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Intake, Energy Expenditure and Methane Emissions of Grazing Dairy Cows at Two Pre-Grazing Herbage Masses

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Abstract

A grazing experiment was undertaken to assess the effects of two levels of herbage mass (HM) on herbage DM intake (DMI), fat and protein corrected milk yield (FPCM), grazing behaviour, energy expenditure (HP), and methane emissions (CH_4) of grazing dairy cows in spring. Treatments were a low HM (1447 kg DM/ha; LHM) or a high HM (1859 kg DM/ha; HHM). Pasture was composed mainly of cocksfoot (*Dactylis glomerata*) and lucerne (*Medicago sativa*), offered at a daily herbage allowance of 30 kg DM/cow, above 5 cm. Eight multiparous Holstein cows were used in a 2 × 2 Latin Square design in two 10-day periods. Despite the differences in pre-grazing HM between treatments, OM digestibility was not different (P = 0.28). Herbage mass did not affect DMI or FPCM. Grazing time was not different between treatments, but cows had a greater bite rate when grazing on LHM swards. However, HP did not differ between treatments. Daily methane emission (per cow), methane emission intensity (per kg FPCM) and methane yield (as percentage of gross energy intake) were not different. The lack of effect of the amount of pre-grazing HM on energy intake, confirms that the difference between HM treatments was beyond the limits that impose extra energy expenditure during grazing.

Keywords

Dairy Cow, Herbage Mass, Intake, Grazing Behavior, Methane

1. Introduction

Grazing systems remain a major source of greenhouse gases (GHG) to the livestock sector. However, these systems stand as the most cost-efficient source of nutrients for dairy cows, and they also provide additional benefits for animal welfare [1]. The promotion of sustainable grazing practices is among the interventions that might reduce GHG emissions per unit of product (carbon footprint) without sacrificing the associated benefits of grazing systems [2]. It is well known the effect of greater nutrient quality and digestibility of forages is positively correlated with reduced methane (CH_4) emissions. Pasture characteristics that determine forage quality, such as botanical composition [3] [4] [5], regrowth period of pasture [6] [7] [8] and season [9], affect herbage digestibility, daily intake, milk production and enteric CH4 emissions. However, the effects of pre-grazing herbage mass (HM) on the dairy industry's carbon footprint have not been studied. Reduced herbage mass and/or sward height, are important extrinsic factors limiting herbage intake on dairy cows, because of their effect on the ease of prehension of the herbage [10] [11]. The difficulty to access the most easily harvestable material forces the cow to graze the deeper strata of the swards. This, demands a greater strength for prehension and cutting, leading to smaller bite mass, resulting in a decrease of the herbage instantaneous intake. Cattle will try to compensate this reduction in bite mass by increasing biting rate and grazing time [12] [13] [14]. Higher grazing activity increases energy requirement for maintenance and could reduce efficiency of production of dairy cows in grazing systems [15]. On a low height ryegrass sward, [16] registered 59 bites per minute, which approaches the upper biting rate limit reported for grazing cattle [17]. In these conditions, the increment in energy expenditure, regarding the same animals at rest, was 52%, during the grazing period [16].

Although production efficiency is essential to maintain profitability and reduce the environmental impacts of grazing dairy production system, information on the effect of herbage mass on energy expenditure and methane emission per litter of milk produced is limited for our country. The aim of the present study was to quantify the effect of the level of pre-grazing herbage mass at the same daily herbage allowance, on herbage intake, grazing behaviour, milk production, energy expenditure, and daily methane emission of grazing dairy cows.

2. Materials and Methods

2.1. Experimental Treatments and Design

The experiment was carried out in "Bernardo Rosengurtt" research station, Universidad de la República, Uruguay (32˚22'S, 54˚26'W), in mid-spring (October-November 2015). The effect of the pre-grazing herbage mass at the same herbage allowance on the performance of grazing dairy cows was examined in a repeated 2 × 2 Latin Square design. Animal procedures were approved by the Honorary Commission on Animal Experimentation of Universidad de la República (Exp. # 021130-001143-14). Treatments consisted of two target levels of herbage mass: 1000 kg of DM/ha above 5 cm (low herbage mass, LHM) and 1500 kg of DM/ha above 5 cm (high herbage mass, HHM). Eight multiparous Holstein Friesian dairy cows with an initial mean body mass of 537 ± 12.9 kg were used in this study. The cows were first blocked by pre-experimental milk yield (21.1 ± 0.5 kg/d) into two groups. Within each group, cows were paired based on their calving dates (190 ± 12.4 DIM) and within pairs randomly allocated to one of both treatments. Cows on both treatments were offered the same total herbage allowance (30 kg DM/cow/d, 5 cm above ground level). Similar herbage allowances per animal were obtained by adjusting the grazing area of each treatment according to their pre-grazing herbage mass. Each grazing period lasted 10 days, 5 days of adaptation, followed by 5 days of animal data collection. Period length was minimized in order to prevent large changes in the herbage conditions within and between periods. For this study it was more important that the chemical composition did not change so as not to confuse possible differences in consumption related to pre-grazing herbage mass (and more specifically its canopy structure), without differences in quality.

2.2. Pasture and Grazing Management

The study was conducted during the Spring (from October 27 to November 15, 2015) on a total area of 8 ha, 1 km away from the milking parlor. The forage mixture used was composed of 85% cocksfoot (*Dactylis glomerata*) and 15% lucerne (*Medicago sativa*). The area had been sown three years earlier and was subdivided into four equal paddocks. Two paddocks, corresponding to the HHM treatment were cut 35 days (5 cm height above ground level) prior to the beginning of Period 1. An additional cut on the other two paddocks was done 15 days before Period 1, in order to reach the target herbage mass for the LHM treatment. The same procedure was followed on the other two paddocks to prepare the grazing area for Period 2.

Before the experimental periods, the cows grazed a non-experimental pasture (composed of the same grass/legume mixture) as a single herd for two weeks, to allow previous adaptation to a diet based only on pastures. During the experiment, both swards were strip-grazed, at the same herbage allowance (30 kg DM/cow/d, 5 cm above ground level). After morning milking, the cows were offered a fresh strip once a day, using temporary electric fences. The area of each strip was calculated based on the pre-grazing herbage mass and the established daily allowance.

2.3. Pasture Measurements

Pre-grazing herbage mass and mean sward height were measured before each plot was grazed (days 1, 5, 7 and 9). Herbage mass was measured by harvesting at random, three diagonal strips (10 m × 0.5 m), using a motor scythe at a cutting height of 5 cm above ground level. Herbage samples were weighed fresh and a bulked sample of 500 g was dried in an oven, at 60˚C for dry matter determination and chemical analysis. The extended tiller height of 50 tillers taken randomly on the same area used for the determination of pre-grazing herbage mass, were measured to estimate the mean sward height (measured from ground level to the uppermost extended point). The same procedure was followed for the determination of the post-grazing herbage mass and sward height after grazing (days 2, 6, 8 and 10). The differences between the pre-grazing and post-grazing height values were used to calculate the mean depth of defoliation for each treatment.

The botanical composition of the pasture was determined three times per treatment and per period, (days 5, 7 and 9). Next to each pre-grazing herbage strip cut for the herbage mass determination, three handfuls of herbage were randomly selected and cut with scissors at ground level. Samples were bulked and arranged in plastic bags in order to keep the herbage structure unaltered and stored at −20˚C. For botanical analysis, a subsample with its original structure still preserved, was used to determine the proportion of grass and legume (on DM basis) in the laboratory. A second subsample was cut at the height corresponding to the mean post-grazing sward height and the upper portion, considered as representative of the defoliated herbage, was dried before chemical analysis.

2.4. Animal Measurements

Cows were milked twice daily at 08:00 and 18:30 h, individual milk yields were recorded automatically at each milking. Milk composition (fat, protein, lactose) was determined four times per period from samples taken at morning and evening milking, from days 6 to 9. The milk yield and composition reported correspond to the CH_4 measurement (days 6 to 10). Fat Protein Corrected Milk (FPCM) yield was calculated using the equation proposed by [18]. Cows were weighed on the last day of each period (day 10).

Individual herbage OM intake was determined using chromic oxide (Cr_2O_3) to estimate faecal organic matter (OMf) output, and nitrogen (Nf) and acid detergent fiber (ADFf) contents in the faeces (g/kg OM) to estimate OM digestibility (OMd) of ingested herbage, according to the equation established by [19] for herbage-based diets without supplements. However, chromic oxide was offered mixed in concentrate pellets (ca. 20 g Cr_2O_3 per day, in portions of 200 g at each milking). Therefore, for forage intake calculations, OMf output from herbage was estimated by subtracting the indigestible OM attributable to the supplied concentrate containing the chromic oxide (92 g/kg OM) from the total measured faecal OM output. The supply of concentrate pellets containing chromic oxide started on day 1 of the experimental period in order to achieve a ruminal steady state. On days 6 to 10 of each experimental period, faeces were rectal-sampled after morning milking and after evening milking and oven dried at 60˚C during 72 h in order to measure the DM content, the Cr_2O_3 concentration, and the chemical composition.

On day 9, grazing and ruminating time, and biting rate were determined at the grazing session between milking (9:00 to 18:00). The cows were observed in the plot, recording every 5 min the behavior of each animal (ingestion, rumination, rest) and counting (by chronometer) the number of bites per minute during the periods of ingestion as described by [20]. Time spent per activity (min) was calculated assuming that the activity recorded was maintained during the 5 min until the next observation.

Energy expenditure as heat production (HP), was estimated using the heart rate and O_2 pulse method reported by [21]. The heart rate (HR) was recorded by a heart rate Radio transmitter (Polar Electro Oy, Kempele, Finland), fitted to the thorax of each animal, behind the forelegs by specifically designed belts, for 4 days in each experimental period (days 6 to 9). O_2 consumption was measured using an open respiratory system in day 2 of each period, as described by [21] and each cow's O_2 pulse was calculated as the O_2 consumption per heart beat. Then the HP throughout the day was calculated by multiplying the HR (beats/min) as the mean value of measures throughout the day from days 6 to 9 in each period by the measured O_2 pulse (L/beat) and by the constant value of 20.47 kJ/L O_2 consumed [22], according to the following equation:

$$\text{Daily HP (MJ/cow/day)} = \text{HR} \times \text{O}_2\text{P} \times 20.47/1000 \times 60 \times 24 \quad (1)$$

where: HR is heart rate (beats/min); O_2P is mL of O_2 per beat and 20.47 is the energy consumed by O_2P (kJ/L O_2).

The enteric CH_4 emission was measured using the sulfur hexafluoride (SF_6) tracer gas technique reported by [23] and adapted by [24], for 5-day collection period. Methane sampling equipment and procedures were as reported by [5]. Seven days before the beginning of the experiment, a SF_6 permeation tube was introduced *per os* into the rumen of each animal. The permeation rate of the tubes containing the SF_6 gas used in this study was 8.5 ± 0.32 mg/d. The breath gas sampling system consisted of two 0.5 L stainless steel collecting vessels per cow, with a ball-bearing inflow restrictor adjusted to accumulate 0.5 bar of air sample during a 5-day period and a short tube used to connect both. Both inflow restrictors were placed above the animal's nostrils and protected against water and dust. The two collecting vessels were fitted to each animal's head by means of especially designed halters. This way, it is possible to obtain two repetitions of CH_4 emission per cow and per period. Immediately prior to the sampling period, each collecting vessel was evacuated (<0.5 mb) after cleaning with high purity nitrogen gas (N_2). The breath gas samples were measured over five days in each period (days 6 to 10). Additionally, an identical set as used with the cows was used to collect background air samples during each 5-day period. The breath gas samples collected were analyzed immediately after the end of the experimental period. Daily CH_4 emissions were calculated from SF_6 release rate and the ratio between CH_4 and SF_6 concentrations in breath samples, after correction for background gas concentrations, according to the equation:

$$\text{CH}_4\ (\text{g/d}) = \text{PRSF}_6 \times [\text{CH}_4]/[\text{SF}_6] \quad (2)$$

where: $PRSF_6$ is the SF_6 permeation rate from the permeation tube and $[CH_4]$ and $[SF_6]$ are the concentrations of these gases (ppm and ppt, respectively) above atmospheric concentration.

2.5. Chemical Analysis

All chemical analyses were conducted at the Animal Nutrition Laboratory (Faculty of Agronomy). All the dried samples were ground through a 1 mm screen before chemical analysis. The dry matter (DM) concentration was determined by drying at 105˚C in an oven for 24 h and ash content was determined by incineration at 600˚C for 4 h for organic matter (OM) calculation.

The total nitrogen was assayed using the Kjeldahl method (Method 984.13; AOAC 2000) and expressed as crude protein (CP, nitrogen × 6.25). Content of neutral detergent fiber (NDFom) and acid detergent fiber (ADFom) were determined as described by [25], except that the samples were weighted into filter bags and treated with neutral detergent solution that included heat-stable amylase, in ANKOM equipment (ANKOM Technology, Macedon NY, USA), and expressed as ash-free residues. Gross Energy (GE) was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, Leics, UK).

Milk samples at every milking of each collection period were analyzed for fat, protein, and lactose content with infrared spectroscopy (Milkoscan 203, Foss Electric, Hillerød, Denmark). Chromium (Cr) concentration in faecal samples was determined by atomic absorption spectrophometry (Perkin-Elmer 2380, Norwald, CT, USA), using air and an acetylene flame according to [26]. Cr standards were prepared using pre-trial faecal collections that contained no Cr.

The concentrations of CH_4 and SF_6 were determined by gas chromatography on an AGILENT 7890 chromatograph. The samples were injected at once in two different setups. For CH_4, a 3 mL loop, a HP-PLOT Q column and an FID detector were used. For SF_6, a 10 mL loop, a HP-MOLSIV column, and an ECD detector were used. Each sample was analyzed at least twice, and the average values were used to obtain CH_4 concentration and CH_4 emission. Maximum delay between the collection and the determination of CH_4 and SF_6 concentrations was 15 days.

2.6. Statistical Analysis

Sward characteristics and herbage chemical composition were analyzed with ANOVA including the fixed effects of treatment and period:

$$Y = \mu + T_i + P_j + \varepsilon_{ij} \tag{3}$$

where μ was the overall mean, T_i is the fixed effect of treatment ($i = 1$ to 2), P_j is the fixed effect of period ($j = 1$ to 2) and ε_{ij} is the associated error.

Herbage intake, live weight, energy expenditure and CH_4 emission were analyzed with a mixed model including the fixed effects of treatment and period, and the random effect of the cow:

$$Y = \mu + T_i + P_j + A_k + \varepsilon_{ijk} \quad (4)$$

where μ was the overall mean, T_i is the fixed effect of treatment (i = 1 to 2), P_j is the fixed effect of period (j = 1 to 2), A_k is the random effect of animal (k = 1 to 8) and ε_{ijk} is the associated error.

Milk yield and composition were analyzed as repeated measures over time, according to an autoregressive model of order one (AR 1) reported by [27]. The milk yield and composition reported correspond to the CH_4 measurement period.

The interaction treatment × period was initially included, but as interaction was not significant, it was excluded in the final models, following the recommendations of [28]. Significance was declared at $P < 0.05$ and tendencies at $0.05 \le P \le 0.10$. All statistical analyses were performed using SAS program (SAS Institute Inc., Cary, NC). Data are presented as least square means ± pooled standard errors.

3. Results

According to INUMET (National Institute of Meteorology), total rainfall over all the experimental period was similar to the 10-year average for the region (77 vs 79 mm), while daily mean temperature (19˚C) was 1˚C lower than the 10-year average (2005-2014). However, Period 2 (6-11-2015 to 15-11-2015) was characterized by a higher daily mean temperature (+2˚C) and higher precipitation (+21 mm) compared to Period 1 (27-10-2015 to 05-11-2015).

3.1. Sward Characteristics and Defoliation

Pre-grazing herbage mass above 5 cm was higher for HHM (+412 kg DM/ha; P = 0.050), as well as the pre-grazing sward height (+11 cm; P = 0.037). However, the botanical composition of the pasture, expressed as the proportion of grass (89% of the herbage biomass on average) and its chemical composition above 5 cm, did not show significant differences between treatments (**Table 1**).

Post-grazing sward height was lower in LHM (−8 cm; P = 0.002). However, the depth of defoliation did not differ between treatments (20.5 cm on average), representing 44% and 54% of the initial height for the HHM and LHM treatment, respectively. The chemical composition of defoliated herbage did not differ between treatments (**Table 2**).

3.2. Herbage Intake

Both faecal output (4.4 kg OM/d on average) and digestibility of the defoliated herbage (740 g/kg OM on average) did not show significant differences between treatments. As a consequence, daily OM intake and DM intake did not differ between treatments (16.7 kg OM and 18.3 kg DM/d on average) (**Table 3**).

3.3. Grazing Behavior

No differences were found between treatments for grazing time (324 min, on

Table 1. Pre-grazing pasture characterization of high herbage mass (HHM) and low herbage mass (LHM) swards.

	Herbage mass		SEM	P-value
	HHM	LHM		
Herbage mass above 5 cm (kg DM/ha)	1859	1447	160	0.050
Height (cm)†	48	37	3.9	0.037
Grass/Legume ratio‡	86	91	2.5	0.119
Chemical composition# (g/kg DM)				
DM (g/kg)	272	279	10.5	0.605
OM	909	905	5.2	0.552
CP	137	142	2.4	0.263
aNDFom	588	566	9.8	0.266
ADFom	321	294	3.3	0.251

†measured as extended tiller height; ‡expressed as percentage of grasses; #above 5 cm.

Table 2. Depth of defoliation and chemical composition of the herbage defoliated by dairy cows grazing high herbage mass (HHM) and low herbage mass (LHM) treatments.

	Herbage mass		SEM	P-value
	HHM	LHM		
Post-grazing height (cm)†	27	19	1.2	0.002
Depth of defoliation (cm)	21	20	3.0	0.776
Chemical composition (g/kg DM)				
DM (g/kg)	242	237	9.8	0.680
OM	903	913	1.6	0.728
CP	137	147	3.5	0.349
aNDFom	577	599	3.9	0.153
ADFom	271	280	0.4	0.033
GE (MJ/kg DM)	16.6	16.1	0.57	0.644

†measured as extended tiller height; ‡expressed as percentage of grasses; #above 5 cm.

Table 3. Effect of herbage mass on faecal output, herbage OM digestibility and daily intake by dairy cows grazing high herbage mass (HHM) and low herbage mass (LHM) treatments.

	Herbage mass		SEM	P-value
	HHM	LHM		
Faecal output (kg OM/d)	4.6	4.2	0.28	0.389
Herbage OM digestibility (g/kg OM)	737	743	5.5	0.281
Herbage OM intake (kg/cow/d)	17.0	16.4	0.63	0.510
Herbage DM intake (kg/cow/d)	18.6	18.0	0.69	0.510
Digestible OM intake (kg/cow/d)	12.5	12.2	0.46	0.584

average) during the daytime period observation (9:00 to 18:00). However, ruminating time was higher for animals in HHM (+35 min; P = 0.047), while resting time tended to be longer in LHM (28 min; P = 0.093). The biting rate was higher for the LHM treatment (+8 bites/min, P = 0.030) (Table 4).

3.4. Milk Production and Milk Composition

The analysis showed no significant effect of pre-grazing HM on milk production. Individual milk yield was 21.1 kg FPCM/d, on average (Table 5). However, significant differences were found in milk composition as fat content (+1.8 g/kg, P = 0.005) and protein content (+0.7 g/kg, P = 0.006) being higher in LHM (Table 5). Regarding the live weight variation, there were no significant differences between treatments, animals gained weight in both treatments (+0.60 kg/d on average).

3.5. Energy Expenditure

In this experiment, there were difficulties in measuring the volume of O_2 in P1. However, in P2 the measurements of the O_2 volume were within the expected values for this type of animal. According to [29], for animals that are not subject

Table 4. Grazing behavior of dairy cows on high herbage mass (HHM) and low herbage mass (LHM) treatments during the observation period, from 9:00 to 18:00 h.

	Herbage mass		SEM	P-value
	HHM	LHM		
Grazing time (min)	321	327	13.7	0.639
Ruminating time (min)	133	98	15.6	0.047
Resting time (min)	47	75	10.6	0.093
Biting rate (N˚/min)	36	42	9.5	0.030

Table 5. Milk yield and milk composition by grazing dairy cows on high herbage mass (HHM) and low herbage mass (LHM) treatments.

	Herbage mass		SEM	P-value
	HHM	LHM		
Milk yield (kg/cow/d)	21.3	20.9	0.48	0.347
Fat content (g/kg)	38.6	40.4	0.64	0.005
Protein content (g/kg)	30.8	31.5	0.79	0.006
Lactose content (g/kg)	47.7	47.9	0.33	0.553
Fat yield (g/d)	821	839	21.5	0.387
Protein yield (g/d)	656	662	15.7	0.687
Lactose yield (g/d)	019	1000	23.7	0.417
Fat and protein corrected milk (kg/cow/d)	20.6	20.7	0.47	0.753
Live weight variation (kg/cow/d)	0.55	0.65	0.422	0.858

to a high heat load or intensive exercise, there is a small variation in O_2P during the day. Hence, measuring the O_2P of an individual animal only once daily on short periods of time (days) could bias the individual energy expenditure (HP) calculations below 5%. Considering that the duration of the experiment was only 20 days, HP was estimated by multiplying the HR measured in each period by the oxygen pulse (O_2P) determined at the beginning of P2 (0.382 mL O_2/MW/beat). The heart rate (HR) did not differ between treatments (87.0 beats/min on average), and neither did HP (979 kJ/kg MW on average) (**Table 6**).

Differences between daily HR records were not significant between treatments in the daytime period, with an increase in the heart rate during the sessions of grazing, from values of 77 beats/min at 5:00 to values of 88 (HHM) and 95 (LHM) beats/min at 18:00 (**Figure 1**).

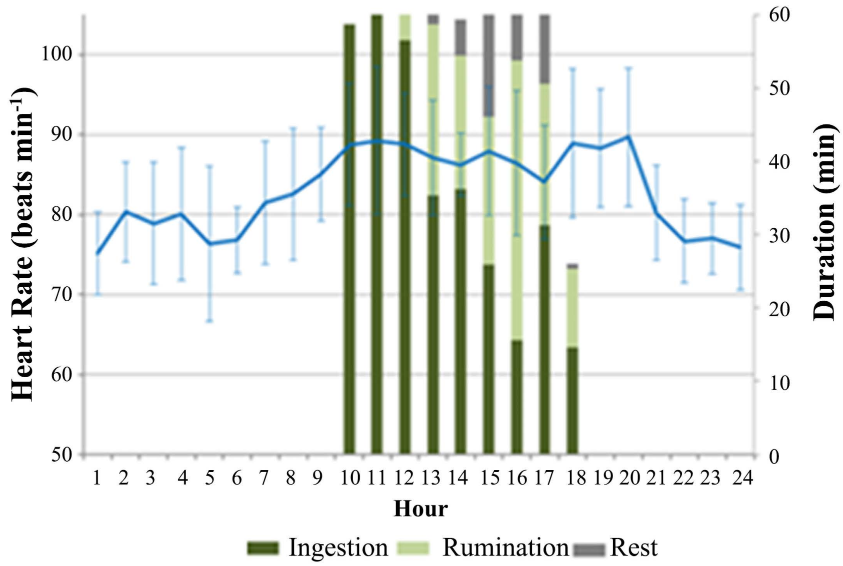
3.6. Methane Emission

Pre-grazing herbage mass did not affect enteric CH_4 emission, and it was 363 g/d (equivalent to 551 L per day), on average. Neither emissions expressed per unit of product (18.3 g kg FPCM/d, on average), per unit of DMI (21.0 g/kg DM on average) or as a percentage of GE intake (Y_m = 6.7% on average) were different (**Table 7**).

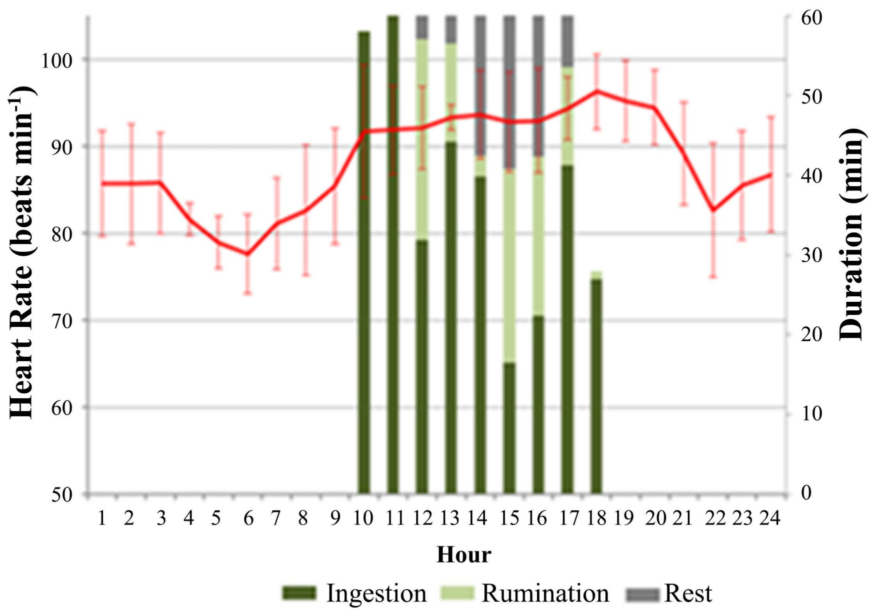
Table 6. Heart rate (HR) and energy expenditure (HP) of cows grazing high herbage mass (HHM) and low herbage mass (LHM) treatments.

	Herbage mass		SEM	P-value
	HHM	LHM		
HR (beats/min)	86.9	87.0	1.09	0.963
Heat Production (kJ/kg MW)	978	981	49.6	0.958

MW = metabolic weight (117.8 kg for HHM and 118.2 kg for LHM, P = 0.699).



(a)



(b)

Figure 1. Evolution of heart rate and the ingestive behavior during the observation period (9:00 to 18:00) for (a) HHM sward and (b) LHM sward.

Table 7. Daily methane emission and methane yield by grazing dairy cows on high herbage mass (HHM) and low herbage mass (LHM) treatments.

	Herbage mass		SEM	P-value
	HHM	LHM		
Methane emission (kg/cow/d)	374	353	34.9	0.685
Methane emission (g/kg FPCM/d)	19.1	17.6	2.52	0.580
Methane yield				
as Gross Energy intake (Y_m)	6.6	6.8	0.87	0.788
as g methane/kg DMI	20.5	21.5	2.73	0.712

4. Discussion

The aim of this study was to investigate if increasing pre-grazing herbage mass without changes in nutritive value during late spring offered at the same herbage allowance could reduce enteric methane emission intensity (g CH_4/kg FPCM) by lowering energy expenditure of grazing dairy cows.

4.1. Sward Characteristics

The pasture management imposed had an effect on the level of pre-grazing herbage mass and on the sward height between HHM and LHM treatments, although the difference was smaller than initially planned. This occurred mainly as a result of the weather conditions (temperature and precipitation) registered during Period 2, that improved herbage growth, principally on LHM sward. It is worth mentioning that the botanical and chemical compositions did not differ between treatments. Cocksfoot, the main species of both swards, blooms relatively late in the season, and may allow high digestibility of the herbage to be maintained in spite of the advance of the Spring [30]. Studies assessing the effect of the available HM, while maintaining the same chemical composition of the forage are scarce [10]. Several authors report variations in DMI, milk production and methane yield between treatments, but the pastures also differed in quality [7] [8]. However, one might expect that the chemical composition of the forage consumed by the cows, would be different from that of the offered forage, since the post-grazing height resulted substantially higher (23 cm on average) than the cutting height of the motor scythe (5 cm on average). In the present study, a herbage subsample, cut at the height corresponding to the mean post-grazing sward height and the upper portion for each sward, was taken as a representative sample of the defoliated forage for quality evaluation. From this analysis, it was possible to estimate that the chemical composition of this fraction did not differ between treatments, and the OM digestibility was high (74% on average). Regarding this, it should be noted that this experiment was carried out with pastures in vegetative stage before initiation of stem elongation and lignification and, consequently, low and high herbage mass were of similar quality with no differences in OM digestibility.

4.2. Herbage Intake and Grazing Behavior

The level of pre-grazing herbage mass (1859 vs. 1447 kg DM/ha, $P = 0.050$) offered at the same daily herbage allowance, had no effect either on the amount of forage ingested by the cows (18.3 kg DM/d on average) or on the milk yield (20.6 kg FPCM/d on average). The high herbage intake is in agreement with that reported by several authors [10] [31] [32], stating that herbage intake was not affected by herbage mass when measured above 5 cm. These authors show that forage intake reaches a maximum when pasture herbage allowance is 25 - 30 kg DM/cow/d above 5 cm or 60 kg DM/cow/d at ground level (similar values to those of pasture allowance expressed at ground level documented in this experiment, not shown here). The aforementioned literature show responses that tend to be asymptotic above these forage allowances, reaching a maximum pasture intake and milk production of approximately 18 kg DM/d and 21 kg/d respectively, similar to the results reported here. Nevertheless, milk composition differed among treatments, which might be most likely related to a milk dilution effect, as differences in milk fat yield (830 g/d on average) and milk protein yield (659 g/d on average) were not significant between treatments.

Time spent grazing did not differ among treatments and it was 324 min on average, 60% approx. of the total observation time (nine hours). However, the biting rate was higher in LHM, which could indicate a compensation mechanism for lower bite weight in this treatment. Intake per bite is the variable most directly influenced by sward conditions, and normally falls as herbage mass or sward height declines [17] [33] [34] [35]. In this regard, [35] found that, at lower pasture height, animals increased both biting rate and grazing time, as a compensation strategy to a lower ingestion rate. Another interpretation, given by several authors, attributes the difference in the bite frequency to a reduction in the number of manipulative jaw movements required on shorter swards, and a consequent increase in the ratio biting/manipulative movements [31] [36]. The longer time required to chew heavier bites explains the increase in time between successive prehension bites. Based on this interpretation, a time of 1.7 seconds per bite could be estimated in HHM compared to 1.4 seconds per bite in LHM. Additionally, when the number of bites of prehension is increased, particle size tends to be shorter [37] and, as chemical composition was similar (NDF content), adaptation is made not on the number of bites during rumination chewing, but on the rumination time (133 vs 98 min, $P = 0.047$, for HHM and LHM respectively).

4.3. Energy Expenditure

Energy expenditure analysis was made based on the technique developed by [21] to evaluate if the level of herbage mass (at the same herbage allowance) could have an effect on the energy cost, indicating a greater physical activity, despite similar herbage intake. This method estimates energy expenditure by heat production (HP) through the O_2P-HR ratio, being HP the sum of HP for maintenance (HPm) and HP for production (HPp). According to [15], there is a negative relationship between the herbage mass and the energy expenditure, that can be explained by the increase in grazing activity (and so in HPm) when the amount of easily harvestable herbage is low. The lack of effect of the amount of pre-grazing mass on energy intake obtained in the present study, confirms that the difference between biomass treatments was beyond the limits that impose extra energy expenditure at grazing. In fact, even if there was an effect of pre-grazing biomass on feeding behavior (bite rate), it was not reflected in a different HP value due to differential activity between treatments. In temperate pastures with non-limiting biomass for herbage intake, the cost of harvest is negligible compared to the energy harvested in each bite according to [38] and [16]. Relating the values of HR to the ingestive behavior (**Figure 1**), it was observed that HR is lowest at 5:00, and increased after 10:00 and towards evening for both treatments, in agreement with the period of greatest activity of ingestion. Comparable results in dairy cows are reported by [21] and [39], associated to an increase in ingested DM throughout the day. In fact, as reported by [40], the maintenance energy requirement in lactating dairy cow increases at grazing compared to zero-grazing dairy cows, which is at least partly caused by more physical activity.

4.4. Methane Emission

Finally, no difference in total enteric CH_4 emissions (363 g/d on average) or CH_4 emission intensity (18.4 g/kg FPCM on average) were observed, and the values are in agreement with those reported by previous international [8] [41] [42] and national literature [5], for dairy cows with similar levels of intake and production. This study constitutes the second study of measured enteric CH_4 emissions from grazing dairy cattle in Uruguay. The average CH_4 emission per unit of estimated feed intake (21 g/kg DMI) and CH_4 emission as a percentage of gross energy intake (6.7%) obtained are aligned with previous national findings [5] and with values reported in the meta-analysis presented by [43] for grazing dairy cows on temperate pastures.

5. Conclusions

The ranges of pre-grazing biomass evaluated in this experiment did not affect forage intake, milk production or methane emission from grazing dairy cows. Although intake was not affected, the cows that grazed the sward with lower biomass adapted their behaviour by means of a higher biting rate. This was not accompanied by an increase in the grazing time during the observation period in this study. The energy cost of prehension bites is very low in temperate pastures with herbage with high digestibility, which could explain why a greater number of bites in LHM treatment did not result in significant differences in terms of energy expenditure due to extra activity of grazing.

It is possible that, at lower daily herbage allowance, animals express behavior adaptations to reach higher intakes that are mediated by the HM and pasture height, with consequences in harvest efficiency and in energy expenditure by extra activity.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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