Restricting time at pasture: Effects on dairy cow herbage intake, foraging behavior, hunger-related hormones, and metabolite concentration during the first grazing session

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ABSTRACT

This study investigated the effect of restricting grazing time on circulating concentrations of ghrelin, nonesterified fatty acids (NEFA), and glucose before, and foraging behavior of dairy cows during the first grazing session of the day (GS, 0800–1200 h). Forty-eight Holstein-Friesian cows (470 ± 47 kg of BW; 35 ± 9 d in milk) were strip-grazed on a perennial ryegrass pasture for either 4 h after each milking (2×4), 8 h between milkings (1×8), or the 24-h period excluding milking times (CTL). Cows were bled before the GS; plasma was analyzed for ghrelin and serum for glucose and NEFA. Herbage mass was measured pregrazing (0730 h), during and at the end of the GS (1200 h), and postgrazing (24 h after the first measurement). Herbage mass data were fitted to a model to estimate herbage disappearance rates. Herbage intake and bite mass were calculated using herbage mass disappearance and behavioral measurements. Bite rate, eating, searching, ruminating, and idling time were determined during the GS for each cow. No difference in glucose concentration was found between treatments. Concentrations of NEFA and ghrelin were the greatest for cows in the 1×8 treatment. Daily herbage intake did not differ between treatments; however, during the GS 1×8 had a greater herbage intake than 2×4 and CTL. Bite mass differed between treatments and throughout the GS. Bite mass was smallest for CTL during the first 60 min and greatest during the last 90 min, when cows in the 2×4 treatment had the smallest bite mass. Cows in 1×8 spent the longest time eating and the least time searching and ruminating. Eating time was greatest for 1×8 during the first 60 and last 90 min of the GS. Searching time only differed in the second 60 min, when it was the lowest for 1×8 . Cows from all treatments did not ruminate during the first 120 min. Cows in CTL had the greatest rumination time during the last 90 min. The model fitted to represent dynamics

of herbage mass disappearance presented differences in the fractional herbage disappearance rate. There was an interaction between treatment and time in herbage depletion rate. The results of this study present a fuller picture of foraging dynamics during the first 4 h of grazing and its potential relationship with physiological markers of hunger as affected by grazing management.

Key words: dairy cow, available grazing time, foraging behavior, hunger-regulated hormones and metabolites

INTRODUCTION

The grazing process has been described as a series of interactions between ingestive and digestive processes (Allden and Whittaker, 1970) and hormonal and metabolic stimuli to herbage intake (Illius and Jessop, 1996; Roche et al., 2008a). However, most of the advances in the understanding of the plant–animal interface have come from research determining how sward structure influences the instantaneous rate of herbage intake (Laca et al., 2001). Although recent integrative research (Gregorini et al., 2009) has focused on the interaction between plant, rumen, and endocrine-metabolic factors influencing grazing behavior, until now few related field studies have been undertaken.

Hunger motivates animals to eat. The key stimuli determining the degree of such a motivation are time available to eat and time elapsed from the last meal (i.e., deprivation; Jensen and Toates, 1993; Staddon, 2003). Hunger level motivates particular behavioral actions, determining foraging tactics and strategies (Pittroff and Soca, 2006). As deprivation increases, instantaneous intake rate increases by increments of grazing time, bite mass, and bite rate (Patterson et al., 1998; Chilbroste et al., 2007). Consistent with these increments, Gregorini et al. (2007a) and Gregorini et al. (2009) reported dynamic changes in foraging tactics and strategies by beef heifers and dairy cows, respectively, as hunger level (represented by rumen fill) increased. These changes appear to be mediated by energy status of the animal and hormonal stimuli such as the orexigenic agent ghrelin (Gregorini et al., 2009). Changes in ingestive behavior relate to satiety factors

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and feeding behavior stimuli/motivation at a discrete meal level (Ivlev, 1961). This is extensively studied in humans, rats, and swine (Day and Rogers, 1997; Day et al., 1998), but little research has been carried out in grazing dairy cows.

In a context of strip-grazing management, cattle consume the bulk of the day's allocated herbage during the first hours of grazing. Consequently, foraging strategies during this period are key determinants of daily herbage intake (Galli and Cangiano, 1999; Soca et al., 2002a; Soca, 2006). Recent research (Kennedy et al., 2009; Perez-Ramirez et al., 2009) investigated the effects of timing and availability of time for grazing (1 vs. 2 periods of available time for grazing, and restricted vs. nonrestricted time to graze, respectively) on milk production and daily herbage intake. However, there is a lack of detailed information about how cows adapt to different herbage allocations and restrictions. The objective of the present study was to investigate and describe the foraging behavior of dairy cows during the first grazing session of the day (GS, 0800–1200 h) in response to restrictions in available grazing time, and its association with changes in circulating concentrations of hunger-related hormones and metabolites.

MATERIALS AND METHODS

This study was approved by the Committee of Animal Care and Use from AgResearch (Hamilton, New Zealand).

Site, Animals, and Treatments

The study was conducted at Scott Farm, DairyNZ (Hamilton, New Zealand) during September and October 2008. Forty-eight Holstein-Friesian cows (470 ± 47 kg of BW; 35 ± 9 DIM; 24.2 ± 0.2 kg/d; $4.5 \pm 0.15\%$ fat; $3.5 \pm 0.06\%$ protein) were randomly assigned to 6 groups ($n = 8$ /group) and grazed according to the following treatments (2 groups per treatment): either 4 h after each milking (2×4), 8 h between milkings (1×8), or the 24-h period excluding milking times (CTL). Adaptation to dietary regimen is important in this case of studies. Animals need to strategize their foraging behavior according to their accessibility to pasture. Therefore, although cows in all treatments grazed the same sward, cows were adapted to treatments for a period of 16 d. Measurements took place during the last 3 wk (1 d/wk) of the experimental period.

Pasture and Grazing Management

During the treatment adaptation and experimental period, cows were strip-grazed on a perennial ryegrass

dominated pasture (*Lolium perenne* L., 79.4%; *Trifolium repens*, 3.3%; *Poa annua* and *trivialis*, 7.3%; and broadleaf weeds, 10%). Chemical composition of herbage was CP, 20.3%; ADF, 22.5%; NDF, 41.9%; NSC, 12.9%, and lipids, 3.7%. Herbage allowance was 0.07 kg of herbage DM/kg of BW per day for all treatments. Mean pregrazing herbage mass for the experimental period was 3,191 kg of DM/ha. The new daily strip of pasture (herbage) was allocated at 0800 h (after morning milking). Cows in treatments 1×8 and 2×4 were restricted to access pasture (taken off from pasture) in a wood chip-covered pad according to the timing, and available grazing time, of each treatment. All cows were milked at 0700 and 1600 h. Cows did not receive feed or mineral supplements and had ad libitum access to fresh water in each daily strip of pasture and on the "stand-off" pad.

Measurements and Calculations

Blood Collection and Ghrelin, NEFA, and Glucose Analysis. Each cow was blood sampled between 0600 and 0700 h before the GS (0800–1200 h). Samples were collected by coccygeal venipuncture into 10-mL evacuated blood tubes (140 IU of sodium heparin and 0.117 mL of 15% K_3EDTA). Samples were placed on ice until centrifuged ($1,500 \times g$, 15 min, $4^\circ C$). Plasma from the K_3EDTA tubes was acidified using 1 N HCl and treated with a protease inhibitor (10 μ L of phenylmethylsulfonyl fluoride) before storage for further analysis. Plasma active (acetylated) ghrelin concentrations were determined using a commercially available RIA (multispecies ghrelin active kit/GHRA-88HK, Linco Research Inc., St. Charles, MO). This assay uses ^{125}I -labeled ghrelin and a ghrelin antiserum to determine plasma concentration of ghrelin by the double antibody-polyethylene glycol technique and was validated for cows by Roche et al. (2008b). The lowest level of ghrelin that can be detected by this assay is 7.8 pg/mL when using a 100- μ L sample size. Intraassay coefficient of variation (CV) was $<10\%$. Analyses for NEFA (colorimetric method) and glucose (hexokinase method) were performed on a Hitachi 717 analyzer (Roche, Basel, Switzerland) at $30^\circ C$ by Alpha Scientific Ltd. (Hamilton, New Zealand). The inter- and intra-assay CV were $<2\%$ for NEFA and glucose.

Foraging Behavior. Behavioral activities were determined during the GS. Six trained observers were randomly assigned to each group of cows to record eating, searching, rumination, and idling behavior every 2 min by scan-sampling as suggested by Hirata et al. (2002). From these data, times for each activity were calculated by multiplying each behavior activity frequency by a 2-min interval; when grazing, cattle search,

acquire into the mouth, masticate, and swallow herbage (Gibb, 1998). For the purpose of this experiment, eating behavior was defined as cows with heads down and engaged in acquiring herbage into the mouth. Walking head down without eating was considered a searching activity. Observers counted bites of each cow during one continuous minute at 0, 30, 60, and 120 min after the GS started. Times spent eating, searching, ruminating, and idling, along with bite rate and herbage intake, were summarized into means for the GS. Eating, searching, ruminating, and idling times were also summarized into 3 periods within the GS: 0–60, 60–120, and 120–210 min.

Herbage Intake, Bite Mass, and Pasture Disappearance Rate. These variables were estimated using data from herbage mass measurements. Herbage mass was measured using a rising plate meter fitted with an electronic counter (Farmworks, Palmerston North, New Zealand). Herbage mass was recorded pregrazing, during (at 30, 60 and 120 min after GS started) the GS, at the end of the GS, and postgrazing (end of 24 h). Each recording was based on 0.075 plate meter readings per square meter of the area (strip) being grazed at the time. Plate meter readings followed the same transect path in all recordings. Pasture strips were always rectangular with an average area of 800 m².

Daily herbage intake was estimated by difference between pre- and postgrazing herbage mass (Macon et al., 2003; McEvoy et al., 2007). Herbage intake for the GS was calculated as the difference between pregrazing herbage mass and herbage mass at the end of the GS. The mean bite mass for GS was calculated by dividing herbage intake from the GS by the calculated total number of bites taken per cow during the GS. The latter was calculated by multiplying bite rate and eating time. Bite mass for the 3 periods within the GS were calculated in a similar way to the GS herbage intake. In this case, herbage intake per period was divided by the number of bites per cow at each period.

Herbage mass disappearance rate was calculated by fitting herbage mass data (y) taken from each group of cows to the model of Ørskov and McDonald (1970) [$y = a - b [1 - \exp(-c \times \text{time})]$]. For the present study, a represented initial available herbage mass; b , potential herbage mass disappearance; and c , fractional disappearance rate (%/h) of herbage mass. Parameters were estimated using GenStat 11.1 (Payne et al., 2008). Instantaneous herbage mass disappearance rates (kg of herbage DM/ha per hour) were estimated for 0, 60, 120, and 210 min after the GS started as the first derivative of the function $y' = -b \times c \times \exp(-c \times \text{time})$.

Statistical Analysis

Data from individual cows were averaged to obtain 6 experimental unit values per day of sampling, which were then analyzed as follows. Treatment effect on circulating concentration of ghrelin, NEFA, and glucose, and mean eating, searching, ruminating, and idling time, bite rate, bite mass, herbage intake during the GS, and daily herbage intake were analyzed using ANOVA, including group of cow as a random effect. Group of cows was the experimental unit. Repeated measurements through the GS were analyzed by mixed models, with treatment, time, and interaction of treatment with time as fixed effects, and cow group as a random effect. A compound symmetry covariance structure was used for within-group repeated measurements, and heterogeneity of variance at the time points was allowed for. Significant interactions between time and treatment were investigated further using ANOVA at each time point. Pasture depletion was analyzed by fitting the model of Ørskov and McDonald (1970) to the data from each group, and then analyzing the parameter estimates and herbage mass disappearance rates at the different time points using ANOVA with cow group as the experimental unit. GenStat 11.1 was used for all statistical analysis (Payne et al., 2008).

RESULTS

Herbage Intake and Grazing Behavior

Herbage intake during the GS was greater ($P < 0.05$) for cows in 1×8 than for cows in 2×4 or CTL, whereas daily herbage intake did not differ ($P < 0.05$) between treatments (Table 1). During the GS, cows in 1×8 had greatest ($P < 0.05$) mean eating time, cows in CTL had the lowest, and 2×4 cows were intermediate (Table 1). Mean searching time was lower ($P < 0.05$) for cows in 1×8 than for cows in 2×4 and CTL, which did not differ from each other (Table 1). Cows in CTL had the greatest mean rumination time, with cows in 1×8 and 2×4 not different from each other ($P < 0.05$). Mean idling time did not differ among treatments ($P > 0.05$). Mean bite rate was least ($P < 0.05$) for cows in CTL and greatest ($P < 0.05$) for cows in 2×4 (Table 1). Mean bite mass was greater ($P < 0.05$) for cows in CTL than cows in 2×4 .

Eating time during the first 60 min of the GS was greatest ($P < 0.05$) for cows in 1×8 (Table 2). During the second 60 min of the GS, treatment did not affect ($P > 0.05$) eating time. During the last 90 min of the GS cows in 1×8 spent more ($P < 0.05$) time eating

Table 1. Effects of allocation of available grazing time on herbage intake; eating, searching, ruminating, and idling times;¹ and bite rate and mass during the first grazing session (GS) of Holstein-Friesian dairy cows grazing a perennial ryegrass pasture

Item	Treatment ²			SED ³	Treatment effect (P-value)
	1 × 8	2 × 4	CTL		
Herbage intake during GS, kg of DM/cow	11.5 ^a	9.2 ^b	9.4 ^b	0.340	0.012
Daily herbage intake, kg of DM/cow	12.5	13.9	13.7	1.233	0.528
Eating	0.81 ^a	0.68 ^b	0.58 ^c	0.021	0.004
Searching	0.02 ^b	0.05 ^a	0.07 ^a	0.008	0.018
Ruminating	0.009 ^b	0.012 ^b	0.112 ^a	0.032	0.079
Idling	0.13	0.21	0.21	0.027	0.119
Bite rate, bites/min	52.1 ^b	54.9 ^a	46.47 ^c	0.541	0.001
Bite mass, g of DM	1.28 ^{ab}	1.10 ^{bc}	1.46 ^a	0.064	0.026

^{a-c}Means in the same row with different superscripts are different ($P < 0.05$)

¹Proportion of the available grazing time (3.5 h) in the first grazing session of the day.

²1 × 8 = 8 h of available grazing time allocated between morning and afternoon milkings; 2 × 4 = 8 h of available grazing time allocated in 2 sets of 4 h after morning and afternoon milking; CTL (control) = 24-h period excluding milking times. Cows spent 4 h/d in the milking process.

³Standard error of difference.

than cows in 2 × 4 or CTL. Searching time during the first 60 min of the GS did not differ between treatments ($P > 0.05$; Table 2). Treatment affected ($P > 0.05$) the time cows spent searching during the second 60 min of the GS, with cows in 2 × 4 and CTL searching for longer than cows in 1 × 8 ($P < 0.05$). During the last 90 min of the GS, treatment did not affect searching time ($P > 0.05$). Cows did not ruminate during the first 120 min of the GS, and CTL cows ruminated longer ($P < 0.05$) than cows in either 1 × 8 or 2 × 4 during the last 90 of the GS (Table 2). Idling time only differed for the first 60 min of the GS; cows in 1 × 8 and 2 × 4 spent less time idling than CTL cows ($P < 0.05$). Bite mass also differed at the beginning and the end of the GS (Table 2). During the first 60 min, cows in 1 × 8 and 2 × 4 had a greater bite mass than cows in CTL ($P < 0.05$). In comparison, cows in CTL had the greatest bite mass and cows in 2 × 4 had the smallest bite mass during the last 90 min of the GS ($P < 0.05$). Cows in 2 × 4 started (min 0) at a greater ($P < 0.05$) bite rate than cows in 1 × 8 and CTL. At 30 min, cows in 2 × 4 had the greatest bite rate and cows in CTL the lowest ($P < 0.05$), with cow 1 × 8 cows having an intermediate rate. At 60 min, cows in 1 × 8 and 2 × 4 had greater ($P < 0.05$) bite rate than cows in CTL. At 120 min, cows in 2 × 4 had the greatest bite rate, cows in CTL had the least, and 1 × 8 cows were intermediate ($P < 0.05$).

Pasture Disappearance

The treatment effect on pasture disappearance dynamics is presented in Figure 1. Table 3 provides the parameter estimates for the model used to represent herbage mass disappearance rate and rates at differ-

ent time points of the GS. Fractional disappearance of herbage (parameter c) differed ($P < 0.05$) between treatments, whereas parameters a and b did not ($P > 0.05$). Herbage disappearance rate differed ($P < 0.05$) between treatments at times 0, 60, and 120 min. At time 0, 1 × 8 and 2 × 4 showed greater ($P < 0.05$) herbage disappearance rate than CTL. At 60 min, 1 × 8 had greater ($P < 0.05$) disappearance rate than 2 × 4 and CTL. At 120 and 210 min, 1 × 8 and CTL showed greater disappearance rate than 2 × 4.

Ghrelin, NEFA, and Glucose Concentrations

Cows in 1 × 8 had the greatest ghrelin concentration ($P < 0.05$) at 0700 h, whereas ghrelin concentration did not differ between cows in 2 × 4 and CTL (Table 4). Cows in 1 × 8 also had greater ($P < 0.05$) NEFA concentrations than cows in 2 × 4, but were not different ($P > 0.05$) from cows in CTL. Plasma glucose was not affected by treatment ($P > 0.05$).

DISCUSSION

Daily Herbage Intake

Cows integrate internal stimuli (i.e., products of digestion) and external incentives (i.e., grazing management/opportunities to eat) to regulate herbage intake, which may explain how and why cattle change short-term foraging tactics and strategies (Houston, 1993; Soca et al., 2002a,b) to maintain a target daily food intake.

In the present study, daily herbage intake was not affected by timing or availability of time to graze. The lack of effect of the timing of pasture restriction confirm

the reports of Kennedy et al. (2009) and Perez-Ramirez et al. (2009), who did not report an effect of timing of available grazing time (1×9 vs. 2×4.5 and 1×9 vs. 2×2.75 , respectively) on daily herbage intake of dairy cows. However, these studies reported a reduction of daily herbage intake for cows restricted in available grazing time when compared with the daily herbage intake of unrestricted cows. Conversely, Mattiauda et al. (2004), Chilibraste et al. (2004a), and Kristensen et al. (2007) reported no reduction in daily herbage intake of dairy cows when available grazing time was reduced from 22 to 16, 8, and 4 h/d, as did Gregorini et al. (2007b) and Gregorini et al. (2008) with beef heifers. Chilibraste et al. (2004a) and Mattiauda et al. (2004) evaluated the effect of available grazing time and herbage allowance on daily herbage intake. According to these studies, unrestricted levels of herbage allowance overcome reductions in available grazing time. Poppi et al. (1987) set a threshold of herbage allowance of 0.04 kg of DM/kg of BW per day below which herbage intake would be constrained. The level of herbage allowance in the present study and that of Kennedy et al. (2009) were similar and both levels were greater than the threshold suggested by Poppi et al. (1987). It appears that the lack of difference between restricted and nonrestricted cows in the present experiment may have been related to differences, compared with Kennedy et

Figure 1. Herbage mass disappearance during the first grazing session (GS) of the day for Holstein-Friesian dairy cows grazing a perennial ryegrass pasture with differences in allocation of available grazing time. $1 \times 8 = 8$ h of available grazing time allocated between morning and afternoon milkings; $2 \times 4 = 8$ h of available grazing time allocated in 2 sets of 4 h after morning and afternoon milking; CTL (control) = 24-h period excluding milking times. Cows spent 4 h/d in the milking process.

al. (2009), in hunger drive and herbage accessibility, as well as the interaction between these two factors. Because cows in the present study were in the first stage of lactation, a greater motivation to eat can be expected. In addition, they were not fed supplements.

Table 2. Effects of allocation of available grazing time on the dynamics of eating, searching, ruminating, and idling times, bite mass, and bite rate during the first grazing session of the day for Holstein-Friesian dairy cows grazing a perennial ryegrass pasture

Item	Time interval, ¹ min	Treatment ²			SED ³	P-value
		1×8	2×4	CTL		
Eating time, min	0–60	57.08 ^a	52.79 ^b	53.75 ^b	1.210	0.07
	60–120	51.12	46.20	42.45	6.118	0.46
	120–210	57.95 ^a	33.39 ^b	35.75 ^b	6.934	0.06
Searching time, min	0–60	1.45	3.62	4.29	1.188	0.18
	60–120	0.68 ^b	3.29 ^a	5.08 ^a	0.606	0.01
	120–210	3.33	3.39	5.66	1.723	0.41
Ruminating time, min	0–60	0	0	0		
	60–120	0	0	2.00	1.089	0.25
	120–210	1.79 ^b	2.22 ^b	14.91 ^a	1.146	0.002
Idling time, min	0–60	0.16 ^b	0.41 ^b	0.70 ^a	0.123	0.04
	60–120	1.85	3.39	9.04	3.371	0.22
	120–210	26.79	34.97	28.54	5.464	0.40
Bite mass, g of DM	0–60	1.99 ^a	1.98 ^a	1.428 ^b	0.138	0.04
	60–120	0.97	0.75	0.86	0.437	0.88
	120–210	0.88 ^b	0.34 ^c	2.10 ^a	0.326	0.02
Bite rate, ⁴ bites/min	0	55.22 ^b	60.21 ^a	53.42 ^b	0.815	0.007
	30	55.33 ^b	58.22 ^a	48.07 ^c	0.590	0.0009
	60	53.83 ^a	51.42 ^a	44.31 ^b	0.821	0.002
	120	44.07 ^b	49.91 ^a	39.97 ^c	0.434	0.0004

^{a–c}Means in the same row with different superscripts are different ($P < 0.05$).

¹Interval of time during the first meal, 0 min (start) and 210 min (end).

² $1 \times 8 = 8$ h of available grazing time allocated between morning and afternoon milkings; $2 \times 4 = 8$ h of available grazing time allocated in 2 sets of 4 h after morning and afternoon milking; CTL (control) = 24-h period excluding milking times. Cows spent 4 h/d in the milking process.

³Standard error of difference.

⁴Fixed point in time (min after meal started).

Table 3. Effects of allocation of available grazing time on herbage disappearance dynamics (calculated parameters and rates of disappearance at different times) during the first grazing session of the day for Holstein-Friesian dairy cows grazing a perennial ryegrass pasture¹

Item	Treatment ²			SED ³	Treatment effect (<i>P</i> -value)
	1 × 8	2 × 4	CTL		
<i>a</i>	3,188.3	3,291.5	3,317.3	146.1	0.682
<i>b</i>	1,206.5	865.1	1,649.8	387.4	0.262
<i>c</i>	0.689 ^b	1.314 ^a	0.277 ^c	0.158	0.016
Herbage disappearance rate, kg of DM/ha per hour					
At time 0 (0 min)	821.6 ^a	1,137.9 ^a	397.2 ^b	125.2	0.022
At time 1 (60 min)	410.7 ^a	304.8 ^b	294.3 ^b	25.9	0.036
At time 2 (120 min)	207.9 ^a	82.2 ^b	221.9 ^a	23.7	0.017
At time 3.5 (210 min)	76.7 ^a	11.7 ^b	150.1 ^a	33.0	0.056

^{a-c}Means in the same row with different superscripts are different ($P < 0.05$).

¹Herbage mass taken at different times during the meal was fitted to the model of Ørskov and McDonald (1970) $\{y = a + b[1 - \exp(-c \times \text{time})]\}$, where a = available herbage (at time 0); b = potential herbage disappearance; c = fractional disappearance rate (%/h).

²1 × 8 = 8 h of available grazing time allocated between morning and afternoon milkings; 2 × 4 = 8 h of available grazing time allocated in 2 sets of 4 h after morning and afternoon milking; CTL (control) = 24-h period excluding milking times. Cows spent 4 h/d in the milking process.

³Standard error of difference.

Based on area allocation and pregrazing herbage mass, the pasture grazed by the cows in the present study experiment was taller, which may have facilitated herbage ingestion by increasing herbage accessibility (Rook et al., 1994). The interaction of these two factors may partially explain why the restricted cows in the present study have similar intakes to the CTL cows. This argument is also supported by Iason et al. (1999) and Soca et al. (2002b) in sheep and beef cattle, respectively, who reported a positive interaction between length of available grazing time restriction and herbage accessibility on daily herbage intake as determined by sward surface height.

Herbage Intake and Foraging Behavior During the First GS

Although herbage intake differed between treatments during the GS, cows in all treatments consumed the bulk of their daily herbage intake during the GS (Table 1). This reinforces the importance of the first meal after a natural (i.e., night) or management-generated (i.e.,

pasture standing-offs) food deprivation on daily herbage intake and, therefore, the foraging strategies used during this period.

Deprivation is defined as the number of hours since the last meal, and its magnitude is positively related to hunger level (Forbes, 1995). The major mechanism by which cattle respond to restrictions in grazing time is to increase eating time during the next available time for grazing (Chilibroste et al., 2004b). In the present study, eating time during the GS was clearly affected by the restriction of total available grazing time and the magnitude of deprivation (Tables 1 and 2). The latter had the strongest effect as motivational stimulus to eat. The way cows allocated eating time during the GS (Table 2) provided evidence that eating motivation for cows in 1 × 8 lasted longer than that for cows in 2 × 4 or CTL. This greater eating motivation for cows in 1 × 8 is also observable and supported by the lower idling time during the first 60 min of the GS. These results and the difference in searching time (Tables 1 and 2) are consistent with the incremental increase in eating time and reductions in searching time, with increas-

Table 4. Effects of allocation of available grazing time on circulating ghrelin, NEFA, and glucose at the start of the first grazing session of the day for Holstein-Friesian dairy cows grazing a perennial ryegrass pasture

Item	Treatment ¹			SED ²	Treatment effect (<i>P</i> -value)
	1 × 8	2 × 4	CTL		
Ghrelin, mmol/L	230.4 ^a	152.6 ^b	178.8 ^b	10.05	0.009
NEFA, mmol/L	0.42 ^a	0.19 ^b	0.27 ^{ab}	0.071	0.10
Glucose, mmol/L	3.89	3.85	3.82	0.051	0.51

^{a,b}Means in the same row with different superscripts are different ($P < 0.05$).

¹1 × 8 = 8 h of available grazing time allocated between morning and afternoon milkings; 2 × 4 = 8 h of available grazing time allocated in 2 sets of 4 h after morning and afternoon milking; CTL (control) = 24-h period excluding milking times. Cows spent 4 h/d in the milking process.

²Standard error of difference.

ing hunger levels reported in beef heifers (Gregorini et al., 2007a). This response is reinforced by the results of Wertz-Lutz et al. (2006), who reported increased eating time associated with greater ghrelin concentrations when beef steers were nutritionally restricted. Incremental increases in eating time are often at the expense of ruminating time (Abrahamse et al., 2008). The pattern of both eating and rumination time during the GS support this concept, as well as the view of rumination as an active rather than a passive digestive process. In other words, cattle may decide when to ruminate according to foraging strategy, integrating not only nutritional or internal stimuli but also learned eating expectations (Soca et al., 2002a).

Unexpectedly, CTL cows had a greater average bite mass (Table 1), which could have been related to a high bite mass estimated for the end of the GS (Table 2). Bite mass decreased from the beginning to the end of the GS for both 1×8 and 2×4 cows. However, the degree of this reduction was smaller for cows in 1×8 than in 2×4 . Bite mass is a key variable determining short-term herbage intake rate (Hodgson 1985; Newman et al., 1994) as well as a compensatory foraging tactic in response to food deprivations. Chilbroste et al. (1997) observed a linear decrease in bite mass through a grazing bout as well as a negative effect of artificially filling the rumen. Gregorini et al. (2007a) and Gregorini et al. (2009) reported increases in bite mass by beef heifers and dairy cows, respectively, as hunger level increased. Similarly, Patterson et al. (1998) found that the effect of deprivation on bite mass by dairy cows was greater as the length of deprivation increased. These studies and the bite mass differences throughout the GS between cows in 1×8 and 2×4 treatments not only point out the importance of bite mass as a compensatory mechanism for reductions in available grazing time, but also as the immediate response to changes in hunger level. The smaller rate of decline in bite mass from cows in 1×8 compared with 2×4 corroborates the magnitude and persistency of the motivational stimulus to keep eating.

Bite rate differed between treatments and appeared to decrease during the GS (Table 2). Deprivation has been reported to increase bite rate (Dougherty et al., 1989; Patterson et al., 1998; Gregorini et al., 2007b). This was evident in the present study, with CTL cows having lower bite rates than 1×8 and 2×4 cows. However, there was not a clear effect of deprivation (time since the last meal) on bite rate when comparing 1×8 and 2×4 . In fact, bite rate was greatest for 2×4 cows for most of the GS. This difference and the fact that cows in 1×8 had a greater herbage intake during the GS diminish the importance of bite rate as a compensatory mechanism and emphasize the importance of

bite mass and eating time in modulating intake rate. These results are supported by the findings of Kennedy et al. (2009), who reported no effect of restricting access time to pasture on mean grazing bites per min and the previous works of Gregorini et al. (2007a) and Gregorini et al. (2009), where beef heifers and dairy cows utilized eating time and bite dimensions, instead of bite rate, as compensatory mechanisms to increase intake rate.

The dynamics of herbage disappearance (Table 3 and Figure 1) can be interpreted and cautiously used as an estimate of herbage intake rate dynamics during the GS. At the beginning of the GS (time 0), restricted cows started consuming herbage at a rate twice as fast as CTL cows. This difference may be supported by variations in bite mass, bite rate, and eating time at the beginning of the GS. Despite the likely lower hunger level of cows in 2×4 compared with the 1×8 cows at the start of the GS, they were not different in herbage disappearance rate. The lower ghrelin and NEFA concentrations in 2×4 cows (Table 4) may indicate that temporal perception (learned expectations) rather than a hunger-related hormone-metabolic signal plays an important role in driving greater herbage intake rates at the beginning of a GS, when a new pasture is allocated. By time 1 (1 h after GS started) the herbage disappearance rate decreased for all treatments, which also matches changes occurring in bite mass, bite rate, and eating time. In this case, cows in 1×8 showed the highest herbage disappearance. This supports previous comments regarding the longer lasting effect of deprivation compared with a net number of hours of available grazing time on the animal's motivation to eat at the time to face the GS. Moreover, this evidence shows the short-term effect of the temporal perception mentioned before on initial herbage intake rates. At time 2 (2 h after GS started), cows in 1×8 continued having greater (2.5 times) herbage disappearance rates than cows in 2×4 . This trend further increased toward the end of the GS. At time 3.5, the herbage disappearance rate for 1×8 cows was 6.5 times greater than for cows in 2×4 . Such a dynamic may indicate a differential balance and strength of stimuli or motivation for cows to keep eating or to stop. Finally, CTL cows' smooth reduction of herbage disappearance rate could be explained by a gradual increase in satiety signals as the GS progressed, as suggested by Gregorini et al. (2007a) and Gregorini et al., (2009).

Ghrelin and NEFA Concentrations Before the First GS

The treatments applied in the present study led cows to face the GS with different hunger levels, which is

consistent with the differences in ghrelin and NEFA concentrations before the GS (Table 4) and the differences in herbage intake and foraging behavior during the GS. Ghrelin is a powerful orexigenic agent (Wren et al., 2000; Roche et al., 2008a), whereas NEFA is an index of the “readiness” to consume more food (Van Itallie and Hashim, 1960), an indirect indicator of energy status (Fox et al., 1991) and feeding frequency (Scrimgeour et al., 2008). For example, Scrimgeour et al. (2008) reported increased concentrations of NEFA when feeding pigs twice a day and further elevated concentrations when feeding them once a day compared with an ad libitum feeding regimen. Kolver and Mac-Millan (1993) reported that, under conditions of unrestricted access to pasture, grazing dairy cows might not have diurnal fluctuations in blood NEFA. These data highlight further the treatment effect found in this study. Nakazato et al. (2001) and Sugino et al. (2004) hypothesized about the possibility that the orexigenic property of ghrelin may be involved in determining feeding behavior mechanisms. The observable changes in foraging behavior during the GS in response to treatment are consistent with this hypothesis, which is supported further by the results of Gregorini et al. (2009). Within the context of the GS, the present results add more detail to these potential relationships.

CONCLUSIONS

Moderate restriction of available grazing time per se may have no effect on daily herbage intake if the amount of herbage allocated does not constrain cattle to accomplish compensatory foraging strategies. However, at the same restriction level, the timing of such a restriction may lead to differential food deprivation. This establishes a different internal state (set of positive and negative internal stimuli to eat) at the time of the first and most important grazing session, which changes their reaction (tactics of a determined/planned foraging strategy) to the perception of the same feed resource. Strategic management of such a reaction may help dairy farmers to better allocate time on pasture.

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