Estimation of carbon dioxide production and energy expenditure of grazing cattle by the sulphur hexafluoride (SF_6) tracer gas technique

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Stewart, A. A., Undi, M., Wilson, C., Ominski, K. H. and Wittenberg, K. M. 2008. **Estimation of carbon dioxide production and energy expenditure of grazing cattle by the sulphur hexafluoride (SF_6) tracer gas technique.** Can. J. Anim. Sci. **88**: 651–658. The purpose of the study was to explore the suitability of the sulphur hexafluoride (SF_6) tracer gas technique to estimate CO_2 production and energy expenditure (EE) for grazing animals on pastures typical of western Canada. During each year of a 3-yr study, CO_2 emissions were collected from 60 yearling steers on grass pastures receiving one of three fertility treatments: no manure, liquid hog manure applied as a split application (74 kg available N ha^{-1}) in the spring and fall, and a single application of liquid hog manure applied at a rate of 155 kg available N ha^{-1} each spring. The mean CO_2 production estimate across all treatments was 5424 ± 2218 L d^{-1} (mean ± SD) with a range of 1099 to 11548 L d^{-1}. When compared across three grazing periods in June, July and August, steers produced more ($P < 0.05$) CO_2 in June than in either July or August. Metabolic body weight ($BW^{0.75}$), average daily gain (ADG), standing forage biomass, and forage neutral detergent fibre (NDF) and crude protein (CP) contents explained 33% of the variation in CO_2 production. Energy expenditure (EE) was estimated from CO_2 production by adopting an energetic equivalent of 22 kJ L^{-1} CO_2 and EE was calculated for animals that were not losing body weight at time of measurement. The average EE estimate was 1.2 ± 0.5 MJ kg^{-1} $BW^{0.75}$ d^{-1} with a range of 0.2 to 2.8 MJ kg^{-1} $BW^{0.75}$ d^{-1}. Energy expenditure declined as ADG and forage biomass declined, but EE was not influenced ($P > 0.05$) by fertility treatment. The EE estimate was higher ($P < 0.05$) early in the grazing season (June) and declined as the grazing season progressed. The SF_6 tracer gas technique was able to estimate EE of individual animals without interfering with herd dynamics. The technique was also able to show differences in EE in response to pasture conditions. The results of this study suggest that the SF_6 tracer gas technique shows potential as a simple and non-invasive method of estimating CO_2 production and EE for grazing animals. Further validation under different grazing conditions and with animals undertaking different degrees of activity is required. Comparisons with other field techniques of estimating EE are also important.

Key words: Energy expenditure, carbon dioxide, sulphur hexafluoride, grazing animals

Stewart, A. A., Undi, M., Wilson, C., Ominski, K. H. et Wittenberg, K. M. 2008. **Estimation de la production de dioxyde de carbone et de la dépense d'énergie par les bovins en paissance par détection de l'hexafluorure de soufre (SF_6).** Can. J. Anim. Sci. **88**: 651–658. L'étude devait établir si la détection de l'hexafluorure de soufre (SF_6) permet d'estimer la production de CO_2 et la dépense d'énergie des animaux mis à l'herbe dans les pâturages caractéristiques de l'Ouest canadien. Chaque année pendant trois ans, les auteurs ont recueilli les émissions de CO_2 de 60 bouvillons d'un an mis à l'herbe sur des pâturages bonifiés de trois façons : aucune application de fumier, application fractionnée de purin de porc (74 kg de N disponible par hectare) au printemps et à l'automne, et application simple de purin de porc à raison de 155 kg de N disponible par hectare au printemps. On estime la production moyenne de CO_2 pour les trois traitements à $5\,424 \pm 2\,218$ L par jour (moyenne ± É.-T.), la fourchette allant de 1 099 à 11 548 L par jour. Lorsqu'on compare les périodes de paissance de juin, de juillet et d'août, on constate que les bouvillons libèrent plus ($P < 0{,}05$) de CO_2 en juin que les deux autres mois. Le poids corporel actif ($PC^{0,75}$), le gain quotidien moyen, la biomasse fourragère et la concentration des fourrages en fibres au détergent neutre et en protéines brutes expliquent 33 % de la variation de la production de CO_2. La dépense d'énergie a été estimée en fonction de la quantité de CO_2 produite, pour un équivalent énergétique de 22 kJ par litre de CO_2, et a été calculée pour les animaux qui ne perdaient aucun poids au moment de la mesure. On estime la dépense moyenne d'énergie à $1{,}2 \pm 0{,}5$ MJ par kg de $PC^{0,75}$ par jour, pour une fourchette de 0,2 à 2,8 MJ par kg de $PC^{0,75}$ par jour. La dépense d'énergie diminue avec le gain quotidien moyen et la biomasse fourragère, mais elle n'est pas affectée ($P > 0{,}05$) par la méthode de fertilisation. Néanmoins, la dépense d'énergie estimée est plus élevée ($P < 0{,}05$) au début de la saison de paissance (juin) et diminue à mesure que la saison progresse. La technique de détection du SF_6 permet d'estimer la dépense d'énergie des animaux sans changer la dynamique du groupe. Cette technique permet aussi de discerner la variation de la

Abbreviations: **ADG**, average daily gain; **BW**, body weight; **$BW^{0.75}$**, metabolic body weight; **CO_2**, carbon dioxide; **CP**, crude protein; **DM**, dry matter; **EE**, energy expenditure; **NDF**, neutral detergent fibre; **SF_6**, sulphur hexafluoride

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dépense d'énergie d'après l'état du pâturage. Les résultats de l'étude laissent croire que la technique de détection du SF_6 pourrait devenir une méthode simple et non invasive d'estimer la production de CO_2 et la dépense d'énergie des animaux en paissance. Il conviendrait de valider ces résultats dans d'autres conditions de paissance et avec des animaux dont le degré d'activité diffère. Il faudrait aussi comparer cette technique avec d'autres techniques servant à évaluer la dépense d'énergie sur le terrain.

Mots clés: Dépense d'énergie, dioxyde de carbone, hexafluorure de soufre, animaux en paissance

Grazing animals are active much of the day, harvesting forage or walking in search of forage, water or shelter. The energy expenditure (EE) of grazing animals is related to grassland terrain, amount of time spent grazing, weather, and muscular work associated with eating, digestion, standing and walking (Osuji 1974; Caton and Dhuyvetter 1997). Estimates of energy expended by animals, such as those made by the Agricultural Research Council (1980), use information obtained from calorimetric studies (Havstad and Malechek 1982; Di Marco and Aello 2001). Early attempts to estimate EE of grazing animals depended on values obtained by calorimetry and on regression analysis of data relating to feed intake, liveweight and liveweight change (Whitelaw 1974).

In recent years, effort has been invested in the identification of techniques to estimate EE of grazing ruminants that are simple and do not alter the grazing behaviour of animals. Recent techniques utilize either oxygen (O_2) consumption or carbon dioxide (CO_2) production to estimate EE of animals. Depending on time after feeding, approximately 70 to 82% of CO_2 emission in ruminants comes from respiration and only a small proportion originates from enteric fermentation (Hoernicke et al. 1965). The CO_2 so produced can be used to estimate EE from established equations. The CO_2 entry rate technique (CERT), which involves infusing either ^{13}C-bicarbonate or ^{14}C-bicarbonate into the animal at a constant rate has been used to estimate CO_2 production and EE in grazing animals (Havstad and Malechek 1982; Di Marco et al. 1996, 1998; Lachica and Aguilera 2003). In turn, the doubly-labelled water technique calculates total CO_2 production and estimates EE by measuring turnover rates of isotopic hydrogen and oxygen (Fancy et al. 1986). This technique was found to overestimate CO_2 production in rapidly growing caribou and reindeer (Fancy et al. 1986). Energy expenditure has also been estimated from measurements of heart rate and O_2 consumption (Brosh et al. 1998; Brosh 2007). However, the relationship between EE and heart rate must be determined for the diet consumed at the time the O_2 pulse is determined (Brosh et al. 2002). Also, basal heart rate differs among individuals (Derno et al. 2005). Another technique utilized to estimate EE is the respiratory gas exchange method, which measures O_2 consumption and CO_2 production in tracheostomized animals (Whitelaw 1974). Problems encountered with this technique relate to the use of tracheostomized animals, which is expensive, has limitations in field application, creates animal welfare considerations and disrupts normal animal behaviour (Brosh et al. 2002).

Most techniques to estimate EE of grazing animals are not easily adaptable for use with large numbers of animals. The SF_6 tracer gas technique was originally developed to estimate CH_4 production in free-ranging animals (Johnson et al. 1994). Preliminary work from this laboratory (Boadi et al. 2002), which compared the SF_6 tracer gas technique and open-circuit calorimetry to estimate CO_2 production in crossbred yearling heifers, showed that, even though the SF_6 tracer gas technique overestimated CO_2 production, animal rankings were similar for the two techniques. This work suggested the potential use of the SF_6 tracer gas technique in energy balance studies for grazing animals. Compared with other techniques of estimating CO_2 production, the SF_6 tracer gas technique is relatively easy to carry out and has been used successfully to estimate CH_4 production in grazing animals (Boadi et al. 2002; Chaves et al. 2006). The SF_6 tracer gas technique offers the further advantage that CO_2 production can be estimated from a large group of grazing animals simultaneously. The purpose of this study was to explore the suitability of the SF_6 tracer gas technique to estimate CO_2 production and energy expenditure of grazing animals on pastures typical of western Canada.

MATERIALS AND METHODS

The study was located in southern Manitoba, near La Broquerie (SE20-5-8E), which has an average annual precipitation of 510 mm and annual temperature of 8.5°C (Province of Manitoba 2000). The selected grassland area was composed of mixed grasses and was divided into six paddocks, two of which were assigned to each of three treatments. The predominant grass species in the pastures were quackgrass [*Elytrigia repens* (L.) Nevski], timothy (*Phleum pratense* L.) and Kentucky bluegrass (*Poa pratensis* L.). This study was imposed on an existing pasture trial with three pasture fertilization strategies and across a 3-yr timeframe, which provided an opportunity to collect data from a large number of animals. The pasture trial evaluated the effect of liquid hog manure on the productivity of grass pastures. The three fertility treatments that were tested in the pasture trial were: (1) no manure (Control), (2) liquid hog manure applied as a split application (74 kg available N ha^{-1}) in the spring and fall (Split), and (3) a single application of liquid hog manure applied at a rate of 155 kg available N ha^{-1} each spring (Full).

The study extended over 3 yr, with animals turned onto a continuously grazed pasture in late May or early June, when standing forage biomass was estimated to be 1000 kg dry matter (DM) ha^{-1}. The grazing period was divided into three 28-d periods (June, July, and August) with measurements taken once each period. Ten British-continental cross yearling beef steers (338 ± 32 kg) were assigned to each treatment at the beginning of each grazing season. Animals in each group were assigned such that the final total weight of each pasture group was similar. Different animals were used each year, and the stocking rate varied from year to year and from period to period as "put and take" animals were used to keep standing forage biomass at approximately 1000 to 1500 kg DM ha^{-1}. Depending on forage biomass, stocking rates in any period could range from 0.7 to 5.6 head ha^{-1} over 3 yr.

Body weight (BW) and average daily gain (ADG) measurements were collected from animals in every period. Animals were weighed upon entry to the pasture at the start of each trial year and also at their removal at the end of the grazing season. Animals were also weighed on day 1 of each sampling period and again at the end of the gas sample collection period. Average daily gain was calculated as the weight at sampling time minus the weight at the beginning of the period divided by the number of days involved. Insecticide (Ectoban^{TM}) was applied when fly pressure was deemed excessive as determined by the presence of flies settling on the backs of the cattle. Animal handling and care procedures in this study were approved by the University of Manitoba Animal Care Committee according to guidelines of the Canadian Council on Animal Care.

Forage samples were clipped to a height of 3.75 cm from nine 0.25-m^2 quadrats in each pasture at the beginning of each period and again during gas collection. These clipped samples were used to determine standing forage biomass. The quality of consumed forage was estimated by following grazing animals and taking grab samples of forage comparable to that eaten.

Estimations of CO_2 Production and Energy Expenditure

The SF_6 tracer gas technique involves the release of SF_6, an inert and non-toxic tracer gas, at a known, steady rate from a permeation tube inserted in the rumen (Johnson et al. 1994). A diluted fraction of eructated and respired gases is collected, and total amounts of gases produced by the animal are calculated based on the CO_2/SF_6 molar ratio of concentrations (Johnson et al. 1994). During each sampling period, gas was collected continuously for two 24-h durations as described by Johnson et al. (1994) and Boadi et al. (2002). On day 1, 10 animals in each treatment were fitted with a gas collection apparatus. An additional collection apparatus was placed in each paddock to measure background levels of SF_6 and CO_2. On day 2, the collection apparatus was removed and the steel collection spheres were pressure-tested. A second gas sample was collected if the collection apparatus was compromised (e.g. disconnected hose) or if pressure was atmospheric. All collection equipment was removed on day 3.

Concentrations of SF_6 and CO_2 in collected breath and background samples were determined by injecting pressurized samples into the sample loop of a gas chromatograph (year 1: Star 3600, Varian, Mississauga, ON; years 2 and 3: CP-3800, Varian, Mississauga, ON), equipped with an electron capture detector for SF_6 and a flame ionization detector for CO_2 (Boadi et al. 2002). The gas chromatograph was calibrated with prepared standards (20.73 ppt (vol/vol) SF_6 – Scott-Marrin Inc., Riverside, CA; 1562 ppm (vol/vol) CO_2 – Praxair Gas, Welder's Supply, Winnipeg, MB). Concentrations of SF_6 and CO_2 were determined by the sample's peak area and retention time. The production of CO_2 was calculated using the equation developed for CH_4 estimation (Johnson et al. 1994) and modified for CO_2 estimation (Boadi et al. 2002):

$$CO_2(\text{L min}^{-1}) = \text{permeation tube } SF_6 \text{ releaser rate } (\text{L min}^{-1}) \times [CO_2]/[SF_6]$$

Where $[CO_2]$ and $[SF_6]$ are concentrations of CO_2 and SF_6 in breath samples above background concentrations. Average CO_2 concentration in breath samples was 460 ppm (vol/vol), with a range of 121 ppm to 2287 ppm. Average CO_2 concentration in background samples was 202 ppm (vol/vol), with a range of 158 ppm to 394 ppm. Daily CO_2 production was estimated as follows:

$$CO_2(\text{L d}^{-1}) = CO_2(\text{L min}^{-1}) \times \text{sampling duration } (\text{min d}^{-1})$$

Carbon dioxide production was estimated during the third period of 2004 and all three periods in 2005. In 2006, data were only collected for the first two periods because animals were removed from pasture early due to a shortage of forage associated with excessively dry conditions.

Estimation of EE from CO_2 production estimates was made by adopting an energetic equivalent of 22 kJ L^{-1} CO_2 (Sahlu et al. 1988; Di Marco et al. 1996). Data from animals that had negative weight gain were not included in the analysis because the calorific equivalent of CO_2 is dependent on the respiratory quotient (RQ). Calculation of heat production for animals catabolizing body tissue will affect RQ from CO_2 production resulting in erroneous EE estimation (Sahlu et al. 1988).

Chemical Analysis

Forage samples representing the material eaten were analyzed for neutral detergent fibre (NDF), using an

ANKOM 200 fibre analyzer (Fairport, NY) as described by Komarek (1994), and for crude protein (CP) using a Leco NS 2000 (LECO Corporation, St. Joseph, MI).

Statistical Analysis

The study provided an opportunity to estimate CO_2 production and EE in a range of environments, as reflected by pasture treatment, time of grazing season and year. Data were analyzed using PROC MIXED procedures of SAS software (Version 9.1, SAS Institute, Inc. 2003). The fixed effects in the model were treatment, period and treatment × period interaction. The Satterthwaite option was selected for the denominator degrees of freedom for tests of fixed effects. The model used a repeated measures design with period as the unit of time and animal (treatment × replicate × year) as the unit measured repeatedly. Animal (treatment × replicate × year) was included in the model as a random effect. Least squares means were calculated where appropriate and mean separation was done using Bonferroni's multiple comparison tests at the $P < 0.05$ probability level. Three CO_2 production estimates were determined to be outliers from Box plots constructed using Proc Univariate of SAS software and were removed from the analysis.

Multiple regression was used to estimate the variables that influence CO_2 production. The original regression equation tested the effects of metabolic body weight ($BW^{0.75}$), ADG, forage CP and NDF content, standing forage biomass, and interactions of these on CO_2 production. Least significant factors were removed one at a time from the model until the remaining factors were significant. Deviations from the mean of each variable were used in the regression. Dominant analysis (Azen and Badescu 2007) was used to compare variables and to determine which variables contributed significantly to the regression equation.

RESULTS AND DISCUSSION

Grazing Conditions, Forage Quality and Animal Performance

The range of pasture conditions and animal parameters observed over the course of the 3-yr study are shown in Table 1. Standing forage biomass, CP and NDF contents of the eaten material differed over the course of the grazing season. Standing forage biomass across treatments averaged 3242 kg DM ha^{-1} in June and declined by approximately 65% by August. The highest standing forage biomass (4079 kg DM ha^{-1}) occurred on the fertilized pastures in June. Values for unfertilized pastures, 1560 kg DM ha^{-1}, were lower ($P < 0.05$) in June. By August, standing forage biomass averaged 1157 kg DM ha^{-1} and did not differ ($P > 0.05$) among fertility treatments. Average forage CP content for the Full treatment (176 g kg^{-1}, DM basis) in June was higher ($P < 0.05$) than that of the other treatments. As the grazing season progressed, unfertilized pasture CP content declined from 109 to 72 g kg^{-1}, DM basis. By August, fertilized pastures contained 171 g CP kg^{-1}, DM basis, with no differences between them. Average forage NDF content (598 g kg^{-1}, DM basis) did not differ ($P > 0.05$) among treatments in June. In August, forage NDF content of fertilized pastures (563 g kg^{-1}, DM basis) was lower ($P < 0.05$) than NDF content of unfertilized pastures (618 g kg^{-1}, DM basis).

Average daily gain data used in this study were restricted to those animals that were not losing body weight at time of measurement. Across treatments, ADG declined from 2.5 kg d^{-1} in June to 0.5 kg d^{-1} in August. In June, ADG was higher ($P < 0.05$) in the Control and Split treatments compared with the Full treatment (Fig. 1). By July, ADG was similar across treatments, averaging 1.2 kg d^{-1}. In August, ADG was higher ($P < 0.05$) in the Control and Full treatments, averaging 0.84 kg d^{-1}.

Estimates of Carbon Dioxide Production

Two hundred and fifteen observations for CO_2 production estimates were included in the data set. The mean CO_2 production estimate across treatments and years was 5424 ± 2218 L d^{-1} (mean ± SD), with a range of 1099 to 11 548 L d^{-1}. The five lowest and five highest CO_2 production estimates averaged 1610 L d^{-1} and 10 542 L d^{-1}, respectively. When expressed relative to metabolic body weight, mean estimated CO_2 production was 70 ± 30 L kg^{-1} $BW^{0.75}$ d^{-1} with a range of 13 to 157 L kg^{-1} $BW^{0.75}$ d^{-1}.

Carbon dioxide production estimates (mean ± SD) in this study were similar to those reported in other studies (Kinsman et al. 1995; Boadi et al. 2002; Chaves et al. 2006; Pinares-Patiño et al. 2007). Boadi et al. (2002), using the SF_6 tracer gas technique, reported CO_2 production estimates with a range of 1541 to 3330 L d^{-1} for 400 kg beef heifers fed at 1.3 × maintenance requirements, whereas Kinsman et al. (1995) using an infrared gas analyzer to estimate whole barn CO_2 emission reported CO_2 production by lactating cows of 6137 L d^{-1} (range = 5032 to 7427 L d^{-1}). Using the SF_6 tracer gas technique, Chaves et al. (2006) reported CO_2 production estimates of 2327 to 3440 L d^{-1} for heifers weighing 380 kg and grazing alfalfa pastures and 2823 to 3541 L d^{-1} on grass pastures. Recently, Holstein heifers kept on low [1.1 livestock units (LU) ha^{-1}] or high (2.2 LU ha^{-1}) stocking rates produced 4655 and 4860 L d^{-1} of CO_2, respectively (Pinares-Patiño et al. 2007). At both stocking rates, CO_2 production ranged from 4306 to 6305 L d^{-1} and varied with grazing season.

In this study, there was considerable range in estimated CO_2 production, as would be expected when sampling a large number of animals over three fertility treatments, three periods and 3 yr. Thus, greater variation in CO_2 production was observed compared with previously reported studies. Extreme values may not realistically represent CO_2 production and may be

Table 1. Means, standard deviations, and range of variables used in the regression analysis of CO_2 production estimates

Variable	N	Mean	Standard deviation	Minimum	Maximum
Crude protein (g kg^{-1} DM)	36	130	49	70	260
Neutral detergent fibre (g kg^{-1} DM)	36	590	37	490	650
Standing forage biomass (kg DM ha^{-1})	36	1850	996	323	4180
Body weight (kg)	218	338	31.7	272	430
Metabolic body weight ($BW^{0.75}$) (kg)	218	79	5.5	67	94
Average daily gain (kg d^{-1})	218	1.3	0.8	0	4.7
Carbon dioxide (L d^{-1})	215	5424	2218	1099	11548
Carbon dioxide (L $kg^{-1}BW^{0.75}$ d^{-1})	215	70	30	13	157

attributed to compromised collection apparatus, as well as other factors such as animal congregation during the 24-h sampling period resulting in uptake of a portion of gases expired by adjacent animals.

The SF_6 tracer gas technique has been reported to overestimate CO_2 production (Boadi et al. 2002; Pinares-Patiño et al. 2007). Comparing the SF_6 tracer gas technique and open-circuit calorimetry to estimate CO_2 production in crossbred yearling heifers, Boadi et al. (2002) showed that the SF_6 technique consistently overestimated CO_2 production by 20%. The higher CO_2 estimate by the SF_6 tracer gas technique was attributed to, among other factors, more activity by penned animals versus those in the respiratory chamber. Pinares-Patiño et al. (2007) speculated that the SF_6 tracer gas technique may overestimate CO_2 production by as much as 65%. In the current study, only statistical outliers were removed from the data set. This resulted in a wider variation in CO_2 estimates than previously reported. However, the mean ± SD are within estimates for CO_2 emission reported in other studies.

Pasture treatment had no influence ($P > 0.05$) on CO_2 production estimates expressed as L d^{-1} or as L kg^{-1} $BW^{0.75}$ d^{-1}. Grazing period, however, influenced ($P < 0.05$) CO_2 production estimates in that more CO_2 was produced in June than in July or August (Table 2). The same trend was observed when CO_2 production was expressed relative to metabolic body weight (Table 2).

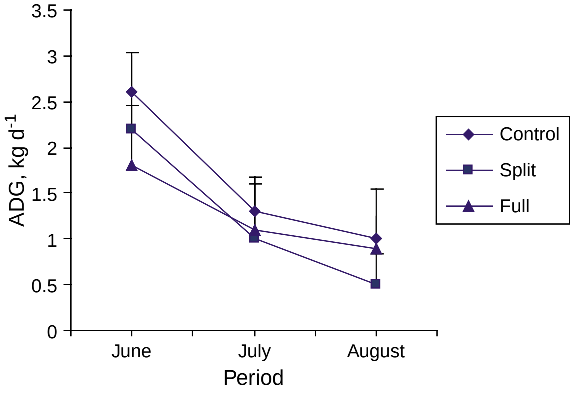

Fig. 1. Average daily gains (kg d^{-1}) of steers from which EE was estimated. Animals with negative ADG were not included.

Metabolic body weight, ADG, standing forage biomass, forage NDF and CP content and their interactions accounted for 33% of the variation in estimated CO_2 production. Approximately 40% of the explained variation in estimated CO_2 production was accounted for by the animal variables, of which $BW^{0.75}$ was most important. Of the remaining 60%, forage NDF and CP content and their interactions accounted for 48% of the variation in CO_2 production estimate. When estimated CO_2 production was expressed relative to $BW^{0.75}$, 37% of the variation in CO_2 production estimate was accounted for by $BW^{0.75}$, ADG, standing forage biomass and forage NDF and CP contents and their interactions. Carbon dioxide production by grazing animals increased linearly ($P < 0.05$) with increasing forage NDF content. However, there was an interaction effect of forage NDF and CP on the CO_2 production estimates (Fig. 2). For example, when forage CP content was high (1 SD above its mean), rate of increase of CO_2 production with increasing NDF content was greater than when forage CP was lower. Other environmental factors such as temperature, precipitation, and insect pests would be expected to influence CO_2 production in grazing animals. However, data for these parameters was not collected in this study.

Estimates of Energy Expenditure

Grazing animals spend 7 to 10 h d^{-1} harvesting forage or walking in search of forage, water or shelter and grazing time increases as quantity of available forage decreases (Havstad and Malechek 1982; Di Marco et al. 1996). The EE associated with grazing is related to grazing time, forage availability, and muscular work associated with eating, digestion, standing and walking (Osuji 1974; Caton and Dhuyvetter 1997). Environmental conditions, level of production, and season of the year also can affect the EE of animals (National Research Council 1981). Additional unknown stress-related factors may also contribute to increase in EE when animals are forced to search for feed under conditions of scarcity (Havstad and Malechek 1982).

In this study, EE estimates ranged from 0.2 to 2.8 MJ kg^{-1} $BW^{0.75}$ d^{-1}, with a mean of 1.2 ± 0.5 MJ kg^{-1} $BW^{0.75}$ d^{-1}. Adopting a daily maintenance energy requirement (ME_m) of 0.416 MJ kg^{-1} $BW^{0.75}$ d^{-1} reported for Hereford steers (286 ± 5 kg) in confinement (Derno et al. 2005), the 0.2 MJ kg^{-1} $BW^{0.75}$ d^{-1} EE

Table 2. Effects of pasture N fertilization treatments, period and their interactions on estimated CO_2 production, energy expenditure (EE) and ratio of energy expenditure to maintenance energy requirement (EE/ME_m)

	Treatment (T)^z				Period (P)				Significance		
	Control	Split	Full	SE^y	June	July	August	SE^y	Treatment	Period	T × P
CO_2 (L d^{-1})	5674	4765	5640	495	6071*a*	5350*b*	4657*b*	487	0.16	0.01	0.28
CO_2 (L kg^{-1}BW$^{0.75}$ d^{-1})	72	62	73	6.6	82*a*	68*b*	57*b*	6.6	0.20	0.004	0.19
EE (MJ kg^{-1}BW$^{0.75}$ d^{-1})	1.3	1.1	1.3	0.12	1.4*a*	1.2*b*	1.0*b*	0.12	0.20	0.001	0.19
EE/ME_m^{x}	3.0	2.6	3.1	0.28	3.4*a*	2.9*b*	2.4*b*	0.28	0.20	0.001	0.19

[z] Treatments were no manure (Control), liquid hog manure applied in split applications (74 kg available N ha^{-1}) in the spring and fall (Split), and a single application of liquid hog manure applied at a rate of 155 kg available N ha^{-1} each spring (Full).
[y] Standard error of least square treatment means.
[x] Ratio of EE to maintenance energy requirement (ME_m). ME_m was assumed to be 0.416 MJ kg^{-1} BW$^{0.75}$ d^{-1} (Derno et al. 2005)
a, *b* Means in the same row followed by different letters differ ($P < 0.05$).

estimate reported at the lower extreme does not meet energy requirements for maintenance and represents approximately 50% of maintenance energy requirement (0.48 × EE at maintenance). Such an EE estimate would be considered an aberration in the data. The EE estimate at the other extreme (2.8 MJ kg^{-1} BW$^{0.75}$ d^{-1}) is equivalent to 6.7 × EE at maintenance and is far above expected heat losses for a beef steer on pasture, where level of intake would be approximately twice maintenance. However, based on results of an outlier test, there was no statistically justifiable reason to remove these estimates from the larger data set.

Pasture treatment did not influence ($P > 0.05$) EE estimates of grazing steers. There was, however, a period effect ($P < 0.05$) in that EE was highest ($P < 0.05$) in June compared with subsequent grazing periods (July and August, respectively; Table 2). The highest EE occurred when ADG was highest (2.5 kg d^{-1}) and this was also the period when standing forage biomass was highest, averaging 3242 kg DM ha^{-1}. Pasture treatment also did not influence ($P > 0.05$) the EE/ME_m ratio (Table 2). This suggests that animal activity or behaviour was similar across pastures. There was, however, a period effect ($P < 0.05$) on the EE/ME_m ratio. In July and August, the EE/ME_m ratio was lower ($P < 0.05$) than in June, suggesting that more EE in June was associated with activities related to grazing and weight gain. This would explain, in part, the higher ADG in June relative to July and August.

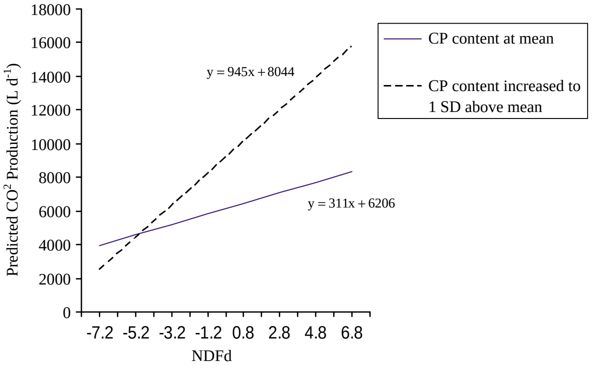

Fig. 2. Effect of forage NDF content on steer predicted CO_2 production when deviation from the mean for all variables (BW$^{0.75}$, ADG, standing forage biomass, forage NDF and CP) equals zero and when deviation from the mean for CP content is changed to one standard deviation above the mean and all other variables remain the same. Variables in the regression equation were expressed as deviations from the mean. That is, NDF_d = NDF deviation from the mean.

Brosh et al. (2004) attributed changes in EE of grazing cows over the course of the year to differences in herbage quality and intake. Herbage quantity is important since the EE of grazing cattle also depends on the rate of biting (Di Marco et al. 1996). The higher EE estimates that were reported by Sanchez and Morris (1984) occurred when pasture was of high quality and cows were gaining body weight. The lack of differences in EE estimates among fertility treatments in the current study may be due to the amount of available forage. By attempting to maintain standing forage biomass at 1000 to 1500 kg DM ha^{-1}, it can be speculated that the animals spent a similar amount of time grazing. The contribution of eating to the daily EE of an animal grazing pasture with low levels of standing forage would be considerably greater since the time spent grazing is greatly increased (Osuji 1974).

In the foothills of California, Sanchez and Morris (1984) reported an EE estimate of 1.1 MJ kg^{-1} BW$^{0.75}$ d^{-1} when pasture was of high quality and animals were gaining body weight (Table 3), with EE declining towards the end of the grazing season with decreased pasture quality. In lactating beef cows on lush grass pasture in early spring, Brosh et al. (2004) reported an EE estimate of 1.3 MJ kg^{-1} BW$^{0.75}$ d^{-1} when stocking rates were low. From the work of Pinares-Patiño et al. (2007) who used the SF_6 tracer gas technique to estimate CO_2 production, it can be calculated that EE estimates of Holstein heifers grazed at two stocking rates ranged from 1.0 to 1.4 MJ kg^{-1} BW$^{0.75}$ d^{-1} with a mean of 1.1 MJ kg^{-1} BW$^{0.75}$ d^{-1}. This calculation was made with the assumption that no animals had negative weight

Table 3. Summary of estimates of energy expenditure (EE) for grazing animals as determined by different methods

Animal	Pasture type	Method of estimation	EE (MJ kg^{-1} $BW^{0.75}$ d^{-1})	Reference
Lactating beef cows	Lush pasture in early spring	HR[z]	1.3	Brosh et al. (2004)
Lactating beef cows (3–6 yr old)	High quality lush herbage	CERT[y]	1.1	Sanchez and Morris (1984)
Yearling beef steers (338 kg)	Mixed grass pastures with different fertility treatments	SF_6[x]	1.2	Current study
Beef cows (3–6 yr old; towards the end of lactation)	Poorer quality pasture	CERT	0.8	Sanchez and Morris (1984)
Beef heifers (305 kg)	Crested wheatgrass	CERT	0.7	Havstad and Malechek (1982)

[z] Heart rate measurement and oxygen consumption technique.

[y] Carbon dioxide entry rate technique.

[x] Sulphur hexafluoride (SF_6) tracer gas technique.

gains during the course of that study. Havstad and Malechek (1982) reported lower EE estimates for heifers grazing crested wheatgrass (Table 3).

The EE estimate under conditions of high forage biomass reported in this study agrees with that observed by Sanchez and Morris (1984) for cows on lush pasture in early spring and by Brosh et al. (2004) for cows kept at a low stocking rate. In all these cases, the high EE estimate can be attributed partly to high standing forage biomass. Although physiological state differed, animals in the current study achieved relatively high rates of gain, averaging 1.3 kg d^{-1}. Studies that restricted feed availability have reported lower EE estimates. For example, Di Marco et al. (1996) reported that 18- to 20-mo-old Angus steers allowed short periods of grazing (1 to 5.5 h d^{-1}) had EE estimates of 0.06 to 0.18 MJ kg^{-1} $BW^{0.75}$ d^{-1}. Direct comparisons of EE estimates across studies are hampered by differences in location, time spent grazing, type of diet, type of animals used in terms of age, weight, sex, and physiological status.

CONCLUSIONS

Estimation of EE in grazing animals has been hampered by lack of a technique that can be used for many grazing animals simultaneously. The SF_6 tracer gas technique was able to estimate EE of individual animals without interfering with herd dynamics. The technique was also able to show differences in EE in response to pasture conditions, as was evidenced for June versus July and August pastures. The results of this study suggest that the SF_6 tracer gas technique shows potential as a simple, non-invasive, and field-friendly method of estimating EE. Further validation under different grazing conditions and with animals undertaking different degrees of activity is required. Comparisons with other field techniques of estimating EE are also important.

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