, or walking, and number of steps taken by each cow. Additionally, a motion index was calculated, which is defined as a summed indication of motion in all 3 dimensions. Data were summarized on a per minute basis.

To investigate eating and ruminating behavior, jaw movement recorders [IGER Behavior Recorder, Institute of Grassland and Environment Research, North Wyke, UK, as described by Rutter et al. (1997)] were attached to each cow for 3 d from 0700 to 1300 h. Data were summarized and analyzed as described by Graf et al. (2005) using the Microsoft Windows TM software Graze (Rutter, 2000).

Prior to the beginning of the experimental periods, animals were accustomed to the backpack, the blood sampling system, the activity meter, and the behavior recorder. All procedures were in accordance with the Swiss guidelines for animal welfare and were approved (No. 86/08) by the Animal Care Committee of the Canton Fribourg, Switzerland.

Laboratory Analysis

Feces and grass samples were lyophilized (Christ Delta 1-24 LSC, Osterode, Germany) and concentrate samples were dried at 60°C for 12 h. All samples were milled through a 1.0-mm screen (Brabender mill with titanium blades, Brabender, Duisburg, Germany), dried at 105°C for 3 h and subsequently incinerated at 550°C for 4 h to determine DM and ash contents. In the ash

residue of feed samples, minerals were digested with nitric acid and then analyzed for Ca, P, Ma, Na, and K using an inductively coupled plasma optical emission spectrometer (ICP-OES Optima 2000 DV, Perkin Elmer, Shelton, CT), as described by Boss and Fredeen (1999). The N content was measured using the Dumas method (AOAC, 1995; method number 968.06) and a C/N analyzer (type FP-2000, Leco Instruments, St. Joseph, MI). The CP content was calculated as $6.25 \times \text{N content}$. Ether extract was determined by pressurized solvent extraction with petrol-ether (fast PSE, Pressurized Solvent Extractor, Applied Separations, Allentown, PA) by heating at 125°C , 100 bar, during 3×12 min. Starch was analyzed with a 241 polarimeter (Perkin Elmer, Wellesley, MA) according to Naumann and Bassler (2007). The ethanol soluble carbohydrates were analyzed, as described by Shannon (1972), using a colorimetric autoanalyzer (Type II, Bran + Luebbe, Norderstedt, Germany). Cell wall constituents in feed and feces samples were analyzed using an Ankom 200/220 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Acid detergent fiber (AOAC, 1995; method number 973.18) was determined, with correction for residual ash obtained after incineration at 500°C for 1 h, and NDF was assayed with the addition of heat-stable amylase and sodium sulfite and with correction for residual ash (Mertens, 2002). The N-alkanes contents (C_{31} , C_{32}) in feed and feces were determined by gas chromatography with a flame ionization detector (GC-FID, Agilent 6850, Agilent Technologies, Santa Clara, CA), as described by Mayes et al. (1986). Milk samples were analyzed by infrared spectrometry (CombiFoss 6000, Foss, Hillerød, Denmark) for fat, protein, and lactose content (FIL-IDF, 2000; method number 141C). Urea was determined using a differential pH-analyzer (Eurochem, Ardea, Italy) before and after hydrolysis with urease (FIL-IDF, 2000; method number 145). For milk acetone determination, acetone and an internal standard (2-butanone) were transferred via static headspace directly from the milk into the gas phase. The composition of the gas phase was determined using a flame ionization detector on a gas chromatograph (HP 5890 Series II, Agilent Technologies, Santa Clara, CA). The extraction of the CO_2 fraction from the blood and the subsequent determination of the $^{13}\text{C}/^{12}\text{C}$ ratio were done according to Junghans et al. (2007). Briefly, 0.5 mL of thawed blood was placed in a 10-mL syringe that had previously been filled with argon. Two mL of 10% lactic acid were added and the syringe was locked by means of a multidirectional stopcock (Discofix-3, B. Braun Melsungen AG, Melsungen, Germany). After 2 h, the released CO_2 in the headspace of the syringe was transferred into an evacuated 13 mL

screw-cap Exetainer (Labco Limited, Buckinghamshire, UK) for dispatch. The $^{13}\text{C}/^{12}\text{C}$ ratio 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.

Calculations and Statistical Analysis

The dietary contents of NE_L and absorbable protein in the duodenum were calculated according to ALP (2008). Feed intake and digestibility were estimated using the ratio of the alkanes C_{31} and C_{32} , according to the equation proposed by Mayes et al. (1986). The NE_L balance was estimated as the difference between the NE_L intake and the NE_L output (NE_L requirements for maintenance and milk production, according to ALP, 2008). Energy expenditure over 6 h was calculated using a modified equation of Brouwer (1965), simplified by omission of the adjustment for excreted urinary N:

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

where **RQ** = respiration quotient. The RCO_2 term was calculated according to Elia et al. (1992) and Junghans et al. (2007):

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

where RCO_2 is the CO_2 production rate (L/6 h), D is the administered ^{13}C dose (mol), and AUC is the calculated area under the ^{13}C enrichment (atom percent excess, APE)-time curve ($\text{APE} \cdot \text{h}$). The recovery factor (RF) 0.7 was used to correct for the incomplete ^{13}C recovery in breath from the rate of CO_2 appearance in blood (Junghans et al., 2007) and a RQ of 1.0 was used, according to Elia and Livesey (1992). In detail, the sum of 2 exponentials was applied to fit the kinetics of ^{13}C enrichments. Nonlinear regression analysis based on the computer program TableCurve (2007 version, SYSTAT Software Inc., Chicago, IL) was used to determine the best-fit curve and to estimate the parameters of the following equation:

$$^{13}\text{C enrichment} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t},$$

where t is time and A_1 , A_2 , k_1 , and k_2 are coefficients in the two exponential equations fitting the time curve of ^{13}C enrichment in blood CO_2 (Figure 1). By integrating from $t = 0$ to ∞ , the area under the ^{13}C enrichment-time curve results from

$$\text{AUC} = A_1/k_1 + A_2/k_2.$$

Decay curves of ^{13}C enrichment in blood CO_2 of cows in the 2 treatments are shown in Figure 1, illustrating that the ^{13}C enrichment was sufficient to calculate the CO_2 production. Data collected over several days were averaged before statistical analysis.

Paired comparisons of the data from the preceding experiments were carried out using PROC TTEST in SAS (version 9.1, SAS Institute Inc., Cary, NC). Data from the main experiment were analyzed as a crossover design using PROC MIXED of SAS according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + E_{ijk},$$

where Y_{ijk} is the observation, μ is the overall mean, T_i is the treatment ($i = 1$ and 2 ; pasture or barn), P_j is the period ($j = 1$ and 2), C_k is the cow ($k = 1$ to 14), and E_{ijk} is the residual error. The model included treatment as fixed effect and cow as random effect. The relationship between EE and physical activity and EE and feeding behavior were examined using Pearson correlations. Effects were considered significant at $P < 0.05$.

RESULTS

Preliminary Experiments

No ($P = 0.29$) differences in CO_2 production of dairy cows were observed between automatic and manual sampling. Furthermore, CO_2 production estimated from blood samples frozen immediately after collection and blood samples cooled in the sampler for the whole 6-h period did not differ ($P = 0.89$). The CO_2 production ratio of manual to automatic sampling was 1.10 (SD 0.18) and that of cooled to frozen storage was 1.00 (SD 0.11).

Main Experiment

The composition of the grass offered was similar for the 2 treatment groups (Table 2). Negligible differences were observed for ADF, ether extract, and starch content.

Cows on pasture and in the barn consumed the same amount of grass and concentrate (Table 3). Consequently, total DM, fiber, and energy intake did not differ between treatment groups. Furthermore, no differences in OM and NDF digestibility were observed.

Neither BW nor milk yield differed between treatments (Table 4). The same held true for the percentage and yield of milk fat and lactose, whereas milk protein percentage was higher ($P < 0.001$) for cows on pasture compared with those in the barn. However,

Table 2. Composition of the experimental feeds

Item	Grass (barn) ¹		Grass (pasture) ²		Concentrate	
	Mean	SD	Mean	SD	Mean	SD
DM (g/kg of wet weight) ³	162	13.23	169	13.33	884	2.82
Analyzed nutrient and mineral composition (g/kg of DM) ⁴						
OM	910	3.91	910	7.34	921	0.21
CP	176	10.44	181	2.94	116	0.42
NDF	404	57.83	409	70.10	178	4.40
ADF	262	47.03	249	43.82	60	0.84
Ethanol-soluble carbohydrates	114	29.91	116	20.31	42	0.83
Starch	7.1	1.67	10.4	1.76	515.3	1.80
Ether extract	47.0	5.10	44.1	8.91	58.1	9.94
Ca	5.09	0.81	5.53	0.76	12.7	0.20
P	4.49	0.46	4.56	0.48	6.37	0.02
Mg	1.79	0.28	1.80	0.21	5.47	0.04
Na	0.23	0.02	0.24	0.03	6.15	0.12
K	32.9	0.52	33.2	0.97	6.37	0.12
Calculated energy and protein supply per kg of DM ⁵						
NE _L (MJ)	6.05	0.07	6.10	0.14	8.21	0.01
Absorbable protein (g)	104	0.01	105	0.02	95	0.02
Analyzed n-alkane contents (mg/kg of DM) ³						
C ₃₁	166	23.03	154	17.64	4	0.73
C ₃₂	3.76	0.75	3.27	0.50	0.20	0.18

¹Samples were collected from the cut grass offered to the cows in the barn.

²Samples were collected on pasture by following the cows and mimicking their grazing behavior.

³Means of 6 (grass) and 2 (concentrate) determinations.

⁴Means of 2 determinations per feed.

⁵According to ALP (2008).

this difference became negligible when milk protein was expressed as yield/d. Urea concentration tended to be higher ($P = 0.06$) in the milk of grass-fed cows in the barn compared with that of grazing cows. Milk acetone concentration remained unaffected. The NE_L balance

was negative for both treatments and did not differ between treatment groups.

During the 6-h EE measurement period, cows on pasture produced more ($P < 0.01$) CO₂ and consequently expended more ($P < 0.01$) energy than did grass-fed

Figure 1. Time curves of ¹³C enrichment in blood CO₂ of cows on pasture (squares) and cows in the freestall barn (circles) after intravenous bolus administration of NaH¹³CO₃ [n = 14, mean (SD)]. APE = atom percent excess.

Table 3. Effects of feeding system on nutrient intake and digestibility

Item	Treatment		SEM	Treatment effect (<i>P</i> -value)
	Barn ¹	Pasture ²		
Intake (per cow per day)				
DM (kg)				
Grass	16.1	15.0	0.95	0.42
Concentrate	6.39	6.39	0.26	0.98
Total	22.5	21.4	0.97	0.44
OM (kg)				
Grass OM	14.7	13.7	0.85	0.42
Concentrate OM	5.88	5.89	0.24	0.98
Total OM	20.5	19.6	0.87	0.43
NDF (kg)				
Grass	6.66	6.20	0.54	0.55
Concentrate	1.14	1.14	0.05	0.97
Total	7.80	7.34	0.55	0.56
Digestibility (%)				
OM	81.9	81.3	0.01	0.56
NDF	78.9	78.8	0.02	0.95

¹Cows were kept in a freestall barn and fed grass cut once daily.²Cows grazed full-time.

cows in the barn (Table 5). Within this time period, the number of steps and the motion index increased ($P < 0.001$) for grazing cows (Table 6). As a result, the proportion of time spent walking increased ($P < 0.001$) for these cows compared with those in the barn, which, conversely, spent more time standing ($P < 0.001$) and lying ($P < 0.05$). The feeding behavior of the treatment groups changed in such a manner that the proportion of time spent eating was higher ($P < 0.001$) and the proportion of time spent ruminating was lower ($P < 0.01$) for grazing cows compared with grass-fed cows in the barn. The amount of time spent idle was unaffected by treatment.

DISCUSSION

As pasture grass is a cost-effective forage, in regions with plentiful and regular grass growth, pasture-based production systems are economical strategies to produce milk. Furthermore, consumers prefer traditional pasture-based production systems because they convey a feeling of safety and proximity to nature and appear to be a more animal-friendly husbandry system. However, pasture-based feeding may increase energy requirements due to additional locomotion and the physical effort of eating feed from the sward (Holmes et al., 1978). Furthermore, potential higher energy demands for digestion and metabolism may exist because of

Table 4. Effects of feeding system on BW, milk performance, and NE_L balance

Item	Treatment		SEM	Treatment effect (<i>P</i> -value)
	Barn ¹	Pasture ²		
BW (kg)	658	658	16.3	0.97
Milk yield and composition				
Yield (kg/cow per day)	43.1	42.4	0.72	0.40
Fat (%)	3.62	3.76	0.11	0.24
Fat (kg/cow per day)	1.56	1.59	0.05	0.33
Protein (%)	3.02	3.12	0.04	<0.001
Protein (kg/cow per day)	1.3	1.32	0.02	0.49
Lactose (%)	4.94	4.93	0.03	0.75
Lactose (kg/cow per day)	2.13	2.09	0.04	0.46
Urea (mg/kg)	208	186	7.8	0.06
Acetone (mg/kg)	1.14	1.11	0.07	0.78
NE _L balance ³ (MJ)	-15.1	-21.9	5.82	0.41

¹Cows were kept in a freestall barn and fed grass cut once daily.²Cows grazed full-time.³According to ALP (2008).

Table 5. Effects of feeding system on CO₂ production and energy expenditure measured from 0700 to 1300 h

Item	Treatment		SEM	Treatment effect (<i>P</i> -value)
	Barn ¹	Pasture ²		
CO ₂ production (L/kg of BW ^{0.75})	12.0	14.6	0.60	<0.01
Energy expenditure (kJ/kg of BW ^{0.75})	253	307	2.63	<0.01

¹Cows were kept in a freestall barn and fed grass cut once daily.

²Cows grazed full-time.

the characteristics of pasture-based diets (i.e., energy-to-protein imbalance and high fiber content; Agnew and Yan, 2000; Bruinenberg et al., 2002). The actual energy costs of these factors are variable and poorly documented.

Assessment of the ¹³C Bicarbonate Dilution Technique to Measure EE in Grazing Cows

Indirect calorimetry performed in respiration chambers is the classical method to measure EE in dairy cows. However, this technique is not feasible to determine EE in grazing cows. Therefore, efforts have long been made to develop techniques that allow the measurement of EE of free-ranging animals. In 1958, Flatt et al. (1958) used tracheal cannulated cows and portable dry gas meters. However, Brockway (1978) questioned whether the system used could deal with the high pulmonary ventilation rates of adult cattle during exercise or of cows in warm climates. Furthermore, Young and Webster (1963) criticized the method, stating that bypassing the nasopharynx disturbs olfaction, communication, and thermoregulation. The required surgery is another disadvantage of this method. To avoid surgical invasion, de C. R. Ribeiro et al. (1977) equipped steers with respiratory masks. However, these masks are unsuitable for grazing animals (Brockway, 1978) due to the position of the apparatus, which hampers grazing

activity. Recently, heart rate monitoring has been used for grazing beef cattle (Brosh et al., 2004) and dairy cows fed a TMR in a free-stall barn (Aharoni et al., 2005) to estimate EE. This method has to be combined with individual measurements of oxygen consumption per heart beat (O₂ pulse) in constrained standing animals using an open-circuit system (Brosh et al., 2004). One approach for the indirect measurement of CO₂ production as a basis to estimate EE is the doubly labeled water technique (Speakman, 1998). However, tracer and analytical costs are very high, which limits its application in large animals. Alternatively, the CO₂ entry rate technique with the isotope ¹⁴C has been used by several researchers. A tracer solution has either been infused subcutaneously (Havstad and Malechek, 1982) or peritoneally (Di Marco and Aello, 1998) using a portable pump. The CO₂ production was calculated from the concentration and specific activity of CO₂ in continuously-sampled urine (Havstad and Malechek, 1982) and in periodic saliva samples (Di Marco and Aello, 1998). Increased awareness of the health hazards of radionuclides and a greater availability of stable isotopes has decreased the use of radioisotopes (Lachica and Aguilera, 2003) and has led to the use of ¹³C-labeled tracers. Junghans et al. (2007) validated a ¹³C dilution technique to estimate EE in young bulls using indirect calorimetry. They concluded that blood is a suitable medium to determine ¹³C dilution at equilib-

Table 6. Effects of feeding system on physical activity and eating pattern recorded from 0700 to 1300 h

Item	Treatment		SEM	Treatment effect (<i>P</i> -value)
	Barn ¹	Pasture ²		
Physical activity				
Standing (%)	46.9	34.9	2.39	<0.001
Lying (%)	43.7	37.3	2.32	<0.05
Walking (%)	9.4	27.9	2.24	<0.001
Steps (n)	370	1,224	76.71	<0.001
Motion index	2.2	13.5	0.87	<0.001
Eating pattern				
Eating (%)	36.5	47.1	1.99	<0.001
Rumination (%)	29.3	22.1	1.44	<0.01
Time idle (%)	34.2	30.8	2.18	0.14

¹Cows were kept in a freestall barn and fed grass cut once daily.

²Cows grazed full-time.

rium. However, so far, regular blood sampling without influencing the behavior of grazing animals has been difficult. This could be solved by the application of an automatic blood sampling system (Fønss and Munksgaard, 2008) that was previously used to determine cortisol concentration in dairy cows over a 2.5-h period. In preliminary experiments, we showed no differences in the CO₂ production determined by the ¹³C dilution technique, regardless of whether blood was sampled automatically or manually.

The EE was calculated according to the equation of Brouwer (1965), as modified by Junghans et al. (2007), which requires a given RQ. In the present study, the following considerations about the RQ were made: cows in the present lactation stage were in negative energy balance and mobilized fat from adipose tissue which would shift the RQ toward 0.7 (Elia and Livesey, 1992). Concomitantly, cows synthesized milk fat, which would result in a RQ of 1.3 (Elia and Livesey, 1992). Therefore, a RQ of 1 was used for further calculations. Nevertheless, although the RQ is an essential criterion, variations in the RQ have a relatively small effect on the estimated EE. According to Junghans et al. (2007), an error of +0.1 in absolute RQ units underestimates EE by only 8%. An alternative is to use the food quotient, which can be calculated from the diet composition (Black et al., 1986). However, RQ only equals the food quotient when animals are in energy balance (Black et al., 1986).

Due to the ¹³C kinetics of blood CO₂, after the intravenous bolus dose of NaH¹³CO₃, EE could only be estimated over a 6-h period in the present experiment. Compared with other techniques, this is a weakness that may be eliminated by several tracer bolus doses throughout the day or by the continuous infusion of NaH¹³CO₃ using a portable infusion pump. Nevertheless, the ¹³C bicarbonate dilution technique was suitable to distinguish between cows on pasture and in the freestall barn. The EE of grazing cows was 21% higher than that of grass-fed cows in the barn. To our knowledge, no data about the EE of lactating grazing dairy cows are available. When the present data were extrapolated to 24 h, the EE of cows on pasture was similar to that of lactating beef cattle on lush pasture in early springtime, measured by heart rate and oxygen consumption (Brosh et al., 2004). The EE of cows in the freestall barn was in the range of those reported by Bruinenberg et al. (2002), who reviewed EE data of grass-fed dairy cows, determined in respiration chambers in 3 different laboratories (908, 917, and 1044 kJ/kg of BW^{0.75}, respectively). Furthermore, the values of barn-housed cows were comparable to those of high-yielding dairy cows kept in an open freestall barn in hot weather and fed at night (Aharoni et al., 2005). However, for a final

evaluation of the EE measured using the ¹³C dilution technique, it has yet to be determined if these data can be extrapolated from 6 h to 24 h. Variation in the ambient temperature and changes in the behavior pattern of cows over 24 h might influence the estimation of EE.

Effect of Physical Activity and Feeding Behavior on EE

The energy costs of eating and walking were higher than those of ruminating, standing, and lying (Susenbeth et al., 1998; Aharoni et al., 2009). Therefore, the positive correlation between EE and time spent eating (Figure 2), and EE and time spent walking (Figure 3) confirmed that the higher EE of pasture cows is at least partly influenced by feeding behavior and physical activity. Grazing cows spent more time eating and less time ruminating, which agrees with the observations made by Gibb et al. (1999) for dry and lactating cows on pasture. Furthermore, similar to the present study, cows on pasture spend more time eating compared with grass-fed cows in the barn (Oshita et al., 2008). Similar DMI and longer eating time suggest that cows on pasture need more time to meet their nutritional demands. The same has been observed for cows grazing full-time on a pasture providing good quality herbage compared with cows grazing part time that were supplemented with hay or corn silage (Graf et al., 2005). It can, thus, be concluded that cows on pasture spend more time foraging or that cows on pasture have a smaller bite size due to more selective grazing compared with grass-fed cows in the barn (Oshita et al., 2008). Both patterns of behavior can explain the higher physical activity of grazing cows compared with barn cows that spent more time standing and lying. Similarly, Legrand et al. (2009) found lower daily lying time for cows on pasture. Hernandez-Mendo et al. (2007) observed the same behavior, but also showed that cows on pasture lie down more often than cows kept indoors. Lying time is strongly connected with ruminating (Schirmann et al., 2009), which supports the findings in the present study that grazing cows spent less time ruminating than grass-fed cows in the barn. The smaller bite size of grazing cows may result in smaller particles in the rumen and a higher passage rate (Oshita et al., 2008), resulting in less ruminating time. Gibb et al. (1999) suggested that less ruminating time for grazing cows decreases nutrient digestibility due not only to a higher passage rate, but also to decreased physical processing and breakdown of the feed particles. However, this could not be confirmed by the present results, where OM and NDF digestibility did not differ between grazing cows and grass-fed cows in the barn.

Figure 2. Correlation between time spent eating and energy expenditure of all cows measured from 0700 to 1300 h ($r = 0.42$, $P < 0.05$). Squares = cows on pasture; circles = cows in the freestall barn.

Inconsistency Between EE and Feed Intake and Milk Performance

The milk protein percentage was higher in grazing cows compared with cows in the barn. However, this difference became negligible when the milk protein yield was expressed on a daily basis, which suggests a dilution effect. Milk production and DMI were similar between treatments, although cows on pasture expended more energy than did grass-fed cows in the barn. Several reasons might exist for this discrepancy. The EE was measured for 6 h from 0700 to 1300 h, whereas milk production and feed intake were recorded for a 24-h period. It is known that feeding behavior and the physical activity of grazing cows may change over 24 h. Cows typically spend more time ruminating and less time eating at night (Graf et al., 2005). As ruminating is strongly connected with lying (Schirrmann et al., 2009), cows presumably spent more time lying than walking at night. Both activities, ruminating and lying, cost less energy than eating and walking (Susenbeth et al., 1998; Aharoni et al., 2009). Thus, EE might be different during a 24-h period and differences between pasture cows and barn cows might be somewhat less than observed here. However, this change in behavior alone cannot explain the inconsistency between EE and DMI and milk performance. Mohammed et al. (2009) and Oshita et al. (2008) hypothesized that grazing cows

can more readily reject unsuitable forage and preferentially consume the leafy and more easily digestible portions of grass compared with grass-fed cows in the barn. Furthermore, although chemical analyses showed no difference in grass quality for cows on pasture and in the barn, a bias in nutrient intake is possible due to diurnal variations in nutrient content. Daily grass samples were taken by following and mimicking cow selection on pasture during 1 h in the morning. However, this period might be too short; Orr et al. (2001) reported that concentrations of water-soluble carbohydrates increased by 17% when grass was offered in the afternoon instead of in the morning. The trend toward lower milk urea concentration in grazing cows compared with cows in the barn indicates that a higher content of highly fermentable carbohydrates may improve N utilization and milk production, despite similar DMI. A further explanation why grazing cows were able to cover the higher energy requirements (production + EE) might be body fat mobilization, which could not be reliably assessed in the present experiment. The calculation of the NE_L was too approximate to identify differences between grazing cows and grass-fed cows in the barn. Furthermore, milk acetone concentration, an indicator used to assess the mobilization of body fat (Pedernera et al., 2008), is blurred by the fact that ketone bodies may be used during muscular activity (Bergman, 1971).

Figure 3. Correlation between time spent walking and energy expenditure of all cows measured from 0700 to 1300 h ($r = 0.60$, $P < 0.01$). Squares = cows on pasture; circles = cows in the freestall barn.

CONCLUSIONS

The ^{13}C bicarbonate dilution technique, combined with an automatic blood sampling system, is a suitable technique to identify differences in EE between grazing cows and grass-fed cows in a freestall barn. It can be hypothesized that the higher EE of grazing cows may result from more eating and more physical activity. Before a specific recommendation on additional energy supply can be provided, it still has to be evaluated if EE measured for 6 h can be extrapolated to 24 h. In addition, more research is required to assess other factors, such as lactation stage and grazing management, to determine their effect on the EE of grazing cows.

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