



Effect of animal activity and air temperature on heat production, heart rate, and oxygen pulse in lactating Holstein cows

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ABSTRACT

A linear relationship between heart rate (HR) and oxygen consumption (VO_2) has been reported in homeothermic animals, indicating that is possible to estimate heat production through HR measurements. This relationship may depend on the animal activity and environmental conditions. The main objective of the present study was to evaluate the effect of the air temperature and animal posture and activity on heat production and VO_2 in relation to HR. In addition, as a secondary objective, the energy cost of eating and ruminating versus idling and standing versus lying down was determined. Twelve Holstein lactating cows were housed inside climate-controlled respiration chambers for 8 d, where the air temperature was gradually increased from 7 to 21°C during the night and from 16 to 30°C during the day with daily increments of 2°C for both daytime and nighttime. During the 8-d data collection period, HR and gaseous exchange measurements were performed, and animal posture and activity were recorded continuously. The oxygen pulse (O_2P), which represents the amount of oxygen that is consumed by the cow per heartbeat, was calculated as the ratio between VO_2 and HR. Results showed that heat production and VO_2 were linearly and positively associated with HR, but this relationship largely varied between individual cows. Within the range tested, O_2P was unaffected by temperature, but we detected a tendency for an interaction of O_2P with the temperature range tested during the night versus during the day. This indicates that the effect of air temperature on O_2P is nonlinear. Standing and eating slightly increased O_2P (1.0 and 2.5%) compared with lying down and idling, respectively, whereas rumination increased O_2P by

5.1% compared with idling. It was concluded that the potential bias introduced by these effects on the O_2P for the application of the technique is limited. The energy cost of eating and ruminating over idling was 223 ± 11 and 45 ± 6 kJ/kg^{0.75} per day, respectively, whereas the energy cost of standing over lying down was 53 ± 6 kJ/kg^{0.75} per day. We concluded that O_2P in dairy cows was slightly affected by both animal posture and activity, but remained unaffected by air temperature within 8 to 32°C. Nonlinearity of the relationship between the O_2P and air temperature suggests that caution is required extrapolating O_2P beyond the temperature range evaluated in our experiment.

Key words: heart rate–oxygen pulse method, energy expenditure, climate respiration chambers, indirect calorimetry

INTRODUCTION

During the last decades, several studies were carried out to quantify the heat production (HP) of modern dairy cows to improve their energy efficiency. The HP has been mostly determined using indirect calorimetry, (i.e., measuring O_2 consumption; VO_2) and production of CO_2 (VCO_2) and CH_4 inside of climate respiration chambers with high accuracy. However, this type of measurement does not usually reflect the conditions under which animals are managed in commercial production systems.

The oxygen pulse and heart rate technique (O_2P -HR) is an alternative method for measuring the HP of animals under the usual conditions of production. Measurements of HP of yearling bulls at different feed intake levels using the O_2P -HR technique showed that the values are comparable to those obtained using respiration chambers or comparative slaughter in the same animals (Oss et al., 2016). The O_2P -HR method is based on the relationship between VO_2 and heart rate (HR) because, in homeothermic animals, most of

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Table 1. Number of cows, BW, parity, average milk production of the current lactation, DIM, and days of pregnancy per treatment (mean \pm SD)

Item	Treatment ¹				
	RH_l + AV_l	RH_m + AV_l	RH_m + AV_m	RH_m + AV_h	RH_h + AV_l
Number of cows	4	1	2	3	2
BW, kg	695 \pm 47	712	751 \pm 45	682 \pm 29	633 \pm 9
Parity	2.3 \pm 0.4	2.0	3.5 \pm 0.5	2.3 \pm 0.5	3.0 \pm 1.0
Milk production, kg/d	27.2 \pm 6.2	28.5	33.2 \pm 0.9	31.2 \pm 1.9	31.5 \pm 5.6
DIM	212 \pm 30	199	230 \pm 37	202 \pm 25	180 \pm 55
Days of pregnancy	100 \pm 24	102	94 \pm 33	108 \pm 42	106 \pm 68

¹Levels of relative humidity (RH) and air velocity (AV; l = low; m = medium; h = high).

the O₂ consumed is transported to the tissues by the work of the heart. Based on the Fick's equation (Fick, 1870), the O₂P-HR method assumes that stroke volume and arterio-venous oxygen extraction remain constant or vary in a predictable and systematic manner to reliably estimate VO₂ from HR measurements (Butler et al., 2004). The O₂P-HR technique consists of long-term measurements (24-h period or longer) of the HR of animals, usually under free-ranging conditions, and short-term measurements (20 min) of the oxygen pulse (i.e., the measurement of oxygen consumption by the cow; O₂P; μ L of O₂/kg^{0.75} per heartbeat), usually with a facemask, and simultaneously with HR (Brosh, 2007).

Subsequently, the long-term VO₂ is calculated by multiplying the O₂P with the continuously recorded HR. It is assumed that the variation of HR is the major component of the cardiovascular system response to an increase in the demand for O₂; thus, O₂P is stable along the day independently of the animal activity, feed intake, or environmental conditions. Therefore, this assumption together with the reliability of O₂P measurement are key factors to obtain high accuracy of the HP measurement using this technique. In this regard, previous research has reported a maximum deviation of a single O₂P measurement of 5% from the whole-day average O₂P for growing calves and lambs (Aharoni et al., 2003) or goats (Puchala et al., 2007), but those experiments did not identify the source of variation in O₂P. During short-term exposure to heat, cows increase HR to increase peripheral blood flow to increase heat loss, but this increase in HR is not accompanied by an increase in VO₂ (Kadzere et al., 2002), indicating that air temperature would affect O₂P in a nonlinear pattern. On the other hand, during exercise, HR increases but VO₂ is also altered by O₂ extraction and stroke volume to meet O₂ demand, and thus the relationship between HR and VO₂ is nonlinear (Brosh et al., 1998). Recently, variations in HR of dairy cows related to different routine activities have been reported (Talmón et al., 2022), but it is not known if the relationship

between VO₂ and HR was linear under the different activities.

Therefore, the main objective of the present work is to evaluate and quantify the effect of air temperature and animal posture and activity on the O₂P. Such relationships could be implemented to improve the accuracy of HP estimates using the O₂P-HR technique. Also, a secondary objective is to quantify the energy cost of eating and ruminating versus idling and standing versus lying down, which may be used to improve predictions of maintenance energy requirements in lactating dairy cows.

MATERIALS AND METHODS

The experiment was carried out from January until April 2021 at the Carus research facilities of Wageningen University and Research (Wageningen, the Netherlands). All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Wageningen University and were conducted under the Dutch Law on Animal Experiments (Project No. 2019.D-0032).

Experimental Design, Animals, and Feed

Twelve multiparous (2.6 \pm 0.8 lactations) Holstein lactating cows were used from the experiment reported by Zhou et al. (2022). At the first day of the experiment, cows were at 206 \pm 38 DIM with an average of 30.0 \pm 4.9 kg of milk per day in the currently lactation and weighed 692 \pm 50 kg. All cows were pregnant with 102 \pm 40 d of gestation (Table 1). Cows were subjected to different treatments to evaluate the effect of air temperature, relative humidity (RH), and air velocity (AV) on their physiological and productive response (Zhou et al., 2022).

Briefly, 5 different treatments based on different combinations of temperature, RH, and AV were applied to the cows during an 8-d research period while they were

individually housed in climate respiration chambers. Treatments consisted of 3 RH levels (low RH: 30–50%, medium RH: 45–70%, and high RH: 60–90%) and 3 AV levels (low AV: 0.1 m/s, medium AV: 1.0 m/s, and high AV: 1.5 m/s) while air temperature inside the chambers was increased, during the 8-d research period, from 7 to 21°C during the night and from 16 to 30°C during the day (daily increments of 2°C for both daytime and nighttime) for low AV, and from 9 to 23°C during the night and from 18 to 32°C during the day for medium AV and high AV.

All cows had an acclimation period of 10 d. In the first 7 d, cows were placed in individual tiestalls where they received the experimental diet and were in routine contact with the caretakers, whereas in the last 3 d, cows were moved to the climate respiration chamber where they were also subjected to the initial temperature, RH, and AV corresponding to each treatment, and the experimental measurements were performed.

Cows were fed *ad libitum* with a TMR formulated to meet or exceed their nutritional requirements according to the Dutch System (CVB, 2018). The diet consisted of 6 kg/d of concentrate and enough forage (62% corn silage and 38% grass silage on fresh basis) to ensure the *ad libitum* intake. The TMR was offered twice a day at 0500 and 1530 h, and fresh water was always available through drinking bowls. More details about the experimental design were published by Zhou et al. (2022).

The original data set consisted of 20 cows, but due to mechanical connection problems between the device and the receptacle of the HR monitors that caused noisy electrocardiogram signals and failures in the HR recordings, data for 8 cows were not used for the analysis to avoid outcome bias. Thus, the number of cows was not evenly distributed among the treatments (Table 1). Preliminary comparison of treatments did not reveal numerical or statistically significant differences on the variables of the data set used in the present study (12 cows), nor on the manually measured HR and VO_2 of the 20 cows used by Zhou et al. (2022; see Appendix Table A1). Therefore, effects of AV and RH were not considered for further analysis.

Climate Controlled Respiration Chamber

Two climate respiration chambers were used, each one was split into 2 airtight rooms of 12.8 m² with 43.5 m³ using removable wall panels with inflated surrounding tubing. The chambers were equipped with thin walls and transparent windows to allow audio-visual contact between 2 cows and thereby minimize the effects of social isolation on their behavior and performance (Heetkamp et al., 2015). During all the experiments, the RH and air temperature were moni-

tored using automatic sensors (Novasina Hygro-dat100, Novasina AG and PT100 temperature sensor, Sensor Data BV, respectively). The chambers were artificially lit from 0500 to 2100 h with 390 to 440 lux, and from 2100 to 0500 h, the light was dimmed to 35 to 40 lux. More details about the RH, air temperature, and AV measurements, as well as a schematic diagram of the climate respiration chamber, were provided by Zhou et al. (2022).

Data Collection

While the cow was inside the respiration chamber, the HR was measured at 1-s intervals using HR monitors (BioHarness 3.0, Zephyr Technology Corporation). The HR monitor was attached to the cow using a specific strap placed around the chest of the cow, just behind of the forelegs. Heart rate data were downloaded using the software developed by the manufacturer (BioModule 3.0, Zephyr Technology Corporation) and unreliable records were deleted based on an algorithm that considers a worn detection indication and the signal-to-noise ratio of the electrocardiogram signal.

The CO_2 and CH_4 concentrations were measured with a nondispersive infrared gas analyzer (ABB Advance Optima AO2000 systems, ABB) and the O_2 concentration was measured using a paramagnetic gas analyzer (ABB Advance Optima AO2000 systems, ABB). Inlet and exhausted air samples from each chamber were taken every 12.6 min on average (4 12 min intervals for each chamber and one 15 min interval for the inlet air) as described by van Gastelen et al. (2020). The VO_2 and VCO_2 , as well as the CH_4 production, were calculated between the inlet and exhausted gas volumes. The ventilation rate in the chamber was 55.8 ± 1.74 m³/h. Ventilation rates and gas concentrations were corrected for pressure, temperature and humidity to obtain standard temperature pressure dew point volumes of inlet and exhausted air. Calibration gases were used daily instead of inlet air to correct the measured gas concentrations from the inlet and exhaust air samples. The respiration chambers were checked before the experiment injecting known amounts of CO_2 in each compartment and comparing them with the data coming from the gas analysis to calculate CO_2 recovery. The average recovery of CO_2 was $102.35 \pm 0.27\%$ (between 101.9 and 102.6%).

Cow activity was recorded by noseband sensors (RumiWatchSystem, ITIN+HOCH) and the data were processed using the software developed by the manufacturer (RumiWatch Converter, ITIN+HOCH) to generate summaries for idling, rumination, eating, and drinking activity at 1-min intervals. Moreover, cow posture information (standing or lying down) were

Table 2. Descriptive data of 12 cows, individually housed for 8 d in climate respiration chambers under conditions of increasing air temperature, and varying relative humidity and air velocity

Item	Mean	SD	Minimum	Maximum	Median	Observation
Heat production, kJ/kg ^{0.75} per d	895	96	615	1218	891	1,906
Heart rate, beats/min	68	8	50	101	67	1,906
O ₂ consumption, L/kg ^{0.75} per d	41.3	4.2	28.5	54.5	41.1	1,906
CO ₂ production, L/kg ^{0.75} per d	47.2	6.0	31.9	73.2	46.6	1,906
CH ₄ production, L/kg ^{0.75} per d	4.2	0.7	2.0	6.7	4.1	1,906
Oxygen pulse, μL O ₂ /kg ^{0.75} per beat	425	38	304	529	428	1,906
Heat production pulse, J/kg ^{0.75} per beat	9.22	0.82	6.72	11.70	9.27	1,906
Respiratory quotient	1.14	0.06	1.01	1.38	1.14	1,906
Light ¹	0.58	0.49	0.00	1.00	1.00	1,906
Temperature, °C	21.5	5.2	8.2	32.2	21.6	1,906
Relative humidity, %	58	13	29	93	57	1,906
DMI rate, kg/h	0.69	0.92	0.00	6.18	0.24	1,906
Eating time proportion	0.17	0.22	0.00	1.00	0.06	1,848
Ruminating time proportion	0.39	0.32	0.00	1.00	0.35	1,848
Idling time proportion	0.43	0.31	0.00	1.00	0.41	1,848
Drinking time proportion	0.01	0.02	0.00	0.23	0.00	1,848
Standing time proportion	0.48	0.38	0.00	1.00	0.45	1,388

¹Light on = 1; light off = 0.

obtained through continuous visual observation of the video-camera recordings to know when each cow was lying down or standing. Feed intake was calculated from data recorded by an automatic weighing scale built into each feed bunk (Technical Development Studio, Wageningen University & Research) at 30-s interval during the whole experiment.

The experimental staff entered the chamber 4 times a day for approximately 30 min, at 0500 h and 1530 h for feeding and milking and at 1000 and 1800 h (in addition to 0500 h) for measuring skin and rectal temperature, respiratory rate, and manual measurement of HR of the cows (Zhou et al., 2022). All the data recorded while the staff was inside the chamber was not considered for the analysis.

Data Processing and Calculations

A complete cycle of gaseous exchange measurements took 12.6 min, whereas HR measurements, activity, and posture were recorded continuously. With deconvolution procedures detailed by Alferink et al. (2015), the instantaneous gaseous exchange data at cow level were calculated from data generated at respiration chamber level. Typically, these procedures lead to noisy data, depending on the chamber washout time, the frequency of measurements, and variation in chamber gas volume (Gerrits et al., 2015). This noise can be dampened by considering averages of subsequent measurements. For the current data, gas exchange measurements were averaged over 3 data points (37.8 min intervals). To synchronize HR and gas exchange data sets for further analysis, HR was also averaged at 37.8 min intervals; additionally, if the HR mean was obtained with less

than 40% of the potential HR observations (37.8 min × 60 HR observations/min = 2,268 possible HR observations), it was discarded to avoid bias considering a balance between keeping as many observations as possible without significantly affecting the model fit between HR and the gas exchange data.

Feed intake rates were calculated as kilograms of DM consumed during the time elapsed to take the 3 air samples (37.8 min), and were expressed in kilograms of DM/h. Animal posture and activity were expressed as the proportion of time that the cow spent in each posture and performing each activity during this period of time (i.e., eating = 0.5 means that the cow spent 50% of the time eating; 37.8 min × 0.5 = 18 min and 54 s).

Heat production was calculated, according to Brouwer's equation (Brouwer, 1965), as

$$HP(kJ) = 16.18 \times O_2 \text{ consumption}(L) + 5.02 \\ \times CO_2 \text{ production}(L) - 2.17 \times CH_4 \text{ production}(L),$$

whereas O₂P and heat production pulse (**HPP**), which represent the amount of oxygen consumed and heat produced per heartbeat, respectively, were calculated as

$$O_2P = \frac{VO_2}{HR},$$

$$HPP = \frac{HP}{HR}.$$

In Table 2, descriptive statistics are presented for each variable in the data set.

Statistical Analysis

Data were analyzed using the SAS Studio 3.8 (SAS Institute Inc.). Due to incomplete HR data, data from 12 of the 20 cows were available, and a preliminary analysis was conducted to evaluate treatment differences. This was done for HP, HR, VO_2 , VCO_2 , respiratory quotient (RQ), O_2P , and HPP data using the GLIMMIX procedure, considering the treatment as a fixed effect and the cow as a random effect. For HP, HR, VCO_2 , and RQ, lognormal distribution was specified in the model, whereas for VO_2 , O_2P , and HPP, it was Gaussian. This preliminary analysis revealed no significant, nor numerically interesting, treatment differences (Appendix Table A1); therefore, effects of RH and AV were not considered for further analysis.

To evaluate the effect of air temperature on HP, HR, VO_2 , VCO_2 , O_2P , HPP, and RQ, regressions of each variable versus air temperature were performed for daytime and nighttime independently in each cow using the REG procedure. Regression slopes were tested for deviance from 0 using the MIXED procedure and considering the time of the day (daytime and nighttime) as fixed effect. One cow in the high RH + low AV treatment was not considered for this analysis because records from the first 4 d of the experiment were not available due to failure of HR measurement.

Mixed-effect regression models considering the cow as a random effect were used to evaluate the relationship between HR and HP and VO_2 , as well as the effect of feed intake rate, animal posture, and activity on HP, HR, VO_2 , VCO_2 , O_2P , HPP, and RQ. For evaluating effects of animal posture and activity on HP, HR, VO_2 , VCO_2 , O_2P , HPP and RQ as response variables, the proportion of time eating, ruminating, and standing were considered as independent variables.

In addition, due to the high correlation between the proportion of time eating and standing ($r = 0.588$; $P < 0.001$) that could have affected the results of the previous regression models, a new data set was generated with different animal posture and activity combinations to evaluate their effect on HP. For these calculations, all observations with proportions of each activity/posture exceeding 0.8 for the interval evaluated [more than 80% of the time (30 min)] were used and considered as 1 observation ($n = 1$) in the new data set. The combinations of posture and activity obtained were lying down + idling ($n = 154$; **a**), lying down + ruminating ($n = 83$; **b**), standing + idling ($n = 13$; **c**), standing + ruminating ($n = 35$; **d**) and standing + eating ($n = 36$; **e**). To evaluate the effect of animal posture and activity combination on HP, O_2P , and HPP, the MIXED procedure was used considering the combination of animal

posture and activity as fixed effect, whereas the cow was specified as random effect. The HP, O_2P , and HPP for different postures and activities were evaluated by least squares means difference as follows: standing versus lying down comparing b versus d; ruminating versus idling comparing b versus a and eating versus ruminating comparing e versus d. The combination of standing + idling (c) was not used for the analysis due to the small number of observations that did not allow to represent all the cows and then eating versus idling was calculated as e versus d + b versus a, assuming that the effect of ruminating standing is equal to the effect of ruminating lying down.

In both GLIMMIX and MIXED procedures, the Kenward-Rogers method was used to adjust denominator of degrees of freedom. For all the analysis, the UNIVARIATE procedure was performed to check the normality of the residuals and to identify outlier data. Pearson correlation coefficients were performed using the CORR procedure.

RESULTS

HP and VO_2 in Relation to Heart Rate

Both HP and VO_2 were positively associated with HR ($r = 0.66$ and 0.64 ; $P < 0.001$, respectively), but these relationships were highly dependent on individual cow variation (Figure 1). The mean O_2P and HPP values for all cows was $424 \pm 32 \mu\text{L } O_2/\text{kg}^{0.75}$ per heartbeat and $9.19 \pm 0.68 \text{ J}/\text{kg}^{0.75}$ per heartbeat, respectively (mean \pm SD, $n = 12$).

Air Temperature

Increasing air temperature during subsequent nights ($7\text{--}21^\circ\text{C}$) did not affect any of the response variables, whereas increased air temperatures during subsequent days ($16\text{--}32^\circ\text{C}$) decreased HP, VO_2 , and VCO_2 , whereas HR and RQ tended ($P = 0.058$ and 0.078 , respectively) to decrease. Neither O_2P nor HPP were affected by the increase in air temperature during subsequent days (Table 3).

Animal Posture, Activity, and Feed Intake Rate

Mixed-effect regression model showed that HP at resting (idling + lying down) was $811 \pm 11 \text{ kJ}/\text{kg}^{0.75}$ per day. The HP, HR, and VO_2 increased with eating, ruminating, and standing, whereas VCO_2 increased with eating and standing, but it was unchanged during ruminating. The RQ increased with eating and decreased with ruminating and standing. As changes

Table 3. Regression slopes of HP, HR, VO₂, VCO₂, RQ, O₂P, and HPP on temperature during daytime and nighttime¹

Item	Slope			P-value		
	Daytime, 0500–2100 h	Nighttime, 2100–0500 h	SEM	Daytime ²	Nighttime ²	Daytime vs. nighttime ³
HP, kJ/kg ^{0.75} ·d ⁻¹ per °C	-2.0794	0.3372	0.6663	0.005	0.618	0.019
HR, beats/min per °C	-0.1162	-0.04507	0.05772	0.058	0.444	0.394
VO ₂ , mL/kg ^{0.75} ·d ⁻¹ per °C	-89.35	12.41	30.13	0.008	0.685	0.027
VCO ₂ , mL/kg ^{0.75} ·d ⁻¹ per °C	-135.4	30.79	39.69	0.003	0.447	0.008
RQ, units per °C	-0.00081	0.000337	0.000435	0.078	0.448	0.078
O ₂ P, μL O ₂ /kg ^{0.75} ·beat ⁻¹ per °C	-0.250	0.402	0.281	0.390	0.167	0.118
HPP, J/kg ^{0.75} ·beat ⁻¹ per °C	-0.00707	0.009486	0.006108	0.261	0.136	0.070

¹HP = heat production; HR = heart rate; VO₂ = oxygen consumption; VCO₂ = carbon dioxide production; RQ = respiratory quotient; O₂P = oxygen pulse; HPP = heat production pulse.

²P-value shows the significance level that the slope differs from zero.

³P-value for statistical difference between daytime and nighttime slopes.

in VO₂, HR, and HP due to animal activity and posture were not in proportion, O₂P decreased for eating, but it increased for ruminating and standing, whereas HPP only increased for ruminating (Table 4; Figure 2).

The correlation between proportion of time spent eating and feed intake rate was high ($r = 0.885$; $P < 0.001$); thus, as feed intake rate increased, HP, VO₂, VCO₂, HR, and RQ also increased. However, O₂P slightly decreased with increasing feed intake rate, whereas HPP was not affected (Table 5).

As was expected, the proportion of time standing was correlated positively with the proportion of time eating ($r = 0.588$, $P < 0.001$), but it was related negatively with the proportion of time idling or ruminating ($r = -0.368$ and -0.079 , respectively; $P < 0.004$).

DISCUSSION

The present work studied the ratio between HP or VO₂ and HR (HPP and O₂P, respectively) throughout the day in lactating dairy cows, evaluating these relationships under different air temperatures, animal posture (lying down or standing), and activities (idling, ruminating or eating). We demonstrated that it is possible to estimate accurately the HP based on HR measurements because a change in HR was the major component in response to an increase in the energy demand due to animal activity.

The mean O₂P value is in line with previous reports for confined lactating Holstein cows [450 ± 14 and 421 ± 14 μL O₂/kg^{0.75} per heartbeat (mean ± SE) from Brosh, 2007 and from Aharoni et al., 2003, respectively]

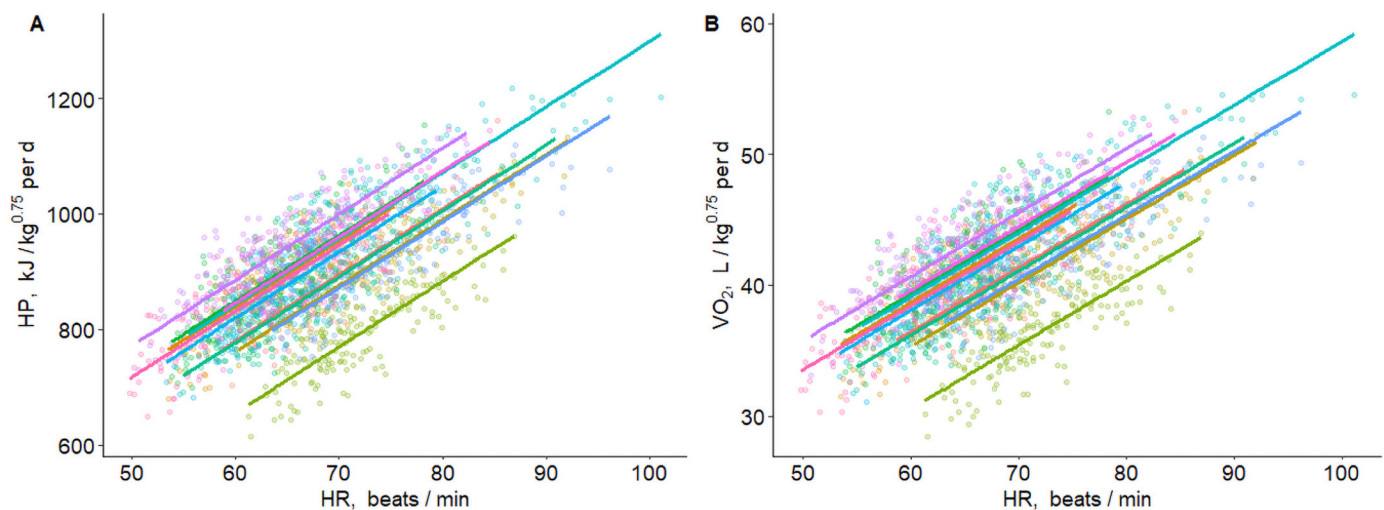


Figure 1. Relationship between heart rate (HR) and (A) heat production (HP) or (B) oxygen consumption (VO₂). Different colors represent different cows ($n = 12$). Dots represent observed values ($n = 1,906$), whereas solid lines represent predicted values of the model. HP (kJ/kg^{0.75} per d) = $120.7 \pm 21.1 + 11.4 \pm 0.2$ HR (beats/min; $R^2 = 0.77$); VO₂ (L/kg^{0.75} per d) = $8.06 \pm 0.95 + 0.49 \pm 0.008$ HR (beats/min; $R^2 = 0.75$).

Table 4. Differences in HP, HR, VO₂, VCO₂, RQ, O₂P, and HPP for eating versus idling, ruminating versus idling, and standing versus lying down based on mixed-effect regression models and LSM differences¹ (mean ± SEM)

Item	Activity			P-value		
	Eating vs. idling	Ruminating vs. idling	Standing vs. lying down	Eating vs. idling	Ruminating vs. idling	Standing vs. lying down
Mixed-effect regression model ²						
HP, kJ/kg ^{0.75} per d	223 ± 11	45 ± 6	53 ± 6	<0.001	<0.001	<0.001
HR, beats/min	16.4 ± 0.8	1.1 ± 0.5	4.0 ± 0.4	<0.001	0.016	<0.001
VO ₂ , L/kg ^{0.75} per d	8.72 ± 0.49	2.64 ± 0.28	2.81 ± 0.27	<0.001	<0.001	<0.001
VCO ₂ , L/kg ^{0.75} per d	16.96 ± 0.71	0.40 ± 0.40	1.56 ± 0.38	<0.001	0.324	<0.001
RQ	0.147 ± 0.007	-0.063 ± 0.004	-0.037 ± 0.004	<0.001	<0.001	<0.001
O ₂ P, μL O ₂ /kg ^{0.75} per beat	-10 ± 4	21 ± 2	4 ± 2	0.006	<0.001	0.045
HPP, J/kg ^{0.75} per beat	0.08 ± 0.08	0.33 ± 0.05	0.02 ± 0.05	0.328	<0.001	0.729
LSM difference ³						
HP, kJ/kg ^{0.75} per d	217 ± 16	49 ± 9	57 ± 11	<0.001	<0.001	<0.001
O ₂ P, μL O ₂ /kg ^{0.75} per beat	-22 ± 6	19 ± 3	10 ± 5	<0.001	<0.001	0.031
HPP, J/kg ^{0.75} per beat	-0.20 ± 0.13	0.31 ± 0.06	0.16 ± 0.10	0.119	<0.001	0.105

¹HP = heat production; HR = heart rate; VO₂ = oxygen consumption; VCO₂ = carbon dioxide production; RQ = respiratory quotient; O₂P = oxygen pulse; HPP = heat production pulse.

²Mixed-effect regression models considering the cow as a random effect and the proportion of time eating, ruminating, and standing as independent variables.

³Mixed model considering the combination of animal posture and activity as fixed effect and the cow as random effect. Different postures and activities were evaluated by LSM difference of the model.

and it is a bit lower than those reported for grazing Holstein cows during mid to late lactation [521 ± 48 and 473 ± 45 μL O₂/kg^{0.75} per heartbeat (mean ± SD) by Talmón et al., 2020 and 2022, respectively].

Ratio between HP or VO₂ and HR

The VO₂ was positively correlated with HR and because VO₂ contributes for 75% of the total HP according to equation by Brouwer (1965), HP was also correlated with HR. However, the relationship between VO₂ and HR, as well as between HP and HR, were

highly dependent on each cow because 33% of the variation was explained by the cow intercept, whereas the regression coefficients were similar, suggesting that the use of individual values of O₂P or HPP are indeed necessary to estimate HP based on HR. When the relationship between HP or VO₂ and HR were adjusted by cow effect, HR explained 77 and 75% of the variation in HP and VO₂, respectively. This is in line with Brosh et al. (1998) and Puchala et al. (2007), who reported a greater variation in HPP between animals than within-animals and concluded that to increase the accuracy of the estimation of HP by HR, the relationship between

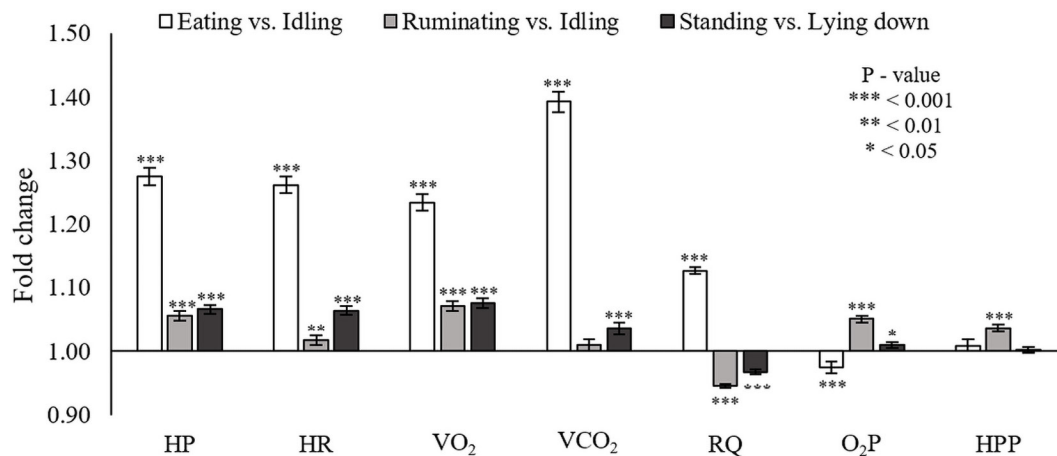


Figure 2. Fold change of heat production (HP), heart rate (HR), oxygen consumption (VO₂), carbon dioxide production (VCO₂), respiratory quotient (RQ), oxygen pulse (O₂P), and heat production pulse (HPP) from eating versus idling, ruminating versus idling, and standing versus lying down. Error bars represent mean standard error.

Table 5. Coefficients (mean \pm SEM) from the model with increasing feed intake rate (kg DM/h) on HP, HR, VO₂, VCO₂, RQ, O₂P, and HPP¹

Item	Intercept		Feed intake, kg DM/h		R ²
	Estimate	P-value ²	Estimate	P-value ²	
HP, kJ/kg ^{0.75} per d	853 \pm 12	<0.001	59 \pm 2	<0.001	0.53
HR, beats/min	64.7 \pm 1.3	<0.001	4.5 \pm 0.1	<0.001	0.61
VO ₂ , L/kg ^{0.75} per d	39.6 \pm 0.6	<0.001	2.3 \pm 0.1	<0.001	0.49
VCO ₂ , L/kg ^{0.75} per d	44.0 \pm 0.6	<0.001	4.4 \pm 0.1	<0.001	0.61
RQ	1.115 \pm 0.006	<0.001	0.038 \pm 0.001	<0.001	0.48
O ₂ P, μ L O ₂ /kg ^{0.75} per beat	426.3 \pm 9.2	<0.001	-3.7 \pm 0.6	<0.001	0.64
HPP, J/kg ^{0.75} per beat	9.19 \pm 0.20	<0.001	0.001 \pm 0.012	0.932	0.64

¹HP = heat production; HR = heat rate; VO₂ = oxygen consumption; VCO₂ = carbon dioxide production; RQ = respiratory quotient; O₂P = oxygen pulse; HPP = heat production pulse.

²P-value shows the significance level differing from zero.

HR and HP should be established for each animal. In the present experiment, however, the variation between animals was less than the variation within-animal for HP, VO₂, and HR, which could be explained by the similarity, in terms of DIM, milk yield, and BW between cows selected to perform the experiment, and the use of only 1 diet for all cows. However, for O₂P and HPP, the variation was larger between animals than within-animal, reaffirming that to improve the accuracy of HP estimation by measuring HR, the ratio between HP or VO₂ and HR must be calculated for each animal (see Appendix Table A2).

Effect of Air Temperature

The increase in temperature decreased HP, VO₂, and HR only during daytime probably due to the higher temperatures (16–32°C) than during nighttime (7–23°C). During short-term exposure to heat, cows increase HR to increase peripheral blood flow to increase heat loss, but this increase in HR is not accompanied by an increase in VO₂. However, when cows are subjected to chronic and moderate heat stress, as in the present experiment, HR typically decrease, which is associated with a reduction of HP in response to long-term exposure to high ambient temperature (Kadzere et al., 2002). In addition, HR is not influenced to the same extent as rectal temperature and respiration rate by the increment of the temperature (Muller and Botha, 1993). Cows in this experiment increased their respiratory rate as temperature increased (Zhou et al., 2022), whereas HR decreased and O₂P remained unchanged, indicating that the increased respiratory rate did not affect the linearity of the relationship between HR and VO₂. Increased respiratory rate is related to increased minute ventilation (volume of air inhaled or exhaled from the lungs per minute) to increase heat dissipation, but this mechanism probably did not greatly affect the arterial-venous oxygen difference or the stroke volume

because the decrease in VO₂ could be explained by a reduction in cardiac output (L/min) related to the reduction in HR.

Brosh et al. (1998) found that the O₂P of growing beef heifers was not affected by the high heat load imposed by the exposure to solar radiation during summer in Queensland, Australia (black globe temperature of 45°C), whereas Aharoni et al. (2003) reported that for high-yielding Holstein dairy cows, the O₂P decreased moderately at temperature humidity index (NRC, 1971) values above 75, explained by decreased VO₂ and unchanged HR. With the range of temperatures tested, O₂P and HPP were unaffected, but the tendency for an interaction for HPP and O₂P and the temperature range tested (night vs. day) points in the same direction as the work reported by Aharoni et al. (2003), who had more extreme heat loads. This would indicate that the effect of air temperature on O₂P and HPP is nonlinear and must be considered so as not overestimate the HP of cows under extreme heat stress.

Effect of Animal Posture, Activity, and Feed Intake Rate on the O₂P

Eating was the activity that most increased HP, VO₂, and HR in agreement with previous research that reported a marked increase in HR during eating for fed-indoor (Purwanto et al., 1990) and grazing dairy cows (Talmón et al., 2022). Eating is associated with cardiovascular changes, contributing to elevate the metabolic rate of the animals (Osuji, 1974). Increasing HP and VO₂ by animal tissues demand an increase in the O₂ extraction (arteriovenous O₂ difference) or an increase in cardiac output, which in turn requires an increase in the stroke volume of the heart or an increase in HR (Purwanto et al., 1990); therefore, it could affect the ratio between HP or VO₂ and HR during eating. In the present experiment, responses of HR during eating exceeded the responses of VO₂, thus reducing the O₂P

($2.5 \pm 0.9\%$) that may lead to underestimation of the HP using the O₂P-HR method during eating. However, in the practice, this slight decrease in O₂P would not substantially bias the estimations of HP as dairy cows only spent 17% of the day eating.

In addition, HP estimation by O₂P assumes that the contribution of VCO₂ to HP is constant. Based on the assumption that under practical conditions the RQ equals 1.0 (McLean, 1972), a value of 20.47 kJ/L of O₂ has been used to convert the estimates of VO₂ to estimates of HP (Brosh et al., 1998; Talmón et al., 2020, 2022); however, recent studies have been reported that RQ is often greater than 1.0 in dairy cows (van Gastelen et al., 2017, 2020; Morris et al., 2020) associated with ruminal anaerobic fermentation of dietary carbohydrates and de novo synthesis of fatty acids (Gerrits et al., 2015) and, therefore, this value could underestimate the HP of dairy cows. In the present experiment, a value of 21.67 kJ/L of O₂ consumed based on the average RQ = 1.14 should be used to estimate HP correctly, which represents an increase of 5.9% when compared with HP estimated using the coefficient suggested by McLean (1972). Thus, the error associated with the RQ value use in the calculation would lead to a greater bias in the daily HP estimation than the slightly decrease in O₂P during eating.

On the other hand, the decrease in O₂P during eating was compensated for by an increase in RQ ($12.7 \pm 0.6\%$), which led to HPP being not affected during eating. This illustrates the added value of the HPP over the O₂P during eating. Alternatively, considering the O₂P constant, thereby ignoring its decline during eating, would only cause a minor error in HP estimates. In addition, the RQ presented a low coefficient of variation between animals (1.8%, Appendix Table A2), which would indicate that it is possible to use a single value for cows that are in a similar physiological state, production level, and diet. The HPP was unchanged between eating and idling, which indicates that is possible to estimate the increase in energy expenditure for eating above idling simply by measuring HR in dairy cows. Although the O₂P when eating was evaluated in cows fed TMR, based on the work published by Berhan et al. (2006), it could also be expected that the relationship between VO₂ or HP and HR remain stable during grazing, in which eating is combined with walking to search and select the feed.

The high correlation observed in this experiment between the feed intake rate (DMI/37.8 min) and the relative eating time (% time eating/37.8 min) indicates that there were no important differences in the instantaneous intake rates because the composition of the diet was similar for all cows and that higher feed intake rates were explained by longer eating times. This is in

agreement with Susenbeth et al. (2004) who reported that the energy expenditure for eating is mainly affected by the time the animal spent eating and not by the intake rate.

As well as during eating, HP, VO₂, and HR increased during ruminating compared with idling; however, the increment in both HP and VO₂ were larger than the increment in HR, hence both O₂P and HPP increased during ruminating (5.1 ± 0.5 and $3.6 \pm 0.5\%$, respectively). The causes of this increment during ruminating are not clear. Probably the rumination activity generally occurred after eating bouts (Hedlund and Rolls, 1977; Gibb et al., 1997), not allowing to differentiate between the HP of the heat increment of feeding (Blaxter, 1989) and that coming from the muscular activity of ruminating. According to the metabolic theory of ecology, an increase in tissue O₂ demand can be met by changes in either cardiac output or O₂ extraction. Increments in the postprandial cardiac output have resulted from significant increases in both HR and stroke volume (Kelbaek et al., 1989; Waaler et al., 1991; Sidery and MacDonald, 1994), as well as in O₂ extraction (Grant et al., 1997). Thus, the response of the cardiovascular system during a postprandial state could not be related only to a change in HR. Accordingly, McPhee et al. (2003) reported that after a meal, HR did not significantly increase to match the observed increase in VO₂, leading to a higher O₂P, which suggested that HR may not accurately reflect the energy demand during a postprandial state. However, in the present work, it is not possible to properly explain the postprandial effect on the relationship between VO₂ (and HP) with HR as we did not measure the O₂ extraction and stroke volume.

The effect of posture (standing vs. lying down) led to differences in HR ($6.4 \pm 0.7\%$), and although the O₂P was higher when the cows were standing than when they were lying down, the difference ($1.0 \pm 0.5\%$) may be considered negligible. Hence, the posture of the animal barely affects the relationship between VO₂ and HR; therefore, the precision in the estimates of HP using the O₂P-HR technique is hardly affected by the posture of dairy cows because a change in HR is the major component in response to a change in the animal posture.

When applying the O₂P-HR method, the O₂P is measured per cow during a short period when the cow is standing and idling. Based on the observed differences in O₂P among activities and postures, we can estimate the bias in daily HP estimates due to the use of this single O₂P value measured for each cow. For example, when grazing cows spend 37 and 30% of the day eating and ruminating, respectively, and 40% of the time lying down (see Dohme-Meier et al., 2014), it would only generate a bias of 0.2% in the estimation of daily

VO₂. For nongrazing cows, in which eating time was less than (28%), but rumination and idling time were similar to that of grazing cows (31 and 43%, respectively, see Dohme-Meier et al., 2014), the bias in the estimation of daily VO₂ would be 0.5%. This illustrates that it is possible to accurately estimate the daily VO₂ of dairy cows by continuously measuring HR and using a single O₂P value per cow measured when the cow is standing and idling.

Energy Cost of Standing, Ruminating, and Eating

The energy expenditure for standing was in the range of 51 to 60 kJ/kg^{0.75} per day previously reported (ARC, 1980; Susenbeth et al., 2004; Suzuki et al., 2014), but in this study, the percent increase in HP of standing over lying down was less than reported previously for steers and nonlactating Holstein cows (6.5% vs. 14 and 15%; Susenbeth et al., 2004; Suzuki et al., 2014). The HP at resting (idling + lying down) was between 1.8- and 2.3-fold higher in this experiment compared with the studies mentioned above, related to the higher metabolism of lactating cows than growing or nonlactating animals.

The energy expenditure for ruminating was similar to the energy expenditure for standing. The energy expenditure for ruminating was 2.7 and 2.0-fold lower in this study than in sheep and nonlactating cows, respectively (McCGraham, 1964; Suzuki et al., 2014), but was close to the range reported by Susenbeth et al. (1998) for cattle (47 to 56 kJ/kg^{0.75} per day). The variability of the reports in the energy cost of ruminating show the difficulty of measuring this activity because the energy cost of the physical activity of rumination is confused with the heat increment associated with feed intake.

The energy costs of eating in the present study was 4- to 5-fold higher than the energy cost of ruminating and standing. The energy expenditure for eating was in agreement with data published by Susenbeth et al. (1998) based on the average of 8 studies using different diets and types of cattle (179 ± 58 kJ/kg^{0.75} per day), but it was 28% less than the cost of prehension and chewing proposed by CSIRO (2007; 308 kJ/kg^{0.75} per day) based on numerous estimates made mainly on sheep consuming different feeds (Osuji, 1974).

Moreover, HP increased 59 kJ/kg^{0.75} per day per kilogram when the feed intake rate increased with 1 kg of DM/h, but this increase could be highly variable depending on feed characteristics. In agreement with Susenbeth et al. (2004), it is expected that eating time rather than feed intake rate explains variation in HP for eating. However, HP differences between feedstuffs are reduced when they are expressed per unit of time eating, which indicates that the number of chews or time spent eating is the main determinant of the energy

requirement for ingestion (Osuji, 1973; Adam et al., 1984; Susenbeth et al., 2004). In the present experiment, cows consumed the same diet and HP was related to feed intake rate as the latter was highly correlated with proportion of time eating. In addition, HP did not increase when the feed was introduced via a rumen cannula, indicating that the activity of eating and chewing is the dominant factor causing the increased HP during ingestion (Susenbeth et al., 2004). Finally, considering that the energy cost of eating is affected by the activity of eating and chewing and not by the effect of the feed in the gastrointestinal tract, the differences between the energy expenditure for eating and ruminating are likely explained by differences in chewing activities and some skeletal muscle activities (Suzuki et al., 2014).

Cows in the present experiment fed with TMR spent less time in the day consuming (17%) than cows grazing (37%) or that were fed indoors with the same grass previously cut and offered in the barn (28%), but they spent more time ruminating than when those fed only grass (39 vs. 31%; see Dohme-Meier et al., 2014). Based on the energy cost of the activities reported above (eating vs. idling and ruminating vs. idling) and assuming that the cows in this experiment presented a maintenance energy requirement of 628 kJ of ME/kg^{0.75} per day as recently reported by NASEM (2021), grazing cows would increase their maintenance energy requirements by 6.2% (39 kJ/kg^{0.75} per day), whereas for cows fed the same grass in the barn, it would increase by 3.2% (19 kJ/kg^{0.75} per day) compared with those in the present experiment consuming TMR. This shows the effect of eating time in the maintenance energy requirements, and it is important highlight that this increase should be even greater in grazing animals due to walking activity.

CONCLUSIONS

We found HP estimates based on the O₂P-HR technique are only marginally affected by air temperature within the range of 8 to 32°C. Caution is required when applying the approach during short-term heat stress or at heat loads exceeding the conditions outside our experiment because of the potentially nonlinear relationship between the O₂P and air temperature. Standing and eating slightly affected the O₂P compared with lying down and idling, respectively, but these changes do not represent a major bias for HP estimates (<2.5%). However, rumination increased O₂P, probably because during a postprandial state, when much of this activity occurs, changes in the cardiovascular system are not only explained by changes in HR. Hence, it is necessary to be cautious when interpreting HP estimates for ruminating using the O₂P-HR method. The results

demonstrate that it is possible to accurately estimate the HP of dairy cows by continuously measuring HR and using a single O₂P value per cow measured when the cow is standing and idling.

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APPENDIX

Appendix Table A1. Effect of different combinations of RH and AV on HP, HR, VO₂, VCO₂, RQ, O₂P, and HPP¹ (mean ± SEM)

Item	Treatment ²					P-value
	RH_l + AV_l	RH_m + AV_l	RH_m + AV_m	RH_m + AV_h	RH_h + AV_l	
Data set of present study ³						
Cow, n	4	1	2	3	2	
HP, kJ/kg ^{0.75} per d	866 ± 25	870 ± 50	914 ± 37	914 ± 30	882 ± 36	0.718
HR, ⁴ beats/min	68 ± 3	68 ± 6	67 ± 4	67 ± 3	66 ± 4	0.996
VO ₂ , L/kg ^{0.75} per d	40.2 ± 1.2	40.3 ± 2.3	42.3 ± 1.7	42.2 ± 1.4	40.8 ± 1.7	0.731
VCO ₂ , L/kg ^{0.75} per d	45.5 ± 1.3	45.5 ± 2.6	48.0 ± 1.9	48.1 ± 1.6	46.3 ± 1.9	0.668
RQ	1.14 ± 0.01	1.13 ± 0.02	1.14 ± 0.02	1.14 ± 0.01	1.14 ± 0.02	0.995
O ₂ P, μL O ₂ /kg ^{0.75} per beat	410 ± 18	410 ± 36	436 ± 26	438 ± 21	426 ± 26	0.837
HPP, J/kg ^{0.75} per beat	8.88 ± 0.39	8.88 ± 0.78	9.44 ± 0.55	9.50 ± 0.45	9.25 ± 0.55	0.825
Complete data set ⁵						
Cow, n	4	4	4	4	4	
HR, ⁶ beats/min	68 ± 1	70 ± 2	71 ± 1	67 ± 1	70 ± 1	0.569
VO ₂ , L/kg ^{0.75} per d	39.9 ± 0.1	43.5 ± 0.1	42.0 ± 0.1	41.8 ± 0.1	40.3 ± 0.1	0.174

¹RH= relative humidity; AV = air velocity; HP = heat production; HR = heat rate; VO₂ = oxygen consumption; VCO₂ = carbon dioxide production; RQ = respiratory quotient; O₂P = oxygen pulse; HPP = heat production pulse.

²Levels of RH and AV (l = low; m = medium; h = high).

³Data from the 12 cows used in the present study.

⁴Automatic measurements using HR monitors.

⁵Data from the 20 cows used by Zhou et al. (2022).

⁶Manually measurements at 0600, 1000, and 1800 h.

Appendix Table A2. Coefficient of variation of HP, VO₂, VCO₂, HR, O₂P, HPP, and RQ within the day, between cows, and between days¹

CV	Value ²	HP	VO ₂	VCO ₂	HR	O ₂ P	HPP	RQ
Within the day, ³ %	Mean	9.3	8.8	11.3	8.8	5.2	5.1	4.7
	SD	1.0	1.0	1.0	1.4	0.7	0.7	0.3
	Max.	11.5	11.0	13.6	11.5	7.0	6.8	5.3
	Min.	8.1	7.7	10.1	6.9	4.1	4.2	3.9
Between animals, ⁴ %	Mean	5.2	5.3	5.2	6.6	7.4	7.4	1.8
	SD	0.7	0.7	0.8	0.5	0.4	0.4	0.3
	Max.	6.4	6.4	6.6	7.2	8.3	8.2	2.4
	Min.	4.2	4.4	4.0	5.8	7.1	7.1	1.4
Between days, ⁵ %	Mean	2.3	2.3	2.4	2.5	1.4	1.4	0.7
	SD	0.8	0.8	0.9	0.6	0.6	0.5	0.2
	Max.	3.5	3.6	3.5	3.4	2.4	2.2	1.1
	Min.	1.0	1.0	0.5	1.1	0.6	0.7	0.4

¹HP = heat production; HR = heat rate; VO₂ = oxygen consumption; VCO₂ = carbon dioxide production; RQ = respiratory quotient; O₂P = oxygen pulse; HPP = heat production pulse.

²Max. = maximum; min. = minimum.

³Mean variation within a day.

⁴Variation between animal means.

⁵Variation between day means.