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Rumen function and grazing behavior of early-lactation dairy cows supplemented with fodder beet

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ABSTRACT

Fodder beet (FB) is a source of readily fermentable carbohydrate that can mitigate early spring herbage deficits and correct the negative energy balance experienced during early lactation in pastoral dairy systems of New Zealand. However, the low-fiber and high-soluble carbohydrate content of both FB bulb and spring herbage are factors that promote subacute ruminal acidosis, impairing rumen function and limiting the marginal milk production response to supplement. In a crossover experiment, 8 Holstein Friesian \times Jersey early-lactation dairy cows were used to test the effect of supplementing 16 kg of dry matter (DM) of a grazed perennial ryegrass herbage with 6 kg of DM/d of FB bulb (FBH) versus herbage only (HO) on changes in rumen function and grazing behavior. Following 20 d of adaptation to diets, DM disappearance (%) of FB bulb (FBH cows only) and herbage were measured in sacco, separately. Cows were fasted overnight, and the ruminal contents were bailed the following morning (~ 0930 h) again to determine the pool size of volatile fatty acids, ammonia, and particle size of digesta, as well as to estimate the rate of ruminal outflow and degradation of neutral detergent fiber. The FBH diet did not alter DM intake, milk yield, or milk solid (fat + protein) production compared with HO. Supplementation of herbage with FB reduced ruminal pH compared with HO between ~0800 h and 1300 h each day. During each period, 1 cow experienced severe subacute ruminal acidosis (pH <5.6 for >180 min/d) during final adaptation to the target FB allocation. The FBH diet reduced the ruminal pool of acetate and ammonia, but increased the ruminal pool of butyrate and lactate compared with HO. When fed FB, rumination and grazing time increased and grazing intensity declined compared with cows fed HO. Despite increased rumination, the comminution of large particles declined 28% between the first and second rumen bailing when cows were fed FB, and in sacco DM disappearance of perennial ryegrass declined 18% compared with cows fed HO. These results indicate that grazing dairy cows supplemented with FB (40% of daily intake) increase rumination and mastication intensity to counteract reduced ruminal degradation of ryegrass herbage due to low ruminal fluid pH.

Key words: grazing dairy cow, fodder beet, digestion, fractional neutral detergent fiber degradation, particle comminution

INTRODUCTION

Fodder beet bulb (**FB**; *Beta vulgaris* L.) is a sugardense supplement used to increase the energy intake of lactating dairy cows. In Europe and the United States, FB pulp is fed as a byproduct of sucrose, extracted from fresh fodder beets for human consumption or ethanol production. In Europe, older FB varieties such as Mangelwurzels have been grown and fed to livestock since the 18th century. However, the widespread use of FB to mitigate seasonal herbage deficits in New Zealand has occurred within the last 10 yr (Waghorn et al., 2019; Dalley et al., 2020). The popularity of FB in New Zealand is driven by the potential to grow large yields (>20 t of DM/ha) of highly utilizable (>90%) bulb with ~ 4 t of DM/ha of a leaf that senesces in winter. Fodder beet is sown in spring, and moderate allocations (<40% DMI) are grazed during late lactation. In winter, gradual transitioning feeding regimens are used widely by the dairy industry, enabling nonlactating dairy cows to graze large quantities of FB (>70% DMI) that were previously thought to be fatal to livestock (Chakwizira et al., 2013; Gibbs, 2014; Saldias and Gibbs, 2016). The residual FB bulb from winter is also harvested and used to supplement the spring herbage supply to return the paddock to pasture and improve the postpartum negative energy balance of early-lactation dairy cows. In New Zealand, harvested FB bulb is generally fed to dairy cows on the paddock using a silage wagon. Feed

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pads are uncommon in the low-input and minimal-infrastructure pastoral dairy systems, which predominate the industry. However, both the CP (<10% DM) and fiber (<20% DM) content of FB bulb are inadequate for lactating dairy cows. The latter, in conjunction with the high water-soluble carbohydrate content (WSC: >60% DM) of FB, are risk factors for ruminal acidosis (Waghorn et al., 2019; Dalley et al., 2020). Ruminal acidosis interferes with healthy rumen function and can impose considerable animal welfare and economic costs to farmers. The physiological mechanisms that regulate ruminal pH and the effect on cow health and rumen function have been explored extensively (Owens et al., 1998; Plaizier et al., 2008; Zebeli and Metzler-Zebeli, 2012). Briefly, SARA is caused by the rapid accumulation of VFA in the rumen when feeds rich in readily fermentable carbohydrates such as FB, are consumed. The accumulation of VFA causes the pH and buffering capacity of the rumen to decline episodically (Owens et al., 1998; Plaizier et al., 2008), but is generally selfcorrected. Severe ruminal acidosis can occur when the pH declines below 5.0 because the growth of lactic acid (10-fold the acidity of other VFA)-producing bacteria increase and cause a rapid deterioration of ruminal pH that the cow is unable to correct (Owens et al., 1998). Despite careful transitioning and individualized feeding of FB, acute and subacute ruminal acidosis have still been reported in both late-lactation and nonlactating dairy cows fed either straw and FB or harvested herbage and FB (Waghorn et al., 2018, 2019). However, changes to ruminal fluid pH and the risk of ruminal acidosis have not been reported for grazing dairy cows supplemented with FB during early lactation.

Comminution of forage starts physically via oral processing during ingestion and continues with rumination of the regurgitated boli. Physical processing of ingesta aid microbial attachment and the chemical degradation of digesta. Microbial degradation of fiber declines in vitro and in vivo when ruminal pH is below 6.2 (Terry et al., 1969; de Veth and Kolver, 2001; Krajcarski-Hunt et al., 2002). The growth of fibrolytic and cellulolytic microbes decline under low pH conditions due to the increased energy needed to maintain intracellular pH. Reduced growth of cellulolytic and fibrolytic communities can lead to their washout from the rumen and reduces the degradation of structural carbohydrates (Russell and Wilson, 1996). The severity of SARA is defined by the duration below a certain pH, which can impair microbial metabolism in mild cases (pH < 5.8 for >180 min/d), reducing the rate of rumen degradation and limiting milk production (Gozho et al., 2005). However, in severe cases, when pH declines below 5.6 for >180 min/d, long-term structural damage to the rumen epithelium can reduce the cow's ability to neutralize ruminal VFA, further increasing the risk of SARA. Long-term structural damage to the rumen epithelium can also limit the absorption of VFA needed for metabolic and production purposes (Gozho et al., 2005; Zebeli et al., 2008).

Physical comminution of forage encourages microbial adhesion to ingesta and increases the outflow of digesta from the rumen. Mastication and chewing also promote salivation, which contains buffers that neutralize the pH of ruminal fluid and encourage VFA absorption from the rumen (Mertens, 1997). Although grazed herbage may require considerable oral processing and salivation before swallowing, the occurrence of moderate and severe SARA in grazing dairy herds is still widespread (Garrett et al., 1999; Bramley et al., 2013). Supplementation of readily digestible perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.) herbage with FB will further dilute the dietary fiber fractions and its effectiveness. Recent indoor experiments by Waghorn et al. (2019) and Pacheco et al. (2020) suggest that supplementation of perennial ryegrass herbage with moderate FB proportions will impair rumen function and health, affecting performance. However, research on the effects of FB feeding in grazing studies is limited, and the effect on rumen degradation and ingestive and digestive processing of a perennial ryegrass-based diet during early lactation has not been explored.

The objective of this study was to evaluate milk production, grazing behavior, and digestive processes when a perennial ryegrass herbage is supplemented with moderate amounts (40% of total DMI) of harvested FB during early lactation. We hypothesized that supplementing perennial ryegrass even with moderate amounts of FB would increase the duration of time that ruminal fluid pH was below 5.8, impairing rumen function by reducing the ruminal digestion of perennial ryegrass and herbage intake by reducing grazing time.

MATERIALS AND METHODS

The experiment was conducted during October and November of 2018 at the Lincoln University Research Dairy Farm (LURDF) in Canterbury, New Zealand (43°38'S, 172°27'E). All procedures were approved by the Lincoln University Animal Ethics Committee (AEC 2018-22). The current study is a continuation of previous research that investigated the effect of FB supplementation on diet adaptation and milk production where cow management, experimental design, treatments, and management of forage and FB have been described previously (Fleming et al., 2020b).

Animals, Experimental Design, and Treatments

Eight spring-calving, multiparous Holstein Friesian \times Jersey dairy cows fitted with a rumen cannula were stratified into 2 groups based on DIM (30 \pm 11.6, mean \pm SD), milk yield (27.4 \pm 5.25, kg/d), and liveweight (482 \pm 50.0, kg). Two treatments of either (1) an herbage-only (HO) control consisting of ~ 30 kg of DM (above ground) per cow per day of an established perennial ryegrass and white clover sward or (2) 6 kg of DM of harvested FB fed after morning milking and an allowance of 30 kg of DM per cow per day (above ground) of the same herbage (**FBH**) were randomly allocated to each of the 2 groups. The crossover experiment was conducted over two 20-d periods, separated by a 5-d (washout) period, during which all cows were returned to herbage to prevent first-order carry-over effects (Senn, 2002). Individual cows were the experimental unit as they grazed in individual paddocks (~ 60 m^2) and were fed FB individually. During each period, cows on the FBH diet were transitioned to target FB intake ($\sim 40\%$ of DMI) over 12 d by increasing the allocation of 0.5 kg of DM/d following industry guidelines (Gibbs, 2014; Dalley et al., 2020). Cows were then adapted to the FBH diet between 12 and 17 d, and response variables were collected between 18 and 20 d. Cows were milked twice daily at approximately 0700 and 1600 h and had free access to fresh water from portable troughs at all times except during milking.

Management of FB

The FB ('Enermax. D') were sourced from another farm and transported to the experimental site before the commencement of each period to maintain the bulb's chemical composition (Fleming et al., 2020b). Fodder beet was harvested, and residual leaf was removed before transportation. Fodder beet bulbs were allocated to each cow in the FBH group individually, following the morning milking in plastic bins on a concrete feed pad. Cows remained on the feed pad for up to 2 h or until completion of the FB meal before returning to a fresh paddock of herbage. Cows fed the HO diet returned to a new allocation of herbage following milking each morning. Daily refusals of FB were collected and weighed to estimate daily FB intake.

Herbage Management

The sward allocated to all cows was dominated by perennial ryegrass (90.2 \pm 3.41%; mean \pm SE) and contained minor percentages of broad-leaved weeds (6.8 \pm 2.42%; predominantly *Rumex obtusifolius* L. and

Taraxacum officinale), white clover $(3.4 \pm 2.81\%)$, and dead material $(2.9 \pm 1.71\%)$. Pastures (~3.0 ha each) were divided longitudinally into eighths using temporary fencing materials. The areas were used to graze the 8 cows over 6 to 7 d by further dividing each strip into individual paddocks. Each paddock was grazed and fertilized with urea ($\sim 100 \text{ kg/ha}$) between 3 and 4 wk before the experiment. Pre- and postgrazing heights were measured each day to estimate herbage mass and herbage allocation. Herbage mass was estimated using compressed height measured with a rising plate meter (Jenguip Ltd.). The botanical and chemical composition of the sward was determined on d 17, before allocation to cows, by collecting random grab samples of herbage by hand (n = 5 per break) at grazing level (~ 3 cm above ground). Before the beginning of the experiment, approximately 20 rectangular quadrats (0.2 m^2) were collected from each paddock, and an additional 2 quadrats from each allocation were collected every third day of the experiment. Samples were washed, oven-dried, and weighed to determine DM content and to develop a regression equation for estimating herbage mass using sward height, as reported previously (Fleming et al., 2020b).

Daily intake of DM (kg/cow) and herbage were calculated from daily energy output and maintenance requirements minus the average daily loss of body condition (or plus the average daily gain of condition, assuming 1 BCS = 32.5 kg) during the experimental period (Roche et al., 2005). The energy calculations that were used have been reported previously by Holmes et al. (2002). The ME requirements for maintenance of lactating dairy cows was 0.6 MJ/kg of BW^{0.75} (Holmes et al., 2002). The energy output from milk was calculated based on daily fat and protein content and total milk yield (kg), assuming a conversion efficiency of net energy to milk energy of 65%, and the efficiency of liveweight mobilization was 80%:

DMI = {[(Lactation energy + maintenance energy + walking energy) - BC loss + BC gain]
- (FB intake × FB ME)}/Herbage ME concentration,

where BC = body condition. Cows were situated adjacent to the milking shed on flat terrain and were assumed to walk approximately 1 km/d while walking to the milking parlor and grazing [liveweight × $(0.026 \times horizontal \text{ km})/\text{k}_m$], where k_m is the efficiency of ME utilization calculated by ME concentration of the diet [(ME × 0.02) + 0.5] (Nicol and Brookes, 2007). The

calculated ME intake from FB was subtracted from the total apparent energy intake and divided by the ME concentration of herbage to calculate DMI.

Plant Subsampling and Analyses

Hand grab samples of herbage were bulked, homogenized, and separated into thirds to determine DM percent (oven-dried at 60°C for 48 h) and chemical and botanical components. Botanical components were sorted (perennial ryegrass, white clover, weeds, and dead material) and oven-dried to calculate relative abundance in the sward. The third sample was frozen $(-20^{\circ}C)$ and stored until freeze-dried, and then ground through a 1-mm sieve (ZM200 Retsch GmbH). Chemical components (CP, ADF, NDF, and OM) were determined by near-infrared spectroscopy (NIRS; FOSS NIRS Systems 5000). Three FB were randomly selected from the face of the stack to analyze DM and chemical composition (Fleming et al., 2020b). Briefly, FB were cut into 4 equal sections by halving the bulb lengthways and then halving lengthways again. Each section was minced separately using an electric hand blender. One section was weighed and oven-dried (100°C) over 72 h, and the second was frozen $(-20^{\circ}C)$ and stored until freeze-dried, ground through a 1-mm sieve, and analyzed for chemical components (CP, ADF, NDF, and OM) using NIRS. Calibration equations for predicting WSC, CP, ADF, NDF, and OM of FB were developed previously on samples of FB. The R-squared values for CP, OM, WSC, NDF, and ADF of both FB and perennial ryegrass herbage were all above 0.90, and all samples were within the calibration range. The ME content of forages was calculated using the modified ADF method as follows: ME (MJ/kg of DM) = 14.55 $-0.015 \times \text{modified ADF}$ (CSIRO, 2007).

Cow Measurements and Sample Analysis

Liveweight and milk yield (kg) were measured automatically at each milking (DeLaval Alpro Herd Management System, DeLaval). The BCS of each cow was assessed and recorded by a certified BCS assessor (DairyNZ Ltd.) on d 0 and d 20 of each experimental period using a 1 to 10 scale (Roche et al., 2004). Milk samples from individual cows were collected using inline milk meters from 2 consecutive milkings (pm of d 19 and am of d 20) to determine the proportion and yield of protein, fat, lactose, and milk solids, which was analyzed by the laboratory of Livestock Improvement Corporation Ltd. using Milkoscan (Foss Electric). A skim milk sample was frozen at -20° C until analyzed for MUN by Randox RX Daytona analyses.

Ruminal pH and Rumen Sampling

To determine the treatment effect on ruminal contents, the pH of ruminal fluid was measured every 10 min using a wireless bolus (SmaXtec Animal Care GmbH) as described by Fleming et al. (2020b). On d 20 of each period, cows were herded to the vards for rumen sampling every 4 h, a procedure which took approximately 40 min between leaving and returning to herbage areas. Once in the yards, random hand grab samples of rumen digesta were collected from the ventral sac of the rumen. Digesta was filtered through 2 layers of an open-weave cloth (Superwipes, Clorox) into two 2-mL microtubules to measure ammonia $(NH_3; acidified with$ 6 M sulfuric acid) and VFA concentration and were stored at -20° C until assessed. The concentration of VFA was determined by gas chromatography using an SGE BP21 (30 m \times 530 µm \times 1.0 µm wide-bore capillary column) using an autosampler (AOC-20i) fitted to a Shimadzu GC-2010 gas chromatograph (Shimadzu) following the method of (Chen and Lifschitz, 1989). Ammonia and L-lactate concentrations of rumen fluid were determined enzymatically using commercially available kits from Randox Daytona.

In Sacco Incubation

Samples of perennial ryegrass and FB bulb were incubated in separate Dacron bags (Custom Advanced Connections; 10×15 cm with 50-µm pore size) in cows on the FBH treatment, and only samples of ryegrass were incubated in cows on the HO treatment. Before incubation, perennial ryegrass was collected in the morning, mixed, subsampled, and weighed into Dacron bags. One subsample was used to determine DM percent, and a second subsample was stored at -20° C until NIRS determined chemical components. Fodder beet bulbs were processed as described previously. Samples of FB and perennial ryegrass were separately blended to <5 mm (to imitate mastication) and incubated in sacco, following procedures adapted from Barrell et al. (2000). Approximately 40 to 60 g of fresh FB or perennial ryegrass was weighed into each Dacron bag, cable-tied to a galvanized chain, and frozen at -20° C until incubated. Dacron bags were removed after 0, 4, 8, 12, 16, and 20 h of incubation. Each chain could fit within a 5-L bucket to reduce the effect on rumen fill on DMI. Each cow received 1 metal chain anchor suspended inside the rumen at 0400 h on d 20 of each period. The collection of Dacron bags from the rumen occurred simultaneously as the collection of rumen digesta samples at 0400, 0800, 1200, 1600, 2000, and 2400 h. Upon removal, rumen bags were submerged in ice water, and excess digesta was removed. Then, bags were machine rinsed using a cold wash cycle for 10 min and oven-dried at 60° C for 72 h to calculate residual DM.

Rumen Bailing and Particle Distribution

The rumen contents were bailed at ~ 0000 h on d 20, and the digesta was removed from each cow and placed into individual large 50- to 80-L bins to estimate the outflow of solid digesta. Rumen bailing took place at midnight following the method of Taweel et al. (2005). Cows were fasted following the dusk grazing bout's completion to prevent carry-over effects (Gregorini et al., 2009a). During rumen bailing, 1 subsample was collected from approximately every 20 hand grab samples, including liquid contents, to gather a representative sample of the liquid and solid components. Once empty, the rumen digesta was weighed and recorded (including the bulk grab sample) and sequentially returned to the rumen. Cows were fasted indoors until ~ 0930 h, after the morning milking, when the rumen bailing procedure was repeated before the cows were returned to their paddocks. The grab samples collected from each bailing session were filtered through 2 layers of an open-weave cloth (Superwipes, Clorox) to separate liquid and solid fractions and weighed. Samples of the solid fraction were collected to determine DM percent (by oven drying at 100°C for 72 h), chemical components (OM, NDF, ADF, and ADL), and particle size (by wet sieving). A sample of solid rumen digesta was freeze-dried and ground to pass through a 1-mm sieve, and the proportion of DM and OM was determined by oven drying at 100°C and ignition at 550°C. The content of NDF and ADF of rumen digesta were analyzed following previous methods (Van Soest et al., 1991). The ADL component was determined following the ADF step, by mixing the residual pellet with 72%sulfuric acid (Möller, 2009). Components of ADF and ADL were determined from separate samples, and the content of both NDF and ADF is expressed as residual ash.

An estimate of the ruminal NDF outflow and fractional degradation rate was calculated using the logarithmic transformation of the below equation, as described by Taweel et al. (2006).

$$\mathbf{R}_t = \mathbf{R}_o \times \mathbf{e}^{-\mathrm{CL} \times t}, \qquad [2]$$

where R_t is the amount of NDF present at the first bailing session at midnight, R_o is the amount of NDF remaining at the second bailing session, the next morning (0930 h), CL is the fractional outflow of NDF (%/h), and t is the time between the 2 evacuations in hours. The fractional ruminal outflow of NDF was corrected using ADL, assuming that ADL is rumen undegradable and removed from the rumen via passage. Factional degradation of NDF may be underestimated using this method, as ADL may pass through the rumen at a rate greater than NDF (Tamminga et al., 1989). The suitability for using ADL as an internal marker has been previously addressed (Taweel et al., 2005). The pool of rumen fermentation end products was determined by multiplying individual VFA concentration by the rumen liquid pool.

Particle size fractions of rumen digesta were determined by wet sieving using the method of Waghorn et al. (1986). One sample (~ 30 g) was weighed and oven-dried at 100°C for 48 h to determine DM percent. Samples of the rumen digesta collected from each cow at each rumen bailing were duplicated and washed for 5 min under a recirculating flow (Waghorn et al., 1986). Digesta was passed through 6 metal sieves with apertures of 4, 2, 1, 0.5, 0.25 mm, and 75 μ m, in order. Following the 5-min wash period, the water flow was turned off, and the contents of each sieve were transferred to filter paper oven-dried at 100°C over 24 h to measure dry weight. The DM proportion on each sieve was calculated, and soluble fractions were calculated as the difference between pre- and postsieving DM weight. Fractions of particle components were multiplied by the DM pool of rumen digesta at each bailing session to determine the DM pool of large (>2 mm) medium (<2)mm and >0.5 mm), and small (<0.5 mm) particles.

Grazing Behavior

On d 16, once FBH cows had consumed the maximum FB allocation for at least 4 consecutive days, a jaw movement recorder (UltraSound Advice) was fitted to each cow to record individual jaw movements over 24 h. Jaw recorders consisted of a transducer that formed a noseband that recorded the electrical resistance as the jaw opened and closed to a microcomputer containing a data logger, a memory card, and a battery (Rutter et al., 1997). Prehension, mastication, and individual boli were differentiated automatically using the GRAZE software (v. 0.8, Institute of Grassland and Environmental Research 1994–1999), which automatically analyzes jaw movements into bite data (Rutter, 1998). Length of grazing, rumination, or FB bouts was determined by manually analyzing jaw amplitude and identifying the start and end of each bout. The minimum interbout length required between grazing bouts was 420 s (Rutter et al., 1997). Grazing data included the period of grazing, rumination, idling, and eating supplement, and counts of prehension, mastication, and rumination boli while grazing, ruminating, or eating FB.

Statistical Analysis

The statistical analyses compared the 2 treatments using a mixed model ANOVA with the lme function of the lme4 package (Pinheiro and Bates, 2018) in R (v. 3.4.4., https://www.r-project.org/). For discrete data (e.g., the number of mastications, prehensions, and the number of rumination boli per day), a generalized linear mixed model with a Poisson distribution using the glmer function of the nlme package was used to conduct statistical analyses. The remaining data (continuous) was analyzed using a linear mixed-effects model. In both models, the individual cow was the experimental unit; diet, time (when appropriate), and period were fixed effects; and individual cow nested within day was the random effect. Apparent rumen DM disappearance was measured over 20 h of incubation, which was not sufficient for complete degradation of fermentable material and did not provide enough time points to determine the disappearance rate using the model outlined by Ørskov and McDonald (1979). Therefore, rumen DM disappearance in sacco was considered as a factorial arrangement and analyzed using a mixed-effects ANOVA, where period and the interaction between plant (FB, FBH ryegrass, and HO ryegrass) and incubation time were fixed effects, and the cow was treated as a random effect. For all variables, the default, unstructured covariance structure of the nlme package was used because it produced the smallest Akaike information criterion when compared with other covariance structures. Least means squares were determined using the emmeans package (Lenth, 2018) of R, upon the significance of the ANOVA. Pairwise contrasts were determined using Tukey's method in the emmeans package to separate the means of significant interactions (P < 0.05). Differences were declared significant if $P \leq 0.05$, and tendencies were $0.05 < P \le 0.10$.

RESULTS

During each period, 1 cow from the FBH group developed SARA symptoms on d 10 of adaptation in period 1 and d 15 of adaptation during period 2 (pH <5.5 for 110 and 240 min/d, period 1 and 2, respectively). The allocation of FB to the 2 affected individuals was reduced to 3 kg of DM/d for the remainder of the experiment. Data collected for the 2 affected cows were included in statistical analyses as runnial pH stabilized without intervention, a characteristic of SARA.

Intake and Milk Production

Chemical composition of herbage fed to either FBH or HO treatments were not different (P > 0.05; Table 1). Fodder beet bulb contained lower proportions of NDF, ADF, CP, and N, but greater proportions of OM and WSC than herbage (P < 0.001).

Estimated DMI from energy output, liveweight, and milk yield are presented in Table 2. The average herbage allocation over both periods was similar between treatments. Fodder beet bulb represented 38% of daily DMI for the FBH treatment. The ME required (182 and 186 MJ of ME/d, FBH, and HO, respectively) and estimated DMI (15.6 and 16.2 kg of DM/d, FBH, and HO, respectively) were similar between treatments, although herbage intake declined 38% with the FBH diet. Milk yield was not different (P > 0.10) between treatments. The FBH diet did not (P > 0.10) alter the fat or protein proportions or yield in milk. The FBH diet reduced percentage (P = 0.01), but not (P = 0.24) yield of lactose (Table 2).

Rumen Pools of Digesta and VFA

The diurnal variation of ruminal fluid pH on d 20 is displayed in Figure 1. There was a diet effect between 0400 h and 1300 h (P < 0.001). Ruminal pH declined following the allocation of either herbage or FB in the morning. The FBH diet caused the pH of the ruminal fluid to decline to 5.6 by 1100 h compared with HO, in which pH declined to 6.0 by midday (P < 0.001). The pH of ruminal fluid measured in cows fed FBH remained below 5.8 between 0930 h to 1200 h each day, and ruminal fluid pH in cows fed HO remained above 5.8.

Solid, liquid, and fiber components of rumen digesta are presented in Table 3. There were no interactions between diet and time of rumen bailing on the solid or liquid proportion of digesta (P > 0.10). Total digesta weight declined 38% between the first and second rumen bailing (P < 0.001). The liquid and solid proportions of digesta were similar between treatments (P = 0.22), P = 0.43, respectively). The solid and liquid pools of digesta declined by 40% and 38%, respectively, between the first and second rumen bailing (Table 3). The FBH diet tended to increase the pool of NDF at the second rumen bailing session (P = 0.08) compared with HO, but did not alter the pool of DM, OM, ADF, or ADL (P > 0.10). The runnial outflow rate of NDF (48.6 and 60.3 ± 4.88 g/h, P = 0.81, FBH and HO, respectively) and ADL (9.88 and 9.91 \pm 1.98 g/h, P = 0.99, FBH and HO, respectively) were similar between treatments. The effect of dietary treatment on the fractional ru-

^{+e}Means within rows with different superscripts are significantly different (P < 0.05)

 $^{1}SE = standard error of estimated marginal means.$

 3 WSC = water-soluble carbohydrate.

²Herbage allocation above ground.

Table 1. Average pre- and postgraonly diet (HO) or herbage + FB di	azing mass and ch iet (FBH)	emical compos	ition ² (% of D	M unless oth	terwise noted)	of ryegrass her	bage and fodd	er beet bulb	(FB) fed as ei	ther herbage-
		Period 1				Period 2			P_{-V6}	due
	Herl	Jage			Her	bage				
Item	ОН	FBH	FB bulb	SE^1	ОН	FBH	FB bulb	SE	Plant	Period
Pregrazing (kg of DM/ha)	5,497	5453		86	3,478	3596		61	0.14	< 0.001
Postgrazing (kg of DM/ha)	2,823	3050		68	1953	2277		59	< 0.001	< 0.001
Area $(m^2/cow per day)$	53.6	52.2		1.06	76.3	73.4		1.71	0.18	< 0.001
Chemical composition										
DM	14.7°	$14.2^{\rm c}$	12.7^{a}	0.36	17.5^{d}	18.1^{d}	20.7^{b}	0.60	< 0.001	< 0.001
OM	$91.5^{ m b}$	91.4^{b}	94.2^{a}	0.26	91.8^{b}	$91.7^{ m b}$	93.7^{a}	0.29	< 0.001	0.75
ADF	21.0°	21.2°	7.81^{a}	0.123	$23.3^{ m d}$	$23.6^{ m d}$	$8.15^{ m b}$	0.130	< 0.001	< 0.001
NDF	$36.6^{ m d}$	37.7°	13.0^{a}	0.185	41.7^{e}	$41.8^{\rm e}$	14.0^{b}	0.241	< 0.001	< 0.001
WSC^3	$21.1^{ m b}$	$20.6^{ m b}$	63.9^{a}	0.39	20.5^{b}	$20.2^{ m b}$	59.4^{a}	0.41	< 0.001	0.15
CP	15.6	16.0	8.23	0.27	15.7	15.5^{b}	9.39^{a}	0.37	< 0.001	0.29
ME $(MJ/kg \text{ of DM})$	11.1^{a}	11.1^{a}	13.5^{b}	0.05	10.8°	$10.8^{\rm c}$	13.2^{b}	0.07	< 0.001	< 0.001
Ether extract	2.30°	2.72^{b}	0.59^{a}	0.084	2.12°	$2.54^{ m b}$	0.40^{a}	0.088	< 0.001	0.13

men degradation rate of NDF was not significant (P =(0.71), averaging 38.7 and 40.4 g of NDF/h for cows fed FBH and HO, respectively.

The concentrations of fermentation end products have been reported previously (Fleming et al., 2020b), and only a brief description of VFA concentrations have been reported. The FBH diet reduced acetate concentrations by 7%, isovalerate by 20%, and isobutyrate by 15% compared with HO (P < 0.01). The FBH diet increased butyrate concentrations by 21% (P = 0.006), valerate by 44% (P < 0.001) and caproate by 33% (P <0.001) compared with HO. The FBH diet reduced the volume of ammonia (33%, P = 0.04), acetate (10%, P< 0.001), isobutyrate (17%, P < 0.001), and isovalerate (22.2%, P < 0.001) compared with the cows fed the HO diet (Table 3). The FBH diet also increased the pool of butyrate (19%, P = 0.05), valerate (42%, P < 0.001), and caproate (31%, P < 0.001) in the rumen compared with the HO treatment. The pool of lipogenic (acetate + butyrate) VFA declined 18% with the FBH diet at the second rumen bailing session compared with HO (P< 0.01). The pool of glucogenic VFA (propionate and lactate) was not different (P = 0.47). However, diet by time interactions were significant (P = 0.014) for the ruminal pool of total volatile fatty acid (**TVFA**), which declined 15% with the FBH treatment at the second rumen bailing session, compared with HO (Table 3).

Diet by time interactions for lactic acid concentrations or pool in the ruminal fluid were not significant (P > 0.10). Although time by treatment interaction for the ruminal concentration of TVFA was not significant, a significant interaction between diet and time of rumen bailing was detected for the pool of TVFA in ruminal fluid (P < 0.001).

Particulate Pools, Turnover, and DM Disappearance from the Rumen

Particle DM fractions and particulate pools of rumen digesta are presented in Table 4. The DM proportion of large- and medium-sized particles increased, and the DM proportion of small particles declined (P < 0.001)between the first and second rumen bailing. The DM pool of large (P < 0.001) and small (P < 0.001) particle sizes also declined, and medium pools tended (P= 0.06) to decline between the first and second rumen bailing. There was an interaction between diet and time of rumen bailing for DM fractions retained on small, medium, and large sieve sizes (Table 4). Between the first and second ruminal bailing, the FBH diet increased the DM fraction of large particles by 25% (P = 0.01), and the DM pool of large particles increased 27% (P = 0.003) compared with HO. The proportion of small particles declined between the first and second rumen

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	Di	iet		<i>P</i> -value
Item	НО	FBH	SE^1	Diet
BCS (1–10 scale)	4.3	4.1	0.14	0.51
BCS change	0.1	0.3	0.20	0.65
Liveweight (kg)	497	499	6.4	0.45
ME required (MJ/d)	180	186	9.8	0.14
HI^2 (kg of DM/d)	16.2	9.9	0.89	< 0.001
FB refusal (kg of DM)	0	0.99	0.17	< 0.001
FB intake (kg of DM)	0	5.79	0.15	< 0.001
Estimated DMI (kg/d)	16.2	15.6	0.88	0.17
Milk component				
Fat (%)	5.09	5.05	0.364	0.92
Protein (%)	3.87	4.03	0.164	0.10
Lactose (%)	5.17	5.07	0.066	0.01
$MS^{3}(\%)$	8.96	9.07	0.471	0.82
Yield of milk components				
Fat (kg/d)	1.22	1.21	0.086	0.85
Protein (kg/d)	0.93	0.95	0.045	0.55
Lactose (kg/d)	1.26	1.22	0.087	0.24
MS (kg/d)	2.15	2.16	0.116	0.99
Milk (kg/d)	23.6	23.4	1.26	0.81

Table 2. Estimated intake of herbage (HI) and fodder beet (FB), total DMI, and yield of whole milk (kg/d) and milk constituents of cows fed either FB bulb + herbage (FBH) or a herbage-only (HO) diet

 ${}^{1}SE = standard error of estimated marginal means.$

 2 Herbage intake estimated from energy output in milk and maintenance requirements – the ME received from FB/ME content of herbage.

 $^{3}MS = milk solids (fat + protein).$

bailing (P < 0.001), and the FBH treatment increased the disappearance of small particles between the first and second rumen bailing by 12% compared with HO (P = 0.04).

Results of in sacco DM disappearance are presented in Figure 1. Diet by time interactions were significant (P < 0.001). The FBH diet reduced the extent of DM disappearance of perennial ryegrass by 18% following 20 h of incubation (P < 0.001). Plant by time interactions were detected at all time points for DM disappearance of herbage (FBH and HO) and FB. By 16 h of incubation, the FBH diet tended to reduce (P = 0.06) the DM disappearance of perennial ryegrass herbage and was significantly less (P < 0.01) by 20 h of incubation than perennial ryegrass incubated in cows fed the HO diet.

Grazing Behavior

Time spent grazing, ruminating, idling, and consumption of FB are presented in Table 5. Total eating time was 9.16 and 8.42 h/d for HO and FBH, respectively. The time spent eating FB represented 7% of total daily activity or 16% of eating activity. The FBH diet reduced the time spent grazing by 21% (P < 0.001) and increased rumination and idling time by 16% (P = 0.03) and 31% (P = 0.02), respectively, compared with the HO treatment (Table 5). The FBH increased rumination time, and the number of boli regurgitated each day was similar to the HO treatment (P = 0.28). However, cows fed FBH regurgitated an additional 104 boli/d compared with those fed HO. The FBH diet increased total mastication jaw movements per day by 5.5% (P < 0.001) compared with HO. The mastication of FB represented 14.6% of total mastications per day. The FBH diet increased chewing frequency while ruminating by 38% compared with HO (P < 0.001). In the FBH treatment, the number of mastications while grazing was 38% less than HO (P < 0.001). The FBH treatment did not alter the number of grazing, ruminating, or idling bouts compared with the HO diet (Table 5; P > 0.10). However, the duration of grazing bouts declined by 21% (P < 0.001) when cows were fed the FBH diet. During the FB meal, FBH led to ~ 3 bouts, which averaged 42 min each, and the average number of mastications during each FB eating bout was equal to the number of chews experienced during a rumination bout (Table 5). Furthermore, feeding cows FB reduced the mean number of bites during each grazing bout by 46% (P < 0.001) and also reduced the number of mastications per grazing bout by 51% (P < 0.001) compared with HO (Table 5).

DISCUSSION

We hypothesized that supplementation of perennial ryegrass with FB would increase the duration of low



Figure 1. (A) Apparent DM disappearance of ryegrass incubated in cows fed an herbage-only diet (HO_rye) or ryegrass (FB_rye) and fodder beet bulb (FBB) incubated in cows fed FBB and herbage (FBH). (B) Diurnal variation of ruminal fluid pH from cows fed either FBH or HO. Vertical reference lines indicate the time of either FBB or herbage allocation. In A, significant differences (P < 0.05) between FB_rye and HO_rye are indicated by *, differences between FBB and HO_rye are indicated by †, and differences between FB_rye and FBB are indicated by Δ . In B, * is used to indicate that the effect of diet is significant (P < 0.05).

ruminal fluid pH (pH <5.8), impairing the ruminal digestion of perennial ryegrass and herbage intake by reducing grazing time. Based on our ruminal fermentation, particle comminution, and grazing behavior results, we accept our hypothesis.

Milk Production, Rumen Fermentation Patterns, and pH

Though the FBH diet was hypercaloric compared with HO, cows fed FBH consumed a similar amount of ME to HO cows. However, the FBH treatment did not benefit milk production, consistent with previous studies where herbage was supplemented with FB bulb (Fleming et al., 2018; Waghorn et al., 2019; Pacheco et al., 2020). It is important to note that the estimation of herbage mass, using calibration equations, underestimated the herbage mass available to the FBH treatment due to the high herbage mass offered to all treatments, which increased trampling and selective grazing of the FBH cows. Evidence of this error is provided by the similar milk production and energy requirements calculated between treatments.

In agreement with our hypothesis, the reduction of ruminal fluid pH between 0400 h and 1300 h in cows fed FBH may have limited the milk response to FB supplement. Low runnial pH was caused by the accumulation of VFA that occurred following the FB meal. It is important to note that other than the 2 cows that developed SARA, the low ruminal fluid pH of cows fed FBH was not indicative of SARA, but may have reduced pH to suboptimal levels for rumen microbial activity (de Veth and Kolver, 2001; Krajcarski-Hunt et al., 2002). In a previous report (Fleming et al., 2020b), we evaluated the time-dependent changes to rumen fermentation and production during dietary adaptation to FB. We concluded that individual cows might require a more gradual and prolonged adaptation to FB to prevent the decline of ruminal fluid pH (Fleming et al., 2020b). The significant decline of ruminal acetate and TVFA pools following the fasting period, the reduction of in sacco DM disappearance of perennial ryegrass, and the reduced comminution of large particles in cows fed FBH further support our hypothesis.

While the risk of SARA has been attributed to feeding FB management errors related to FB yield estimation, individual accessibility to FB, or poor transitioning methods (Gibbs, 2014), 25% of cows still experienced SARA toward the end of the transitioning period, even though they were under controlled individual feeding conditions. Previous studies have reported acute and SARA occurrence when FB is fed >40% of DMI during late lactation (Waghorn et al., 2019; Dalley et al., 2020; Pacheco et al., 2020). Risk of SARA—at similar feeding proportions of FB—may be enhanced during early lactation both by the reduced absorptive capacity of the rumen and the reduced secretion of saliva, which contrast with the increased energy demands experienced during early and peak lactation (Cassida and

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Table 3. Average total weight and percentage of solids, liquid OM, and fiber components of rumen digesta and pool of fermentation end products collected by rumen bailing at midnight (0000 h) and morning (0930 h) from cows fed either a fodder beet bulb and herbage (FBH) or herbage-only (HO) diet

	Midnight				Morning			<i>P</i> -value			
Item	НО	FBH	SE^1	НО	FBH	SE	Diet	Time	$\stackrel{\rm Diet}{\times \rm time^2}$		
Total rumen weight (kg)	88.4 ^a	$91.0^{\rm a}$	3.39	$55.1^{\rm b}$	56.6^{b}	3.39	0.14	< 0.001	0.66		
Rumen solids (kg of DM)	8.82	8.48	0.473	4.94	5.45	0.473	0.82	< 0.001	0.23		
Rumen liquids (kg)	79.5	82.5	3.05	50.2	51.5	3.05	0.12	< 0.001	0.42		
NDF (kg)	4.60^{a}	$4.73^{\rm a}$	0.467	2.96^{b}	$3.40^{\rm cb}$	0.239	0.06	0.004	0.08		
ADF (kg)	2.64^{a}	2.67^{a}	0.229	1.61^{b}	1.82^{b}	0.116	0.22	< 0.001	0.11		
ADL (kg)	0.40^{a}	0.42^{a}	0.038	0.29^{b}	0.32^{b}	0.027	0.44	< 0.001	0.62		
OM (kg)	7.85^{a}	7.57^{a}	0.657	4.49^{b}	4.99^{b}	0.306	0.75	< 0.001	0.14		
Fermentation end-products											
$NH_3 (mol)$	0.42^{a}	0.28^{b}	0.045	$0.14^{\rm c}$	0.06^{d}	0.012	0.042	< 0.001	0.03		
Acetate (mol)	5.73^{a}	5.15^{b}	0.20	2.94°	2.38^{d}	0.078	< 0.001	< 0.001	0.008		
Butyrate (mol)	1.28^{b}	1.58^{a}	0.017	0.45°	0.40°	0.064	0.05	< 0.001	0.026		
Propionate (mol)	1.96^{a}	1.87^{a}	0.079	0.76^{b}	$0.73^{ m b}$	0.025	0.36	< 0.001	0.80		
Lactate (mmol)	0.87	1.26	0.342	0.21	0.19	0.065	0.59	0.015	0.83		
Valerate (mol)	0.13^{a}	0.22^{a}	0.164	$0.04^{\rm c}$	$0.07^{ m b}$	0.003	< 0.001	0.001	0.016		
Caproate (mol)	0.050^{a}	0.072^{a}	0.0036	$0.021^{\rm b}$	0.025^{b}	0.0013	< 0.001	< 0.001	0.80		
$NG:G ratio^{3}$	2.54^{a}	2.62^{a}	0.193	$3.83^{ m b}$	$3.33^{ m b}$	0.268	0.53	0.003	0.47		
$TVFA^4 \pmod{2}$	9.33^{a}	$9.03^{\rm a}$	0.399	4.30^{b}	3.67°	0.155	0.08	< 0.001	0.014		

^{a–d}Different superscripts within rows are different (P < 0.05).

 $^{1}SE = standard error of estimated marginal means.$

 $^2\mathrm{Diet}$ by sampling time interaction.

 $^3 \mathrm{Nonglucogenic}$ (acetate + butyrate) to glucogenic (propionate + lactate) ratio.

⁴Total volatile fatty acids.

Stokes, 1986; Penner et al., 2007; Dohme et al., 2008). Previous simulation modeling research on the feeding strategies to optimize milk production from herbage and FB during early lactation also suggest that supplementing herbage with FB at 30% of daily DMI would cause suboptimal pH of ruminal fluid and reduce herbage intake of early-lactation dairy cows (Fleming et al., 2020a). Our empirical results, and that of Fleming et al. (2020b), support our previous modeling outcomes and indicate a significant risk of SARA when supplementing a grazed herbage with FB. Although milk production from the FBH treatment was not reduced compared with HO at the group level, individuals who experienced an extended duration of low pH below 5.5 were at risk of developing ruminitis, parakeratosis (Gäbel et al., 2002; Krajcarski-Hunt et al., 2002), increased oxidative stress, and suppressed immune function (Bull et al., 1965; Gozho et al., 2005; Guo et al., 2013). Our results indicated a high variation of response between individuals when spring herbage is supplemented with

Table 4.	The percentage	e of particle si	ze and the	total poo	ol of particl	es in rume	en digesta	collected	from th	e rumen o	f cows fee	1 either	a fodder
beet bulk	and herbage (F	FBH) or herba	ge-only die	t (HO) a	t midnight	(0000 h) ε	and followi	ng fasting	the nex	xt morning	g (0930 h))	

	Midnight			Mor	ning			<i>P</i> -value			
Item	НО	FBH	SE^1	НО	FBH	SE^1	Diet	Time	${\rm Diet} \times {\rm time}^2$		
Particle fraction (%) $\geq 2 \text{ mm}$ $< 2 \text{ and } \geq 0.5 \text{ mm}$ < 0.5 mm	$25.3^{\rm a}$ $15.0^{\rm a}$ $58.4^{\rm a}$	22.9^{a} 17.5^{b} 59.4^{a}	$1.31 \\ 0.70 \\ 2.19$	26.0^{a} 23.7^{c} 49.1^{b}	32.5° 23.7 $^{\circ}$ 43.1 $^{\circ}$	$1.52 \\ 0.96 \\ 1.64$	$0.10 \\ 0.17 \\ 0.16$	<0.001 <0.001 <0.001	$0.01 \\ 0.02 \\ 0.04$		
Particle pool (kg) $\geq 2 \text{ mm}$ $< 2 \text{ and } \geq 0.5$ < 0.5 mm	$2.1^{\rm a}$ $1.3^{\rm b}$ $5.01^{\rm a}$	2.01^{a} 1.52^{a} 5.25^{a}	$\begin{array}{c} 0.138 \\ 0.103 \\ 0.301 \end{array}$	1.31^{c} 1.17^{b} 2.41^{b}	$1.8^{ m b}$ $1.32^{ m ab}$ $2.3^{ m b}$	$\begin{array}{c} 0.184 \\ 0.086 \\ 0.118 \end{array}$	$\begin{array}{c} 0.11 \\ 0.03 \\ 0.77 \end{array}$	<0.001 0.06 <0.001	0.003 0.87 0.34		

^{a-c}Different superscripts within rows differ (P < 0.05).

 $^{1}SE = standard error of estimated marginal means.$

 $^2\mathrm{Diet}$ by sampling time interaction.

P < 0.05, P < 0.01, P < 0.01, P < 0.001.

FB. Therefore, further research of such outcomes and incidence or SARA-related disorders at the herd-scale are still required.

Rumen Degradation and Oral Processing

Our results indicate that the decline of ruminal fluid pH due to feeding FB reduced the rumen's fibrolytic activity. The growth of fibrolytic microbes is impaired when pH is <5.8, and damage to epithelial tissue can occur when pH is <5.6 (Zebeli et al., 2012b). de Veth and Kolver (2001) reported that DM digestibility declined 16.1%, and apparent NDF digestibility declined 11.7% when the ruminal fluid pH was below 5.8. However, the FBH diet did not alter the rate of ruminal NDF or ADL outflow. Though the observed rate of NDF outflow was similar to previous reports (49.5 vs. 56 g/h), the rate of ADL turnover was 3-fold less in the current experiment (10 vs. 30 g/h), and the fractional NDF degradation rate was greater than previously observed for mid-lactation dairy cows that grazed a ryegrass herbage in summer (39.6 vs. 25 g of NDF/h; Taweel et al., 2005). However, the ruminal pool of ADL observed in the present study was similar to that reported by Taweel et al. (2005), which indicates differences may be due to the physiological state of the experimental animals and ryegrass herbage used, as the methods used to estimate ruminal turnover and degradation of NDF are within range of previous reports (Möller, 2009).

Ruminal DM pools were similar between treatments, and total digesta DM weight declined 38% during the fasting period, which is consistent with previous reports for lactating dairy cows 9 to 10 h postprandial (Chilibroste et al., 2000). The DM disappearance of perennial ryegrass from dacron bags inserted in the rumen of cows fed FBH declined 24% at 20 h of incubation compared with those incubated in cows fed HO (56 vs. 80% DM disappearance). The DM disappearance of perennial ryegrass observed in the present study is consistent with the report of Barrell et al. (2000), in which 80% of masticated perennial ryegrass was degraded in sacco following 20 h of incubation. The decline of herbage DM disappearance in sacco in the FBH treatment further supports our conclusion that moderate amounts of FB can reduce the microbial degradation of perennial ryegrass.

In support of our hypothesis, supplementing perennial ryegrass with FB reduced the comminution of large

	D	iet		<i>P</i> -value
Activity	FBH	НО	SE^1	Diet
Rumination (min/d)	539	453	30	0.03
Grazing (min/d)	440	556	18	< 0.001
Supplement (min/d)	82.0	0	8.0	
Total eating (h/d)	8.74	9.69	0.33	0.09
Idle (\min/d)	309	213	35	0.02
Oral processing (no./d)				
Boli	794	690	86	0.28
Grazing mastications	5,341	9,660	795	< 0.001
FB mastications	5,969	,	343	< 0.001
Prehension	18,666	30,260	2020	< 0.001
Rumination chewing	33,095	20,268	2,432	< 0.001
Total jaw movements	60,316	63,897	5,090	< 0.001
Daily bout data				
Grazing bout	14.1	13.8	1.49	0.89
Ruminating bout	16.6	15.5	2.07	0.66
Idle bout	36.9	30.6	2.83	0.104
Supplement bout	3.12	0	0.07	< 0.001
Grazing (min/bout)	30	44	3.1	< 0.001
Rumination (min/bout)	34	32	3.1	0.50
FB (min/bout)	42		2.1	
Idle (min/bout)	8	10	1.2	0.42
Grazing (mastication/bout)	371	765	100.5	< 0.001
Rumination (chewing/bout)	2,117	1,748	279	0.22
FB (mastication/bout)	1,813		200	
Grazing prehension/bout	1,235	2,305	213	< 0.001
Boli/rumination bout	50	52	4.4	0.79

Table 5. Grazing behavior, mean duration of daily activity, oral processing (mastication, prehension, and boli), and bout length of cows fed a fodder beet (FB) bulb and herbage (FBH) or herbage-only diet (HO)

 ${}^{1}SE = standard error of estimated marginal means.$

*P < 0.05, **P < 0.01, ***P < 0.001.

particles in the rumen. The pool of large particles in the rumen was not different between treatments at the first bailing session at midnight, and the pool of large particles following fasting was 27% greater in cows fed FBH than those fed HO. Particle comminution determines the rate of degradation and ruminal passage as smaller particles have a greater proportion of surface area available for microbial attachment, and particles < 1.18 mm can freely pass the ruminal-reticular orifice (Yang and Beauchemin, 2009; Zebeli et al., 2012a). The greater pool of large particles observed in the rumen of FBH cows was surprising, given FBH cows ruminated longer and grazed less intensively by reducing the number of prehensions per grazing bout by 1,070 compared with those fed HO. The reduced comminution of the large particle pool and increased physical degradation of forage in cows fed FBH suggested the microbial degradation of fiber was less than HO. Pacheco et al. (2020) also found that feeding 45% of DMI as FB increased the proportion of large particles by 40% preprandial and by 27% postprandial compared with cows fed a harvested herbage diet. The fraction of large DM particles postprandial of all cows reported by Pacheco et al. (2020) was greater than observed currently (26.5)vs. 37.4 g/100 g, reflecting the different time of digesta collection relative to feeding. The increased time spent ruminating may also explain the lack of effect of the FBH treatment on NDF turnover and degradation rate, which would be expected to decline under low ruminal fluid pH conditions.

Grazing Behavior

Interestingly, the FBH treatment spent just 7% of their daily activity eating FB; yet, the number of mastications while eating FB was 619 greater than the number of mastications counted during grazing each day. The decline of herbage mastication observed in the FBH treatment may also be explained by the variation of NDF across the sward horizon, which may have reduced tensile strength and mastication needed to ingest herbage. There have not been any prior experiments reporting the effect of supplementing herbage with FB on oral processing. Pacheco et al. (2020) hypothesized that feeding harvested and chopped FB bulb would increase the particle size of the boli compared with cows that grazed FB crop in situ. However, our results indicated that cows spend more time per kilogram of DM masticating and processing FB before ingestion than while grazing herbage. Cows consume FB when it is grazed in situ by stabilizing the bulb with their dental pad and scrapping pieces of FB from the bulb using their lower incisors. Therefore, the method used to feed FB (e.g., grazing or feeding out harvested bulbs) may alter oral processing, particle size, the rate of ruminal degradation, VFA accumulation, and the risk of cows developing SARA from FB. Further research is needed to identify the effects of either grazing FB in situ or feeding cows a harvested and chopped FB bulb on the rate of FB degradation in the rumen and the pH of ruminal fluid.

The decline of grazing time and increase of rumination time observed in cows fed FB was expected. However, the reduced time available for grazing due to the FB meal (82 min/d) did not account for the reduced grazing time (116 min/d) and increased time spent ruminating (+86 min/d) compared with the HO diet. Bargo et al. (2003) previously reported the time spent grazing is expected to decline 12 min/kg of concentrate supplement. In comparison, we observed that the grazing time of cows fed FBH declined 20 min/ kg of DM of FB consumed. Furthermore, the shorter bout duration of each grazing and reduced grazing intensity (mastication/grazing bout) indicate that cows fed FB were satiated earlier in the meal than those fed HO (Gregorini et al., 2009b). Cows fed the FBH diet spent more time ruminating, but did not ruminate with greater regurgitation frequency (i.e., the number of boli was not influenced by diet). However, chewing intensity while ruminating increased in the FBH treatment, indicating a greater amount of energy was expended on processing the FBH diet. An alternative explanation may be due to the increased incidence of pseudorumination due to delayed return of fibrous material to the reticulum and the inability to form a solid boli, which may also explain why the FBH cows were idle for 90 min longer each day than the HO treatment (Deswysen and Ehrlein, 1981). However, the current results do not support this conclusion as we did not detect any diet by time interaction for solid and liquid fractions of rumen digesta, which would indicate cows fed FBH may have been unable to form a solid boli. Further research of the particle fractions in the regurgitated boli may help to explain the observed increased time and chewing intensity while ruminating by cows fed FBH. We propose that the greater rumination time observed in the FBH treatment plus the greater chewing per bolus may have helped to improve particle comminution and outflow from the rumen. Although the comminution of large particles over the fasting period was reduced by FBH, the comminution of medium and small particle pools over the fasting period were similar to cows fed HO. Therefore, the increased time spent ruminating, plus the extra chewing per bolus may have helped to

maintain rumen function (due to increased saliva flow Farm to the rumen) and milk production of the FBH treat- techn

ment. Muscle contractions of the rumen act to either mix (primary contractions) or regurgitate (secondary contractions) digesta; however, rumen motility and rumination are often reduced during acute or lactic ruminal acidosis (Huber, 1976; DeVries et al., 2009). The comminution of particles occurs largely through rumination; although masticating while eating, rumen motility, and salivation are important processes that aid microbial adhesion and digestion of feed particles (Maekawa et al., 2002; DeVries et al., 2009). It is expected that the increased mastication caused by the FBH diet would have increased salivation (Beauchemin, 2018). Therefore, the increased rumination of cows fed FBH may have also been a regulatory response to low ruminal fluid pH. Williams et al. (2006) also reported that cows experiencing mild SARA spent more time ruminating and masticating when grazing dairy cows were supplemented with cereal grains. Furthermore, the time spent ruminating has been positively related $(R^2 = 0.98)$ with the time that pH is below 5.8 (DeVries et al., 2009). Therefore, it is possible that supplementation in grazing dairy cows increases rumination time as well as chewing and mastication intensity to aid the decline of microbial activity of the rumen and to increase ruminal fluid pH.

CONCLUSIONS

The results indicated that supplementing spring herbage with moderate amounts of FB bulb ($\sim 40\%$ of DMI) during early lactation reduces the pH of ruminal fluid and ruminal degradation of a perennial ryegrass herbage. The increased time spent ruminating, chewing intensity while ruminating, plus ingestive mastication observed in the FBH treatment provided further evidence that cows respond to low ruminal pH by increasing oral processing. The increase of oral processing in the FBH treatment may also increase salivation of neutralizing buffers, although further investigation is needed to confirm this observation. We conclude that supplementing spring herbage with harvested FB reduces grazing time, causes certain individuals to develop SARA, and does not benefit early-lactation milk production. Dairy producers should consider alternative feed sources if they are available.

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