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ANIMAL PRODUCTION

Different methods and times to estimate heat production in sheep fed with sunflower meal

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ABSTRACT: The objective of this study was to assess the oxygen pulse and heart rate method (O_2P-HR) using a respiration chamber at different measurement times for estimate the heat production (HP) of lambs fed increasing levels of sunflower meal in their diet. Twenty-four lambs were assigned to four experimental diets (0, 100, 200, and 300 g of sunflower meal kg^{-1} DM). Heat production was estimated using the O_2P-HR (HP_{O2P}) method and a respirometry chamber (HP_{RC}). Measurements were obtained by simultaneously measuring heart rate (HR) and oxygen consumption over 3, 6, 9, 12, 15, 18, 21 and 24 h. A flow-through respirometry chamber for small ruminants was used to determine oxygen consumption (VO_2) and carbon dioxide and methane production. Data on dietary treatment, measurement times and their interactions were analyzed as repeated measures using mixed model procedures and Restricted Maximum Likelihood (REML) estimation. The Pearson's correlation coefficient was used to compare techniques. There was no effect of the different levels of sunflower meal inclusion on VO_2 and heat production. The HP_{O2P} (126.16 kcal/ $BW^{0.75}$/day) was 2% higher than that of the HP_{RC} (124.61 kcal/ $BW^{0.75}$/day), and the correlation coefficients was 0.628. The coefficient of variation was greater for the HP_{O2P} (21.33%) than for HP_{RC} (11.44%). HR (beats/min), VO_2 (mL/min/$BW^{0.75}$) and O_2P-HR (mL/beat) required measurement times of 24, 15 and 9 hours, respectively. A measurement time of 24 h was necessary to ensure a more accurate estimate of the heat production using the O_2P-HR method.
Key words: bioenergetic, energy requirements, indirect calorimetry, lamb, ovine.

Diferentes métodos e tempos de medição para estimar a produção de calor em ovinos alimentados com farelo de girasssol

RESUMO: O objetivo com este estudo foi avaliar o método do pulso de oxigênio (O_2P-FC) usando câmara respirométrica em diferentes tempos de medição para estimar a produção de calor de cordeiros alimentados com níveis crescentes de farelo de girassol na dieta. Vinte e quatro cordeiros foram distribuídos em quatro dietas experimentais (0,10, 20 e 30% de farelo de girassol). A produção de calor foi estimada pelo método de O_2P-FC (PC_{O2P}) e por câmara respirométrica (PC_{CR}). As estimativas foram obtidas medindo-se simultaneamente a frequência cardíaca (FC) e o consumo de oxigênio (VO_2) durante 3, 6, 9, 12, 15, 18, 21 e 24 horas. Uma câmara de respirométrica para pequenos ruminantes foi usada para determinar o VO_2 e a produção de dióxido de carbono e metano. Os dados referentes a dieta experimental, tempos de medição e suas interações foram analisados como medidas repetidas usando os procedimentos de modelo misto e estimativa de máxima verossimilhança restrita. A correlação de Pearson foi usada para comparar as duas técnicas de estimativa da produção de calor. Não houve efeito dos diferentes níveis de inclusão de farelo de girassol sobre o consumo de oxigênio e produção de calor dos animais. A PC_{O2P} (126,16 kcal/ $PV^{0.75}$/dia) foi 2% maior que a PC_{CR} (124,61 kcal/ $PV^{0.75}$/dia), e o coeficiente de correlação foi de 62,8%. O coeficiente de variação foi maior para PC_{O2P} (21,33%) comparado com PC_{CR} (11,44%). A FC (batimentos/min), VO_2 (mL/min/$PC^{0,75}$) e o O_2P-FC (mL/batimento) requerem tempos de medição de 24, 15 e 9 horas, respetivamente. É necessário a mensuração por 24 horas para garantir uma estimativa mais precisa da produção de calor usando o método de O_2P-FC.
Palavras-chave: bioenergética, calorimetria indireta, cordeiros, exigências de energia, ovinos.

INTRODUCTION

Energy is the primary nutrient limiting ruminants. It is derived from the oxidation of dietary nutrients and is essential for maintenance of vital processes. This nutrient is dissipated by animals during ingestion and metabolism of food: first, energy is consecutively lost in feces, urine and fermentative gases, and subsequently lost as heat increases. The remaining energy is primarily directed towards maintenance (approximately 70% of the net energy available) and production processes (TEIXEIRA et al., 2017).

The standard method for measuring energy expenditure in ruminants involves the use of open-

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circuit respirometry chambers. In this method, the products resulting from the animal's metabolism, such as gas exchanges with the environment (oxygen consumption, carbon dioxide and methane production), combined with urinary nitrogen excretion, are quantified (SILVA, 2011; OSS et al., 2016). A respirometry chamber is an accurate technique, however it is used under laboratory conditions and is extremely expensive. It also requires significant expertise and infrastructure, wich makes it impractical for small rural properties (RODRIGUEZ et al., 2007; MACHADO et al., 2016).

Measuring the heat production of animals can provide insights into how efficiently they utilize the nutrients in their diet, thereby helping optimize feed efficiency, since heat production is closely linked to the metabolic processes and energy utilization of animals.

Researchers are seeking to estimate heat production in ruminants by using heart rate adjusted for oxygen consumption per beat as there is a linear relationship between heart rate (HR) and oxygen consumption (VO_2) in homeothermic animals, thereby indicating that it is possible to estimate heat production through HR measurements (TALMON et al., 2023). The primary goal is to improve and develop techniques capable of measuring the energy requirements of animals in a shorter time_frame, with cheaper equipment, and without changing the behavior and normal conditions of animal husbandry (BROSH, 2007; CHAVES et al., 2015).

The O_2P-HR technique can be used as an alternative method to determine heat production. However, there are some problems associated with the ideal time to measure oxygen consumption, heart rate, and O_2P-HR, especially considering the intraday changes that interact directly with animals. According to OSS et al. (2016), O_2P-HR is an alternative technique, but has a greater between-animal coefficient of variation, which has a negative effect on the power of the experiments. Further studies should be performed to investigate ways to minimize the errors associated with the O_2P-HR method to increase the precision and statistical power of experiments using this technique.

The objective of this study was to evaluate the O_2P-HR method at different measurement times to estimate heat production in crossbred lambs fed diets containing increasing levels of sunflower meal.

MATERIALS AND METHODS

Twenty-four crossbred (Santa Inês x Dorper) intact male lambs with a mean age of 4 months were arranged into three blocks, four treatments, and two replicates per block using a randomized block design. A 10-day adaptation period was allowed before the data collection.

The lambs received four isoproteic experimental diets formulated according to the NRC (2007) recommendations for lambs on maintenance levels. The diets containing a roughage:concentrate ratio of 40:60 on a dry matter basis (DM). Corn silage was supplied as the roughage source and the concentrate was formulated by replacing soybean meal with increasing levels of sunflower meal (0, 100, 200 and 300 g kg^{-1} DM) (Table 1). The diet was provided in two daily meals at 8 a.m. and 4 p.m.

The chemical composition of the diets and orts was determined by analyzing the dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), non-fibrous carbohydrates (NFC) and total carbohydrate (TC) content according to the procedures of INCT-CA (DETMANN et al., 2012).

Before starting the experiment, the lambs were weighed, drenched and vaccinated against clostridial diseases. During the experiment, lambs were housed in individual metabolic cages provided with feed and water troughs, which allowed the collection of urine and fecal samples. After the adaptation period DM intake was measured, and urine was collected for nitrogen determination for five days. Heat production was estimated by using a respirometry chamber after adaptation to the diet.

The O_2 consumption (VO_2) and CO_2 production data were recorded using a Sable System (Sable Systems International, Las Vegas, NV, USA). The lambs were individually placed in a respirometry chamber for 24 h and the same dietary treatment offered during the adaptation period was administered to each lamb once in the morning.

Ambient air flowed through the chamber at a controlled flow rate based on lamb's weight (0.6 liters/kg of body weight/minute), and it was mixed with the exhaled air. Samples were taken every 5 min for 24 h to determine O_2, CO_2 and CH_4 concentrations. All data were recorded using an automated data acquisition program (Expedata; Sable Systems International).

The maximum allowable concentration of CO_2 in the chamber was 1.0%. Oxygen consumption and CO_2 production were calculated by comparing the composition and volume of the air that flowed through the respirometry chamber with the air released. The temperature was kept at 22 °C by using an air conditioner placed inside the chamber to provide thermal comfort to lambs.

Table 1 - Nutritional composition of the experimental diets.

Item (g kg^{-1})	---------------Sunflower meal level (g kg^{-1} DM)---------------			
	0	100	200	300
Corn silage	400	400	400	400
Soybean meal	264	196	118	18
Corn grain	315	281	256	261.6
Sunflower meal	0	100	200	300
Vitamin-Mineral Premix[1]	10.5	15.0	22.0	36.6
Dicalcium phosphate	10.5	8.0	4.0	0
---------------Nutrients---------------				
DM (g/kg^{-1} NM)	643.4	641.7	653.3	658.8
MM (g/ kg^{-1} DM)	45.0	43.2	45.7	46.1
CP (g/ kg^{-1} DM)	207.9	203.8	195.6	189.5
NDF(g/ kg^{-1} DM)	337.9	364.5	391.2	419.8
ADF(g/ kg^{-1} DM)	176.3	212.5	227.2	302.8
EE (g/ kg^{-1} DM)	65.5	62.9	60.9	60.1
NFC(g/ kg^{-1} DM)	343.7	325.6	306.6	284.5
TC (g/ kg^{-1} DM)	681.6	690.1	697.8	704.3

DM = Dry matter, NM = Natural Matter, CP = Crude Protein, NDF = Neutral detergent Fiber, ADF = Acid detergent fiber, EE = Ether extract, NFC = Non-fibrous carbohydrate, TC = Total carbohydrate.

[1]Composition of Vitamin-Mineral Premix: Calcium (Max.) 150 g, Calcium (Min.) 130 g, Phosphorus (Min.) 65 g, Sodium (Min.) 130 g, Fluorine (Max.) 50 mg, Sulfur (Min.) 12 g, Magnesium (Min.) 10 g, Iron (Min.) 1000 mg, Manganese (Min.) 3000 mg, Cobalt (Min.) 80 mg, Zinc (Min.) 5000 mg, Iodine (Min.) 60 mg, Selenium (Min) 10 mg, Vitamin A (Min.) 50000 IU, Vitamin E (Min.) 312 IU.

Heat production was estimated using the respirometry chamber technique (HP_{RC}) according to Brouwer's equation (1965) as follows:

HP_{RC} (Kj) = 16.18 x VO_2 (L) + 5.02 x VCO_2 (L)- 5.88 x UN (g) – 2.17 x VCH_4 (L)

HP_{RC} = Estimation of heat production using the respirometry chamber technique

VO_2 = Oxygen consumption

VCO_2 = Carbon dioxide production

UN = Urinary nitrogen

VCH_4 = Methane production

Estimation of heat production using the O_2 pulse methodology (HP_{O2P}) was based on a protocol adapted from BROSH et al. (1998). After the adaptation period, the lambs were monitored for four days to record the mean heart rate using a POLAR® RS800 transmitter. The transmitters were attached to the girth of the lambs using elastic strips. Data were recorded at 60 s intervals and subsequently transferred to a computer using an infrared sensor.

After determining the mean heart rate (HR during the four days of measurement), data on heartbeat (HR-RC) and oxygen consumption (VO_2) were collected simultaneously for 24 h using a respirometry chamber, as described above. These data were used to calibrate the O_2 volume per heartbeat. The oxygen pulse and heart rate were calculated as VO_2 per heartbeat.

Daily heat production was obtained by multiplying the total O_2 consumption by the constant 4.89 kcal/L of O_2 (NICOL & YOUNG, 1990). The results were expressed as metabolic weight (kcal/kg $BW^{0.75}$/day). Heat production was estimated using the following equation:

$$HP02P: \frac{kcal}{day \times kg\ BW0.75} = (HR - RC \times 2\ O2P \times 4.89) \times 1440 / (kg\ BW^{0.75})$$

HP_{O2P} = Estimation of heat production using the oxygen pulse and heart rate method

HR-RC: Mean heartbeat (beat/min)

O_2P: Oxygen consumption per heartbeat (L/beat).

Data analysis

The dietary treatments, measurement times and their interactions were analyzed as repeated measures (each treatment was analyzed at eight measurement times: 3, 6, 9, 12, 15, 18, 21 and 24 h) because the observations were interdependent. Data were analyzed using the Proc MIXED procedure in SAS (SAS 9.0 Inst. Inc.) and Restricted Maximum Likelihood (REML) estimation according to the following model:

$yijt = \mu + \alpha i + dj(i) + \gamma t + (\alpha\gamma)it + (b + \beta j) + eijt$

Where, *yijt* = the expected outcome for the dependent variable Y observed at the measurement time *t* for the lamb *j* fed the diet *i*; μ is the overall mean; αi is the fixed effect of diet; *dj*(*i*) is the random effect of lamb *j* nested within diet *i*; γ*t* is the fixed effect of measurement time; (αγ) *it* is the interaction between diet and measurement time; *b* is the regression coefficient; β*j* is the slope deviation (diet *i*) of the regression coefficient *b*; eijt is the random error associated with lamb *j* fed diet *i* at the measurement time *t*, eijt ~ NID ((0, $\sigma^e{}_2$) (data is approximately normally distributed with mean of 0 and variance of $\sigma^e{}_2$); and the values of *dj*(*i*) and eijt are assumed to be independent.

Five variance-covariance matrix structures were tested as follows: variance components (VC - variances are equal and observations are independent, i.e., there is no correlation between observations over time); compound symmetry (CS - equality of variances and covariances); first-order autoregressive model (AR (1) - equality of variances and covariances with higher correlation between adjacent measures); first-order ante-dependence (ANTE (1) - the magnitude of the covariance depends on the values of both correlation and standard deviations associated with them); unstructured (UN – each variance and covariance is estimated exclusively from the data) (SAS, 2004). The best model for each set of variables was selected based on the lowest corrected Akaike Information Criterion (AICc) value.

The variance-covariance matrix structure of the best fit for the measurement time was selected based on the lowest corrected Akaike Information Criterion (AICc) value (LITTELL et al., 2006). The ANTE (1) model provided the best fit for HR, O_2P (mL/beat/$BW^{0.75}$), HPO_2P (kcal/day) and HPO_2P (kcal/day/$BW^{0.75}$), thereby modeling the covariance structure and thus generating valid tests. The AR (1) model provided the best fit for the VO_2 (L/day), VO_2 (mL/min/$BW^{0.75}$) and O_2P (L/beat), whereas the ANTE (1) model did not converge. The UN model did not converge for VO_2 (L/day) or VO_2 (mL/min/$BW^{0.75}$). For the other parameters, problems were encountered when the Hessian matrix was applied, which demonstrates that the UN structure was inappropriate.

After defining the best model for each set of variables, the result of the fixed effect analysis (measurement time) was used as a criterion to test the significance of the treatment effect (α=0.05). The parameters were subjected to regression analysis (PROC REG) using SAS (2004) when the diet was significant. Differences between groups means (each measurement time vs 24 h) were determined by calculating the minimum significant difference for p = 0.05 using the Tukey's test' when measurement time was significant. To express the accuracy and repeatability of the test, the coefficient of variation was calculated using the PROC UNIVARIATE procedure (SAS, 2004). Pearson's correlation was used to compare techniques using PROC CORR (SAS, 2004). Statistical significance was set at P < 0.05.

RESULTS AND DISCUSSION

The inclusion of sunflower meal did not change (P > 0.05) the DM intake, as the animals were fed at a maintenance level, with averages of 612 g/day and 50.48 g/$BW^{0.75}$/day. There was no effect on CP intake owing to the lack an effect on DM intake and the isonitrogenous profile of the diets (Table 2). However, there was a significant difference (P < 0.05) in NDF intake among the treatments, with a linearly increasing effect observed. This behavior can be

Table 2 - Means, coefficient of variation (CV) for dry matter intake (DMI), crude protein intake (CPI) and neutral detergent fiber intake (NDFI) of lambs fed with different levels of sunflower meal inclusion.

Variables	--------------Sunflower meal level (g kg^{-1} DM)----------				CV	---------------------P------------------	
	0	100	200	300	(%)	Linear	Quadratic
DMI (g/day)	637.63	596.57	601.7	613.80	10.70	0.593	0.5440
DMI (g/ $BW^{0.75}$ /day)	50.94	50.16	50.28	50.54	2.63	0.6576	0.5850
CPI (g/ $BW^{0.75}$ /day)	10.9	9.85	10.04	10.04	2.72	0.8721	0.5929
NDFI (g/ $BW^{0.75}$ /day)[1]	17.49	19.58	22.47	25.14	14.17	<.0001	<.0001

[1]y =17.29 + 0.0258x; R² = 0.967.

explained by the increased fiber concentration in the diets resulting from the inclusion of SFM.

The difference in NDF intake was not sufficient to change the VO_2 or heat production ($P > 0.05$). This behavior was possibly due to the animals' energy intake being close to maintenance levels. Typically, animals with higher digestible energy intake consume more oxygen during metabolic processes.

The HR-RC, normal HR, VO_2, HP_{O2P}, and HP_{RC} did not differ bamong sunflower meal inclusion levels ($P > 0.05$), possibly because the lambs were fed near maintenance. Moreover, the interaction between SFM level and time was not significant ($P > 0.05$).

The HR of lambs during the four days of measurement varied by only 2.6% in comparison with the HR-RC (Table 3), wich suggests the absence of stress or lack of exercise during the evaluation period. LANDAU et al. (2006) observed similar results for daily HR and HR-RC (81.1 ± 5.1 and 79.2 ± 5.1, respectively). According to the authors, this response was associated with a lower environmental effect on grazing during the measurement of the O_2 pulse.

The VO_2 (Table 3) was similar to that reported by MACHADO et al. (2015) when evaluating heat production in sheep fed sorghum silages at different maturation stages (18.37 mL/min/kg $BW^{0.75}$). The similarity in the results is likely related to feeding near maintenance and absence of stress during the execution of both studies. This is also corroborated by the heart rate data as variations of less than 20% were observed between normal heart rates.

The O_2P values (Table 3) corroborate those reported by ARIELI et al. (2002) (0.250 mL/beat/kg/$BW^{0.75}$) in sheep fed high or low energy diets (75% and 25% concentrate, respectively). Under conditions where animals are not subjected to stress, physical activity or if the variation in heart rate is less than 20%, O_2P remains constant, and the data are reliable.

The coefficient of variation was greater for HP_{O2P} (21.33%) than for HP_{RC} (11.44%). The repeatability of individual animals over time needs to be high to reliably detect the differences in the heat production of animals through respiration trials. OSS et al. (2016) compared the O_2P method with measurements using a respirometry chamber in crossbred steers (Holstein × Gyr), which was also confirmed by a greater between-animal coefficient of variation (16.6%) compared to RC (7.7%). According to the authors, the O_2P-HR method had a higher coefficient of variation, and the sample size (*n*) must be increased to determine the differences in HP between treatments more accurately.

Despite the differences in the coefficients of variation, the correlation between HP_{O2P} and HP_{RC} was 0.628 (Figure 1), thereby validating the efficiency of the O_2P-HR method in predicting heat production. The HP_{O2P} was 2% higher than the HP_{RC}. In a study evaluating the efficiency of the O_2P-HR method as a tool for determining energy expenditure in sheep, ARIELI et al. (2002) reported that heat production using the O_2P-HR technique was 6.7% higher than that using the comparative slaughter method.

Table 4 presents the differences between the overall means observed at each measurement time and after 24 h. A significant difference was detected between measurement times of up to 6 h *vs*. 24 h for all variables studied. This response is probably due to the initial adjustment phase of gas collection, which is essential for equilibrium between gas production and consumption inside the respirometry chamber.

There was an increase in HR and VO_2 during the first hours, followed by a gradual reduction after feeding. This response is likely related to

Table 3 - Mean, standard deviation, minimum and maximum of heart rate, oxygen consumption and heat production of sheep.

Item	Mean	SD	Min.	Max.
HR[1], bpm	79.52	9.28	65.70	96.80
HR-RC[2], bpm[3]	81.65	11.50	61.84	105.71
VO_2[4], ml/min/kg $BW^{0.75}$	18.09	2.52	12.21	29.42
O_2P[5], ml /beat/kg/ $BW^{0.75}$	0.225	0.039	0.143	0.287
HP_{O2P}[6], kcal/day/kg $BW^{0.75}$	126.16	26.91	68.72	164.92
HP_{RC}[7], kcal/day/kg $BW^{0.75}$	124.61	14.26	95.94	149.03

[1]Mean heart rate during the four days of measurement. [2]Mean heart rate collected for 24 hours using a respirometry chamber. [3]Beat per minute. [4]Oxygen consumption. [5]Oxygen consumption per heart beat. [6]Heat production using the O_2 pulse methodology. [7]Heat production using the respirometry chamber methodology.

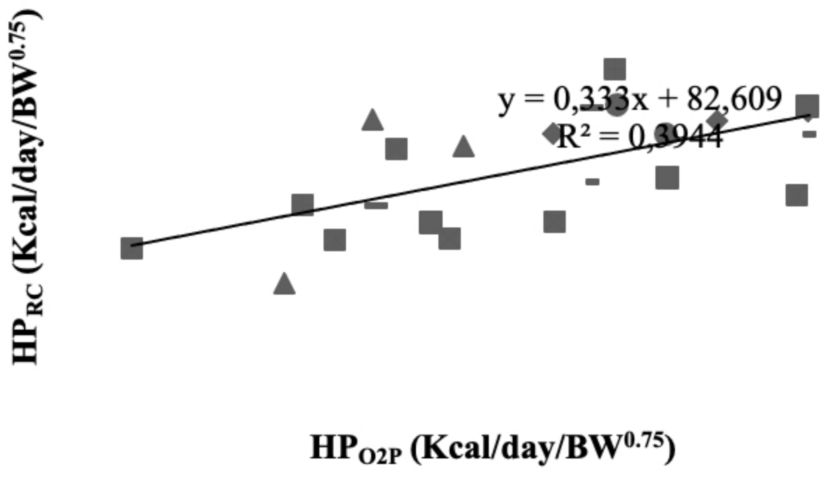

Figure 1 - Relationship between heat production estimated by the O2 pulse methodology (HP_{O2P}) and heat production estimated by the respirometric chamber methodology (HP_{RC}).

($y = 0.333x + 82.609$; $R^2 = 0.6279$; P=0.0013).

reduced stress levels after the initial period and a more extended post-feeding period, as the diet was provided once a day at the beginning of the measurement. The feeding time and the physical activities of chewing and swallowing were the leading causes of the increase in HR and VO_2 during the first few hours, as they showed a gradual reduction after feeding. According to TALMON et al. (2023) eating was the activity that most increased HP, VO_2, and HR.

Measurement time had a significant effect on HR (Table 4) throughout the 24 h period (P < 0.0001). Nevertheless, the variations observed after the 9 h period were lower than 15%. According to BROSH (2007), variations in a normal heartbeat are acceptable for the determination of O_2P, thereby ensuring the reliability of our database.

Based on the statistical analysis, we observed that HR measurements should be performed for 24 h for more complete data collection, avoiding intraday variations such as feeding time and diet quality (metabolic activity increases during digestion and absorption), lower heart rate at night (when

Table 4 - Variation in heart rate, oxygen consumption, oxygen volume per heart beat, and heat production using the O_2 pulse methodology in sheep (n = 23), expressed as the difference between the mean measurement at each time studied and that obtained during 24 hours.

	---Measurement time---							
Variable	3h	6h	9h	12h	15h	18h	21h	24h
HR, beat/min	26.52***	17.08***	12.88***	8.34***	5.17***	2.64***	0.97***	81.65 ± 2.40
VO_2, l/day	5.40***	4.13**	3.26**	2.11*	1.02	-0.16	-0.04	30.53 ±8.92
VO_2, ml/min/$BW^{0.75}$	3.19***	2.43**	1.94*	1.24*	0.60	0.06	-0.02	18.09 ± 0.53
O_2P, ml/beat	-0.30**	-0.20*	-0.14	-0.10	-0.09	-0.09	-0.04	2.6±0.11
O_2P, ml/beat/$BW^{0.75}$	-0.03*	-0.02*	-0.01*	-0.008*	-0.007*	-0.007*	-0.002*	0.225±0.008
HP_{O2P}, Kcal/day	-174.9*	-107.3*	-79.51*	-55.98*	-52.63*	-52.63*	-20.48*	1485.2±74.32
HP_{O2P},Kcal/day/$BW^{0.75}$	-14.51*	-8.75*	-6.48*	-4.60*	-4.23*	-4.23*	-1.63*	126.17±5.61

***P < 0.0001; **P < 0.001; *P < 0.05. HR=Mean heart rate during the four days of measurement. VO_2=Oxygen consumption. O2P= Oxygen consumption. per heart beat. HP_{O2P}=Heat production using the O_2 pulse methodology.

animals are at rest) and excitement resulting from the presence of people. BARKAI et al. (2002), ARIELI et al. (2002), AHARONI et al. (2003), and LANDAU et al. (2006) used the methodology of BROSH et al. (1998) with HR and VO_2 measurements for 15-20 min throughout the day and obtained results similar to those found in the literature. However, according to PUCHALA et al. (2007), the HR and energy expenditure of goats consuming different quality diets varied within 24 h, thereby corroborating our observations.

The measurement time (up to 12 hours) affected VO_2 (L/day) and VO_2 (mL/min/$BW^{0.75}$). From 15 h onwards, the parameters were similar to those obtained after 24 h (Table 4). Oxygen consumption may have varied at the beginning of the gas collection phase owing to the start of feeding and the initial stress associated with the chamber, which resulted in increased O_2 consumption. VAN MILGEN et al. (1997) observed that oxygen consumption varied according to animal behavior when assessing O_2 consumption and CO_2 production during the resting state, feeding and physical activity in pigs. BARKAI et al. (2002) and LANDAU et al. (2006) estimated oxygen consumption for 15-20 min at different times of the day using the methodology of BROSH et al. (1998) and observed no variation in oxygen consumption. However, based on our observations, more accurate measurements of the oxygen consumption require longer measurement times.

Although it was possible to measure O_2P (mL/beat) for 9 h, the effect of time on O_2P (mL/beat/$BW^{0.75}$) and HP_{O2P} (Kcal/day and Kcal/day/$BW^{0.75}$) over the entire measurement period, demonstrated that these parameters should be measured for 24 h when using the O_2P methodology (Table 4). This may be associated with variations in HR or processes involving digestion and diet quality. The roughage:concentrate ratio (40:60) explains the 24 h variations in HP_{O2P} because the degradation of non-fibrous carbohydrates is fast, whereas the digestion of fibrous carbohydrates occurs more slowly owing to the long lag time.

CONCLUSION

The O_2P-HR method is highly correlated with the respirometry chamber methodology for estimating heat production in sheep; however, O_2P-HR should be measured for 24 h to ensure greater accuracy. Sunflower meal inclusion levels did not affect heat production in the animals.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Conceptualization: LCG and ASC. Data acquisition: SSS, ASC and LCG. Design of methodology and data analysis: LCG and ASC. LCG, SSS, ASC and FSM prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experimental procedures involving animals were approved by the Ethics Committee on Animal Use of the Universidade Federal de Minas Gerais (UFMG) under protocol No. 189/15.

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