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A sensory additive alters grazing behavior and increases milk response to concentrate supplementation in dairy cows

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ABSTRACT

The aim of this study was to evaluate the effect of a sensory additive, incorporated into a starch-based concentrate, on milk production and composition and grazing behavior in pasture- fed dairy cows. Forty-five Holstein cows were used in 15 incomplete 3 × 2 Latin squares conducted concurrently with 3 treatments and 2 periods of 28 d At the beginning of the study, cows averaged 60 ± 17.1 d in milk, 2.2 ± 1.51 parity, 27.5 ± 4.52 kg/d of milk, and 504 kg ± 61.9 of BW (mean ± SD). Cows were assigned to the 15 squares by parity, milk yield, d in milk, and BW and within squares randomly assigned to 3 concentrate treatments that were 1 kg/d of a mineral concentrate (MC), 7 kg/d of a starch-based concentrate (CC), and 7 kg/d of CC supplemented with 30 g/d of a sensory additive (PEC; ProEfficient, Lucta SA, Barcelona, Spain). Cows grazed a perennial ryegrass pasture (*Lolium perenne* L.) offered at a daily allowance of 30 kg of DM per cow. Supplementation with the starch-based concentrate increased ($P < 0.05$) milk yield 4.4 kg/d compared with MC (24.3 .vs. 28.7 kg/d), whereas cows supplemented with PEC produced more ($P < 0.05$) milk and energy-corrected milk than CC cows (0.6 and 1.6 kg/d, respectively). As a result, milk response to concentrate supplementation (kg milk/kg concentrate) was improved by PEC. Additionally, PEC increased ($P < 0.05$) milk protein percentage (3.74 vs. 3.43%) and yield (1.08 vs. 0.98 kg/d) compared with CC. Concentrate supplementation increased ($P < 0.05$) total DMI, but reduced ($P < 0.05$) total daily grazing time (GT) and biting rate (BR) in the evening. Compared with CC, PEC did not affect ($P > 0.05$) total and pasture DMI but increased ($P < 0.05$) GT during the first 2 h after the a.m. milking as well as BR and ruminating time during the diurnal hours. The plasma concentration of active ghrelin was similarly reduced ($P < 0.05$) by CC and PEC after 2 h of grazing. In summary, supplementation of a starch-based concentrate with a sensory additive improved milk and protein responses of dairy cows grazing a ryegrass pasture.

1. Introduction

In pasture-based systems, low DM and ME intake are major limiting factors for milk production by dairy cows (Bargo et al., 2003). This limitation is frequently solved by offering supplementary feeds, increasing total DMI beyond that achieved when offering pasture alone (Bargo et al., 2003; Peyraud and Delagarde, 2013). A higher milk response to supplementation depends partly on the impact on pasture DMI, i.e. substitution rate (**SR**; Bargo et al., 2003).

The SR refers to the reduction in pasture DMI per kg of concentrate added to the ration and 50 it is affected by several factors as pasture quality and availability, amount and type of concentrate, etc. (Bargo et al., 2003). There are several theories that explain this reduction in pasture DMI when animals are supplemented, some of them related to changes in ruminal environment (Dixon and Stockdale, 1999) and a decrease in grazing time (McGilloway and Mayne, 1999). In fact, dairy cows can modify their grazing behavior, measured as grazing time (**GT**), biting rate (**BR**), and bite mass, to satisfy their nutritional needs (Taweel et al., 2004). Otherwise, it is accepted that DMI in ruminants is regulated by circulating neuroendocrine factors as ghrelin (Roche et al.,

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2008). Ghrelin is an acylated peptide that acts as an endogenous ligand for growth hormone secretagogue receptor (Sugino et al., 2004). It is produced mainly in the stomach and it is known as an orexigenic hormone (Sugino et al., 2004). In this line, Roche et al., (2007) reported that circulating ghrelin concentrations decline more rapidly in grazing cows that have received supplement before grazing, which is consistent with the more rapid cessation in grazing (Sheahan et al., 2013a). Therefore, neuroendocrine factors may be also involved in the mechanisms that potentially explain the SR.

Recent studies have determined that eating behavior can be modified by sensory additives. 65 Oro-sensorial stimuli may stimulate consumption by enhancing feed acceptability and consequently changes in feeding frequency and mechanisms involved in feed intake regulation can be altered (Villalba et al., 2011). Previous studies have been shown that when a sensory additive was included in a total mixed ration (**TMR**) fed to dairy cows, DMI and milk yield increased (Bargo et al., 2014, 2016). This was in line with the stimulatory action of the sensory additive on eating time (Bargo et al., 2016). In addition, Villalba et al. (2011) have associated changes in DMI of sheep receiving different sensory additives with alterations in the circulating satiety and hunger-regulating peptides. However, none of these previous studies were conducted with grazing dairy cows. We hypothesize that including a sensory additive into the concentrate supplemented to grazing dairy cows increase milk yield and pasture DMI through changes in grazing behavior and plasma ghrelin. Our objectives were to determine changes in milk yield and composition, grazing behavior, and circulating concentration of ghrelin when lactating dairy cows grazing a perennial ryegrass pasture were supplemented with a starch-based concentrate, either plain or treated with a sensory additive.

2. Material and methods

The experiment was conducted at the Experimental Research Station of the Universidad Austral de Chile (Valdivia, Chile; latitude 39°47′ S and longitude 73°14′ W) between October 23 and December 19, 2014. The average daily temperature was 12.5 °C and the average maximum and minimum were 19.4 °C and 6.1 °C, respectively. Procedures undertaken were approved by the Animal Welfare Committee of the Universidad Austral de Chile.

2.1. Cows and treatments

Forty-five Holstein cows [BW, 504 ± 61.9 kg; milk yield, 27.5 kg/d ± 4.52; parity, 2.2 ± 1.51; d in milk, 60 ± 17.1 d (mean ± SD)] were used in 15 incomplete 3 × 2 Latin squares conducted concurrently. This design consisted of three treatments and two 28-d experimental periods. Parity, milk yield, d in milk, and BW at the onset of the study were the criteria used for assigning cows to the 15 Latin squares. Within each square, cows were randomly assigned to treatments. The first period was conducted from October 23 to November 20 and the second period from November 21 to December 19. The incomplete Latin Square design with only 2 periods was chosen because in Southern Chile the spring season is usually short and pastures quality changes considerably after December. During each of the two 28-d periods, the first 21 d were used to adapt cows to treatments and the last 7 *d were used for experimental measur*ements. The three concentrate treatments were 1.0 kg/d of a mineral concentrate (**MC**), 7.0 kg/d of a starch-based concentrate (**CC**), and 7.0 kg/d of CC plus 30 g/d of a sensory additive [(**PEC**); ProEfficient (**PE**); Lucta SA, Barcelona, Spain)]. The PE additive is a mix of sensory additives from natural components (steviol glycosides and molasses flavor). Concentrates were offered in two equal parts at each milking (a.m. milking from 0630 to 0800 h and p.m. milking from 1530 to 1700 h). The MC was composed of 60% wheat/barley/oat mix, 10% rapeseed meal, and 30% of a mineral premix. The concentrate for treatments CC and PEC was composed of 65% corn, 13.5% triticale/oat/wheat mix, 10% soybean meal, 5% rapeseed meal, 2% sugar beet molasses, and 4.5% mineral premix. The composition of mineral premix included in the 3 treatments was 140 g/kg Ca, 30 g/kg P, 40 g/kg Mg, 20 g/kg S, 100 g/kg Cl, 120 g/kg Na, 0.4 g/kg K, 1,950 mg/kg Zn, 260 mg/kg Mn, 720 mg/kg Cu, 18 mg/kg Co, 15 mg/kg Se, 80 mg/kg I, 1,500 mg/kg Fe, 100,000 IU/kg vitamin A, 40,000 IU/kg vitamin D, and 500 IU/kg vitamin E.

A 20-ha perennial ryegrass (*Lolium perenne* L.) pasture was grazed. All treatments received a pasture allowance (PA) of 30 kg DM/cow once daily after the p.m. milking. Pasture allowance was determined by adjusting the daily grazing area according to the pasture mass, which was estimated from 100 compressed sward height measurements using a rising plate meter (Ashgrove Plate Meter, Hamilton, New Zealand). Plate meter measurements were made by walking along the pasture in a “W” pattern. These measurements were also repeated post grazing. The difference between pre- and post-grazing pasture mass was used to calculate pasture utilization.

2.2. Experimental measures and sample analyses

Pasture was sampled at 4 cm height in the afternoon before cows entered the new daily paddocks. Samples were frozen immediately, stored at −20 °C, and then freeze-dried for chemical analysis. Samples of concentrate were collected once a wk during each of the two 28-d periods of the study and dried for 48 h at 60 °C for chemical analysis. Pasture and concentrate were all analyzed for DM, ash, CP, ADF (AOAC, 1996), and NDF (Van Soest et al., 1991). 126 Soluble protein and water soluble carbohydrates were determined using near-infrared spectroscopy (NIRS). In vitro DM digestibility was estimated using an in vitro digestibility method with ruminal liquor according to Tilley and Terry (1963) as modified by Goering and Van Soest (1970). Pasture and concentrate chemical composition is presented in Table 1.

Cows were milked twice daily at 0630 and 1530 h and milk production was recorded daily in both periods. On d 25 and 26 of each period, milk samples were collected at the a.m. and p.m. milking for milk fat, milk total protein, and urea N analyses by infrared spectroscopy (Foss 4300 MilkoScan, FOSS Electric, Hillerod, Denmark). Cows were automatically weighed daily and BCS was recorded at the beginning and at the end of each period by two experienced observers using the five-point scale (Ferguson et al., 1994).

Dry matter intake was estimated using the method of fecal output and diet digestibility (Le Du and Penning, 1982). During the last 2 wk of

Table 1

Nutrient composition (SD in parentheses) of pasture and concentrates offered to dairy cows supplemented with a starch-based concentrate with or without a sensory additive (n = 4).

		Concentrate[a]		
Item[b]	Pasture	MC	CC	PEC
DM, %	20.3 (1.02)	89.1 (0.36)	87.1 (0.27)	87.2 (0.16)
% of DM				
Ash	9.5 (0.16)	29.9 (3.22)	7.1 (0.74)	7.2 (0.43)
CP	22.3 (0.84)	10.4 (0.48)	14.2 (0.62)	14.1 (0.41)
Soluble protein	8.2 (0.57)	–	–	–
Ether extract	3.1 (0.14)	2.3 (0.40)	4.5 (0.16)	4.4 (0.11)
NDF	41.9 (0.56)	17.1 (1.95)	14.7 (0.45)	14.6 (0.13)
ADF	20.1 (0.65)	7.3 (0.04)	5.9 (0.30)	6.1 (0.82)
WSC	10.8 (0.23)	–	–	–
IVDMD	77.7 (0.39)	64.2 (3.16)	88.8 (1.20)	88.9 (0.74)
ME, MJ/kg	11.68 (0.04)	9.91 (0.42)	13.26 (0.16)	13.26 (0.08)

[a] MC = 1.0 kg/d mineral concentrate; CC = 7.0 kg/d starch-based control concentrate; PEC = 7.0 kg/d starch-based control concentrate plus 30 g/d of sensory additive (PE).

[b] DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; WSC = water soluble carbohydrates; IVDMD = In vitro DM digestibility; ME = metabolizable energy.

each period, all cows received daily capsules containing 12 g of the indigestible fecal marker Cr_2O_3. Fecal grab samples were collected after each milking twice daily for 7 d, starting 7 d after administration, and immediately frozen at −20 °C. Fecal concentration of Cr was later determined by atomic absorption spectroscopy (Spectronic Genesys 5 spectrophotometer, Milton Roy, Ivyland, PA, USA). Total DMI was estimated from the fecal output and IVDMD of total diet as follows: total DMI = fecal output / (1 −IVDMD), where daily fecal DM output was calculated as Cr dose (g/d)/fecal Cr concentration in feces DM. Pasture DMI was determined by subtracting the known concentrate DMI from the total DMI as described by Bargo et al. (2002).

On d 22 and 23 of each period, grazing behavior was recorded via scan sampling over a 24-h interval by three trained observers in all cows every 10 min during daylight hours (diurnal: 0530 to 2150 h) and every 15 min at night (nocturnal: 2200–0515 h). The activities recorded were: grazing, ruminating (standing and lying), and idling. Individual cows were identified by large numbers painted on the sides of the cows. Cow's BR was recorded visually over one minute intervals with a hand-held counter to provide 2 measurements per d, one between 0900 and 1100 h and another between 1700 and 1900 h. If more than 10 s passed between bites, the observation was canceled and the counting process was restarted.

Coccygeal blood was sampled using vacutainers containing EDTA in the morning of d 28 of each period before milking and 2 h after feeding the concentrate to determine pre- and post- prandial plasma concentration of active ghrelin, respectively. Plasma was aspirated following centrifugation at 800 x *g* for 10 min at 4 °C. Prior to analysis, 10 μL of PMSF (10 mg/ml) were added to 1 mL of plasma and then centrifuged for 5 min, and 5 μL of protease inhibitors (AEBSF, Aprotinini, Bestatin, Leupeptin, Pepstatin A, E64, Thermo 87,786) were added to 500 μL of plasma. Plasma samples were frozen at −80 °C. Ghrelin was measured using an active ghrelin RIA kit (GHRA-88HK; Millipore Corp.) at DairyNZ (Hamilton, New Zealand). Furthermore, coccygeal blood sampled before the a.m. milking was analyzed for urea N (Stanbio Urea Nitrogen kit 580, Stanbio Laboratory, Inc., San Antonio, TX), NEFA (Wako NEFA C-Kit no. 990–75,401, Wako Chemicals USA, Inc., Richmond, VA), and β-hydroxybutyrate (**BHBA**).

2.3. Statistical analysis

Data were analyzed using a mixed-effect model for a 3 × 2 replicated Latin square that included the fixed effects of treatment, period, the two-way interactions (treatment x period), and the random effect of square and cow within square. Analyses were conducted using the Proc Mixed procedure in SAS v. 9.4 (SAS Institute Inc, Cary, NC). Least squares means and SEM are reported for all data. Treatment effects were compared across squares using the Fisher's LSD procedure in SAS. Differences among treatments were considered to be significant when $P < 0.05$, whilst trends were assumed when $P > 0.05$ and < 0.10.

3. Results

3.1. Nutrient composition of feeds

Nutrient composition of feeds is presented in Table 1. Pre and post-grazing pasture mass averaged 2557 and 1754 kg DM/ha respectively along the trial. According to chemical composition, pasture can be considered as a high quality pasture, with high level of CP and moderate proportion of NDF and ADF (Table 1). The concentrate MC had a high mineral content as expected while CC and PEC were similar since differences between treatments were only related to the inclusion of PE.

3.2. Dry matter intake and grazing behavior

Supplementation with starch-based concentrates increased ($P < 0.01$) total DMI compared with MC treatment by 1.4 kg/d (Table 3), but concentrate supplementation reduced ($P < 0.01$) pasture DMI (11.5 vs. 15.3 kg/d) compared with MC treatment. There was no effect ($P > 0.05$) of the sensory additive PE on pasture DMI (Table 3).

Grazing behavior was affected by treatments (Table 3). Total GT per day was greater ($P < 0.05$) for MC than PEC, but did not differ ($P > 0.05$) between MC and CC groups. Treatments did not affect ($P = 0.593$) diurnal GT, but cows supplemented with PEC grazed 15% more min ($P < 0.05$) than CC cows during the first 2 h after the a.m. milking (101 vs. 88 min). Treatments did not affect ($P = 0.921$) the GT during the first 2 h after p.m. milking (mean = 117 min). The PEC treatment increased ($P < 0.01$) diurnal BR compared with both MC and CC treatment (64 vs. 57 bites/min), but MC cows had the highest nocturnal BR, with CC intermediate, and PEC the lowest. Supplementation with PEC increased total ($P < 0.05$) and diurnal ($P < 0.01$) RT compared with MC and CC (455 vs. 405 min/d and 174 vs.141 min/d, respectively), while nocturnal RT did not differ ($P = 0.562$) among treatments (Table 3).

3.3. Plasma metabolites

Plasma metabolites are presented in Table 4. Concentrate supplementation increased ($P < 0.01$) plasma BHBA concentration, with BHBA from PEC cows also greater than measured in CC cows. In comparison, plasma NEFA concentration was less in cows receiving concentrates ($P < 0.01$), with no effect ($P > 0.05$) of PE addition on plasma NEFA concentration. There was no effect ($P = 0.463$) of treatment on blood urea N. All treatments had similar ($P = 0.174$) pre-prandial concentration of active ghrelin in plasma. Compared with MC, supplementation with both starch-based concentrates (CC and PEC) reduced ($P < 0.01$) the circulating concentration of active ghrelin 2 h post-feeding compared with the MC group (53.9 vs.−41.4 pg/mL).

3.4. Milk production and composition, body Weight, and body condition score

Supplementation with starch-based concentrates increased ($P < 0.01$) milk production (4.1 and 4.7 kg/d for CC and PEC, respectively) and energy-corrected milk (**ECM**) (3.3 and 4.9 kg/d for CC and PEC, respectively) compared with MC (Table 2). Cows supplemented with PEC produced more ($P < 0.01$) milk and ECM than CC cows. Supplementation with PEC also increased milk protein content ($P = 0.0245$), protein yield ($P < 0.01$), and fat yield ($P < 0.01$) compared with CC. Treatments did not affect ($P = 0.421$) milk fat content. The marginal milk (0.78 vs. 0.67 kg milk/kg concentrate) and ECM (0.80 vs. 0.59 kg ECM/kg concentrate) responses to concentrate supplementation increased 16.4% and 35.5%, respectively, when PE was included in the concentrate. Compared with MC, supplementation with starch-based concentrates reduced ($P < 0.01$) milk urea N, which reached the lowest value in the CC group (Table 3). Neither initial nor final BCS and BW were affected by treatments ($P > 0.05$).

4. Discussion

To our knowledge, this is the first study evaluating the effect of adding a sensory additive into the concentrate feed offered to grazing dairy cows. Research on sensory stimulation in dairy cows is limited and it has been mainly focused on animals fed TMR (Merrill, 2013; Iglesias et al., 2014; Bargo et al., 2014, 2016). In this study, cows supplemented with PEC produced more milk and ECM than those fed CC, resulting in an increase in marginal milk and ECM production responses to concentrate supplementation as well as greater milk fat and protein production. These responses were not associated with changes in measured DMI because both total and pasture DMI were similar between CC and PEC supplemented cows. The production increase without the increase in DMI is peculiar to this experiment. In a previous study under heat stress conditions with high producing dairy cows in

Table 2
Milk production and composition, BW, and BCS of grazing dairy cows supplemented with a starch-based concentrate with or without a sensory additive (n = 15).

Item	Treatment[d]			SEM	P-value[e]	
	MC	CC	PEC		Treat	Per
Milk, kg/d	24.3[c]	28.4[b]	29.0[a]	0.44	<0.01	0.64
ECM,[f] kg/d	26.8[c]	30.1[b]	31.7[a]	0.64	<0.01	0.71
Fat, %	3.96	3.75	3.83	0.12	0.42	0.51
Protein, %	3.65[ab]	3.43[b]	3.74[a]	0.08	0.03	0.68
Fat, kg/d	0.96[c]	1.07[b]	1.11[a]	0.02	<0.01	<0.01
Protein, kg/d	0.88[c]	0.98[b]	1.08[a]	0.03	<0.01	0.79
Milk urea N, mg/dL	12.6[a]	9.8[c]	10.9[b]	0.26	<0.01	<0.01
BW,[g] kg						
Initial	494	487	494	5	0.13	<0.01
Final	510	515	509	5	0.15	<0.01
Change	16[b]	27[a]	15[b]	3	0.01	<0.01
BCS,[h] 1 to 5						
Initial	2.92	2.88	2.93	0.03	0.32	0.44
Final	2.90	2.95	2.97	0.03	0.32	0.01
Change	−0.02	0.07	0.03	0.04	0.34	0.01

[a,b,c]Means within a row with different superscripts differ (P < 0.05).
[d] MC = 1.0 kg/d mineral concentrate; CC = 7.0 kg/d starch-based control concentrate; PEC = 7.0 kg/d starch-based control concentrate plus 30 g/d of sensory additive (PE).
[e] Treat = treatment effect, Per = period effect.
[c] Energy-corrected milk (ECM) = 0.327 × milk yield (kg) + 12.97 × fat (kg) + 7.20 × protein (kg) (DRMS, 2014).
[f] Body weight.
[g] Body condition score.

Table 3
Pasture DMI and eating behavior of grazing dairy cows supplemented with a starch-based concentrate with or without a sensory additive (n = 15).

Item	Treatment[a]			SEM	P-value[b]	
	MC	CC	PEC		Treat	Per
DMI,[c] kg/d						
Concentrate	0.9	6.1	6.1	–	–	–
Pasture	15.3[a]	11.8[b]	11.2[b]	0.573	<0.01	<0.01
Total	16.2[b]	17.9[a]	17.3[a]	0.574	<0.01	<0.01
Grazing time (GT), min						
Daily total	516[a]	499[a,b]	475[b]	16.6	0.02	0.89
Diurnal (0530–2150 h)	419	426	408	14.1	0.59	0.66
Nocturnal (2200–515 h)	97[a]	73[b]	67[b]	10.1	0.01	0.88
First 2 h after a.m. milking (0900–1100 h)	100[a]	88[b]	101[a]	5.6	0.04	<0.01
First 2 h after p.m. milking (1700–1900 h)	117	118	116	4.6	0.92	0.63
Biting rate (BR), bites/min						
Diurnal (0530–2150 h)	57[b]	56[b]	64[a]	2.8	<0.01	0.05
Nocturnal (2200–0515 h)	72[a]	65[b]	55[c]	1.9	<0.01	0.13
Ruminating time (RT), min						
Total per day	414[a]	405[a]	455[b]	19.5	0.02	0.07
Diurnal (0530–2150 h)	144[a]	137[a]	174[b]	11.3	<0.01	0.01
Nocturnal (2200–0515 h)	270	268	281	15.8	0.56	0.60

[a,b,c]Means within a row with different superscripts differ (P < 0.05).
[a] MC = 1.0 kg/d mineral concentrate; CC = 7.0 kg/d starch-based control concentrate; PEC = 7.0 kg/d starch-based control concentrate plus 30 g/d of sensory additive (PE).
[b] Treat = treatment effect, Per = period effect.
[c] Dry matter intake.

late lactation (Bargo et al., 2014), milk yield increased (P < 0.05) from 33.2 to 35.2 kg/d when the TMR was top-dressed with PE; this response was explained, at least in part, by an increase in total DMI of 1.2 kg DM/d or 5.1%. Merrill (2013) also reported a trend (P < 0.10) towards increased milk production (+3.9 kg/d or 9.4%) and DMI (+1.5 kg/d or 5.9%) in multiparous cows fed a TMR diet including PE. More recently,

Table 4
Plasma metabolites and acylated (active) ghrelin of grazing dairy cows supplemented with a starch-based concentrate with or without a sensory additive (n = 15).

Item	Treatment[d]			SEM	P-value[e]	
	MC	CC	PEC		Treat	Per
β-hydroxyburitate, mmol/L	0.538[c]	0.553[b]	0.582[a]	0.0214	<0.01	<0.01
NEFA,[f] mmol/L	372.5[a]	187.8[b]	163.6[b]	27.98	<0.01	<0.01
Blood urea N, mmol/L	1.918	1.845	1.895	0.6301	0.46	0.63
Acylated (active) ghrelin, pg/ml						
Pre a.m. supplementation	163.5	202.2	194.1	14.91	0.17	<0.01
2 h post a.m. supplementation	217.4[a]	171.4[b]	142.2[b]	15.93	<0.01	0.47
Change	53.9[a]	−30.8[b]	−51.9[b]	17.32	<0.01	<0.01

[a,b,c]Means within a row with different superscripts differ (P < 0.05).
[d] MC = 1.0 kg/d mineral concentrate; CC = 7.0 kg/d starch-based control concentrate; PEC = 7.0 kg/d starch-based control concentrate plus 30 g/d of sensory additive (PE)
[e] Treat = treatment effect, Per = period effect
[f] Non-sterified fatty acids.

Bargo et al. (2016) reported a cubic response (P < 0.01) in DMI and ECM of dairy cows fed with a TMR when PE dose was increased from 0 to 45 g/d. These previous data from literature may reflect differences in the underlying reasons for increased milk production in response to sensory stimulation by PE on grazing situation. In this sense, our results showed differences in terms of BW change, which may reflect a differential energy portioning across treatments. The PEC treatment had a higher BW loss compared to CC, potentially meaning that consumed energy was more partitioned to milk production rather weight gain.

A divergent effect of PE on DMI between feeding systems may, in part, stem from the fact that the sensory stimulation induced by PE is dissociated between the concentrate and forage fraction offered to grazing cows but not to TMR-fed animals. The lack of PE consumption throughout the day in grazing dairy cows could reduce the overall effect on DMI. However, if true, these results indicate a non-DMI mediated effect of PE on milk production efficiency that has not been elucidated. Other possible explanations for the lack of effect of PE on pasture DMI in this study relate to 1) the inability of dairy cows to increase DMI in pasture-based systems, which is dictated by many variables (e.g. pasture allowance, level and type of supplementation, SR, etc.) that ultimately affect grazing behavior (Bargo et al., 2003; Gregorini, 2012), and 2) the inability to measure pasture DMI accurately in grazing dairy cows. To determine whether pasture DMI is actually affected by PE supplementation or whether the increased marginal milk response reflects a non-DMI effect, further research involving feeding cows with different amounts of pasture indoors will need to be undertaken.

In the study reported herein, supplementation with starch-rich concentrates reduced pasture DMI when compared to the MC-fed group. It is well known that supplementation with concentrate increases total DMI and reduces pasture DMI in grazing dairy cows, which is known as SR (Bargo et al., 2003; Peyraud and Delagarde, 2013). Furthermore, it has been proposed that reduced GT is a mechanistic component of SR (Bargo et al., 2002). These points are consistent with the finding that MC cows registered both the longest GT and highest pasture DMI. Interestingly, PEC-fed cows dedicated more time to graze during the first 2 h after the a.m. milking and had a higher diurnal BR than their CC counterparts. These differences in grazing behavior indicate a more intensive grazing session during the morning in the PEC group compared to the CC group. Although no measures were done related to nutrient intake, we hypothesize that these changes may affect the composition of the pasture consumed.

Changes in eating behavior elicited by the feeding of PE were also reported in previous studies with non-grazing dairy cattle. Mereu et al. (2011) found that in 8 out of 19 experimental d, calves fed PE took 6% more ($P < 0.05$) meals than those fed the control diet (10.3 vs. 9.7 meals/d). Furthermore, PE calves increased ($P < 0.05$) intake rate compared with control animals (20.2 vs. 18.9 g/min, respectively). Iglesias et al. (2014) reported that non-lactating Holstein cows reduced ($P < 0.05$) the time dedicated to eating (119 vs. 149 min/day), but increased ($P > 0.05$) eating rate (106.4 vs. 85.3 g/min) in response to PE supplementation of the TMR. In a more recent study (Bargo et al., 2016), lactating Holstein cows fed with a TMR increased the time dedicated to eating (229 vs. 198 min/day) in response to PE supplementation of the TMR. In our study, the lack of differences in grazing behavior during the afternoon between PEC and CC might be related to the fact that grazing activity is much more intense in the afternoon prior to the sunset (Taweel et al., 2004; Gregorini, 2012) and there is less ability to increase activity. Collectively, the data indicate that PE results in changes in sensory stimulation and eating behavior; but, any effect on pasture DMI requires further experimentation.

If we assume that PE did not affect pasture DMI, such a non-DMI related effect of a sensory additive is intriguing. Considering that after the a.m. milking cows returned to a previously grazed paddock, we hypothesize that the PE-driven changes in grazing behavior could have affected nutrient intake via changes in selectivity and grazing pattern as well as pasture chemical composition. More precisely, it is likely that PEC cows consumed less N, less non-fiber carbohydrates, and more effective fiber than CC cows as a consequence of a more intense grazing session during the first 2 hours after the a.m. milking. The increase in effective fiber intake is supported by the observation that feeding PEC rather than CC increased both total and diurnal RT. Concentrate supplementation has been reported to reduce the digestibility of forage fiber (Bargo et al., 2003). An increase in RT as a result of the proposed change in nutrient intake might have resulted in a more favorable ruminal environment for fiber digestion, thereby enhancing the productive response of cows to concentrate supplementation, despite similar pasture and total DMI. Indeed, the increase in milk protein output driven by PEC suggests increased microbial protein synthesis due to a better use of energy and N by ruminal microbes (Pacheco and Waghorn, 2008). However, this hypothesis must be proven.

Previous work suggests that the feeding of sensory additives to ruminants can affect ghrelin secretion (Villalba et al., 2011). Ghrelin achieves its highest concentration in plasma prior to a meal event and begins to decrease as the active GT elapses (Roche et al., 2007; Sheahan et al., 2013a, b). Several experiments have reported a positive correlation between the concentration of ghrelin and DMI in dairy cows (Roche et al., 2006; Sheahan et al., 2013b) and beef cattle (Wertz-Lutz et al., 2006). The decline in acylated ghrelin 2 h after the morning feeding in the supplemented treatments is likely a result of the concentrate supplementation in agreement with Roche et al. (2007). High circulating levels of ghrelin have been associated with a deficient supply of energy (Roche et al., 2006; Wertz-Lutz et al., 2006; Sheahan et al., 2013b), which is consistent with the higher NEFA concentration in MC cows compared with both starch- supplemented groups. Villalba et al. (2011) reported that the feeding of a sensory additive similar 311 to PE to sheep increased plasma ghrelin and DMI. In the study reported herein, PE supplementation did not affect circulating concentrations of acylated ghrelin. This observation is consistent with the lack of differences in DMI and circulating NEFA (indicative of similar energy balance) between cows from those groups and supports the non-DMI effects of PE supplementation on milk production. As proposed in the previous paragraph, the contradictory highest level of plasma BHBA in PEC cows may have resulted from improved ruminal fiber fermentation and absorption of butyrate. These results suggest that the mode of action of PE in grazing dairy cows does not involve changes in plasma ghrelin. However, it is important to consider that because ghrelin concentration varies during the day (Sugino et al., 2004) the times chosen for blood sampling could have masked changes in circulating ghrelin in response to PEC.

5. Conclusions

In line with the literature, the feeding of a starch-rich concentrate to pasture-fed cows increased total DMI and milk production, but reduced GT and pasture DMI. When PE was added to the starch-rich concentrate, milk and ECM production and protein content were increased further, although DMI remained unaltered. These productive responses were associated with increased GT and BR during the first 2 h of grazing after the a.m. milking. As suggested by the longer RT, such changes in grazing behavior may have allowed cows to consume more effective fiber, ultimately improving ruminal fermentation.

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