

# Heat production and body composition of primiparous Holstein cows with or without grazing pastures in early lactation

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## ABSTRACT

The aim of the study was to compare energy partitioning between heat production (**HP**) and retained (milk and body reserves) energy as well as energy efficiency of dairy cows assigned to different feeding strategies (with or without pasture grazing) during early lactation. At calving, 18 primiparous cows ( $528 \pm 40$  kg body weight (**BW**);  $3.2 \pm 0.2$  body condition score (**BCS**); fall calving) were assigned in a randomized block design, during the first 61 days postpartum, to either (**G0**) mixed ration (**PMR**) *ad libitum* (58% forage:42% concentrate) + 4.0 kg DM/d of an energy-protein concentrate in the milking parlor or (**G1**) grazing of alfalfa (6-h grazing in 3 days strips; 20 kg DM/d of pasture allowance) + PMR at 70% of *ad libitum* intake + 4.0 kg DM/d of an energy-protein concentrate in the milking parlor. Diets were composed by 77% PMR and 23% concentrate for G0 cows and 54% PMR, 22% concentrate and 24% pasture for G1 cows. Heat production (**HP**) was measured at  $40 \pm 3$  days postpartum by the  $O_2$  pulse technique and energy retained (**RE**) in milk and body tissue was estimate based on NRC equations for the period between 26 and  $54 \pm 3$  days postpartum. In addition, body composition was determined using the urea dilution technique at  $-7$  and  $40 \pm 3$  days postpartum. Absolute body water, fat and protein mass, and gross RE decreased from  $-7$  to  $+40$  days but the decrease in fat mass and gross RE was 10% greater for G1 than G0 cows. In addition, during this period relative lipid mass and gross energy content decreased only in the G1 cows. During the second month of lactation (from 26 to 54 days), the G0 cows tended to produce 6% more milk and had 0.3 units more of BCS than the G1 cows. Both RE in milk and in body tissue were greater for G0 than G1 cows (7% and 3-fold greater, respectively). No differences were found in metabolizable energy (**ME**) intake and HP measured at  $+40$  days between the cow groups. However, residual HP (difference between HP measured and predicted HP calculated from  $BW^{0.75}$  and total RE on the assumption of constant efficiency coefficients), expressed as percentage of ME intake, tended to be 10% less for G0 than G1 cows. The adjusted gross energy efficiency (total RE divided by ME intake) tended to be greater for G0 than G1 cows. The results indicated that 100% PMR fed cows were more efficient, secreting more energy in the milk and retaining more energy in the body tissue than grazing cows supplemented with PMR. This was probably due to an increase of about 10% in maintenance requirements associated to greater forage content in the diet and/or grazing and walking activities in grazing cows.

## 1. Introduction

Pasture-based dairy production systems have gained interest during the last decades due to their economic, environmental and animal-welfare advantages (Dillon, 2006). In the same way, intensification of global dairy production systems has been based on a significant increase in the use of concentrates, forage reserves in the dairy cow diet (Wales et al., 2013). Indeed, in the last decades feeding systems based on total mixed ration (**TMR**) or that supply mixed rations to grazing

dairy cows (partial mixed rations; **PMR**) have increased to improve intake and milk production when pastures are limiting (Bargo et al., 2002; Wales et al., 2013).

Mobilization of body reserves occurs in early lactation, as high-producing dairy cows cannot consume enough dry matter (**DM**) to meet their nutrient requirements. Cows often mobilize both, fat and protein body reserves (Gibb et al., 1992). Indeed, mobilization of dairy cows during early lactation has been reported to range between 0.52 and 0.66 kg/d of body fat and between 0.04 and 0.09 kg/d of body protein

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(Gibb et al., 1992; Tamminga et al., 1997). However, while fat mobilization occurs from parturition up to 60 to 80 days postpartum, protein mobilization starts before parturition until the first 14 to 30 days of lactation (van der Drift et al., 2012). Increased energy intake reduced both, fat and protein mobilization of dairy cows in early lactation (Chilliard et al., 1991) whereas dietary protein, and particularly rumen undegradable protein, increased tissue mobilization if energy intake was restricted but not when an adequate nutrition was provided (Ørskov et al., 1977; Komaragiri et al., 1998). Thus, to maintain an adequate body condition and a milk production above 30 kg/d, high-yield dairy cows in grazing production systems require supplemental energy (Kolver and Muller, 1998; Dillon et al., 2003).

Moreover, production performance of dairy cows under grazing conditions is frequently lower than the estimated by feeding systems based on pasture nutrient and energy supply (Gruber et al., 2007). Reduced energy density, nutrient imbalances (i.e. dietary protein to energy ratio), reduced efficiency of use of metabolizable energy (ME) for milk production (i.e. due to energy cost of excreting nitrogen) and increased energy requirements for maintenance (i.e. cost of rumination and digestion, grazing and walking activity) could explain, among other factors, the reduced efficiency of production of dairy cows in grazing systems (Kolver and Muller, 1998; Agnew and Yan, 2000; Bruinenberg et al., 2002; Dong et al., 2015a, 2015b).

Although production efficiency is essential to maintain profitability and sustainability of dairy grazing production systems, information about energy expenditure under pasture-based conditions is limited. Kaufmann et al. (2011), using the  $^{13}\text{C}$  bicarbonate dilution technique, reported in early lactation dairy cows greater energy expenditure for grazing vs. grass-fed cows in the barn, even though DM intake and milk production did not differ between treatments. These latter authors suggested that improved nitrogen utilization or greater body fat mobilization may explain that grazing dairy cows maintained milk production despite of their greater energy requirements. Indeed, Dohme-Meier et al. (2014) using the same technique in three moments of lactation, determined unchanged milk production, lower DM intake and increased energy expenditure in grazing vs. grass-fed cows in the barn associated with greater mobilization of body reserves. In addition, Miron et al. (2008) using the  $\text{O}_2$  pulse technique ( $\text{O}_2\text{P}$ ) showed that, although heat production remained constant, retained energy in milk and tissue decreased as fiber content of TMR increased.

In order to correctly estimate energy demands of cows on pasture and mixed dairy systems, more information related to energy expenditure and partitioning under these production conditions is needed. The objective of the current study was to compare energy partitioning between heat production (HP) and retained energy (RE) of primiparous Holstein cows fed different strategies in early lactation (mixed ration vs. grazing plus mixed ration). We hypothesized the decrease of the mixed ration offer to 70% *ad libitum* intake as pasture grazing is included in the diet would allow a milk production similar to *ad libitum* mixed ration fed cows at expenses of greater mobilization of body reserves due to increased energy requirements for maintenance.

## 2. Materials and methods

The experiment was carried out at the Experimental Station Mario A. Cassinoni (Facultad de Agronomía, Universidad de la República, Uruguay) from February to May 2015. The average temperature for this period was  $21.0 \pm 5.9^\circ\text{C}$ , the rainfall was  $67.5 \pm 39.0\text{ mm}$  and the ITH was  $66.8 \pm 8.2$ . Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (Expe # 021130-001914-15).

### 2.1. Experimental design, animals, and treatments

Primiparous Holstein cows ( $n = 18$ ) were grouped by calving date (fall calving; average calving date: 04/18/2015  $\pm 11$  days), blocked

within group according to body weight (BW;  $528 \pm 40\text{ kg}$ ) and body condition score (BCS;  $3.2 \pm 0.2$ , scale 1 (skinny) to 5 (fat); Edmonson et al., 1989) and used in a randomized complete block design with two nutritional treatments from calving to 61 days postpartum: (G0) control cows fed a mixed ration (PMR) *ad libitum* in individual stalls + 4.0 kg DM/d of an energy-protein concentrate in the milking parlor or (G1) cows grazing alfalfa and receiving PMR (offered at 70% of *ad libitum* intake) in individual stalls + 4.0 kg DM/d of an energy-protein concentrate in the milking parlor. During the pre-calving period, from -42 to  $-21 \pm 11$  days relative to calving, cows were managed as a single group and grazed on good-quality pastures to maintain BCS. From  $-21 \pm 11$  days until calving, cows were managed in individual stalls ( $10 \times 4\text{ m}$ ) with water *ad libitum* and shade available and were offered 12.3 kg DM/d of a mixed diet based on corn silage (41.4%), malt sprout (18.4%), pre-calving commercial ration (18.2%; based on grain–soybean meal) and moha (*Setaria italica*) hay (22%).

During the postpartum period, cows were assigned to experimental diets. The G0 cows were offered, in individual stalls (0.8 km from the milking parlor), PMR *ad libitum* (10% feed refusal) once a day in the morning. The PMR had a forage to concentrate ratio of 58/42 (DM basis) and was composed by corn silage (37.7%), alfalfa haylage (20.1%), sorghum grain (33.4%), corn grain (1.8%), wheat grain (1.2%), soybean expeller (3.6%), sunflower expeller (1.8%), and a salt, vitamin and mineral mix (0.4%) with a chemical composition of 403 g/kg of DM, 93 g/kg DM of crude protein (CP), 326 g/kg DM of neutral detergent fiber (NDF), 197 g/kg DM of acid detergent fiber (ADF), 40 g/kg DM of ether extract (EE), and 12.6 MJ/kg DM of ME. The PMR plus concentrate diet was formulated according to NRC (2001) for a milk production target of 30 kg/d. The G1 cows had direct access (1.1 km from the milking parlor) to a second-year alfalfa (*Medicago sativa*) pasture in one morning grazing session (6 h, 7:30 to 13:30 h) and received once a day, after the afternoon milking, 70% of the *ad libitum* PMR intake under the same conditions as G0 cows. Grazing was in 3 days rotational system with a mean herbage allowance of 20 kg DM/cow/d (4 cm above ground level) with 227 g/kg of DM, 160 g/kg DM of CP, 415 g/kg DM of NDF, 297 g/kg DM of ADF, 26 g/kg DM of EE, and 10.5 MJ/kg DM of ME. Herbage mass was determined monthly using the double sampling technique (Haydock and Shaw., 1975), and adjusted weekly using the records of the Rising Plate Meter (Mattiauda et al., 2013). The concentrate that consumed all cows (G0 and G1) in the milking parlor divided in the two milking shifts (4.0 kg DM/d) was composed (DM basis) by corn grain (20.3%), wheat grain (13.6%), soybean expeller (40.7%), sunflower expeller (20.3%), a salt, vitamin and mineral mix (5%), with 898 g/kg of DM, 196 g/kg DM of CP, 285 g/kg DM of NDF, 110 g/kg DM of ADF, 27 g/kg of DM of EE and 12.4 MJ/kg DM of ME.

The proportions of PMR, concentrate and pasture in the diet (DM basis) calculated for each treatment after DM intake of PMR and concentrate (based on difference between feed offered and refused) and pasture (based on NRC requirements) were determined (Ceriani et al., 2018), indicated that diets were composed 77% PMR and 23% concentrate for G0 cows and 54% PMR, 22% concentrate and 24% pasture for G1 cows (Table 1). Cows were milked twice a day (05:00 and 16:00 h), milk yield was recorded daily, and milk samples were collected once a week (composite of am and pm milking samples) from calving to +61 days postpartum to determine protein, fat and lactose. Cow BCS and BW were recorded every 14 days from -28 to +61 days relative to calving.

### 2.2. Urea dilution technique

At  $-7$  and  $+40 \pm 3$  days relative to calving, body composition was determined using the urea dilution technique (Kock and Preston, 1979). Briefly, cows were infused with 0.65 mL/kg BW of a diluted urea solution (20% urea in 0.9% physiological saline, wt/vol)

**Table 1**

Estimated nutrient composition of diets according to feeding strategy in early lactation.

Component <sup>b</sup>	Treatments <sup>a</sup>	
	G0	G1
Dry matter, g/kg of feed	517	488
Crude protein, g/kg DM	117	132
Neutral detergent fiber, g/kg DM	317	339
Acid detergent fiber, g/kg DM	177	202
Ether extract, g/kg DM	37	34
NIDN <sup>c</sup> , g/kg DM	24	27
Ash, g/kg DM	56	71
Metabolizable energy (MJ/kg DM) <sup>4</sup>	12.1	11.8
Net energy of lactation (MJ/ kg DM) <sup>4</sup>	8.0	7.7

<sup>a</sup> Feeding strategies from calving (day 0) to 61 days postpartum were control cows fed PMR *ad libitum* (G0, *n* = 9) or cows grazing alfalfa and supplemented with PMR offered at 70% of *ad libitum* intake (G1, *n* = 9).

<sup>b</sup> Nutrient composition calculated from PMR, concentrate and pasture dry matter intake estimated by Ceriani et al. (2018) and feed sample chemical analyses.

<sup>c</sup> NDIN = Neutral detergent insoluble nitrogen. <sup>4</sup>Metabolizable energy and net energy of lactation were estimated according to NRC (2001).

by jugular venipuncture over a period of 3 min through a polyethylene catheter (1.6 mm × 600 mm). Blood samples were collected of the coccygeal vein in heparin tubes (BD Vacutainer tubes; Becton Dickinson, NJ, USA) before and 12 min after the mean infusion time. Blood samples were centrifuged at 2000 × *g* for 15 min and plasma was frozen at -20 °C to determine plasma urea concentrations. Concentrations of plasma urea-N were determined by a colorimetric assay using a commercial kit (Laboratorios Wiener, Rosario, Argentina) on Vitalab Selectra 2 autoanalyser (Vital Scientific, Dieren, The Netherlands). The intra-assay coefficient of variation did not exceed 10%.

Urea space volume (kg) was calculated by dividing the amount of urea (mmol of urea) infused by the difference in concentrations of plasma urea-nitrogen between blood samples (before and after infusion; mmol urea-N/L) while the urea space (% BW) was calculated by dividing the urea space volume by BW. Urea space was used along with BW, BCS, and milk production to estimate relative body water, protein and fat by regression equations (Agnew et al., 2005).

### 2.3. Heat production

Heat production was measured using the O<sub>2</sub>P technique (Brosh, 2007) at 40 ± 3 days postpartum. This method is based on the measurement of heart rate (HR) and O<sub>2</sub> consumption individually in each cow. The HR was measured continuously for 4 days using a heart monitor (Polar RS400; Polar Electro Oy, Kempele, Finland) with a HR transmitter Polar WearLink® (Polar Electro Oy), and a data logger programmed to record HR at 1-min intervals. The devices were fasted by means of a specifically designed belt to the thorax behind the forelegs. To calculate the O<sub>2</sub>P (mL O<sub>2</sub>/BW<sup>0.75</sup> per beat), simultaneous short-term measurements (10 to 15 min) of HR at 10 s intervals and oxygen consumption (mL O<sub>2</sub>/kg BW<sup>0.75</sup> per h) were measured in each cow using an open respiratory mask circuit. Oxygen consumption measurements were performed 1 day before or 1 day after the HR measurement period between 06:00 and 11:00 h. Nitrogen recovery testing was performed to confirm the entire system calibration and was 101%.

The daily average HP and the HP along the day were quantified from the individual data of HR, the O<sub>2</sub>P and the constant of 20.47 kJ/L of O<sub>2</sub> consumed (Nicol and Young, 1990) according to the following equations (Brosh, 2007): Daily HP (MJ/cow per d) = specific HP (kJ/kg BW<sup>0.75</sup> per d) × BW<sup>0.75</sup> (kg)/1000; where specific HP (kJ/kg BW<sup>0.75</sup> per d) = HR (beat/min) × O<sub>2</sub>P (mL/beat per kg BW<sup>0.75</sup>) × (20.47 kJ/L O<sub>2</sub> consumption /1000 mL/L) × 60 min/h × 24 h/d.

### 2.4. Calculations and statistical analyses

Data from -14 to 14 days around body composition and HP measurements were used for energy balance calculations (from 26 to 54 ± 3 days postpartum). Milk energy output (**RE-milk**) was calculated from milk yield and its composition, using the coefficients of 38.8, 22.8, and 16.5 MJ/kg of fat, protein, and lactose, respectively. Retained energy in body reserves (**RE-tissue**) was estimated based on body composition estimated by urea dilution using the coefficients of 39.3 and 23.2 MJ/kg of fat and protein, respectively or based changes of BW and BCS (Fox et al., 1999). Total RE was calculated as the sum of RE-milk and RE-tissue. Metabolizable energy intake was estimated as the sum of HP + total RE and gross energy efficiency was calculated as RE-milk divided by ME intake and adjusted energy gross efficiency as total RE divided by ME intake. Predicted HP was calculated from BW and total RE using the coefficients of 0.33 MJ/kg BW<sup>0.75</sup>, 0.62 and 0.64 for maintenance requirement, km and kl, respectively (NRC 2001).

Data were analyzed using the SAS System program (SAS® University Edition, SAS Institute Inc., Cary, NC, USA). Univariate analyses were performed on all variables to identify outliers and inconsistencies and to verify normality of residuals. Milk yield change of BCS and BW, and energy balance components and efficiency were analyzed using the MIXED procedure with a mixed model that included nutritional treatment as a fixed effect and block as a random effect. Body composition data were analyzed as repeated measures using the MIXED procedure, the unrestricted covariance structure (UN) and the Kenward-Rogers procedure to adjust the denominator degrees of freedom. The model included day postpartum and nutritional treatment within day as fixed effects and block as a random effect.

The HR and HP data along the day were analyzed as repeated measures using the MIXED procedure with the first-order autoregressive (AR(1)) covariance structure the Kenward-Rogers procedure to adjust the denominator degrees of freedom. The model included nutritional treatment, hour and their interaction as fixed effects and block and cow as random effects. For all analyses calving date was used as a covariate if *P* < 0.20. Tukey-Kramer tests were conducted to analyze mean differences (*α* = 0.05). For all results, means were considered to differ when *P* ≤ 0.05, and trends were identified when 0.05 < *P* ≤ 0.10. Data are presented as least square means ± pooled standard errors.

## 3. Results

### 3.1. Changes in body composition during early lactation

Both cow groups lose empty BW from -7 to 40 days of lactation, however, at +40 days, empty BW was greater for G0 than G1 cows (Table 2). Absolute body water, fat and protein mass, as well as gross retained energy, estimated by the urea dilution technique, decreased (*P* ≤ 0.05) from pre to postpartum. However, the decrease in fat mass and gross retained energy was greater (*P* ≤ 0.04) for G1 than G0 cows. Relative water and protein mass remained unchanged from pre to postpartum and during the postpartum were not affected by the nutritional treatment. In contrast, relative lipid mass and gross energy content decreased (*P* ≤ 0.01) only in the G1 cows from -7 to +40 days. This determined that at +40 days, the relative fat mass and gross energy content were greater (*P* = 0.05) for G0 than G1 cows.

### 3.2. Energy partitioning during the second month of lactation

Average milk production from +26 to +54 days postpartum (second month of lactation) tended to be 1.8 kg greater (*P* = 0.09) for G0 than G1 cows, while percentages of milk fat, protein and lactose were not affected by nutritional treatments (Table 3). Cow BW did not differ between nutritional treatments while BCS was greater (*P* = 0.01) for G0 than G1 cows. The G0 cows secreted more energy in milk

**Table 2**

Changes in empty body weight (EBW), body condition score (BCS) and body composition from pre to postpartum of dairy cows assigned to different feeding strategies in early lactation.

Days relative to calving Treatment <sup>b</sup>	-7	+40			P-value <sup>a</sup>	
		G0	G1	SE	Days	T(Days)
EBW (kg)	486a	462ab	441b	9.8	<0.01	0.17
BCS (units)	3.3a	2.9b	2.6c	0.06	0.01	<0.01
<b>Absolute composition</b>						
Water, kg	247a	237ab	232b	4.9	0.05	0.60
Lipids, kg	75a	70a	62b	2.3	0.01	0.03
Protein, kg	70a	67ab	66b	1.6	0.05	0.61
Gross energy, MJ	4831a	4582a	4167b	124	0.01	0.04
<b>Relative composition</b>						
Water, g/kg EBW	510	511	527	6.2	0.19	0.11
Lipids, g/kg EBW	156a	151a	142b	2.4	<0.01	0.02
Protein, g/kg EBW	145	145	149	1.9	0.47	0.16
Gross energy, MJ/kg EBW	10.0a	9.8a	9.4b	0.09	0.01	0.02

<sup>a</sup> T(Days) = treatment within days.

<sup>b</sup> Feeding strategies from calving (day 0) to 60 days postpartum were control cows fed PMR *ad libitum* (G0, *n* = 9) or cows grazing alfalfa and supplemented with PMR offered at 70% of *ad libitum* intake (G1, *n* = 9). 2 EBW = empty body weight.

**Table 3**

Milk performance and energy partitioning of dairy cows assigned to different feeding strategies in early lactation.

Variable	Treatments <sup>a</sup>		SE	P-value
	G0	G1		
Milk yield (kg/d) <sup>b</sup>	28.1	26.3	0.50	0.09
<b>Milk composition (%)</b>				
Fat	4.09	4.2	0.1	0.40
Protein	3.1	3.2	0.05	0.60
Lactose	4.9	4.9	0.09	0.50
Body weight (kg)	506	494	14.0	0.60
Body condition score (units)	2.9	2.6	0.06	0.01
<b>Energy partitioning (MJ/d)<sup>b,c</sup></b>				
Metabolizable energy intake	182.2	179.7	4.8	0.70
Retained energy in milk	90.2	83.6	1.7	0.04
Retained energy in body tissue	2.8	0.9	0.5	0.02
Total retained energy	92.7	84.8	1.7	<0.01
Measured heat production	90.7	94.4	3.8	0.50
Predicted heat production	110.7	104.1	1.9	0.02
Residual heat production	-19.6	-7.5	3.9	0.06
Gross energy efficiency	0.49	0.47	0.01	0.30
Adjusted gross energy efficiency	0.51	0.47	0.01	0.08

<sup>a</sup> Feeding strategies from calving (day 0) to 60 days postpartum were control cows fed PMR *ad libitum* (G0, *n* = 9) or cows grazing alfalfa and supplemented with PMR offered at 70% of *ad libitum* intake (G1, *n* = 9).

<sup>b</sup> Data referred to the period +26 to +54 days postpartum.

<sup>c</sup> Metabolizable energy intake = Total RE + HP; Measured heat production = heart rate (beats/min) × O<sub>2</sub>P (L of O<sub>2</sub>/beat per kg BW<sup>0.75</sup>) × (20.47 kJ/1000) × 60 × 24; Predicted heat production calculated from metabolic body weight (BW<sup>0.75</sup>) and total retained energy and NRC (2001) efficiency coefficients for production and maintenance; Residual heat production = Measured heat production - Predicted heat production; Gross energy efficiency = NEL/ME intake; Adjusted gross energy efficiency = Total RE/ME intake.

(*P* = 0.04) and retained more energy in body tissue (*P* < 0.02) than did G1 cows. Thus, total RE was greater (*P* < 0.01) for G0 than G1 cows. Retained energy in body tissue at +40 days postpartum calculated based on BW and BCS and estimated by the urea dilution technique were high and positively correlated (*r* = 0.89, *P* = 0.002).

The whole-animal HP (MJ/d) at +40 days postpartum did not differ between nutritional treatments (Table 3). However, the predicted HP, which was calculated from BW<sup>0.75</sup> and total RE on the assumption of constant efficiency coefficients, was greater (*P* = 0.02) for G0 than G1 cows, determining that residual HP tended to be lower (*P* = 0.06) for the former than latter ones. The calculated ME intake, which was the

**Table 4**

Heart rate, oxygen pulse and energy partitioning values per metabolic body weight (BW<sup>0.75</sup>) of dairy cows assigned to different feeding strategies in early lactation.

Variable	Treatments <sup>a</sup>		SE	P-value
	G0	G1		
Heart rate (beat/min)	86.3	90.0	2.3	0.26
O <sub>2</sub> pulse (mL/beat per kg BW <sup>0.75</sup> )	0.336	0.340	0.015	0.88
<b>Energy partitioning (kJ/kgBW<sup>0.75</sup> per day)<sup>b,c</sup></b>				
Retained energy in milk	1720	1711	50.2	0.89
Retained energy in body tissue	826	802	20.0	0.40
Total retained energy	863	814	19.0	0.09
Measured heat production	858	896	40.6	0.51
Predicted heat production	1026	998	10.4	0.09
Residual heat production	-167	-74	33.7	0.07

<sup>a</sup> Feeding strategies from calving (day 0) to 60 days postpartum were control cows fed PMR *ad libitum* (G0, *n* = 9) or cows grazing alfalfa and supplemented with PMR offered at 70% of *ad libitum* intake (G1, *n* = 9).

<sup>b</sup> Data referred to the period +26 to +54 days postpartum.

<sup>c</sup> Metabolizable energy intake = Total RE + HP; Measured heat production = heart rate (beats/min) × O<sub>2</sub>P (L of O<sub>2</sub>/beat per kg BW<sup>0.75</sup>) × (20.47 kJ/1000) × 60 × 24; Predicted heat production calculated from metabolic body weight (BW<sup>0.75</sup>) and total retained energy and NRC (2001) efficiency coefficients for production and maintenance; Residual heat production = Measured heat production - Predicted heat production.

sum of HP and total RE was not different between G0 and G1 cows. Therefore, although gross energy efficiency did not differ between nutritional treatments, adjusted gross energy efficiency tended to be greater (*P* = 0.08) for G0 than G1 cows.

Nutritional treatments did not affect HR or O<sub>2</sub>P (Table 4). When expressed in terms of metabolic BW (kJ/kgBW<sup>0.75</sup> per day), RE-milk did not differ between nutritional treatments while total RE tended (*P* = 0.09) to be greater for G0 than G1 cows. Also, the HP measured per unit of metabolic weight was not different between nutritional treatments, while the predicted HP tended to be greater (*P* = 0.09) and the residual HP (*P* = 0.07) tended to be lower for G0 than G1 cows. Both, HR (beat/min) and HP (kJ/kg BW<sup>0.75</sup> per day) differed along the day (*P* < 0.01) and were affected by the interaction between nutritional treatment and hour (*P* < 0.04). The HR and HP reached minimum values early in the morning, increased throughout the day and decreased markedly after 19:30 h (evening). However, minimum values were reached earlier and maximum values later for G1 than G0 cows. In addition, HR was greater between 3:30 and 5:30 h and at 9:30 h for G1 than G0 cows (Fig. 1).

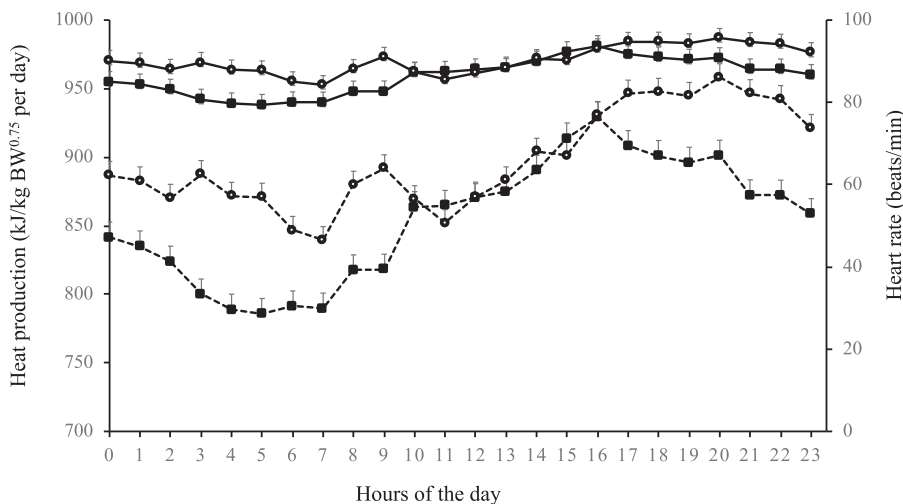
## 4. Discussion

### 4.1. Changes in body composition during early lactation

In agreement with the loss of BCS, body composition determined by means of the urea dilution technique, showed cows mobilized both, body protein and fat mass in early lactation; being mobilization of protein less extensive than fat (van der Drift et al., 2012). Mobilization of body protein mass was similar among treatments (5%; 0.072 kg/d) and was in line with previous reports in early lactation cows using directed slaughter measurements (0.044 kg/d; (Gibb et al., 1992) or mathematical calculations (0.050 to 0.123 kg/d; Chilliard et al., 1991; Tamminga et al., 1997). Moreover, in the present study, protein mobilization was in relation to empty BW loss as relative body protein mass did not change from pre to postpartum. Mobilization of protein reserves is regulated by hormonal changes (ie, reduced plasma insulin) and changed more drastically with limited energy intake than with changes in protein supply (Andrew et al., 1994; Komaragiri and Erdman, 1997; van der Drift et al., 2012).

In contrast, mobilization of body fat was 10% greater for G1 than G0 cows (7 and 18%, respectively) as G1 cows mobilized predominantly





**Fig. 1.** Diurnal pattern of heat production (kJ/kgBW<sup>0.75</sup> per day; dashed lines) and heart rate (beats/min; solid lines) of dairy cows fed PMR *ad libitum* (G0,  $n = 9$ ; black squares) or cows grazing alfalfa and supplemented with PMR offered at 70% of *ad libitum* intake (G1,  $n = 9$ ; open circles). The vertical bars above the symbols represent the standard error for the group by hour interaction.

body lipids as both, absolute and relative fat mass decreased from pre to postpartum. This was reflected in decreased both, body retained energy as well as energy content per kg of empty BW. The greater fat mobilization of G1 than G0 cows indicated a more severe negative energy balance of early lactation in the former ones. Indeed, previous research (Meikle et al., 2013; Astessiano et al., 2015) showed greater plasma concentrations of non-esterified fatty acids or  $\beta$ -hydroxybutyric acid in grazing than TMR-fed cows, indicative of fat mobilization in the former ones. Nevertheless, fat mobilization for both treatments in our study (0.107 and 0.277 kg/d for G0 and G1 cows from -7 to 40 days of lactation) was less than those previously reported (0.520 to 0.668 kg/d; Chilliard et al., 1991; Gibb et al., 1992; Andrew et al., 1994; Tamminga et al., 1997). Differences in parity may explain differences between previous studies performed in multiparous cows (Chilliard et al., 1991; Gibb et al., 1992; Andrew et al., 1994; Tamminga et al., 1997) and our results in primiparous cows as fat tissue is reduced both, in absolute and relative terms) in the latter ones (Belyea et al., 1978). In addition, the low percentage of protein in the diets in the present study (124 g/kg DM) may have limited milk yield and milk protein production (Law et al., 2009) reducing fat mobilization.

#### 4.2. Energy partitioning during the second month of lactation

The inclusion of pasture in the diet (G1 cows), by direct grazing, decreased milk yield and RE-milk in early lactation by almost 10% when compared to G0 cows. This was consistent with previous studies, that reported milk yield increased 5 to 20% when 100% TMR-fed cows were compared with grazing (30 to 60% of pasture inclusion) cows supplemented with TMR (Bargo et al., 2002; Vibart et al., 2008; Meikle et al., 2013; Fajardo et al., 2015). In addition, although changes in milk composition were variable, similar increases (5 to 19%) were observed in RE-milk when TMR-fed were compared to PMR-supplemented grazing cows (Bargo et al., 2002; Vibart et al., 2008; Meikle et al., 2013; Fajardo et al., 2015). In contrast, Kennedy et al. (2005) did not report differences in daily milk yield or DM intake between spring-calving cows grazing high quality pastures (82% of pasture in the diet) when compared to cows fed TMR. Nevertheless, in the latter study both, RE-milk (+26%) and cow BW were greater for TMR than grazing cows.

Although we did not find differences in cow BW between nutritional treatments, in agreement with previous authors (Meikle et al., 2013; Fajardo et al., 2015) average BCS was 0.3 units greater for G0 than G1 cows. Washburn et al. (2002) reported BCS between 0.3 and 0.6 units lower for cows on pasture-based than on TMR-based feeding systems throughout lactation. Similarly, Kolver and Muller (1998) in a short-term study (4 wk) reported that high-producing dairy cows, that consumed only pasture, lose 0.5 units of BCS more when compared to cows

that consumed a TMR-based diet which maintained BCS. Thus, in the present study the greater total RE for G0 than G1 cows indicate a better energy balance which impacted also in an improved reproductive performance (Astessiano et al., 2018).

However, cow ME intake (total RE + HP) was not affected by nutritional treatments as neither DM intake (Ceriani et al., 2018) nor dietary ME concentration differed between cow groups. In agreement with the high correlation ( $r = 0.87$ ) between ME intake and HP (Brosh, 2007), HP did not differ between G0 and G1 cows as both, HR and O<sub>2</sub>P were similar between groups. Previous studies in dairy cows fed TMR or pasture, using the O<sub>2</sub>P (Aharoni et al., 2005, 2006; Miron et al., 2008) or other techniques (Dohme-Meier et al., 2014; Dong et al., 2015a, 2015b) to measure HP, reported values of HP, expressed as MJ/cow per day or MJ/kg BW<sup>0.75</sup>, 30% greater than those estimated in this study. However, our HP estimations were in line with the ones predicted by NRC (2001) as well as the ones reported for low-producing cows (Aharoni et al., 2006) or for cows fed high-forage diets (Dong et al., 2015b). In the present work, measured HR were in the range of the HR reported in dairy cows in previous studies that used the O<sub>2</sub>P technique (Aharoni et al., 2005, 2006; Miron et al., 2008). However, O<sub>2</sub>P values were less than the expected ones and were closer to the O<sub>2</sub>P reported in beef cows (Brosh, 2007). Decreased O<sub>2</sub>P values would indicate a lower metabolic rate in our cows which would probably reflect, among other factors, differences in parity (primiparous vs. multiparous), milk production (~10 kg/d lower in the present study), feed intake and diet quality between this and previous studies.

Total HP is the sum of HP for maintenance (HP<sub>m</sub>) and HP for production (HP<sub>p</sub>) (Miron et al., 2008). As mentioned above, total HP did not differ between treatments but total RE was 8% greater for G0 than G1 cows. Thus, it could be expected that HP<sub>p</sub> increased while HP<sub>m</sub> decreased for G0 than G1 cows. The greater adjusted gross energy efficiency estimated for G0 than G1 cows would indicate that the latter ones would require 0.16 additional units of energy intake to retain the same amount of energy in milk and tissue. Although, daily RE-milk was greater for G0 than G1 cows, we did not detect differences between treatments when milk energy output was expressed relative to their BW<sup>0.75</sup>. However, RE-tissue relative to BW<sup>0.75</sup> was 2-fold greater in G0 than G1 cows, which would indicate that the latter cows would partition a greater portion of the ME intake to maintenance and a lower portion was directed to maintain or replenish body reserves as milk production would be prioritized in early lactation cows.

Differences between cow groups in partition of consumed ME between maintenance and production were analyzed with residual HP calculation, which was based on the differences between measured HP and predicted HP from BW and total RE using the NRC (2001) coefficients for maintenance, km and kl, without considering energy

requirement for activity (walking + grazing). In the present study, residual HP was negative for both groups of cows probably as HP was measure in early lactation, when feed intake is restricted and gastrointestinal viscera and liver are still growing in mass and activity (Baldwin et al., 2004) determining reduced maintenance requirements. Indeed, Aharoni et al. (2006) reported that maintenance energy requirement varied along lactation being minimum in early lactation and Ellis et al. (2006) reported an increase of 20% in maintenance requirements from the early to mid-lactation. Nevertheless, residual HP difference between G0 and G1 cows were about 10% of the estimated ME intake. These results could be related to grazing and walking activity as well as percentage of forage in the diet (45 vs. 55% for G0 and G1, respectively). Indeed, previous research using the  $^{13}\text{C}$  and  $^{14}\text{C}$  dilution techniques in dairy cows (Dohme-Meier et al., 2014) and steers (Di Marco and Aello, 2001) grazing cultivated pastures reported that energy expenditure due to grazing and walking increased between 8 and 30% above maintenance requirement depending on, among other factors, forage mass and quality, bite rate and topography. In addition, Dong et al. (2015a, b) summarizing information from 32 experiments (more than 900 cows) in calorimetric chambers, determined that the maintenance requirement varied with the proportion of forage in the diet, it was 10% more when the diets included more than 60% of forage in comparison with those with less than 30%.

Although we observed that the HP per unit of  $\text{BW}^{0.75}$  did not differ between treatments, it did vary along the day, associated with the daily routine of each treatment group. Previous authors (Brosh et al., 1998; Aharoni et al., 2005; Brosh et al., 2006) reported that the daily patterns of HP of confined TMR-fed cows depend mainly of the time of feed supply and consumption while for grazing cows this pattern would also depend on other activities. Indeed, the daytime pattern of HP in the present study showed that minimum values of HP were reached just before the morning milking and it increased immediately after they started to consume first the concentrate in the milking parlor and later pasture or mixed ration. However, this rise was earlier in G1 than not G0 cows as the former ones access the pasture and started to graze as soon as they arrive to the paddock which occurred before PMR was offered to the latter ones (Ceriani et al., 2018). After the first grazing session, HP decreased for G1 cows to increase again parallel to HP of G0 cows, reflecting mainly DM intake and nutrient metabolism. However, although after the afternoon milking, when both cow groups were in confinement, HP continued to increase but it did it until later for G1 than G0 cows. This was probably associated with the PMR intake in G1 cows as it was the moment of the day they had access to it. The elevated HP in late afternoon - early night hours in G1 cows would suggest that they consumed a high proportion of the PMR offered (70% of *ad-libitum* mixed ration intake) during this short period of time (16 to 20 h).

## 5. Conclusion

Although the inclusion of pasture in the early-lactation fall-calving cow diet could have a positive effect on farm profitability (Kennedy et al., 2015), in the present study, pasture grazing (30% of the diet) in primiparous cows decreased energy secreted in milk and retained in tissue, by increasing body fat mobilization, when compared to mixed ration fed cows. These results indicated a decreased energy balance of cows with pasture grazing in the diet which was not associated with a reduced ME intake as it did not differ between nutritional treatments, but with about a 10% increase in energy maintenance requirements, probably associated with greater dietary forage content and/or grazing and walking activities.

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## Conflict of interest

None of the authors have any conflict of interest to declare.

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