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Short-term feed intake regulation of dairy cows fed a total mixed ration or grazing forage oats

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Abstract. The integration of feeding behaviour with hepatic and endocrine–metabolic signals provides insights for a better understanding of short-term intake in dairy pasture-based systems. Therefore, the objective was to quantify hepatic and endocrine–metabolic signals before and after the first daily feeding event relating to feeding behaviour in a total mixed ration (TMR) versus a grazing pasture-based diet. During 15 days of adaptation and 5 days of measurements, 14 multiparous Holstein cows (days in milk = 148 ± 12.7; liveweight = 535 ± 10.9 kg; body condition score = 2.8 ± 0.08 (1–5 scale); milk yield = 28.9 ± 3.32 kg) were assigned to two treatments in a randomised block design: PAS = pasture (herbage allowance = 45 kgDM/cow.day; dry matter (DM) = 21%, net energy requirements for maintenance and lactation = 6.7 MJ/kgDM) + concentrate (0.9% of liveweight) or TMR (55 : 45 forage : concentrate ratio, as-dry basis; DM = 40%, net energy requirements for maintenance and lactation = 7.2 MJ/kgDM) *ad libitum* in a free stall facility. The DM intake of the first feeding event, feeding behaviour, and total DM intake and milk production, were measured. Blood and liver samples were taken before and after the first feeding event for hormones and metabolites determination. Comparing TMR versus PAS cows, total DM and net energy requirements for maintenance and lactation intake, milk production, and energy balance were greater ($P < 0.05$), eating and rumination activities were lower (9.2%, $P < 0.01$; 2.4%, $P = 0.06$ respectively) and resting activity was greater (11.6%, $P < 0.01$), whereas duration and DM intake of the first feeding event did not differ. The insulin : glucagon ratio and liver adenosine triphosphate : adenosine diphosphate ratio increased ($P < 0.05$), and plasma glucose decreased ($P < 0.05$) after the first feeding event only in TMR cows, probably due to greater flux of propionate to the liver. A negative correlation between post-feeding liver adenosine triphosphate : adenosine diphosphate ratio and post-feeding liver acetyl coenzyme A ($r = –0.82$, $P = 0.045$) was also observed only in TMR cows. It is concluded that hepatic and metabolic signals known to support the hepatic oxidation theory in TMR-fed cows appear not to affect the cessation of the first feeding event in mid-lactation cows grazing a pasture-based diet. Further research is required to relate intake rate, flux of nutrients to liver and its response in hepatic metabolism in grazing dairy cows.

Additional keywords: dairy pastures, feeding behaviour, hormones.

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Introduction

In pasture-based dairy systems, dry matter (DM) intake is the major limitation of milk production (Kolver and Muller 1998; Bargo *et al.* 2002). However, independent of the production system and feeding conditions, dairy cows consume individual and discrete meals throughout the day, also called feeding and/or grazing events (Forbes 1995; Gibb *et al.* 1998). At pasture, three main feeding events are generally observed close to sunrise, in the afternoon and prior to sunset (Gregorini *et al.* 2006; Sheahan *et al.* 2013*a*). The intensity of each feeding event (intake rate and length of the feeding event) can change throughout the day, as well as the interval between them, affecting total DM intake and, therefore, milk production (Allen 2000).

The decision of when to start or finish a feeding event is affected by the integration of internal and external signals of the animal in the brain feeding centres (Allen 2000; Gregorini *et al.* 2006). In pastures, this process is even more complex, and is affected by grazing management. Access time to pasture (Kennedy *et al.* 2009), herbage allowance and herbage mass (Chilibroste *et al.* 2012) has been reported as the main factors affecting feed intake. Moreover, herbage characteristics, such as neutral detergent fibre (NDF) concentration and its digestibility (Oba and Allen 1999), and DM content of pasture (Bargo *et al.* 2002), have been reported to affect feed intake due to effects on rumen fill. However, in highly digestible pastures, these factors are not be the main determinant of feed intake (Chilibroste *et al.* 1997; Hills *et al.* 2015). Osmolarity and pH (Faverdin 1999), and the rate of generation of fermentation products or N-NH3 in the rumen (Chilibroste *et al.* 1998; Chilibroste 1999) play roles in

the regulation of feed intake. In addition, feed intake is also regulated by changes in circulating concentrations of hormones and metabolites in response to the ingestion and digestion of feed (Sheahan *et al.* 2013*a*), fasting period (Patterson *et al.* 1998; Chilibroste *et al.* 2007) or the physiological state of the animal. These factors define the demand of nutrients for milk production (Gibb *et al.* 1999; Allen 2014) and the sensitivity of tissues to hormones. Moreover, according to the optimal theory of grazing, cows optimise their input and output, by fulfilling their energy and nutrient requirements at the lowest cost in energy and time spent grazing (Phillips 2002).

In contrast, studies of the use of total mixed ration (TMR) diets with a high proportion of fermentable starch show that a feeding event could be concluded by signals generated in the liver by oxidation of fuels (Allen 2014). The production of adenosine triphosphate (ATP) within meals is affected by the rate of production of anapleurotic fuels (as propionate), and contents of acetyl coenzyme A (CoA) and reducing equivalents in the liver, and it is highly variable by the physiological state and glucose demand of the cow and by the diet (Allen and Piantoni 2013). The metabolic signals from fuel oxidation likely predominate during the transition period (Allen and Piantoni 2013). However, when glucose demands are increased (peak of lactation), propionate is mainly used for gluconeogenesis, reducing the possibility for propionate oxidation within meals, and extending the length and size of meals. On the contrary, when glucose demand is decreased (e.g. past peak of lactation, mid-to-late lactation), propionate could be oxidised within or during actual meals, resulting in signals of satiety (Oba and Allen 2003). The higher levels of non-fibre carbohydrates in a TMR diet in comparison with a grass pasture (Waghorn 2002), and so the rapid increase of propionate in the rumen, might cause a change in the energy status of the liver when a meal is finalised. However, although this may vary between sward type, age of regrowth, and between and within days (Chilibroste *et al.* 1998), dairy cows grazing on high digestible grass pastures can reach a high propionate concentration and/or acetate : propionate ratio of 2.6–2.8 in the rumen (Chilibroste *et al.* 1998; Stakelum and Dillon 2003; Ribeiro Filho *et al.* 2012). According to our knowledge, we have not found studies that have focused on the role of liver signals that may contribute to understanding the regulation of short-term feed intake in grazing dairy cows during mid-lactation. We hypothesised that the energy status of the liver, determined by the rate of production and utilisation of ATP, is associated with short-term feeding behaviour and to the endocrine–metabolic status of dairy cows at grazing. Therefore, the aim of this study was to quantify hepatic oxidation signals before and after the first daily feeding event, and relate them to feeding behaviour during the event, to blood metabolites and hormones before and after the first daily feeding event, and to daily total DM intake in cows fed TMR and at grazing temperate grass pasture.

Materials and methods

The experiment was conducted at the Experimental Research Station "Dr M.A. Cassinoni" (EEMAC) of the Facultad de Agronomía (Paysandú, Uruguay, 32°22′52″S, 58°03′10.25″W) during August 2016, at the end of the winter season. Animal procedures were approved by the Animal Experimentation Committee of Universidad de la República (UdelaR, Montevideo, Uruguay), application number 021130-002616-14.

Experimental design, animals and treatments

A total of 14 Holstein cows were used in a 20-day trial, 15 days for adaptation to experimental management and diets, followed by 5 days for experimental determinations. A total of 12 cows were in their second lactation and two in their third lactation. At the start of the experiment, their average (±s.d.) days in milk (DIM) was 148 ± 12.7, liveweight (LW) was 535 ± 10.9 kg, body condition score (BCS; 1–5 scale, 1 = thin and 5 = fat, Edmonson *et al.* 1989) was 2.8 ± 0.08 and milk yield was 28.9 ± 3.3 kg. Cows were selected from an autumn calving herd, blocked by DIM, LW, BCS, number of lactations and previous milk yield, and randomly assigned to two treatments: (1) TMR (non-fresh pasture, control), and (2) pasture + concentrate (PAS).

Management and feeding

Cows were milked twice daily, at 0400 and 1500 hours. After morning milking, they were kept in a pen with access to water until 0800 hours. Access time to treatment was from 0800 to 1430 hours, and from 1600 to 0330 hours. Cows in PAS were fed concentrate (0.9% of LW; Table 1), which was offered in two equal meals at each milking in the milking parlour, and were allowed to graze an oat pasture in one group (*Avena byzantina*). Herbage allowance was 45 kg of DM/cow.day (Table 1). Pasture was offered in daily strips marked off by an electric fence. Fresh daily strips were accessed in the morning and none were re-grazed during the experiment. The forage availability was estimated to determine herbage allowance, using the technique described by Haydock and Shaw (1975), cutting grass at ground level in a 30 × 30-cm frame, with electric garden hand shears (combined shears GSL35; Black & Decker, Towson, MD, USA) and using a rising plate meter (Mattiauda *et al.* 2013). Oat pasture was sown in March 2016 with 120 kg/ha of seed, and fertilised with 46 kg of N in April and June. Cows in the TMR treatment were individually housed in a free stall facility (wood-frame barn) and fed *ad libitum* TMR (Table 1) distributed once daily in the morning.

Measurements and sample analyses

Milk production, liveweight and body condition score

During the 5 days of measurements, individual milk yields (kg) were recorded at each milking (Waikato MKV Milk Meter, Hamilton, New Zealand). Milk fat, protein and lactose concentrations were determined from one successive a.m. and p.m. milk sample taken on the third day (MilkoScan Foss FT2, Hillerød, Denmark). Fat-corrected milk yield (4%) was calculated using the equation of Tyrrell and Reid (1965).

Animal behaviour

Through the first three consecutive days of the measurement period, animals eating, ruminating or resting (not eating or ruminating) were recorded. Cows were observed every 5 min during diurnal access time to treatment. Time spent per activity

Table 1. Ingredients and nutrient composition (% as-dry basis) and estimated net energy for lactation (NE_L; MJ/kg DM) of experimental diets

TMR, total mixed ration; PAS, pasture plus concentrate at 0.9% of liveweight/day; DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein; EE, ether extract; NE_L, net energy for lactation estimated according to NRC 2001

Item	TMR	Treatments PAS	
		Concentrate	Pasture
Ingredients (%)			
Corn silage	42.6		
Lucerne hay	8.5		
Sorghum grain	14.8		
Corn grain	5.8	32.0	
Barley grain	8.8	31.0	
Soybean meal	11.6	32.0	
Sunflower grain	6.4		
Insalmix premium	1.2		
Salt (NaCl)	0.3	1.0	
Compound salt		4.0	
Analysed composition (%)			
DM	40.0	86.9	21.1
OM	93.3	91.2	89.8
Starch[A]	29.8	40.1	
NDF	40.2	30.0	46.9
ADF	20.8	10.2	28.2
CP	14.4	16.5	14.8
EE	5.2	3.0	3.5
NE_L (MJ/kg DM)	7.2	7.4	6.7

[A]Starch: estimated by Dairy One (2019).

(min) was calculated assuming that the activity recorded was maintained during the 5 min until the next observation. The length of the first daily feeding event and the length of the first daily non-eating event were calculated when the activity was maintained for at least two consecutive recordings.

Total DM and energy intake estimation

The total DM intake was estimated during the experimental period using Cr_2O_3 as an external indigestible faecal marker to estimate faecal production (Peyraud 1998) together with acid insoluble ashes (AIA) in faeces and diet (Sales and Janssens 2003) as an internal marker. All cows were dosed twice daily, after each milking, for 12 consecutive days (7 days for adaptation and regulation of marker excretion flow, and 5 days for faecal collection) with a bolus of paper containing 7.5 g of Cr_2O_3 (60% purity). After dosing, animals were observed to ensure that there was no regurgitation. Faecal grab samples were collected from the rectum of each cow twice daily after milking. Sward grazing horizon was representatively sampled using electric garden hand shears before the first feeding event, from Day 7 to 11 during the intake measurement period. Concentrate and TMR samples were collected from each feeder immediately after feeding during the intake measurement period, as well as feed refused. All samples were frozen at –20°C until dried at 60°C for 48 h and composited. The DM concentration was determined by drying at 105°C for 24 h. The ash was determined by combustion in a muffle furnace at 300°C for 5 h. The organic matter was determined by mass difference among DM and ash. The total nitrogen was assayed using the Kjeldahl method (Method 984.13; AOAC 2000) and expressed as crude protein (nitrogen × 6.25). The ether extract was determined using Soxhlet extraction (Method 920.39; AOAC 2000); samples were packed in cartridges of filter paper and the extraction lasted 16 h using petroleum ether. The content of AIA was determined according to Tejada de Hernandez (1983), and NDF and acid detergent fibre as described by Van Soest *et al.* (1991), 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. Starch concentration was estimated by Dairy One (2019). The Cr_2O_3 of faecal samples was determined as described by Parker *et al.* (1989).

The total DM intake of TMR treatment, pasture DM intake of PAS treatment and total DM intake of PAS treatment were calculated as:

$$\text{Total DM intake of TMR treatment (kgDM/cow.day)} = (F \times [\text{AIA}_\text{F}])/[\text{AIA}_\text{TMR}]$$

$$\text{Pasture DM intake (kgDM/cow.day)} = \{(F \times [\text{AIA}_\text{F}]) - (\text{C} \times [\text{AIA}_\text{C}])\}/[\text{AIA}_\text{PASTURE}]$$

$$\text{Total DM intake of PAS treatment (kgDM/cow.day)} = \text{C intake} + \text{Pasture DM intake}$$

where F = faecal production (kg DM); C = concentrate (kg DM); $[\text{AIA}_\text{F}]$, $[\text{AIA}_\text{TMR}]$, $[\text{AIA}_\text{C}]$ and $[\text{AIA}_\text{PASTURE}]$ = concentration of insoluble ashes (%) in faeces, TMR, concentrate and pasture respectively.

Net energy requirements for maintenance and lactation (NE_L; MJ) calculations were based on NRC (2001). The NE_L concentration of TMR, pasture and concentrate were estimated to calculate total NE_L intake. Daily energy balance was expressed as an amount of NE_L requirements satisfied by NE_L intake.

First feeding event intake and intake rate

First feeding event intake (kgDM) and intake rate (gDM/min) were estimated at the first daily feeding event (after morning milking) during two consecutive days. For PAS, DM intake was calculated by weighing each cow pre- and post-voluntary cessation of a grazing bout (post-feeding), and corrected for 1 h of insensible weight loss, according to the procedure described by Penning and Hooper (1985). Before grazing, animals were fitted with faecal and urine collecting bags to avoid excreta losses. Cows were weighed using a precision balance (accurate to 50 g) in a location protected from wind, 100 m away from the paddock. Three measurements per second were recorded using a complement of Windows (Hyper Terminal private edition v.7.06 electronic download) until a measurement was repeated at least 10 consecutive times. The LW was calculated as the mode (the most frequent value) of the recorded data. Animals were familiarised with harnesses, faecal and urine collecting bags, and the presence of observers during the adaptation period. The intake rate was calculated by dividing DM intake by feeding event duration (min). The DM of pasture

consumed was estimated from hand clipping samples of each cow (Jones and Moseley 1993). For TMR cows, DM intake was measured by the difference between feed offered and feed remaining, and intake rate was calculated as described before. The DM of feed consumed was estimated from representative samples of each feeder taken before DM intake estimation. To estimate NE_L intake at the first feeding event, NE_L of TMR and hand clipped samples were estimated as described for total intake. In addition, the first feeding event DM intake : total DM intake ratio (%) and first feeding event NE_L intake : total NE_L intake ratio (%) were calculated.

Blood and liver sampling and analysis

Pre-feeding blood and liver sampling of the first daily feeding event were taken the day before the measurement period, and post-feeding samples were taken at the precise moment when each cow had voluntarily finished its first feeding event on the last day of measurements in order not to interfere with normal behaviour. Blood samples were collected by venipuncture of the coccygeal vein into two vacuum tubes, one for serum (BD Vacutainer REF 367820), and the other for plasma containing sodium fluoride and potassium oxalate (BD Vacutainer REF 367922) as a glycolytic inhibitor. Both tubes were centrifuged (2000g for 15 min at 4°C) within 2 h after collection, and plasma and serum were stored at −20°C until analysed. After blood collection, liver biopsies (500 mg) were obtained using a 14-G biopsy needle (TruCore-II Biopsy Instrument; The Hague, the Netherlands), as described by Carriquiry *et al.* (2009). Liver samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Each metabolite and hormone was determined in a single assay. Plasma glucose, serum non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentrations were determined by colorimetric assays on a Vitalab Selectra II autoanalyzer (Vital Scientific, Dieren, the Netherlands) using commercial kits (glucose: BioSystems, Barcelona, Spain; NEFA: Wako NEFA-HR (2), Wako Pure Chemical Industries, Osaka, Japan; BHB: Randox Laboratories, Antrim, United Kingdom). Intra-assay coefficient of variation for all determinations was ≤10%. Serum concentrations of insulin were measured using an immunoradiometric assay with a commercial kit (DIAsource INS-IRMA Kit, Louvain-la-Neuve, Belgium) previously used in cattle (Astessiano *et al.* 2015). The assay detection limit was 1.518 μIU/mL, and intra-assay coefficients of variation for control 1 (23.4 μIU/mL) and 2 (76.3 μIU/mL) were 6.2 and 2.2% respectively. Serum glucagon was measured using a radioimmunoassay kit (#GL-32K; Millipore, Billerica, MA, USA) specific for glucagon in serum or plasma in most mammals (Sheahan 2014). Intra-assay coefficients of variation for control 1 (62 pg/mL) and control 2 (109 pg/mL) were 9.7 and 9.1% respectively.

In the liver samples, ATP, adenosine diphosphate (ADP) and acetyl-CoA concentrations were determined using commercial kits from Abcam (Cambridge, UK; ATP assay kit, Colorimetric/Fluorometric, ab83355; ADP assay kit, Colorimetric/Fluorometric, ab83359; and PicoProbe Acetyl CoA Assay Kit, ab87546 respectively) according to the manufacturer's instructions. The ATP and ADP liver tissue homogenates absorbance were colorimetric measured using a Thermo Scientific Multiskan GO (Waltham, MA, USA), and the fluorescence signal of liver acetyl-CoA tissue homogenate was measured using a Thermo Scientific Varioskan Flash. The relative concentrations were normalised with the fresh tissue mass. In the case of ATP and acetyl-CoA measurements, homogenates were deproteinised using perchloric acid 2-4 N, and neutralised with potassium chloride 2 M.

Statistical analyses

All statistical analyses were performed using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA), except daily feeding behaviour, which was undertaken using the GLIMMIX procedure assuming a binomial distribution and estimating the probability of occurrence of the different activities. Residuals were tested for normality distribution using the Shapiro–Wilk test (PROC UNIVARIATE statement).

Total DM intake, components of energy balance, daily milk and milk constituents yield, milk composition, and first feeding event behaviour variables were analysed by the mean of each individual variable using ANOVA. The model included treatment and block as fixed and random effect respectively.

$$\text{Model}: \text{Yij} = \mu + \text{Ti} + \text{Bj} + \text{Eij}$$

where μ = mean; Ti = treatment (i = 1 to 2); Bj = block (j = 1 to 7); and Eij = residual error term.

Blood hormone, and blood and liver metabolite concentrations were analysed with repeated measurements using the following model:

$$\text{Yijl} = \mu + \text{Ti} + \text{Bj} + \text{Eij} + \text{Hl} + (\text{T} \times \text{H})\text{il} + \text{dijl}$$

where μ = mean; Ti = treatment (i = 1 to 2); Bj = block (j = 1 to 7); Eij = residual error term; Hl = moment of sampling (pre- and post-feeding); (T × H)il = treatment × moment of sampling; and dijl = residual error of the repeated measure.

The model included treatment and moment of sampling as fixed effects, and block as a random effect using the first order autoregressive as the covariance structure. To improve the accuracy of the models, LW and BCS were tested as covariates specific to the variables being analysed. Tukey–Kramer tests were conducted to analyse differences between groups ($\alpha = 0.05$). For each treatment, the relationship between first feeding event behaviour variables and pre- and post-feeding metabolic variables were analysed by Pearson correlations using PROC CORR (SAS Institute). For all results, means were considered to differ when $P < 0.05$, and trends were identified when $0.05 < P \leq 0.10$. Data are presented as least square means ± pooled standard errors.

Results

Total DM intake, energy balance, milk yield and milk composition

Data of total DM intake, energy balance and milk yield, and composition for TMR and PAS treatments are shown in Table 2. Cows fed TMR consumed more ($P < 0.05$) DM and more NE_L than PAS cows (19.8% and 25.9%, DM and NE_L, respectively) with lower ($P < 0.001$) NE_L maintenance

Table 2. Effect of diet on total DM and energy intake, feeding behaviour, and first feeding event behaviour
Values for treatments are least square means (n = 14). TMR, total mixed ration; PAS, pasture plus concentrate at 0.9% of liveweight/day; s.e.m., standard error of the mean; DM, dry matter; NE_L, net energy for lactation estimated according to NRC 2001; NE_L balance, amount of NE_L requirements satisfied by NE_L intake

	Treatments			*P*-value
	TMR	PAS	s.e.m.	treatment
Total feed intake (kg DM)	23.0	19.2	1.16	0.025
Pasture intake (kg DM)	–	14.1	–	–
Estimated NE_L balance				
Intake (MJ/day)	166.9	132.6	8.53	0.012
Maintenance (MJ/day)	39.7	42.3	0.75	<0.001
Lactation (MJ/day)	99.6	85.8	2.34	0.001
Balance	27.0	3.2	7.59	0.030
Yield (kg/day)				
Milk	31.2	26.6	0.88	0.001
Fat-corrected milk (4%)	31.4	27.5	0.83	0.006
Fat	1.3	1.1	0.04	0.045
Protein	1.1	0.9	0.04	0.005
Lactose	1.6	1.3	0.04	<0.001
Total milk solids	3.9	3.3	0.08	0.001
Milk composition (%)				
Fat	4.1	4.2	0.16	0.443
Protein	3.4	3.5	0.07	0.607
Lactose	5.0	4.8	0.07	0.014
Feeding behaviour[A]				
No. of feeding events	5.6	5.8	0.31	0.444
Feeding event duration (min/event)	40.5	48.7	2.53	0.002
First non-feeding event duration (min)	54.6	84.8	13.16	<0.001
Eating time (min)	229	281	13.00	<0.001
Rumination time (min)	156	169	9.00	0.059
Resting time (min)	179	114	10.20	<0.001
First feeding event behaviour				
Intake (kg DM)	4.0	3.3	0.40	0.233
NE_L intake (MJ)	29.6	22.5	2.51	0.074
Feeding event duration (min)	74.2	84.1	7.75	0.137
Intake rate (g DM/min)	54.9	40.2	0.18	0.004
First feeding event intake/total intake (%)	18.1	17.9	2.21	0.940
First feeding event NE_L intake/total NE_L intake (%)	20.2	22.7	1.60	0.174

[A]Daily feeding behaviour was recorded during 9.4 h in daylight hours.

requirements. Milk yield was also greater for TMR than PAS cows ($P < 0.001$) with greater lactose content ($P = 0.014$), and without differences in fat and protein content. These differences were also observed in NE_L output for lactation, which was greater ($P = 0.001$) in TMR than PAS cows. The NE_L balance was positive in both treatments and 17% greater ($P = 0.05$) in TMR than PAS cows.

Daily feeding behaviour and first feeding event behaviour

Results describing daily feeding and first feeding event behaviour are shown in Table 2. The probability of finding cows eating during the observation time was greater ($P < 0.001$) for PAS than TMR cows, equivalent to 52 min. The number of feeding events did not differ between treatments, determining greater ($P = 0.002$) feeding event duration for PAS than TMR cows. In contrast, the probability of finding cows resting was lower ($P < 0.001$) for PAS treatment, despite spending 30 min more ($P < 0.001$) time in the first non-feeding event than TMR cows. This non-feeding event was positively correlated ($r = 0.98$, $P < 0.01$) to the duration of the first feeding event only in PAS cows. In addition, the probability of rumination tended to be greater ($P = 0.059$) for PAS than TMR cows.

When the first feeding event behaviour was considered, NE_L intake tended ($P = 0.074$) to be greater for TMR than PAS cows. Although neither DM intake nor feeding event duration differed between treatments, the increase in DM intake (+0.64 kg) and decrease in feeding event duration (–9.9 min) resulted in a greater ($P = 0.004$) intake rate of TMR compared with PAS cows. Furthermore, the DM intake tended to be positively correlated to intake rate ($r = 0.77$, $P = 0.071$) in PAS cows, whereas in TMR it was correlated to the duration of the event ($r = 0.95$, $P < 0.01$).

Pre- and post-feeding blood and hepatic metabolic variables

No differences between treatments were found in pre- and post-feeding NEFA concentrations. However, as was expected, the concentration of NEFA decreased ($P < 0.01$) in both treatments

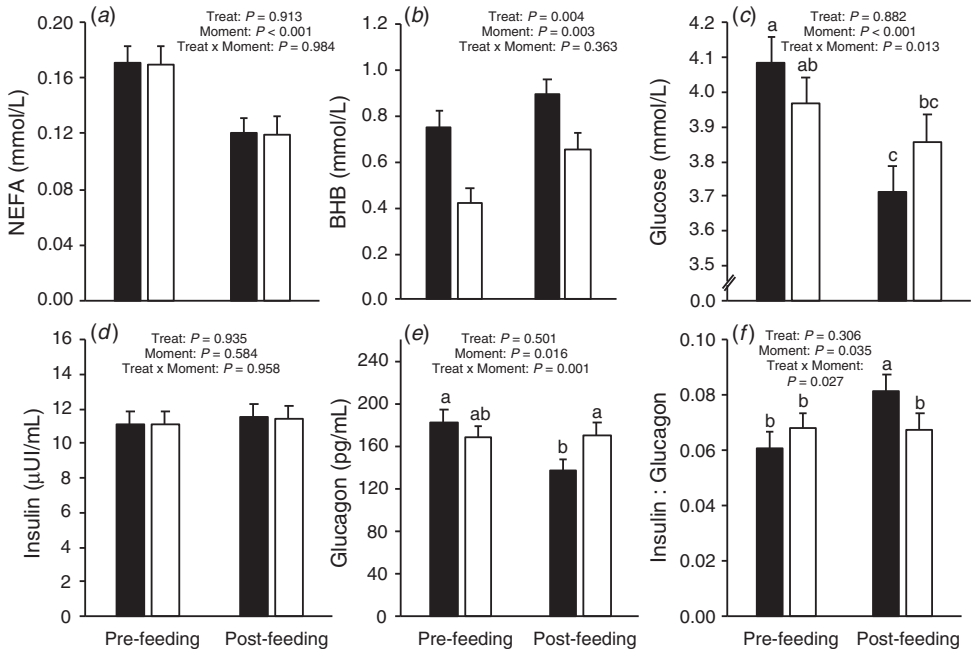

Fig. 1. Pre- and post-feeding blood concentration (+s.e.m.) of (*a*) non-esterified fatty acids (NEFA; mmol/L), (*b*) β-hydroxybutyrate (BHB; mmol/L), (*c*) glucose (mmol/L), (*d*) insulin (µUI/mL), (*e*) Glucagon (pg/mL) and (*f*) the relationship of the insulin : glucagon ratio of the first daily feeding event for total mixed ration (black bar) and pasture (white bar) treatments. Different letters (a,b,c) indicate significant differences ($P < 0.05$) between treatments and moment of sampling. Moment, moment of sampling (pre-feeding and post-feeding); Treat, treatments (total mixed ration and pasture).

after feeding (Fig. 1*a*). The pre- and post-feeding BHB concentrations were greater ($P < 0.05$) for TMR than PAS cows, and increased ($P < 0.05$) significantly with feed intake in both treatments (Fig. 1*b*). Glucose did not differ between treatments in the pre- and post-feeding concentrations, and decreased ($P < 0.001$) with feed intake only in TMR (Fig. 1*c*). Insulin concentration did not differ neither between treatments nor between the moment of sampling (Fig. 1*d*), whereas glucagon concentration decreased at the post-feeding sampling only in TMR cows ($P < 0.01$), thus generating differences ($P = 0.048$) between treatments post-feeding (Fig. 1*e*). The insulin : glucagon ratio differed ($P = 0.022$) between treatments only post-feeding, explained by an increase ($P = 0.005$) of this ratio in TMR cows after feeding (Fig. 1*f*).

Hepatic ATP concentration did not differ between treatments or moment of sampling (Fig. 2*f*). Pre-feeding ADP concentration did not differ between treatments, and tended ($P = 0.070$) to decrease with feed intake only in TMR cows, leading to differences ($P = 0.008$) between treatments in the post-feeding concentration (Fig. 2*b*). The inverse response was observed in the ATP : ADP ratio, increasing significantly ($P = 0.005$) after feeding in TMR cows and generating differences ($P = 0.001$) between treatments in the post-feeding ratio (Fig. 2*c*). Liver acetyl-CoA concentration was not affected by the first daily feeding event in either treatments; however, the post-feeding concentration of this metabolite in TMR cows was greater ($P = 0.007$) than PAS cows (Fig. 2*d*).

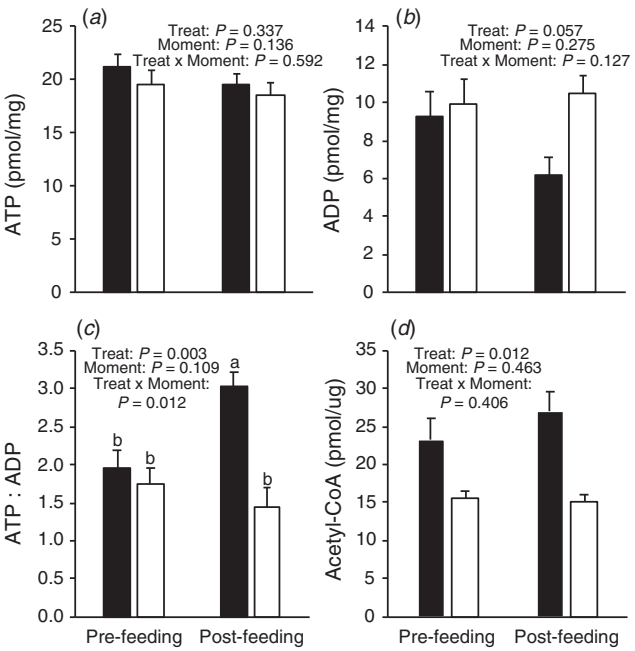

Fig. 2. Pre- and post-feeding hepatic concentration (+s.e.m.) of (*a*) adenosine triphosphate (ATP), (*b*) adenosine diphosphate (ADP), (*c*) ATP : ADP ratio (pmol/mg) and (*d*) acetyl coenzyme A (Acetyl-CoA; pmol/µg) of the first daily feeding event for total mixed ration (black bar) and pasture (white bar) treatments. Different letters (a,b) indicate significant differences ($P < 0.05$) between treatments and moment of sampling. Moment, moment of sampling (pre-feeding and post-feeding); Treat, treatments (total mixed ration and pasture).

For TMR cows, the post-feeding concentration of serum BHB and liver ATP were positively correlated to the first non-feeding event duration (r = 0.78, P = 0.040; r = 0.82, P = 0.023 respectively).

Relationship between behaviour and pre- and post-feeding metabolic variables of the first feeding event

In TMR cows, the first feeding event DM intake was negatively correlated with the decrease in plasma glucose concentration (r = –0.84, P = 0.017) and tended to be negatively correlated to the pre-feeding liver ATP : ADP ratio (r = –0.74, P = 0.059). In addition, the post-feeding liver ATP : ADP ratio was positively correlated with the decrease in plasma NEFA concentration (r = 0.76, P = 0.045), and was negatively correlated to the post-feeding serum insulin : glucagon ratio (r = –0.96, P < 0.001), serum BHB (r = –0.75, P = 0.052) and liver acetyl-CoA concentration (r = –0.82, P = 0.045).

In PAS cows, the first feeding event DM intake was negatively correlated to the pre-feeding serum insulin : glucagon ratio (r = –0.87, P = 0.024), and tended to be positively correlated to the pre-feeding liver ATP : ADP ratio (r = 0.76, P = 0.078). The post-feeding liver ATP : ADP ratio was positively correlated with the decrease in serum NEFA (r = 0.96, P = 0.008) and the increase in serum BHB (r = 0.96, P = 0.009), and tended to be negatively correlated with intake rate (r = –0.83, P = 0.078). Additionally, the increase in serum BHB during the feeding event was negatively correlated to intake rate (r = –0.83, P = 0.034).

Discussion

Our hypothesis that the energy status of the liver, determined by the rate of production and utilisation of ATP, was associated with short-term feeding behaviour and to the endocrine–metabolic status of mid-lactation dairy cows grazing a pasture-based diet could not be confirmed. Although the type of diet modified feeding behaviour, endocrine and hepatic factors, and total DM intake, the energy status of the liver does not seem to be the main factor affecting short-term feeding behaviour in PAS treatment.

Improvements in DM intake are usually associated with increased milk yield due to an increase in energy intake (Kolver and Muller 1998; Moallem *et al.* 2000; Bargo *et al.* 2002). The greater total DM intake and milk production observed in TMR cows of the current experiment was consistent with the findings of Bargo *et al*. (2002), who compared dairy cows fed TMR and cows at grazing with supplementation. Grazing as such produces an increase in energy expenditure for more intensive walking required (Bargo *et al.* 2002) compared with animals with feed easily available as a TMR diet (Roca-Fernández *et al.* 2013). Therefore, as expected, the potential increase in maintenance energy of PAS cows and lower NE_L intake compared with TMR explain a large part of the differences in milk and solids production between dietary treatments.

Daily feeding behaviour and first feeding event behaviour

The pattern of feeding activities observed in the current study was in line with data from other researchers who studied mid-lactation cows fed TMR (Mendoza *et al.* 2018) and grazing with supplementation (Kennedy *et al.* 2009). The longest time spent eating by PAS cows was related to longer feeding event duration, despite the fact that there were no differences between treatments in the length of the first feeding event. This is also consistent with the lower intake rate observed in the PAS treatment, which supports the longer time needed by grazing dairy cows to meet their energy requirements throughout the day. A possible explanation of this could be the lower DM content of pasture, which takes longer to harvest per unit of DM consumed (Cabrera Estrada *et al.* 2004), longer time for bite manipulation and chewing during ingestion (Thorne *et al.* 2003), and greater searching time (Chilibroste *et al.* 1997; Gregorini *et al.* 2007). The longer duration of the first feeding event compared with the average feeding event in both treatments could have been related to the stimulus of fresh feed delivery for TMR and new pasture strip for PAS cows, as well as to the fasting time during the early morning (Patterson *et al.* 1998; Chilibroste *et al.* 2007; King *et al.* 2016; Miller-Cushon and DeVries 2017). Interestingly, the DM and NE_L intake in the first feeding event of both treatments was nearly 20% of the total DM and NE_L intake, emphasising the importance of the factors regulating the first feeding event intake in the control of total intake (Gill and Rommey 1994). Additionally, the positive correlations observed between DM intake in the first feeding event with feeding event duration in TMR, and DM intake in the first feeding event with intake rate in PAS show the different ways to optimise feed intake in response to diet, and suggest that different signals could be involved in its regulation (Gill and Rommey 1994).

Control of the first feeding event intake

Previous to the first feeding event, the lack of differences between treatments in most metabolites and hormones is consistent with previous data reported for grazing (Sheahan *et al.* 2013*a*) and TMR-fed dairy cows (Sutton *et al.* 1986; Wylie *et al.* 2008; Nikkhah 2014). It can probably be associated with the natural fasting during the night and increased rumination activity (Forbes 1995), which reduces the rumen pool size, and increases the passage rate and nutrient absorption (Tóthi *et al.* 2003). However, the greater serum concentration of BHB in TMR cows was not expected, and could be associated with greater ruminal pool size by the greater total DM intake during the day compared with PAS cows, and, therefore, greater ketone bodies synthesis in the ruminal epithelium (Sheahan *et al.* 2013*a*; Nikkhah 2014; Piantoni *et al.* 2015).

Post-feeding, the reduction in serum NEFA concentration in both treatments showed a change in the energy status from a tissue state of catabolism to anabolism (Lafontan *et al.* 2009). This is also supported by the increase in BHB, which reflects the uptake of ruminal volatile fatty acids during a feeding event (Chilibroste *et al.* 1998). The highest post-feeding BHB concentration reached by TMR cows, and the lack of differences in the first feeding event duration and DM intake between treatments, suggest that serum BHB at this concentration would not be a main signal of feeding event

cessation. This is consistent with Zarrin *et al*. (2013), who found no effect on feed intake in dairy cows increasing plasma BHB concentration from 0.59 to 1.74 mmol/L by intravenous infusion. However, the higher post-feeding BHB concentration in TMR cows would probably maintain the sensation of satiety for longer (Rossi *et al.* 2000) due to the positive correlation observed with the first non-feeding event.

For TMR treatment, the post-feeding decrease in plasma glucose was consistent with other studies (Ametaj *et al*. 2009; Iqbal *et al*. 2012). This variation in glucose concentration could be related to an increase in the insulin : glucagon ratio, which increases glucose uptake by peripheral tissues (Derno *et al.* 2013). It is likely that the observed increase in the insulin : glucagon ratio post-feeding may have increased the oxidation rate of propionate in the liver (Derno *et al.* 2013), which is also supported by the positive correlation between the ATP : ADP ratio and insulin : glucagon ratio in this treatment. The secretion of these hormones is affected, among others, by the rate of glucose synthesis (Roche *et al.* 2008) and by variation of blood volatile fatty acids, mainly propionate (Bines and Hart 1984), which its flux to the liver is increased after feeding in early and mid-lactation cows (Benson *et al.* 2002). The increase in the hepatic ATP : ADP ratio post-feeding in this treatment, and the negative correlation between the ATP : ADP ratio and acetyl-CoA may be indicating hepatic oxidation of fuels in the TCA cycle. Taken together, the results from TMR treatment are consistent with the hepatic oxidation theory, which suggests that the satiety signal generated from the liver to the brain during a feeding event seems to be more related to a balance between ATP production and utilisation than ATP concentration *per se* (Allen and Piantoni 2013; Allen 2014). Nevertheless, the positive correlation observed between post-feeding ATP concentration and the duration of the first non-feeding event may also reflect an effect of hepatic ATP concentration in the maintenance of satiety state. In contrast, the negative correlation between the pre-feeding ATP : ADP ratio and DM intake in the first feeding event may indicate that low pre-feeding energy load of the liver could also be a stimulatory signal for intake (Friedman 1997). Furthermore, the negative correlation between DM intake in the first feeding event and the decrease in plasma glucose could be associated with individual cow responses to insulin (Bradford and Allen 2007).

In PAS treatment, unlike TMR, the lack of variation in plasma glucose between pre- and post-feeding sampling was consistent with the unchanged circulating insulin and the insulin : glucagon ratio. These results were in agreement with data reported by Sheahan *et al*. (2013*b*), who observed that glucose concentration remained constant during the first ~60 min after pasture was offered in the a.m. grazing bout. In addition, we observed a lower rumen volatile fatty acids pool in PAS than TMR treatment and a delay in reaching the highest volatile fatty acids pool after the first feeding event in PAS than TMR cows (data not shown, estimated by run CTR model, Chilibroste *et al.* 2008). These could explain the lack of variation between pre- and post-feeding liver ATP : ADP ratio in PAS cows. Interestingly, the negative correlations observed between intake rate and the post-feeding liver ATP : ADP ratio, and between intake rate and the increase in serum BHB concentration, as well as the positive correlation between the increase in serum BHB concentration and the post-feeding liver ATP : ADP ratio, highlight the importance of the intake rate in the availability of nutrients under grazing condition (Ulyatt *et al.* 1986). Collectively, the results from PAS treatment do not support the hepatic oxidation theory, as to be a primary signal controlling the first feeding event cessation under grazing highly digestible pastures.

Conclusion

The type of diet affects the DM intake feeding strategy and the availability of nutrients during a feeding event. Hepatic and metabolic signals known to be associated with intake regulation in TMR-fed dairy cows appear not to have a primary role in the cessation of the first feeding event in grazing dairy cows in this experiment. Further research relating to intake rate, flux of nutrients to the liver during meals, and its response in endocrine–metabolic signals and hepatic metabolism is needed to better understand the metabolic control of short feed intake in grazing dairy cows.

Conflict of interest

The authors declare no conflicts of interest.

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