



Estimation of the energy cost of activities in grazing dairy cows using the oxygen pulse–heart rate method

Daniel Talmón,^{1*} Alejandra Jasinsky,¹ Federica Marin,¹ Gabriel Menegazzi,^{1,2} Pablo Chilbroste,¹ and Mariana Carriquiry¹

¹Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay 12900

²Animal Nutrition Group, Department of Animal Sciences, Wageningen University and Research, Wageningen, the Netherlands 6700 AH

ABSTRACT

Grazing dairy production systems have gained interest due to the increasing consumer preferences associated with environmental care and animal welfare perception. Nonetheless, grazing dairy cows have lower feed conversion than those fed indoors with a TMR, partly because of increased maintenance energy costs associated with grazing and walking activity. However, to our knowledge, the energy cost of grazing has not been quantified for dairy cows. The objectives of the present work were to evaluate the effects of grazing on the oxygen pulse (O_2P ; mL O_2 consumed per heartbeat; experiment 1) and to quantify the energy cost of grazing in dairy cows using the oxygen pulse–heart rate (O_2P -HR) technique (experiments 1 and 2). In both experiments, heat production (HP) was measured using the O_2P -HR technique and synchronized with animal activity records. In experiment 1, 15 dairy cows were used to measure the O_2P when resting and then immediately after the first grazing session to assess the effect of grazing on O_2P . Grazing activity increased HR and oxygen consumption compared with when the cows were resting. However, the increase in both variables was proportional; therefore, O_2P was similar, indicating that the change in HR was the main component in the response to an increased O_2 demand due to grazing. Hence, it is possible to use the O_2P -HR method to measure HP during grazing in dairy cows. The energy cost of grazing was estimated by the difference in HP between when the cow was grazing and when it was not grazing (i.e., idling or ruminating) and ranged from 6.63 ± 1.12 to 7.85 ± 0.68 kJ/kg^{0.75} per hour and 1.31 ± 0.28 to 1.59 ± 0.14 kJ/kg per hour, respectively. In addition, the energy cost of walking was calculated as the difference in HP when the cow was walking to and from the pasture and the milking parlor versus when

it was idling or ruminating. The energy cost of walking was 24.03 ± 1.12 kJ/kg^{0.75} per hour or 4.72 ± 0.28 kJ/kg per hour. Hourly energy expenditure (kJ/h) was similar between grazing and consuming TMR at the feed bunk, indicating that the extra energy required by dairy cows when fed in a grazing system is mostly explained by the longer time spent to harvest the pasture and the energy cost of walking between the pasture and the milking parlor compared with confined cows fed TMR. Finally, the results of this study suggest that O_2P is not affected by grazing and therefore that the O_2P -HR technique is a promising methodology to estimate the energy cost of grazing animals without substantially affecting their behavior within the production system.

Key words: energy expenditure, indirect calorimetry, grazing cattle, maintenance energy cost

INTRODUCTION

Interest in grazing dairy farms has recently increased due to their economic, environmental, animal welfare, and product quality benefits currently demanded by consumers. In pasture-based dairy systems, achieving a high forage consumption per cow to satisfy its nutrient requirements is indispensable to increase milk production (Dillon, 2006). However, decreased milk yields due to reduced feed intake and increased maintenance energy cost have been reported when comparing high-yielding dairy cows managed under grazing versus confinement systems (Kolver and Muller, 1998; Bargo et al., 2002).

Grazing cattle expend more energy harvesting feed (walking to selected feeding stations, prehension, and chewing of selected bites) than cattle fed TMR indoors (NASEM, 2021) due to the greater physical effort of the grazing animal to harvest feed from the sward (Agnew and Yan, 2000). However, to our knowledge, reports on the energy cost of grazing cattle, particularly in dairy cows, are scarce. Indeed, the model of the energy cost of walking and grazing in the eighth and latest edition of Nutrient Requirements of Dairy Cattle (NASEM, 2021)

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*Corresponding author: danieltalmon94@gmail.com

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

is based on a few studies in beef cattle (di Marco et al., 1996; Aharoni et al., 2009; Brosh et al., 2010). Few studies have reported the extra energy requirement for grazing, probably due to the complexity of quantifying it because grazing is a process that occurs on a spatial scale that is not possible to replicate within respiration chambers, which are considered the gold standard method for measuring heat production (HP). Therefore, alternative methods such as the oxygen pulse (O_2P ; mL of O_2 consumed/heartbeat)–heart rate (HR) technique could be used to estimate the energy expenditure for grazing cattle (Brosh, 2007).

The O_2P -HR method is an indirect calorimetry method based on the physiological relationship between oxygen consumption (VO_2) and HR. With this method, if these 2 variables are calibrated against each other under controlled conditions, VO_2 and therefore HP can be estimated through HR measurement in free-range animals (Butler et al., 2004; Brosh, 2007). Oss et al. (2016) reported that daily HP estimates using the O_2P -HR method were comparable to those estimated using respiration chambers, whereas Talmón et al. (2023), also using respiration chambers, reported that O_2P was barely affected by animal activity (eating at the feed bunk, ruminating, or idling) or posture (lying or standing) in dairy cows. Based on these works, it can be concluded that it is possible to accurately estimate HP for those activities and postures by measuring HR and using a single O_2P value per cow measured when the cow is standing and idling. Although several studies have reported the energy cost of grazing in beef cattle by measuring HR (Brosh et al., 2006, 2010; Aharoni et al., 2009), the relationship between VO_2 and HR during grazing, and therefore also the applicability of the O_2P -HR technique to estimate grazing HP, have not been evaluated. Thus, the objectives of the present work were to evaluate the effect of grazing on O_2P and to quantify the energy cost of grazing and other activities in dairy cows using the O_2P -HR technique. We hypothesized that grazing activity would not affect O_2P and that it would therefore be similar to the O_2P of a standing idling cow. In addition, we hypothesized that HP would be greater during grazing than when the cow is ruminating or idling but would be lower than when the cow is walking, reflecting changes in HR.

MATERIALS AND METHODS

This study consisted of 2 experiments. In experiment 1, O_2P was measured at rest and immediately after the first grazing session to assess the effects of grazing on O_2P in dairy cows, and in addition, the energy cost of grazing was measured by determining the difference in HP between grazing and resting using the O_2P -HR technique. In experiment 2, HP records were synchronized with animal

posture and activity records throughout the day to quantify the energy cost of different activities of dairy cows managed in 2 grazing systems with TMR supplementation. Experiment 1 was conducted chronologically after experiment 2; however, it is presented first to improve the coherence and understanding of this publication because the results of experiment 1 provide methodological support to the results obtained in experiment 2.

Experiment 1

The experiment was carried out in April 2024 at the dairy research facilities of the Instituto Nacional de Investigación Agropecuaria—La Estanzuela (INIA; Colonia, Uruguay). All the experimental procedures were approved by the INIA Commission on Ethics in Use of Experimental Animals (file #INIA2017.2).

Experimental Design, Animals, and Feeds. Fifteen nonpregnant Holstein dairy cows (6 primiparous and 9 multiparous) weighing 601 ± 58 kg of BW and producing 23.8 ± 3.2 kg of milk per day at 217 ± 41 DIM (mean \pm SD) were used to evaluate the effect of grazing on O_2P and to estimate the energy cost of grazing through measurements of VO_2 and HR. The minimum number of animals to be used in the experiment was previously evaluated to detect differences greater than 10% of the mean with 95% confidence and 80% statistical power in a repeated measures design. The mean value of O_2P and the maximum variation between animals reported by Talmón et al. (2023) for dairy cows in respiration chambers were considered for the power analysis and indicated that a minimum of 8 cows were needed, which is in line with previous reports (Butler et al., 2004; Brosh, 2007). All cows used in the experiment were in good health, as they came from the general herd of the experimental station, which was subjected to regular health checks before the experiment.

Because O_2P during grazing cannot be quantified in a respiration chamber, O_2P was measured immediately before and after a grazing session to assess the effect of grazing on O_2P (Figure 1). Cows were placed in a pen for at least 2 h after the morning milking (0500 h) without access to water or feed to warrant a baseline resting state (**RES-state**) in which the VO_2 and HR were measured. Immediately after its RES-state measurement, each cow grazed in a 1-ha paddock of a pasture composed of *Cynodon dactylon* and *Pennisetum clandestinum* (4,538 kg of DM/ha and 22 ± 3 cm height from ground level; 19.1%, 64.7%, and 28.6% of CP, NDF, and ADF, respectively) during the first grazing session in which grazing time, bite rate, and HR were recorded. The facilities used to measure the VO_2 were located adjacent to the paddock (20 m) to minimize time and physical activity of walking to and from the pasture.

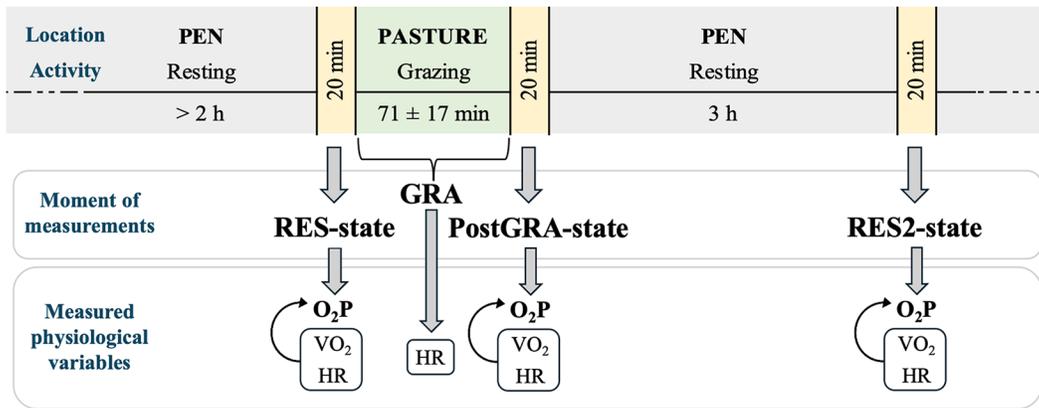


Figure 1. Experimental diagram of the moments of measurements (RES-state = resting state; GRA = grazing; PostGRA-state = post grazing state; RES2-state = resting after grazing) and the physiological variables measured (HR = heart rate; VO_2 = oxygen consumption; O_2P = oxygen pulse) in experiment 1.

Immediately after the first grazing session (**PostGRA-state**), VO_2 and HR were measured again in each cow while it remained in a pen without access to water or feed. The VO_2 and HR measurements were repeated 3 h after the end of the grazing session (**RES2-state**) to compare with the RES-state. Only 9 of the 15 cows were measured in RES2-state due to the time available before the afternoon milking. Nevertheless, the number of cows evaluated in RES2-state was greater than the minimum number of cows required to evaluate O_2P mentioned previously. The 9 cows evaluated in RES2-state are representative of the total group of cows (Appendix Table A1), so the results are not expected to be affected.

The VO_2 was measured using a facemask open-circuit respirometry system (Fedak et al., 1981). During the VO_2 measurement, the cow was immobilized using a cattle squeeze chute where cow weight was recorded, and a facemask was mounted covering the snout to ensure that the exhaled air was collected. To obtain reliable O_2P values, this procedure was performed by people experienced in the methodology and with calm handling to minimize stress to the cows, which was evidenced by HR monitoring (Kovács et al., 2014). A mass flow system (Flow-Kit 500, Sable Systems International) was used to control and measure the air flow rate through the mask and to deliver air samples from the facemask to a fully integrated respirometry system combining O_2 and H_2O analysis with data acquisition and subsample flow control and measurement (field metabolic system, FMS; Sable Systems International). Subsamples from the facemask air were collected by the FMS at 1-s intervals over 20 min, and ambient air samples were collected during 4 min before and after the 20-min measurement to set the baseline. Before VO_2 measurements, calibrations for the O_2 analyzer

(span) and water vapor sensor (zero and span) were performed following the methods described in the user manual provided by the manufacturer (Sable Systems International). The air flow and O_2 concentration data were processed and corrected to obtain standard temperature-pressure dew point (STD) values using the software ExpeData (Sable Systems International).

The VO_2 was calculated according to the following equation [Equation 1]:

$$VO_{2(L/min)} = STD FR_{(L/min)} \times (O_2fm - O_2b), \quad [1]$$

where $STD FR$ is the STD flow rate, O_2fm is the O_2 concentration of the air from the facemask, and O_2b is the O_2 concentration of the ambient air.

The respirometry system was checked before and after the measurement by injecting known amounts of N_2 and comparing them with the data coming from the FMS to calculate the N_2 recovery (McLean and Tobin, 1988). The average N_2 recovery was $99.7\% \pm 1.1\%$ (mean \pm SD).

The HR was recorded every 5 s when the cow was immobilized in the cattle chute (RES-state, PostGRA-state, and RES2-state; 20 min for each time point) for the VO_2 measurement and while it was on the pasture during the first grazing session (GRA; 71 ± 17 min, mean \pm SD). The recording of HR was performed using Polar devices (Polar Electro Oy), with a model H10 HR transmitter and a watch model RCX3 data logger. Both electronic devices were mounted on the animal using an elastic belt fitted around the thorax and behind the forelegs, and conductive gel was used to ensure conductivity.

Heat production during GRA was estimated using the O_2P -HR technique (Brosh, 2007). The O_2P was determined by the average VO_2 ($mL/kg^{0.75}$ per minute) over the average HR (beats/min) using the 20-min records where

both variables were measured simultaneously. The HP was calculated using the following equation [Equation 2]:

$$HP_{\left(\frac{\text{kJ}}{\text{kg}^{0.75} \cdot \text{h}^{-1}}\right)} = HR_{\left(\frac{\text{beats}}{\text{min}}\right)} \times O_2P_{\left(\frac{\text{mL}}{\text{kg}^{0.75} \cdot \text{beat}^{-1}}\right)} \times 20.47_{\left(\frac{\text{J}}{\text{mL O}_2}\right)} \times \frac{60_{\left(\frac{\text{min}}{\text{h}}\right)}}{1,000_{\left(\frac{\text{J}}{\text{kJ}}\right)}}, \quad [2]$$

where 20.47 represents the amount of heat produced per milliliter of O₂ consumed assuming that the respiratory quotient is 1 (McLean, 1972), and O₂P is the average value of RES-state and PostGRA-state.

When VO₂ was measured, HP was calculated using the following equation [Equation 3]:

$$HP_{\left(\frac{\text{kJ}}{\text{kg}^{0.75} \cdot \text{h}^{-1}}\right)} = VO_2_{\left(\frac{\text{mL}}{\text{kg}^{0.75} \cdot \text{min}^{-1}}\right)} \times 20.47_{\left(\frac{\text{J}}{\text{mL O}_2}\right)} \times \frac{60_{\left(\frac{\text{min}}{\text{h}}\right)}}{1,000_{\left(\frac{\text{J}}{\text{kJ}}\right)}}. \quad [3]$$

Bite rate was registered by a trained operator through visual observation by counting the number of bites made during 1 min (Gibb et al., 1997). Bites were identified from the ripping noise as leaves were fractured (Illius et al., 1995). This procedure was repeated for each cow through scan sampling every 10 min from when the cow entered the pasture until the end of the first grazing session when it was moved back to the animal chute for PostGRA-state measurement. Therefore, the grazing bite rate of each cow was determined as the average of 8 ± 2 one-minute observations.

Samples of the grazed pasture were collected by hand clipping (Coates and Penning, 2000), dried at 60°C for 96 h, and composited in a unique sample. The composited sample was analyzed at the Laboratorio de Nutrición Animal (Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay) for DM (AOAC 934.01), CP (AOAC 990.03), ash (AOAC 942.05; AOAC, 2023), and ADF and NDF (Ankom Technology Methods 5 and 6, respectively; Ankom Technology, 2017a, b).

The mean temperature during measurements was 20.2°C (maximum and minimum of 23.5 and 16.9°C, respectively), and the mean relative humidity was 80% (maximum and minimum of 96% and 64%, respectively). The mean temperature-humidity index (Mader et al., 2006) was 67 (maximum and minimum of 71 and 62, respectively); therefore, no heat stress conditions were evidenced (Armstrong, 1994).

Statistical Analysis. Data were analyzed using SAS software (SAS University Edition, SAS Institute Inc.). All variables were analyzed with a mixed model using

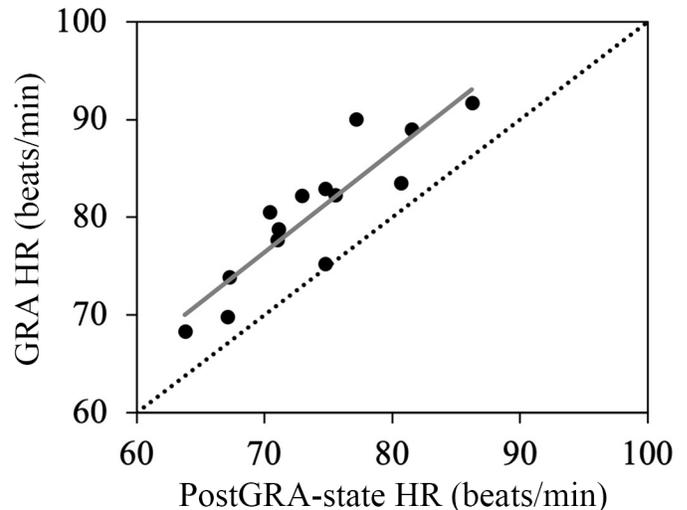


Figure 2. Relationship between heart rate (HR; beats/min) immediately after grazing (PostGRA-state) versus during grazing (GRA). The solid line represents the linear regression between the variables (GRA HR = $4.64 \pm 11.18 + 1.03 \pm 0.15$ PostGRA-state HR [$R^2 = 0.79$]), and the dotted line represents $y = x$.

repeated measurements within cow by the MIXED procedure. The model used to analyze VO₂, O₂P, HR, and HP included the time of measurement as a fixed effect, being RES-state, PostGRA-state, and RES2-state for the first 2 variables and RES-state, GRA, PostGRA-state, and RES2-state for the latter 2 variables. The compound symmetry covariance structure was specified, and the Kenward–Rogers procedure was used to adjust the denominator of df. Regression analyses were performed using the PROC REG procedure between RES- and PostGRA-state for HR, VO₂, and O₂P as well as between GRA and PostGRA-state for HR. Univariate analyses were performed on all variables to check the normality of residuals and identify outlier data. Data were discarded when the studentized residuals were greater than 2 or less than -2; only 2 VO₂ records were discarded (1 for RES-state and 1 for PostGRA-state) and were coincident with symptoms of stress in the animal during the measurement. In addition, a GRA HR record was discarded due to mechanical connection problems between the HR transmitter and the data logger that caused noisy electrocardiogram signals and failures in HR recording. Means were considered to differ when $P \leq 0.05$, and tendencies were identified when $0.05 < P < 0.10$.

Experiment 2

The experiment was carried out during the winter of 2023 at the dairy research facilities of the Estación Experimental “Dr. Mario A. Cassinoni” (Universidad de la República, Paysandú, Uruguay) as part of a larger grazing

Table 1. Heart rate (HR), oxygen consumption (VO₂), and oxygen pulse (O₂P) at rest (RES-state; n = 15), immediately after grazing (PostGRA-state; n = 15), 3 h after the end of the grazing session (RES2-state; n = 9), and during grazing (GRA; n = 14; mean ± SEM)

Variable	Time point of measurement				P-value
	RES-state	PostGRA-state	RES2-state	GRA	
HR (beats/min)	66.2 ± 1.6 ^d	74.1 ± 1.6 ^b	69.9 ± 1.8 ^c	80.6 ± 1.6 ^a	<0.001
VO ₂ (mL/kg ^{0.75} per minute)	29.6 ± 0.7 ^b	32.4 ± 0.7 ^a	31.5 ± 0.8 ^a	—	0.004
O ₂ P (μL/kg ^{0.75} per beat)	447 ± 10	438 ± 10	458 ± 11	—	0.175
HP ¹ (kJ/kg ^{0.75} per hour)	35.9 ± 0.9 ^d	40.1 ± 0.9 ^b	37.9 ± 1.0 ^c	43.7 ± 0.9 ^a	<0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹For RES-state; for GRA-state and RES2-state, HP (kJ/kg^{0.75} per hour) = VO₂ (mL/kg^{0.75} per minute) × 20.47 (kJ/L O₂) × 60 (min/h)/1,000 (mL/L); and for Gra, HP (kJ/kg^{0.75} per hour) = HR (beats/min) × O₂P (mL/kg^{0.75} per beat) × 20.47 (kJ/L O₂) × 60 (min/h)/1,000 (mL/L).

trial to evaluate the effects of 2 defoliation intensities on dairy cow behavior, productive performance, and energy partitioning (Menegazzi et al., 2024). In this experiment, HP estimates were synchronized with a detailed record of each cow's posture and behavior throughout the entire day, allowing a comparison of the energy cost of the different activities carried out daily by cows fed in a grazing system with TMR supplementation. All the experimental procedures were approved by the Animal Experimentation Committee of the Universidad de la República (file #311170-000044-23).

Experimental Design, Animals, and Feeds. Eleven multiparous, fall-calving Holstein dairy cows (3.7 ± 0.7 lactations, 92 ± 17 DIM, 34.1 ± 5.7 kg/d milk yield, 666 ± 66 kg BW, mean ± SD) were selected from a 32-cow experimental herd (Menegazzi et al., 2024) as they were the only ones that had simultaneous HR, cow behavior, and posture registers. Cows were managed under a grazing system with TMR supplementation with 2 treatments representing different defoliation intensities (traditional vs. lax) imposed throughout lactation. All cows started grazing in a new 5-d occupation paddock of tall fescue (*Lolium arundinaceum* cv. INIA Fortuna) when 3 new extended leaves were developed (Fulkeron and Donaghy, 2001; 2,630 kg of DM/ha and 23 cm high; 23.8% DM, 13.9% CP, 57.9% NDF, and 27.4% ADF), but 5 cows grazed until reaching a postgrazing sward height of 12 to 15 cm (lax), and 6 cows grazed until reaching a postgrazing sward height of 5 to 7 cm (traditional; Menegazzi et al., 2024). The different postgrazing sward heights were generated by adjusting the area assigned to each treatment. Cows were on pasture from 0800 to 1400 h and in a compost barn from 1630 to 0400 h, where they consumed 12.5 kg DM/cow of a TMR (45.5% DM, 21.6% CP, 39.0% NDF, and 22.2% ADF). From 1400 to 1630 h and from 0400 to 0800 h, cows were at the milking parlor or walking to and from the pasture to the milking parlor (750 m).

The O₂P-HR technique (Brosh, 2007) was used to determine HP, similar to experiment 1, but HR was recorded for 5 consecutive days at 5-s intervals, O₂P was measured during the 20 min immediately before and after the 5-d HR recording, and the average O₂P value was used to estimate HP. The open-circuit respirometry system was checked through the N₂ recovery method (McLean and Tobin, 1988), and the average value was 99.7 ± 1.5% (mean ± SD).

Animal posture (lying down or standing) was recorded using an Onset Pendant G data logger (Onset Computer Corporation) attached on the medial side of the hind leg above the metatarsophalangeal joint (Ledgerwood et al., 2010). The logger was placed on the leg such that the x-axis was perpendicular to the ground pointing downward and the y-axis was parallel to the ground pointing cranially. Onset Pendant G data loggers were set to record the g force on the x-axis at 1-min intervals, and the data were downloaded using Onset HOBOWare software (Onset Computer Corporation). A cut-off value of 0.65 from the x-axis was used to determine whether the cow was lying down (≤0.65) or standing (>0.65); this value represents the equivalent of the cut-off value reported by Ledgerwood et al. (2010) considering differences in the position in which the data logger was placed on each cow's leg and data processing.

Animal activity was recorded by halters containing a noseband pressure sensor to detect jaw movements, a 3-axis accelerometer to detect 3-dimensional head movements, and a data logger (RumiWatchSystem, ITIN+HOCH). The collected data were processed using the software developed by the manufacturer (RumiWatch Converter V0.7.4.05, ITIN+HOCH) to generate summaries for idling (noningestive-related activities), rumination (chewing and swallowing of a ruminating bolus), eating at the feed bunk (intake, chewing, and swallowing of TMR) or grazing (intake, chewing, and

swallowing of pasture) activity at 10-min intervals (Zehner et al., 2017; Rombach et al., 2018). During the time when the cows were walking to and from the pasture and the milking parlor, the walking activity was specified manually in the dataset. Finally, the animal posture, activity, and HR records were synchronized with a 10-min resolution to generate a database containing the following posture + activity combinations (P+A): lying down idling, lying down ruminating, standing idling, standing ruminating, grazing or eating at the feed bunk, and walking.

Statistical Analysis. Data were analyzed using SAS software (SAS University Edition, SAS Institute Inc.). The HR and HP were first analyzed using the MIXED procedure including the combination of animal P+A as a fixed effect, cow as a random effect, and day hours as a fourth-degree polynomial covariate to consider the effect of the circadian rhythm on the daily variation of HP (Brosh, 2007). Based on this model, individual HR and HP values were obtained for each P+A and day of measurement. The effects of the P+A on HR and HP were evaluated using a MIXED model including the P+A, postgrazing sward height treatment, and their interaction as fixed effects and day of measurement as a random effect. The MIXED procedure was used to analyze the effect of the bite rate (bites/min) on the HP during grazing. The model included the bite rate, postgrazing sward height treatment, and their interaction as fixed effects and day of measurement and cow as random effects. The Kenward–Rogers procedure was used to adjust the denominator of df. Univariate analyses were performed to check the normality of residuals and identify outlier data. Data were discarded when the studentized residuals were greater than 2 or less than -2; ~4% of the observations were discarded. Means were considered to differ when $P \leq 0.05$, and tendencies were identified when $0.05 < P < 0.10$.

RESULTS

Experiment 1

The HR was greater during GRA than PostGRA-state; however, the proportionality was maintained as the regression of GRA HR on PostGRA-state presented an intercept and slope that were not different from 0 ($P = 0.686$) and 1 ($P = 0.893$), respectively (GRA HR = $4.64 \pm 11.18 + 1.03 \pm 0.15$ PostGRA-state HR; $R^2 = 0.79$; Figure 2). Cow HR was the highest during GRA, the lowest during resting before and after grazing (RES-state and RES2-state, respectively), and intermediate during PostGRA-state; HR was 1.22- and 1.15-fold greater during GRA than during RES- and RES2-states, respectively. Similarly, VO_2 was 1.10-fold greater dur-

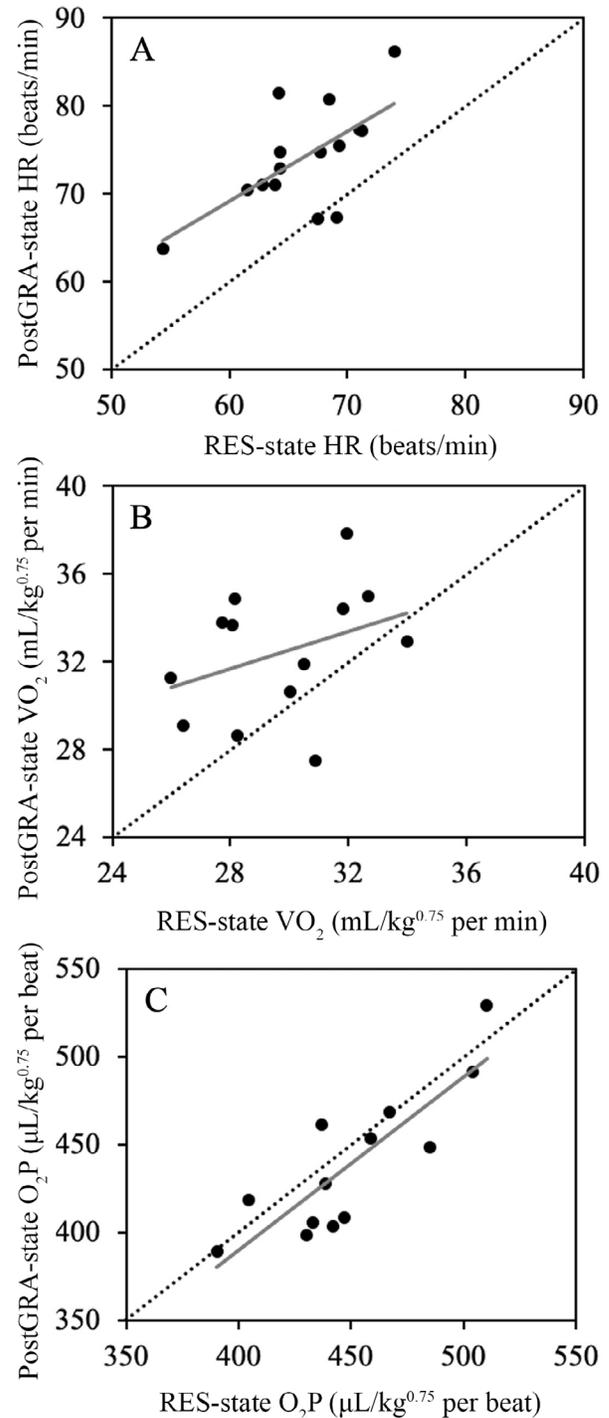


Figure 3. (A) Relationship between heart rate (HR; beats/min) at rest (RES-state) and immediately after grazing (PostGRA-state), where PostGRA-state HR = $21.29 \pm 17.58 + 0.80 \pm 0.26$ RES-state HR ($R^2 = 0.41$). (B) Relationship between oxygen consumption (VO_2 ; mL/kg^{0.75} per minute) at RES-state and PostGRA-state, where PostGRA-state $VO_2 = 19.70 \pm 9.90 + 0.43 \pm 0.33$ RES-state VO_2 ($R^2 = 0.13$). (C) Relationship between oxygen pulse (O_2P ; $\mu\text{L}/\text{kg}^{0.75}$ per beat) at RES-state and PostGRA-state, where PostGRA-state $O_2P = -6.99 \pm 86.90 + 0.99 \pm 0.19$ RES-state O_2P ($R^2 = 0.71$). The solid lines represent the linear regression between the variables, and the dotted lines represent $y = x$.

ing PostGRA-state than RES-state, but RES2-state presented a VO_2 similar to that in PostGRA-state. As HR and VO_2 varied together in the same direction, the O_2P ($449 \pm 36 \mu\text{L}/\text{kg}^{0.75}$ per beat; mean \pm SD) was not affected by the time of measurement (Table 1). The regression of PostGRA-state O_2P on RES-state O_2P presented an intercept not different from zero ($-7 \pm 86 \mu\text{L}/\text{kg}^{0.75}$ per beat; $P = 0.937$) and a slope equal to 1 ($0.99 \pm 0.19 \mu\text{L}/\text{kg}^{0.75}$ per beat; $P = 0.970$; Figure 3).

As HP was calculated based on VO_2 , HP for RES-state and PostGRA-state followed the same pattern as VO_2 , being greater for PostGRA-state than RES-state, whereas the HP for RES2-state presented an intermediate value (Table 1). During access to pasture, cows grazed with a bite rate of 37 ± 4 bites/min (mean \pm SD), and HP during GRA, calculated using the O_2P -HR method, was the greatest during this time, representing an increment ($P < 0.001$) of $7.85 \pm 0.68 \text{ kJ}/\text{kg}^{0.75}$ per hour when compared with that in RES-state.

Experiment 2

Daytime hours used as a fourth-degree polynomial covariate to account for the effect of circadian rhythm explained a major part of the variation in HR and HP throughout the day ($R^2 = 0.71$). Heat production expressed per unit of $\text{BW}^{0.75}$ or per unit of BW was affected by animal P+A ($P < 0.001$), but no interaction was observed between animal P+A and the postgrazing sward height treatment ($P = 0.658$), indicating that the postgrazing sward heights evaluated in this work (5–7 and 12–15 cm) did not affect the energy cost of activity.

Considering the mean values for each P+A, the HP during rumination and idling did not differ regardless of animal posture. However, HP for eating activity (grazing or consuming at the feed bunk) showed a 1.2-fold increase when compared with the HP during ruminating or idling. There was no difference between grazing or consuming TMR at the feed bunk ($P = 0.420$). The bite rate was 41 ± 15 bites/min during grazing and 16 ± 12 bites/min during TMR consumption at the feed bunk (mean \pm SD), but neither the bite rate nor its interaction with the postgrazing sward height treatment affected HP during grazing ($P = 0.958$ and $P = 0.467$, respectively) or consuming TMR ($P = 0.780$ and $P = 0.542$, respectively). The energy cost for grazing, calculated as the difference in the HP between during grazing and during nongrazing (idling + ruminating), was $6.63 \pm 1.12 \text{ kJ}/\text{kg}^{0.75}$ per hour or $1.31 \pm 0.28 \text{ kJ}/\text{kg}$ per hour. Walking was the activity that involved the greatest HP, representing a 1.6-fold increase ($24.03 \pm 1.12 \text{ kJ}/\text{kg}^{0.75}$ per hour or $4.72 \pm 0.28 \text{ kJ}/\text{kg}$ per hour) when compared with ruminating or idling and a 1.4-fold increase when compared with grazing or eating at the feed bunk (Table 2).

DISCUSSION

Using the O_2P -HR Method to Estimate the Energy Cost of Grazing

Both HR and VO_2 were basal during the fasted rest but increased when measured immediately after the first grazing session (12% and 9%, respectively), indicating that grazing activity increased O_2 demand, with the change in HR being the main component that would explain its variation (Butler et al., 2004). In fact, both variables varied proportionally, resulting in a consistent O_2P , in agreement with Talmón et al. (2023), who reported negligible O_2P variations when cows changed posture (standing vs. lying down) or activity (idling vs. ruminating vs. eating at feed bunk) in a respiration chamber experiment. In this study, HR was greater when cows were grazing (GRA) than when VO_2 was measured immediately after grazing (PostGRA-state), indicating an instantaneous response in HR due to the energy demand of grazing that was not entirely reflected in the PostGRA-state. There is evidence that O_2P typically increases during exercise (Brosh et al., 1998), however, grazing would not represent a high-intensity exercise to significantly affect the relationship between VO_2 and HR. This is supported by Berhan et al. (2006), who reported that the HP:HR ratio of goats walking at grazing pace did not differ from that when they were resting. Thus, based on our results and previous reports (Berhan et al., 2006; Talmón et al., 2023), it could be expected that O_2P during grazing would be similar to that during resting.

The O_2P -HR technique is an indirect measurement of gas exchange (relationship between VO_2 and HR) that is used to indirectly estimate HP with the consequent risk of including errors from the monitoring of HR to obtain HP. Moreover, the relationship between HP and VO_2 depends on the metabolic substrate (carbohydrate, fat, or protein) of oxidation, which would require known CO_2 production in relation to VO_2 (respiratory quotient; **RQ**). Under situations where RQ is unknown, it is assumed that RQ is 1.0 to estimate HP in ruminants from open-circuit calorimetric measurements (McLean, 1972); however, values of RQ greater than 1.0 in dairy cows have been recently reported (Morris et al., 2020; van Gastelen et al., 2020; Talmón et al., 2023), and such values can lead to underestimation of HP based on the O_2P -HR method. However, Talmón et al. (2023) reported that when RQ increases, O_2P decreases, indicating that the relative difference in HP between cow activities is closely related to variations in HR. Therefore, the relative increase in HP during grazing compared with resting or ruminating would not be affected when measured using the O_2P -HR method.

Table 2. Heart rate (HR) and heat production (HP) during different combinations of animal posture and activity in Holstein dairy cows

Item	Cow posture						SEM	P-value ¹	
	Lying down		Standing						
	Idling	Ruminating	Idling	Ruminating	Grazing	Eating			Walking
HR (beats/min)	61.6 ^c	63.2 ^{dc}	64.4 ^d	64.2 ^d	74.3 ^c	77.0 ^b	103.5 ^a	1.0	<0.001
HP (kJ/kg ^{0.75} per h)	36.8 ^c	37.6 ^c	38.5 ^c	38.2 ^c	44.4 ^b	45.3 ^b	61.8 ^a	1.1	<0.001
HP (kJ/kg per h)	7.14 ^c	7.30 ^c	7.49 ^c	7.42 ^c	8.65 ^b	8.83 ^b	12.06 ^a	0.28	<0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹P-values indicate the significance level of differences between different combinations of animal posture and activity. There was no interaction between the animal posture and activity combination and the postgrazing sward height ($P = 0.628$).

Energy Cost of Grazing and Other Activities

In the present study, the energy required for grazing (compared with nongrazing activities, such as idling or ruminating, for experiments 1 and 2) or consuming TMR (compared with idling in experiment 2) was lower than the energy costs reported for adult cattle eating TMR or fresh grass (9.04 kJ/kg^{0.75} per hour or 1.88 kJ/kg per hour, respectively) in respiration chambers (Susenbeth et al., 2004; Talmón et al., 2023). Previous reports have demonstrated that HP during eating is mainly determined by the eating time, indicating that the energy cost for eating, when expressed per unit of consumed feed, would be explained by feed intake rate and dietary DM content (Osuji, 1973; Adam et al., 1984; Susenbeth et al., 2004). Thus, a greater energy cost per unit of feed consumed (kJ/kg of DM) is expected for grazing cows than TMR-fed cows due to differences in the presentation and physical form of the diet, as well as the reduced DM content of fresh pastures, which leads to a relatively low ingestion rate (Adam et al., 1984; Susenbeth et al., 2004; Kaufmann et al., 2011).

The postgrazing sward heights evaluated in experiment 2 (12–15 vs. 5–7 cm) did not affect the energy cost of grazing per unit of time, supporting the concept that the energy cost of eating is more a function of the time spent eating than a function of the amount of feed ingested (Adam et al., 1984). Nonetheless, lower sward heights increase the grazing time and decrease the ingestion rate (Boval and Sauvart, 2021), which would lead to cows that grazed until reaching a postgrazing sward height of 5 to 7 cm having a greater energy cost of grazing expressed per day and per kg of pasture consumed than cows that grazed until reaching a postgrazing grass height of 12 to 15 cm.

The energy cost per unit of BW^{0.75} for grazing obtained in this study was numerically greater than data compiled from the literature for grazing cattle (Table 3). It was 32% greater than that measured for beef cows grazing rangelands using the same technique as in this

study (Aharoni et al., 2009; Brosh et al., 2010). Thus, the energy cost for grazing of dairy cows appears to be higher than for beef cattle. Although the explanation for this is not clear, a possible hypothesis could be related to the greater carcass protein-to-fat ratio in dairy than in beef cattle (Nour et al., 1983), which leads to a greater energy cost per unit of metabolic BW for leaner animals and vice versa (Birnie et al., 2000). However, estimating the energy cost of grazing as the difference between HP when the cow is grazing versus when it is not grazing is entirely a reflection of the difference in HR between these 2 moments multiplied by the O₂P and the HP to VO₂ ratio. Dairy cows have a greater O₂P than beef cows as consequence of a higher metabolic rate due to a greater energy intake and requirements (Brosh, 2007). Therefore, an increase of 15 beats/min during grazing compared with nongrazing will represent a greater HP for dairy cows than for beef cows due to the greater leverage of O₂P, which could lead to an overestimation of the energy cost of grazing in dairy cows.

Moreover, the energy cost of grazing expressed per kilogram of BW reported in the present study was lower than previously reported values for dairy calves (Holmes et al., 1976, 1978). However, if the cost of grazing is expressed as a function of metabolic BW, these differences decrease, and even the coefficients obtained in this work would be higher in some cases than those reported for calves. This raises the need to know whether the costs of grazing are related to animal BW or metabolic BW to improve the precision of the models used to estimate the energy cost of grazing. Until then, caution is needed when estimating the cost of grazing based on coefficients generated in a type of cattle other than that for which the estimates will be made.

The HP during grazing was not affected by the bite rate, which varied between 11 and 72 bites/min, indicating that mandibular movements might not demand high energy; additionally, this is in agreement with the fact that rumination represents a minor part of the daily energy requirements of a cow, as demonstrated in this

Table 3. Compilation of literature data on energy requirements for grazing in cattle¹

Source	Energy cost of grazing		Type of cattle	Method
	$\text{kJ/kg}^{0.75}$ per hour	kJ/kg per hour		
Holmes et al. (1976)	5.56 ²	1.88	Jersey calves	HR measurement ³
Holmes et al. (1978)	7.41–11.66 ^{2,4}	2.18–3.43	Friesian calves	VO_2 in a ventilated box
Adam et al. (1984)	—	2.34 (1.88–3.43)	—	Simulation
Marco et al. (1996) ⁵	2.3–7.7	0.55–1.92 ²	Angus steers	¹⁴ C bicarbonate dilution technique
CSIRO (2007)	—	2.50	—	—
Aharoni et al. (2009)	5.52	1.23 ²	Beef cows	$\text{O}_2\text{P-HR}$ method
Brosh et al. (2010)	5.47	1.18 ²	Beef cows	$\text{O}_2\text{P-HR}$ method
NASEM (2021)	3.14	—	—	—
Experiment 1 (present study)	7.85 ± 0.68	1.59 ± 0.14	Dairy cows	$\text{O}_2\text{P-HR}$ method
Experiment 2 (present study)	6.63 ± 1.12^6	1.31 ± 0.28^6	Dairy cows	$\text{O}_2\text{P-HR}$ method

¹HR = heart rate; VO_2 = oxygen consumption; $\text{O}_2\text{P-HR}$ = oxygen pulse–heart rate.

²Calculated based on the BW reports.

³Regression of CO_2 and heat production on HR.

⁴Grazing between 55 and 65 bites/min.

⁵Grazing at 28 and 59 bites/min, respectively.

⁶Calculated as grazing – nongrazing (idling and ruminating).

study (Table 2) and previous reports (Susenbeth et al., 1998; Talmón et al., 2023). In contrast, di Marco et al. (1996) reported an increase from 2.3 to 7.7 $\text{kJ/kg}^{0.75}$ per hour when Angus steers grazed at 28 or 59 bites/min, respectively. However, unlike the present study, the variation in bite rate recorded by di Marco et al. (1996) was obtained from 2 different experiments, and therefore, confounding effects related to year, pasture composition and morphology, animals, and sampling time could also explain the differences between the 2 experiments performed by di Marco et al. (1996).

In the present study, there was no significant increment in HP for standing versus lying down or ruminating versus idling, unlike in the study by Talmón et al. (2023), who did report an increase for cows housed in respiration chambers. The lower precision for measuring HP of the $\text{O}_2\text{P-HR}$ technique compared with the respiration chamber technique probably did not allow the detection of these differences. Moreover, when the animal is confined within the respiration chamber, idling is synonymous with resting; however, when collecting measurements from free-range cows, this is not necessarily true, as idling may occur simultaneously with other behaviors, such as movements on the pasture or in the compost barn associated with moments of recreation or social interaction with other cows. Therefore, standing + idling does not imply exactly the same activity in both experiments. Despite this, standing or ruminating would not represent an energy cost greater than 2.2 $\text{kJ/kg}^{0.75}$ per hour, which is in line with Talmón et al. (2023). In addition, the variation in HR indicates that changing posture or ruminating represent significantly lower costs than eating (grazing or eating TMR) or walking (Figure 4).

Finally, in this study, the energy cost of walking was estimated as the difference in HP between walking and when the cow was ruminating or idling, regardless of posture (considered part of basal activity). This value was $24.03 \pm 1.12 \text{ kJ/kg}^{0.75}$ per hour or $4.72 \pm 0.28 \text{ kJ/kg}$ per hour at a speed of approximately 3 km/h, in agreement with Ribeiro et al. (1977), who estimated the energy cost of walking using a ventilated hood to measure the VO_2 of steers walking on a treadmill. However, our estimates and those of Ribeiro et al. (1977) are greater than the values of 1.00 and 1.42 kJ/kg per hour reported by di Marco and Aello (1998) using the ¹³C bicarbonate dilution technique in steers walking at 2 and 4 km/h, respectively. The differences between these reports are not clear. The results obtained could be related to the measurement method, the type of cattle, or the walking conditions, but more research is needed to understand the differences between the results.

Contribution of Grazing to Maintenance Energy Requirements

Dairy cattle managed in a grazing system have a greater energy cost of maintenance due to the additional energy cost of walking and grazing (Agnew and Yan, 2000). On the one hand, the average energy cost of grazing in the present study was 7.24 $\text{kJ/kg}^{0.75}$ per hour, which is 2.3-fold greater than the energy cost of grazing recently proposed by NASEM (2021) based on studies using a similar methodology in beef cows (Aharoni et al., 2009; Brosh et al., 2010). On the other hand, the energy cost of walking in this study was 6.86 $\text{kJ/kg}^{0.75}$ per kilometer assuming a walking speed of 3.5 km/h for a healthy

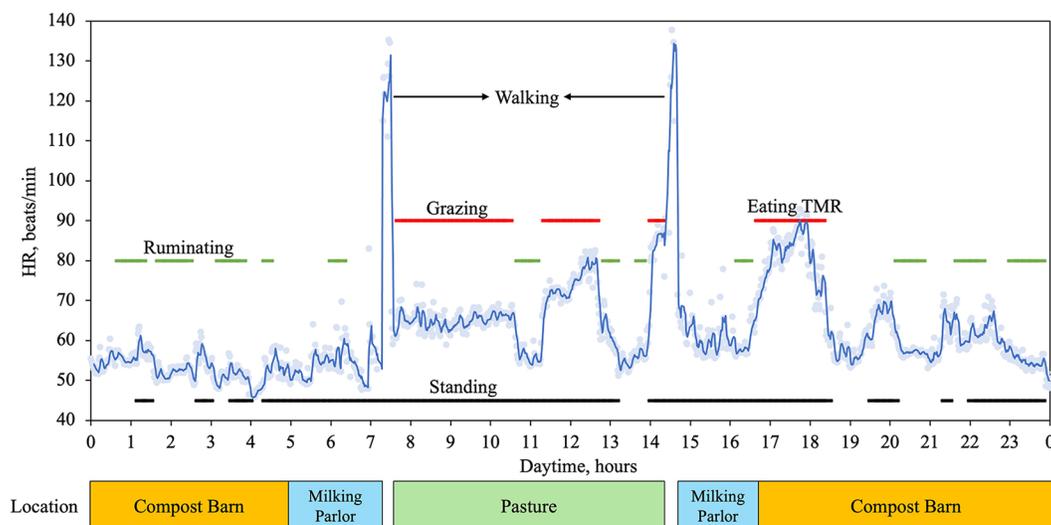


Figure 4. Heart rate (HR) pattern of a single cow (cow 402) throughout a day (July 5, 2023). Blue dots represent HR averaged over 1-min intervals ($n = 1,440$), whereas the blue solid line represents the 5-min moving average HR. Solid black lines represent standing posture; solid green lines represent ruminating activity; solid red lines represent grazing or eating TMR at the feed bunk, depending on whether the cow is on pasture or in the compost barn, respectively. The 2 highest HR peaks represent walking to and from the pasture.

cow (Zillner et al., 2018), which is in agreement with the proposed value of 1.46 kJ/kg BW per kilometer for an adult dairy cow (NASEM, 2021). Assuming a 600-kg BW dairy cow that is milked twice a day and fed only on a pasture located 750 m from the milking parlor, the cow will graze ~ 10 h/d (Bargo et al., 2003) and walk 3 km/d to move from the pasture to the milking parlor and back. In this situation, the daily energy cost of grazing and walking would be 72.4 and 20.7 kJ/kg^{0.75} per day, respectively, representing 22% of the maintenance energy cost (418 kJ/kg^{0.75} per day; NASEM, 2021).

Otherwise, to calculate the extra energy cost of activity for a dairy cow fed in a grazing system without supplementation versus a cow in a TMR indoor-fed system, the above estimates were compared against a daily energy cost of eating of 37.2 kJ/kg^{0.75} per day reported for dairy cows fed TMR in respirometry chambers (9.3 kJ/kg^{0.75} per hour \times 4 h/d; Talmón et al., 2023). Based on the above, the energy cost of activity for cows managed in grazing systems without supplementation would be 2.5-fold greater than for cows fed TMR indoors and would represent 2.2 kg of 4% FCM for a cow of 600 kg BW.

The NASEM (2021) feeding system estimates the grazing time based on a maximum grazing time of 600 min/d and a decrement of 12 min/kg DM of consumed concentrate (Bargo et al., 2003), assuming cows remain on pasture all day except when they are in the milking parlor or walking to and from the pasture. However, due to unfavorable climatic conditions or low pasture growth rates that make it impossible to meet the nutrients re-

quirements of the animals, pasture access time (**PAT**) is often restricted throughout the year (Stirling et al., 2021; Ortega et al., 2024). It has been reported in dairy cows that the grazing time as a proportion of PAT decreases as the PAT increases ($y = 0.9269e^{-0.037PAT}$, $R^2 = 0.79$; Chilibroste et al., 2015) and that the energy cost of grazing could therefore be estimated as a function of PAT. Thus, the following equation [Equation 4] is proposed:

$$\text{Grazing energy requirements (kJ/kg}^{0.75} \cdot \text{d}^{-1}) = D/WS \times 24.03 + (0.9269 \times e^{-0.037PAT}) \times PAT \times 7.24, \quad [4]$$

where D represents the daily distance walked (distance between the milking parlor and the paddock \times number of trips; km/d), WS represents the walking speed (km/h), and PAT represents the time of access to pasture (h/d).

Regardless of the coefficient of energy cost per hour of grazing used in the model, the equation proposed to estimate the activity energy requirement of a grazing cow (Equation 4) would result in improved predictions of grazing energy cost than the equation proposed by NASEM (2021) when comparing 2 versus 1 grazing turns and the proportion of concentrate in the diet increase (Figure 5). Furthermore, the walking cost for the proposed model is a function of the time the animal spends walking, without considering variations in topography, so it should only be used for flat topographies such as the one in which it was developed. Finally, the model needs to be evaluated and validated against an

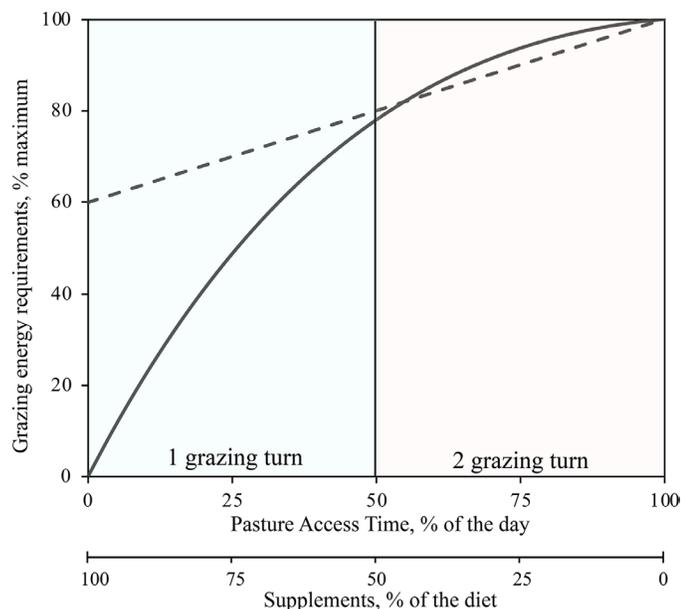


Figure 5. Prediction of the energy cost of grazing from the model proposed by NASEM (2021; dashed line) or from the model proposed in this study, denoted as $(0.9269 \times e^{-0.037PAT}) \times PAT \times 7.24$ (solid line), as a function of the time of access to pasture (*PAT*) or the level of supplementation.

independent database, and adjustments are needed for use in hilly topographies.

CONCLUSIONS

The results of this study suggest that O_2P remains stable under grazing conditions and that it is therefore possible to use the O_2P -HR method to estimate the energy cost of grazing. Furthermore, grazing activity required an average of $7.24 \text{ kJ/kg}^{0.75}$ per hour or 1.45 kJ/kg per hour compared with when cows were not grazing (idling or ruminating). The energy cost of eating per unit of time was similar between grazing and consuming TMR at the feed bunk; therefore, the extra energy requirements in grazing dairy cows are associated with the longer time required to harvest their feed (forage) and the extra cost of walking between the pasture and milking parlor. For a 600-kg dairy cow grazing 10 h/d and walking 3 km/d, the maintenance energy requirements would represent 2.2 kg of 4% FCM when compared with that of a cow fed a TMR.

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Nonstandard abbreviations used: FMS = field metabolic system; GRA = grazing activity during the first grazing session; HP = heat production; HR = heart rate; O_2P = oxygen pulse; P+A = posture plus activity combination; PAT = pasture access time; PostGRA-state = state immediately after grazing; RES-state = baseline resting state; RES2-state = state 3 h after grazing; RQ = respiratory quotient; STD = standard temperature-pressure dew point; VO_2 = oxygen consumption.

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ORCID

- Daniel Talmón, <https://orcid.org/0000-0003-2284-4354>
 Alejandra Jasinsky, <https://orcid.org/0009-0009-3726-1494>
 Federica Marin, <https://orcid.org/0009-0005-3583-355X>
 Gabriel Menegazzi, <https://orcid.org/0000-0003-4133-5563>
 Pablo Chilibroste, <https://orcid.org/0000-0001-9579-9967>
 Mariana Carriquiry <https://orcid.org/0000-0002-1600-2591>

APPENDIX

Table A1. Number of cows, parity, DIM, milk yield, and BW used for measurement of oxygen pulse during rest before grazing (RES-state) and during rest after grazing (RES2-state; mean ± SD)

Variable	RES-state	RES2-state
Number of cows	15	9
Parity	2.4 ± 1.4	2.3 ± 1.5
DIM	217 ± 41	210 ± 39
Milk yield (kg/d)	23.8 ± 3.2	24.1 ± 2.6
BW (kg)	601 ± 58	592 ± 48