The effect of alfalfa (*Medicago sativa*) silage chop length and inclusion rate within a total mixed ration on the ability of lactating dairy cows to cope with a short-term feed withholding and refeeding challenge

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ABSTRACT

The objectives of the study were (1) to test whether 6 h of feed deprivation followed by refeeding induces an acidosis challenge in dairy cattle and (2) to quantify the acidosis challenge mitigation potential of increased alfalfa silage concentration in the diet. Alfalfa silage constituted either 25 or 75% of forage dry matter (DM) replacing corn silage [low (LA) or high alfalfa (HA)] and was chopped to either 14 or 19 mm theoretical length [short (S) or long (L)]. Dietary treatments LAS, LAL, HAS, or HAL were offered to 4 rumen-cannulated Holstein dairy cattle (161 d in milk; 5th or 6th parity) in a 4 × 4 Latin square design study with 21-d periods. Starch concentration was 69 g/kg of DM higher for LA diets than HA diets. Feed was withheld for 6 h followed by ad libitum refeeding on d 18 of each period. Measurements of DM intake, milk yield and composition, rumen pH, and eating and rumination behavior were taken on 1 baseline day, the challenge day, and 2 further recovery days. After refeeding, rumen pH was reduced in cows fed LA diets but not HA diets. Feeding LAL resulted in the greatest subclinical acidosis risk (pH <5.8 for 355 min on the first recovery day). Animals fed LA produced 4.4 L less milk on the challenge day in comparison to baseline. It was concluded that short-term feed deprivation detrimentally affected rumen health and milk yield in dairy cattle normally fed ad libitum, but had no effect on DM intake or milk composition. Feeding alfalfa silage in place of corn silage mitigated acidosis risk due to interrupted feed supply, likely due to a combination of lower starch concentration in HA diets, greater effective fiber concentration, and higher buffering capacity of alfalfa relative to corn silage.

Key words: alfalfa, chop length, acidosis, feed withholding, rumen challenge

INTRODUCTION

Lactating dairy cow diets are often formulated to include a high concentration of rapidly fermented NFC as a source of energy to support milk production (Lechartier and Peyraud, 2011); however, such diets can also decrease rumen pH through greater rate of production of VFA (Allen, 1997). In circumstances where pH remains below 5.8 for 3 consecutive hours, a dairy cow is purported to suffer from SARA, a condition that can reduce milk yield and milk fat concentration (Plaizier et al., 2008). Dietary strategies to increase the resilience of dairy cattle to SARA include feeding forages with high buffering capacities (e.g., alfalfa, *Medicago sativa*) or increasing the concentration of physically effective fiber (peNDF) in the diet by lengthening forage chop length (McBurney et al., 1983; Zebeli et al., 2006). Physically effective fiber is defined as the NDF contained within particles that are longer than the critical particle size for rumen escape [which recent research suggests is 4 mm, although it was historically defined as 1.18 mm (Oshita et al., 2004; Maulfair and Heinrichs, 2012)] and therefore can contribute to the rumen mat (Mertens, 2000). A lower rumen pH has also been linked with changes in cow feeding behavior and the adoption of coping mechanisms, including showing preferences for long particles in the diet (DeVries et al., 2008; Maulfair et al., 2013) or for supplementary hay (Kmicikewycz and Heinrichs, 2015).

Experimentally, the stability of rumen pH can be tested by induction of a rumen fermentation challenge. This is typically achieved through the addition of a large quantity of a rapidly degradable carbohydrate to the diet, such as cereal grains or alfalfa pellets (Krause and Oetzel, 2005; Colman et al., 2013). However, it is unclear whether such a method accurately replicates conditions that cause SARA and, furthermore, may not provide an appropriate model for evaluating dietary mitigation strategies. An alternative approach to instigate a rumen challenge is deprivation of feed for a period of several hours (Oetzel, 2007). A period of

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fasting is then followed by a period of overeating when access to feed is returned, termed refeeding (Chilibroste et al., 2007). Periods of feed deprivation lasting up to 6 h may be relatively common in a commercial setting; for instance, where insufficient feed or pasture is allocated, during feeding equipment failure, or when the animal's access to feed for routine processes such as milking or health checks is removed. However, relatively little is known about the severity of the effect of such events on rumen function and milk production. Studies in the literature have examined the effect of longer periods of fasting, such as 12 to 48 h (Chelikani et al., 2004; Oetzel, 2007; Toerien and Cant, 2007), that generally result in high levels of temporary milk yield loss; however, we are not aware of any studies that have examined the effects of shorter fasting periods in dairy cattle that would be more representative of commercial practice. Therefore, the aims of the present study were (1) to test whether 6 h of feed deprivation followed by refeeding induces an acidosis challenge and (2) to examine the effect of varying inclusion rate (**IR**) and chop length (**CL**) of alfalfa silage replacing corn silage in a TMR on resilience to a feed withholding and refeeding challenge.

MATERIALS AND METHODS

Forage Harvesting and Clamp Sampling

The present study formed part of a larger research trial that used the same dietary treatments and observed their effects on milk yield, DMI, diet digestibility, and rumen function under nonchallenging conditions in a larger cohort of cows and over a longer time period, as reported previously (Thomson et al., 2017a,b). In brief, alfalfa silage was harvested as a second cut crop at an estimated 10% bloom in July 2014 and conserved in concrete-walled clamps. The crop was wilted for 48 h and ensiled, producing a high-DM (576 g/kg of fresh weight) silage. Two CL [long (**L**) and short (**S**)] were created from material collected in alternate swaths by altering the knife arrangement of the precision chop forage harvester (Claas Jaguar, Claas Group, Harsewinkel, Germany) from a theoretical chop length of 14 (shortest setting) to 19 mm (longest setting) that were ensiled in 2 adjacent clamps. An additive was applied (Axcool Gold containing *Lactobacillus buchneri*; 2 L/t; Biotal, Cardiff, UK) to prevent heating in the clamp. Samples for chemical composition analysis (Sciencetec Analytical Services, Cawood, UK) were obtained using a clamp corer. A detailed analysis of the particle length profile of the silages produced (mean 14.3 and 9.0 mm for L and S, respectively) has been published previously (experiment 2; Thomson et al., 2017b). Corn

(*Zea mays*) silage for the study was taken from a commercial crop of mixed varieties harvested in autumn 2014 that was chopped by the forage harvester (Model FR700, New Holland Ltd., Turin, Italy; theoretical chop length of 18 mm) and ensiled as described for the alfalfa clamps [geometric mean particle length of 10 mm determined using a Penn State particle separator (**PSPS**); Heinrichs, 2013].

Diets

Diets comprised a TMR with 50:50 ratio of forage to concentrate on a DM basis (Thomson et al., 2017a,b), in which the forage portion consisted of corn and alfalfa silage at IR (DM basis) of either 25:75 (high alfalfa; **HA**) or 75:25 (low alfalfa; **LA**), respectively. These treatments were combined with the 2 alfalfa silage CL in a 2 × 2 factorial arrangement to give 4 treatments (**HAL**, **HAS**, **LAL**, and **LAS**) that were formulated to be isonitrogenous (170 g of CP/kg of DM) and contain similar levels of NDF (320 g/kg of DM). The reduction in corn starch associated with the lower corn silage inclusion in HA diets was partially offset by increasing the concentration of corn meal (Table 1); however, for the experimental diets fed, starch concentration was still lower in the HA diets (Table 2).

Animals

Four multiparous Holstein dairy cows, previously prepared with rumen fistulae (Bar Diamond rumen cannula; Parma, ID), in mid-lactation (161 DIM, SE ± 23.1), weighing 739 kg (SE ± 13.9), and 7 to 9 yr of age (5th or 6th parity), were randomly assigned to 1 of 4 initial treatments according to a 4 × 4 Latin square design balanced for carryover effects with 21-d periods. All procedures were licensed and monitored by the UK Government's Home Office under the Animal (Scientific Procedures) Act 1986. The experimental design and replication employed was based on variance and expected treatment effects for key variables observed in previous studies (Reynolds et al., 2014). During adaptation weeks (wk 1 and 2 of each period), animals were housed in a cubicle yard and individually fed once daily for ad libitum intake (10% refusals) through Insentec RIC feeders (Insentec B.V., Marknesse, the Netherlands). Continuous access to water was provided. From d 12 of each period, animals were housed and milked in individual tiestalls to facilitate sampling. Animals were allowed to acclimatize to the stalls for 3 d before sampling beginning on d 15. While in tiestalls, animals were offered their daily feed allocation in 2 halves at 1000 and 1600 h. Refusals were taken daily at 0930 h. Between d 15 and 18, measurements of rumen func-

tion under nonchallenging conditions were performed, including rumen VFA and ammonia concentrations, rumen pH, rumen mat particle distribution, and fecal particle distribution, which have been reported previously (Thomson et al., 2017b). The feeding routine differed on d 18 of each period when a refeeding challenge was simulated (described below). While in tiestalls, each cow was also fitted with a rumination head collar (Itin+Hoch GmbH, Fütterungstechnik, Liestal, Switzerland) to measure eating and rumination behavior as described previously (Ruuska et al., 2016).

Experimental Routine

Baseline measurements of all variables were taken on d 16 of each period (other than rumen pH, which was measured on d 15 because other measurements being

performed on d 16 have been reported separately). On d 18 of each period, refusals from the previous day were removed from the cows 1 h early (0830 h) to begin a period of fasting. Feed was withheld for 6 h until 1430 h, when half the daily diet allocation was offered followed by the second half 2 h later at 1630 h. On d 19, refusal and feeding routine was returned to that of d 17. To summarize, the timetable for wk 3 of each period was

- d 15: Basal rumen pH recorded (coinciding with sampling of rumen liquor, reported separately);
- d 16: Basal DMI, milk yield, and eating and rumination behavior measurements;
- d 17: Rest day with refusals removed 1 h early the following morning;
- d 18: Feed withheld until 1430 h followed by refeeding;
- d 19: Recovery d 1, original feeding routine resumed;
- d 20: Recovery d 2; and
- d 21: No measurements, rest allowed before diet change.

Table 1. Ingredients used in diet formulation

Ingredients, g/kg of DM	Diet ¹	
	LA	HA
Alfalfa silage ²	125	375
Corn silage ³	375	125
Concentrate blend ⁴		
Cracked wheat	80	80
Corn meal	54	97
Unmolassed sugar beet feed	40	40
Soy hulls	82	108
Soybean meal	100	65
Rapeseed meal	100	65
Molasses	10	10
Dicalcium phosphate	5	5
Salt	5	5
Dairy mineral ⁵	10	10
Megalac ⁶	15	15

¹LA = low-alfalfa diet; HA = high-alfalfa diet.

²Long chop alfalfa silage composition: 593 g/kg of DM; 164 g of CP/kg of DM; 397 g of NDF/kg of DM; 348 g of ADF/kg of DM; 108 g of ash/kg of DM; and 10 g of water-soluble carbohydrate/kg of DM. Short chop alfalfa silage composition: 566 g/kg of DM; 167 g of CP/kg of DM; 385 g of NDF/kg of DM; 326 g of ADF/kg of DM; 108 g of ash/kg of DM; and 17 g of water-soluble carbohydrate/kg of DM.

³Corn silage composition: 383 g/kg of DM; 63 g of CP/kg of DM; 387 g of NDF/kg of DM; 223 g of ADF/kg of DM; 37 g of ash/kg of DM; 357 g of starch/kg of DM; and 25 g of water-soluble carbohydrate/kg of DM.

⁴HA concentrate composition: 911 g/kg of DM; 199 g of CP/kg of DM; 278 g of NDF/kg of DM; 172 g of ADF/kg of DM; 67 g of ash/kg of DM; 247 g of starch/kg of DM; and 57 g of water-soluble carbohydrate/kg of DM. LA concentrate composition: 884 g/kg of DM; 241 g of CP/kg of DM; 272 g of NDF/kg of DM; 171 g of ADF/kg of DM; 71 g of ash/kg of DM; 195 g of starch/kg of DM; and 67 g of water-soluble carbohydrate/kg of DM.

⁵Contained vitamin A (400,000 IU/kg), vitamin D (80,000 IU/kg), and vitamin E (2,000 IU/kg), manganese (2.2 g/kg), calcium (230 g/kg), zinc (5.2 g/kg), phosphorous (20 g/kg), magnesium (40 g/kg), sodium (95 g/kg), copper (1.2 g/kg), and selenium (30 mg/kg).

⁶Megalac rumen protected fat supplement (Volac International Ltd., Royston, UK).

Intake and Diet Analysis

The weight and DM concentration of feed offered and refused were measured during d 14 to 21 of each period for each cow. A daily grab sample of each TMR and the TMR constituents was bulked across the sampling week for each diet in each period (16 samples in total). Dry matter concentration of feed was determined by oven drying at 100°C for 24 h. Samples of the TMR constituents for each diet in each period were stored frozen at -20°C until analyzed for DM, N (using the macro-Kjeldahl method; AOAC 954.01, AOAC International, 2000), ash (by combustion at 500°C for 16 h), NDF and ADF (expressed inclusive of residual ash; Robertson and Van Soest, 1981; Mertens et al., 2002), starch (Fuller, 1967; Macrae and Armstrong, 1968), and water-soluble carbohydrates as described previously (Reynolds et al., 2014; Kliem et al., 2017). Concentrations (g/kg of DM) of CP, NDF, ADF, ash, starch, and water-soluble carbohydrates in each TMR were calculated based on constituent inclusion rates. A sample of each TMR from each period was analyzed for particle size distribution using a PSPS (sieve apertures measuring 19, 8, and 4 mm in diameter and a bottom pan). A DM correction for material retained on each sieve was obtained (Thomson et al. 2017a). Average particle size of the sample was calculated as described previously (Heinrichs, 2013) and peNDF was calculated as the proportion of particles (DM corrected) greater than the threshold length (4, 8, or 19 mm) multiplied

Table 2. The chemical and physical composition of 4 TMR containing a high (HA) or low (LA) concentration of alfalfa silage at a long (L) or short (S) chop length (Thomson et al., 2017b)

Item	Diet				SEM	P-value ¹		
	LAS	LAL	HAS	HAL		IR	CL	IR × CL
Chemical composition, g/kg DM (unless noted)								
Oven DM, g/kg	555	571	610	632	5.0	0.022	0.065	0.364
Ash	62	63	78	77	0.6	0.001	0.471	0.070
CP	164	163	168	167	3.5	0.200	0.710	0.945
NDF	311	322	335	340	4.8	0.115	0.221	0.510
ADF	202	208	237	245	1.5	0.004	0.007	0.322
Starch	234	235	164	168	7.0	0.039	0.680	0.780
WSC ²	37	35	35	32	0.7	0.006	0.020	0.371
Particle size distribution ³								
Material retained, g/kg DM								
19 mm	32 ^a	50 ^a	53 ^a	121 ^b	7.5	0.001	0.001	0.007
8 mm	364 ^a	419 ^b	374 ^{abc}	391 ^c	5.0	0.129	0.012	0.026
4 mm	165 ^a	135 ^b	187 ^c	126 ^b	2.4	0.033	0.001	0.004
Bottom pan	438	398	379	363	5.0	0.001	0.010	0.094
Mean particle size, ⁴ cm	0.50	0.56	0.54	0.65	0.014	0.001	0.001	0.099
peNDF, ⁵ g/kg DM								
>19 mm	10.3 ^a	16.4 ^a	17.4 ^a	40.4 ^b	2.68	0.001	0.001	0.009
>8 mm	123	148	138	182	2.7	0.056	0.030	0.137
>4 mm	172	199	205	213	3.8	0.003	0.004	0.051

^{a-c}Values within a row with different superscripts differ significantly at $P < 0.05$.

¹IR = inclusion rate; CL = chop length; IR × CL = interaction between IR and CL.

²WSC = water-soluble carbohydrate.

³Particle size distribution measured using a Penn State Particle Separator with 3 sieves: 19, 8, and 4 mm diameter.

⁴Mean particle size was determined using the recommended equation of Penn State University (Heinrichs, 2013).

⁵Physically effective neutral detergent fiber (peNDF) determined as the proportion of particles in the TMR greater than the threshold length multiplied by the NDF concentration of the TMR (Mertens, 1997).

by the NDF concentration of the diet (Mertens, 1997; Farmer et al., 2014). The chemical and physical composition of the diets is shown in Table 2 for reference but has been discussed in detail previously (Thomson et al., 2017b).

Milk Yield and Composition. Cows were milked twice daily at 0630 and 1630 h, and milk samples, preserved using potassium dichromate, were analyzed for fat, protein, casein, lactose, urea, and SCC by mid-infrared spectroscopy on a CombiFoss machine (National Milk Laboratories, Chippenham, Wiltshire, UK). The CombiFoss machine combines both the Fossomatic 5000 and Milkoscan 6000 (both Foss, Hillerød, Denmark) and uses the entire mid-infrared wavelength spectrum. Morning and afternoon milk samples were scanned separately. Only data from d 16 (baseline), 18 (challenge), 19 (recovery d 1), and 20 (recovery d 2) were statistically analyzed.

Rumen pH. An indwelling pH meter (Sentix 41–3 probe, WTW Trifhof, Weilheim, Germany) attached to a weight (200 g) and connected to the rumen cannula using nylon cord (50 cm) was placed within the rumen of each animal for 24 h, beginning just before feeding (0930 h) on d 15 of each period until refusals were removed at 0930 h on d 16, to establish baseline patterns of rumen pH; the pH meter was inserted again

at 0830 h on d 18 (challenge day), remaining within the rumen until 0930 h on d 21. The probe was calibrated before every insertion by immersion in solutions of pH 4 and 7. After use, the probe was reimmersed in the calibration solutions and any drift was calculated as the given value subtracted from the true pH of the solution. Drift greater than 0.3 pH units was considered the upper threshold for inclusion; however, no readings greater than this value were found in the present study and therefore all data were included. The pH probe was attached to a data logger (ph340i, WTW Trifhof) with readings recorded every 10 min. Time spent at pH <6.2 and <5.8 were calculated for each day for each cow in each period. Readings were then averaged over each hour for further analysis, beginning on the hour for baseline, and recovery d 1 and 2, and at the half hour mark for challenge day to coincide with feeding times. Any measurements within the first hour of insertion (0830 to 0930 h) were not included in statistical analysis due to differences in the start time of each cow.

Statistical Analysis

Average daily data starting at morning feeding, 1000 h, were calculated for 4 phases (days) of wk 3: baseline (d 15/16), challenge (d 18), recovery 1 (d 19), and re-

covery 2 (d 20). Averages for each cow, treatment, and day combination were analyzed to determine fixed effects of period, alfalfa IR, alfalfa CL, day (as a repeated measure) and their interactions ($IR \times CL$, $IR \times day$, $CL \times day$, and $IR \times CL \times day$), and random effects of cow using mixed models procedures of SAS (version 9.4, SAS Institute Inc., Cary, NC). The SLICE option was used to show treatment effects for each day. Least squares means for each treatment and effects of IR, CL, and $IR \times CL$ interactions within each day are presented separately. Within each treatment, means for challenge or recovery days were compared with the baseline value for that treatment using the PDIF option within the LSMEANS statement of the Mixed procedure. For measurements of eating time and relative rumen pH within each day, the same model was used except day was replaced with hour (a repeated measure) and each day was analyzed separately. The covariance structure giving the best fit (out of compound symmetry, compound symmetry heterogeneous, unstructured, or spatial power) was chosen for each variable using the Bayesian information criterion. Compound symmetry and spatial power were the most common structures of best fit.

For rumen pH, a baseline value for each hour of a 24-h period (starting at morning feeding, d 15, 1000 h) was taken for each cow on each treatment that was then subtracted from the hourly mean at the same time point for each subsequent phase to analyze and present each hourly value relative to baseline. The data were transformed in this way to ensure the magnitude of any effects could be compared between animals with differing baseline rumen pH levels. For example, the nadir pH observed during baseline varied between cows from 5.76 to 6.22 (mean of all treatments for each animal), and basal daily mean rumen pH similarly ranged from 6.48 to 6.76 between animals. Therefore, presenting data as time below a certain threshold was judged to be of lesser importance than pH change relative to baseline. A mean of relative pH for each day was also analyzed (with the challenge day subdivided into fast and refeeding) to determine fixed effects of period, alfalfa IR, alfalfa CL, and $IR \times CL$ interaction, and random effects of cow using mixed models procedures with each day and subphase tested separately. For rumen pH parameters, we found no effects of period; therefore it was judged that recovery time was sufficient in between challenges to prevent carryover effects.

Effects of treatment on diet chemical and physical composition were analyzed separately using values for each bulked diet sample in each period ($n = 16$ bulked samples originating from d 15–21). Fixed effects of period, alfalfa IR, alfalfa CL, $IR \times CL$ interaction and random effect of cow were also used using mixed

models procedure of SAS with period as a repeated measure.

RESULTS

Baseline Treatment Effects

The effect of treatment on diet chemical composition and particle size in the present study (Table 2) have been reported previously (Thomson et al., 2017a,b). Briefly, the concentration of starch was 69 g/kg of DM greater in LA diets than in HA diets by design ($P < 0.04$), whereas ADF concentration was 36 g/kg of DM greater in HA diets ($P < 0.01$). Increasing CL from S to L increased the proportion of particles retained on both the 8- and 19-mm sieves of the PSPS by 36 and 43 g/kg of DM, respectively, whereas it reduced the proportion that was retained on the 4-mm sieve and in the bottom pan (all $P < 0.02$). Both greater IR and greater CL of alfalfa increased or tended to increase peNDF concentrations using 4, 8, and 19 mm threshold lengths ($P < 0.06$) relative to a low IR and a short CL.

We found no effect of diet on daily mean rumen pH, for which the average across all treatments was 6.36 (Table 3), or on daily time spent at less than pH 6.2 or pH 5.8, nor were there any time points during the baseline day in which we noted an effect of treatment on rumen pH. Following feeding at baseline, rumen pH showed a downwards trend, reaching a nadir between 9 and 13 h after morning feeding followed by a return to prefeeding levels between 15 and 22 h postfeeding (Figure 1a). Baseline eating patterns showed an increase in time spent eating (20–40 min/h) in the first hour after fresh feed was offered (at both 1000 and 1600 h), followed by a reduction in time spent eating in the second hour postfeeding to roughly 10 min/h, a rate that was sustained throughout the daytime hours (Figure 1b). Between 13 and 19 h postfeeding, <5 min/h of eating occurred, corresponding to the rise in rumen pH shown in Figure 1a. Dry matter intake, milk yield, milk composition, and the yield of milk solids showed no effect of treatment during the baseline phase (Table 4). Both daily mean time spent eating (Table 5) and transient eating patterns were similar for all dietary treatments at baseline. Cows fed HAL diets had more daily mean rumination chews and spent more time ruminating per day than cows fed either LAL or HAS, whereas cows fed LAS had an intermediate number of ruminating chews ($IR \times CL$; $P < 0.04$). Cows fed HAL diets also showed a tendency to spend the greatest time ruminating per day compared with other dietary treatments ($IR \times CL$; $P < 0.07$). Hourly patterns of rumination indicate a level profile of rumination for all treatments throughout the day, with 10 to 30 min spent ruminating each hour (Figure 1c).

Table 3. Mean relative rumen pH of lactating dairy cows fed a TMR containing a high (HA) or low (LA) concentration of alfalfa silage at a long (L) or short (S) chop length before, during, and following a rumen challenge that involved a 6-h fast followed by a refeeding challenge

Phase ¹	Diet				SEM	P-value ²		
	LAS	LAL	HAS	HAL		IR	CL	IR × CL
Baseline daily rumen pH	6.30	6.38	6.31	6.43	0.130	0.785	0.396	0.828
Relative rumen pH ³								
Challenge day								
Fast	+0.43	+0.42	+0.46	+0.38	0.098	0.905	0.517	0.592
Refeeding	−0.15	−0.21	+0.04	−0.08		0.007	0.115	0.643
Recovery d 1	−0.20	−0.41	−0.01	−0.11		0.001	0.023	0.443
Recovery d 2	−0.01	−0.17	0.02	0.01		0.241	0.365	0.428
Minutes below pH 6.2 ⁴								
Baseline	531	390	428	348	209.3	0.700	0.561	0.921
Challenge day								
Fast	—	—	—	—		—	—	—
Refeeding	761	463	353	328		0.168	0.398	0.426
Recovery d 1	1,008*	880*	438	408		0.017	0.677	0.097
Recovery d 2	551	612	435	357		0.343	0.965	0.769
Minutes below pH 5.8 ⁵								
Baseline	25	33	23	3	11.9			
Challenge day								
Fast	0	0	0	0				
Refeeding	93	100	45	15				
Recovery d 1	135	355	15	18				
Recovery d 2	35	113	20	57				

¹The fast period combines measurements from 0930 until 1430 h on the day of the challenge, during which time animals were not allowed to access feed (note, the start of the feed withdrawal was 0830 h; however, the time taken to insert rumen pH probes meant that data for this hour was incomplete and not included in the analysis). The refeeding period combines measurements from 1430 h on the day of the challenge until 0930 h the following morning. The subsequent two 24-h periods are termed recovery d 1 and recovery d 2 that both begin at 1000 h.

²IR = inclusion rate; CL = chop length; IR × CL = interaction between IR and CL.

³Relative rumen pH calculated hourly as rumen pH measurement minus the corresponding baseline measurement (Thomson et al., 2017b) at the same hour of the day for each cow on each treatment in each phase.

⁴All cows spent either low or no time below pH 6.2 during the fast subphase, and therefore this subphase was removed from statistical analysis to prevent non-normality of the remaining data set.

⁵For minutes below pH 5.8 a large number of values were 0, and therefore the data did not display a normal distribution nor could a meaningful transformation be achieved. Thus, data are presented as arithmetic means.

*Value differs significantly ($P < 0.05$) from a baseline value for that treatment (not applicable to relative rumen pH).

Challenge Effect on Rumen pH and Eating Patterns

Relative rumen pH increased steadily during the feed withholding period for all diets (Figure 2a). We observed no effect of treatment on the mean relative pH (Table 3) nor at any individual time points over the fasting phase. At the peak of the fasting phase, mean rumen pH across the treatments ranged from 6.8 to 7.2. A steep fall in rumen pH on all treatments occurred with the refeeding event. Over the first hour after refeeding, relative rumen pH in cows fed the LAL diet decreased to the baseline level in comparison to the other 3 diets ($P < 0.03$); in the LAS, HAL, and HAS diets, relative pH remained elevated above baseline levels until 2 h after refeeding, which coincided with the second offering of feed. At 8 to 12 h after refeeding, rumen pH of cows fed LA diets fell to lower levels than HA relative to their baseline values (IR effects $P < 0.04$), whereas HAS remained closer to baseline than HAL (IR × CL interaction; $P < 0.04$). Cows fed HAS diets maintained a rumen pH that was close to baseline pH throughout

the refeeding period, 0.04 pH units higher than baseline over the entire refeeding phase. Cows fed LA diets had a rumen pH 0.16 pH units lower, on average, over the refeeding phase than HA diets relative to their own baseline values ($P < 0.008$; Table 3) and spent, on average, 97 min at pH < 5.8 compared with 30 min for cows fed HA diets.

Cows spent a greater proportion of time eating in the 3 h following refeeding than during the same period after the initial feed was offered at baseline (57 vs. 29% of each hour was spent eating in 0–3 h postfeeding respectively; Figure 2b). At 4 h after refeeding, eating intensity reduced for cows fed all diets, although at 6 h after refeeding cows fed the LAS diet again spent a high proportion of time eating in comparison to cows fed other diets ($P < 0.01$). Following this, cows on all diets continued to eat at a fluctuating rate between 0 and 20 min/h (Figure 2b). Rumination pattern indicated a slightly larger reduction in rumination between 0 and 4 h after refeeding than at mealtimes on other days during the observation period for cows on all treat-

Figure 1. Hourly mean (a) rumen pH (Thomson et al., 2017b), (b) time spent eating, and (c) time spent ruminating of lactating dairy cows fed a TMR containing a high (HA) or low (LA) concentration of alfalfa silage at a long (L) or short (S) chop length over a 24-h baseline period beginning at 1000 h (hour 1). Baseline values were measured over a single 24-h period 2 d (for eating pattern) or 3 d (for rumen pH) before a feed deprivation or refeeding challenge was administered. Black triangles indicate time points at which half a daily allocation of feed was offered. Hours at which a significant effect of alfalfa inclusion rate (IR), alfalfa chop length (CL), or their interaction occurred, analyzed using Mixed Models procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC), are marked.

ments. In the hour before refeeding, cows fed LA diets ruminated very little (<5 min) in comparison to cows fed HA that continued to ruminate for between 15 and 25 min during the hour ($P < 0.04$).

Recovery from the Rumen Challenge

On recovery d 1, the rumen pH of all cows recovered close to baseline levels before morning feeding. However, postfeeding, the rumen pH of cows fed LA diets again decreased relative to their baseline values, leading to multiple hours with effects of IR. At 31 h after refeeding the rumen pH of cows fed LAS diets returned to basal values, whereas cows fed LAL diets continued to show reduced relative rumen pH until 36 h after refeeding (IR $\times$ CL interactions $P < 0.04$). Cows fed HAS diets continued to show a rumen pH pattern close to baseline whereas cows fed HAL diets were marginally lower than baseline values (Figure 2a). Mean relative rumen pH for the recovery d 1 phase demonstrated that cows fed LA and L diets had reduced relative pH in comparison to HA and S diets (effect of IR $P < 0.001$; effect of CL $P < 0.03$), which was also reflected in cows fed LA spending longer at pH <5.8 than cows fed HA.

On recovery d 2, we found no significant differences in relative rumen pH between treatments or any hours in which treatment differences occurred, although the relative rumen pH of cows fed LAL diets continued to be the lowest of the 4 treatments and, on average, 0.17 pH units below baseline values for that diet (Table 3). Over both recovery days, eating and rumination patterns appeared similar to those observed at baseline. Some fluctuation led to significant effects on time spent eating and ruminating during these days, but overall differences were slight and not sustained.

Induction of SARA

Taking the definition of SARA to be a period of 3 consecutive hours where rumen pH is less than 5.8, we observed 6 bouts of SARA within the data set, of which 2 bouts were in the same cow when fed the LAS diet and the remaining 4 were in 3 cows when fed the LAL diet (with 1 cow experiencing 2 separate bouts on this diet). Of these 6 bouts of SARA, 2 occurred on the day of the challenge (1 LAS and 1 LAL) and 4 occurred on recovery d 1 (1 LAS and 3 LAL). No episodes of SARA were observed in cows fed HA diets.

Challenge Effect on Intake and Milk Production

On the day of the challenge, DMI was similar to that consumed on baseline day (Table 4), as was daily mean time spent eating and ruminating (Table 5) despite

Table 4. Daily mean intake, milk production, and milk composition of lactating dairy cows fed a TMR containing a high (HA) or low (LA) concentration of alfalfa silage at a long (L) or short (S) chop length before, during, and following a 6-h fast followed by a refeeding challenge

Item ¹	Diet				SEM	P-value ²		
	LAS	LAL	HAS	HAL		IR	CL	IR × CL
DMI, kg/d								
Baseline day	25.5	21.3	22.5	24.4	1.44	0.998	0.359	0.133
Challenge day	25.2	23.7	24.5	23.6		0.764	0.352	0.810
Recovery d 1	25.3	21.5	23.1	22.2		0.548	0.085	0.251
Recovery d 2	23.1	19.6	23.7	21.8		0.289	0.049	0.130
Milk yield, kg/d								
Baseline day	31.7	30.1	27.8	29.2	5.89	0.654	0.992	0.938
Challenge day	27.2*	25.8*	25.9	27.3		0.985	0.994	0.994
Recovery d 1	31.9	29.5	29.0	30.8		0.879	0.954	0.978
Recovery d 2	30.9	28.2	29.0	27.0		0.775	0.664	0.962
Milk fat, g/kg								
Baseline day	32.6	35.2	35.3	33.2	3.04	0.766	0.832	0.305
Challenge day	38.7	38.4	34.0	34.8		0.218	0.948	0.638
Recovery d 1	34.7	35.3	35.8	35.8		0.588	0.817	0.939
Recovery d 2	37.0* ^a	36.5 ^a	35.3 ^a	40.3* ^b		0.292	0.046	0.033
Milk fat yield, kg/d								
Baseline day	1.07	1.04	0.94	1.03	0.208	0.701	0.897	0.963
Challenge day	1.06	1.00	0.84	0.95		0.485	0.886	0.864
Recovery d 1	1.11	1.05	1.04	1.09		0.946	0.996	0.991
Recovery d 2	1.12	1.01	1.01	1.07		0.892	0.898	0.968
Milk protein, g/kg								
Baseline day	31.3	31.5	30.9	30.9	1.16	0.602	0.923	0.952
Challenge day	31.4	31.0	30.2	29.5		0.209	0.583	0.585
Recovery d 1	30.3	30.8	30.2	29.1		0.364	0.764	0.637
Recovery d 2	30.6	30.4	30.5	29.1		0.546	0.490	0.755
Milk protein yield, kg/d								
Baseline day	0.99	0.95	0.84	0.87	0.173	0.440	0.996	0.869
Challenge day	0.85*	0.80*	0.77	0.80		0.808	0.954	0.990
Recovery d 1	0.96	0.91	0.87	0.89		0.740	0.920	0.982
Recovery d 2	0.94	0.87	0.88	0.75		0.571	0.549	0.857

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

¹Baseline data were collected on d 16 and the challenge day was d 18 (starting at 1000 h) of each period, during which animals spent 4.5 h of the day fasting (after a 1.5-h period during which refusals were removed early to make a total fast of 6 h) and a 17.5-h period in which feed was offered ad libitum. Recovery d 1 and 2 were the subsequent 24-h periods (d 19 and 20, respectively, both beginning 1000 h).

²IR = inclusion rate; CL = chop length; IR × CL = interaction between IR and CL.

*Value differs significantly ($P < 0.05$) from a baseline value for that treatment.

the pattern of eating during the day being altered as described earlier. A numerical decline in intake was observed between the challenge day and recovery day 2 for cows fed LAL and HAL diets, resulting in animals fed L eating 2.7 kg/d less than animals fed S on recovery day 2 ($P < 0.05$).

Milk yield was reduced in cows fed LAS and LAL diets on challenge day relative to milk yield at baseline ($P < 0.05$) by 4.5 and 4.3 kg, respectively, although yield was not significantly lower than that of cows fed the HA diets on the challenge day. The reduction in milk yield on LA diets on this day also led to significant reductions in milk protein yield compared with baseline for these treatments. On recovery d 1 and 2, milk yield for all treatments was not statistically different ($P > 0.05$) from baseline levels. Concentrations of milk protein were unaffected by treatment and day. The milk fat yield of cows fed LAS and HAL diets on recovery d 2 was higher than baseline ($P < 0.05$), and furthermore

the milk fat yield for HAL cows on that day was greater than that of any other dietary treatment (IR × CL; $P < 0.04$).

DISCUSSION

Effect of a Refeeding Challenge on Eating Patterns and Rumen pH

During the fasting phase, before refeeding, we observed increased rumen pH for all animals, likely because of rumen VFA being absorbed and not replaced due to a lack of substrate for fermentation, and perhaps as an effect of salivation while the animals were waiting for feed to be offered. In support of this, cows were shown to continue ruminating during the fasting period. Following refeeding, animals exhibited a 3-h period in which a high proportion of time was spent eating across all treatments in comparison to

Table 5. Eating and rumination behavior of lactating dairy cows fed a TMR containing a high (HA) or low (LA) concentration of alfalfa silage at a long (L) or short (S) chop length before, during, and following a 6-h fast followed by a refeeding challenge

Item ¹	Diet				SEM	P-value ²		
	LAS	LAL	HAS	HAL		IR	CL	IR × CL
Eating chews × 10 ³ /d								
Baseline day	17.8	12.1	12.8	14.0	2.56	0.509	0.351	0.392
Challenge day	16.2	12.0	15.4	14.5		0.707	0.283	0.626
Recovery d 1	15.4	9.8	13.5	11.4		0.950	0.120	0.410
Recovery d 2	14.4	11.1	11.9	15.0		0.791	0.961	0.605
Eating time, min/d								
Baseline day	268	225	339	239	35.0	0.795	0.490	0.817
Challenge day	250	217	267	227		0.681	0.271	0.665
Recovery d 1	241	177	249	189*		0.768	0.073	0.305
Recovery d 2	229	184	217	222		0.703	0.551	0.808
Ruminating chews × 10 ³ /d								
Baseline day	27.6 ^{ab}	27.5 ^a	24.2 ^a	35.4 ^b	3.01	0.414	0.052	0.038
Challenge day	28.7	26.2	26.1	34.2		0.336	0.319	0.124
Recovery d 1	31.0	29.0	27.8	32.0		0.958	0.691	0.673
Recovery d 2	30.1	26.1	28.1	32.7		0.459	0.934	0.479
Ruminating time, min/d								
Baseline day	442	460	421	574	47.1	0.281	0.056	0.065
Challenge day	464	432	439	548		0.303	0.389	0.198
Recovery d 1	499	484	469	520		0.944	0.686	0.835
Recovery d 2	494	438	478	533		0.414	0.991	0.591

^{a,b}Values within a row with different superscripts differ significantly at $P < 0.05$.

¹Baseline data were collected on d 16 and the challenge day was d 18 (starting at 1000 h) of each period, during which animals spent 4.5 h of the day fasting (after a 1.5-h period during which refusals were removed early to make a total fast of 6 h) and a 17.5-h period in which feed was offered ad libitum. Recovery d 1 and 2 were the subsequent 24-h periods (d 19 and 20, respectively, both beginning 1000 h).

²IR = inclusion rate; CL = chop length; IR × CL = interaction between IR and CL.

*Value differs significantly ($P < 0.05$) from a baseline value for that treatment.

the baseline day (57 vs. 29% of each hour was spent eating from 0–3 h postfeeding respectively; Figure 2b). An increase in eating intensity following feed deprivation is consistent with the findings of other studies (Patterson et al., 1998; Oetzel, 2007) and has been linked with low rumen fill before refeeding (Gregorini et al., 2007). This overeating episode resulted in a rapid decrease in rumen pH, such that 3 h after refeeding animals had reached the same rumen pH as observed 7 h after feeding on the baseline day. We attributed this accelerated decline in rumen pH to acid load from the ingested feed and from VFA produced from fermentation of the same. Furthermore, high feed intake in a short time-period would have increased the supply of rapidly degraded starch and sugars to the microbial population, especially within the LA diet that contained a greater concentration of starch from corn silage. Total VFA concentration in the rumen is dependent on the rate at which VFA are produced in comparison to the rate at which VFA can be absorbed through the ruminal epithelium, be neutralized by saliva, or are removed from the rumen by passage. Various absorption mechanisms facilitate VFA removal from the rumen; however, the most predominant are bicarbonate-dependent transport (Aschenbach et al., 2011) and passive diffusion (Chibisa et al., 2016). For

the latter, a low VFA concentration in the rumen, such as that created by short-term feed deprivation, would reduce VFA removal rate initially until a sufficient diffusion gradient was established. Simultaneously, recent research suggests that such conditions are likely to also favor increased production rate of VFA by microbes that benefit from a diffusion gradient that swiftly removes VFA from their boundary layer (Russell et al., 2009; Mason and Stuckey, 2016). Therefore, the swift decline in rumen pH observed is likely to be a combined effect of increased microbial productivity combined with reduced ability to remove VFA from the rumen through absorption. Another longer-term study also noted a reduction in epithelial absorption rate during and after feed restriction that was attributed to reduced blood flow due to feed deprivation (e.g., 5 d of feed restriction followed by refeeding; Zhang et al., 2013); however, this is unlikely to be the case in our study, where feed was only withheld for 6 h. Few previous studies examined withholding and refeeding TMR. Studies have examined effects in grazing animals (Chilibroste et al., 2007), but data are still lacking on rumen kinetics to explain the mechanisms underpinning responses to such a challenge, and further work is required to fully understand responses in TMR-fed animals.

Figure 2. Hourly mean (a) relative rumen pH, (b) time spent eating, and (c) time spent ruminating of lactating dairy cows fed a TMR containing a high (HA) or low (LA) concentration of alfalfa silage at a long (L) or short (S) chop length over a 72-h period beginning at 0830 h on d 18 of the period, when feed was withheld for 6 h followed by a refeeding challenge at 1430 h. The hour beginning 1430 h is represented by 0 on the x axis. Black triangles indicate time points at which half a daily allowance of feed was offered. Hours at which we noted a significant effect of alfalfa inclusion rate (IR), alfalfa chop length (CL), or their interaction, analyzed using Mixed Models procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC), are marked.

Despite the reduced window of time when animals were allowed access to feed on the challenge day (18.5 h), we found no difference in the quantity of feed consumed or total minutes spent eating in comparison to baseline days, again highlighting that eating rate postrefeeding was increased in comparison to basal eating rate. Milk yield was reduced on the day of the challenge for all diets, and significantly so for LA diets, which might indicate a carryover effect of the fasting period for these diets or that the increased rate of feed consumption after refeeding reduced the efficiency of energy capture from the diet. Concentrations of fat and protein within the milk were largely unaffected, other than an unexpected rise in milk fat concentration seen on recovery d 2 in both the LAS and HAL diets; however, this is likely to be due to slightly reduced milk yield on these treatments, as total fat yield was unaffected. It should also be noted that using a single day as a baseline value may not have fully accounted for day-to-day variation in our study.

Acidosis Mitigation Potential of the Dietary Treatments

In the present study, cows fed diets comprising a high IR of alfalfa silage were less affected by the rumen challenge than those with a low IR, despite no difference in rumen pH profile between the diets at baseline. Alfalfa silage provided more effective fiber (Table 2) to the diet than the corn silage and has also been reported previously to have a higher cation exchange capacity than corn (McBurney et al., 1983); therefore, a combination of these 2 factors could explain the increased ability of the cows to buffer against low rumen pH. Furthermore, alfalfa often contains a higher proportion of indigestible, lignified stem in comparison to other forages that may reduce rumen passage rate and maintain rumen fill for longer, providing a better environment for continued microbial activity and facilitating a slow rate of VFA production in the rumen during the period of feed deprivation (Dewhurst et al., 2003). In support of this, the present study showed that cows fed HA diets spent more time ruminating during the fast period than those fed LA. This may have enhanced the rate of microbial adaptation to refeeding, reduced any disruption of epithelial function, and therefore reduced negative effects on milk yield. The LA diets also contained a higher concentration of starch that would have contributed to reduced rumen pH at refeeding. The difference in starch concentration between the 2 diets may also have altered utilization of dietary nutrients, particularly nitrogen. We observed no incidence of SARA in cows fed HA diets, confirming that feeding alfalfa at the higher IR of 375 g/kg diet of DM, and

consequently feeding less corn silage and starch, was successful at mitigating acidosis risk in comparison to the lower inclusion rate. Milk loss in cows fed LA diets on the day of the challenge (4.4 kg/d) was a decrease of 14.3% compared with baseline yield, which represents a cost to the farmer if animals were regularly fasted for similar periods (6 h continuous). Furthermore, the work of Dohme et al. (2008) suggests the severity of acidosis can increase where challenges are repeated in quick succession, although this was not evident in our study, as we observed no significant or numerical ($P > 0.2$) effect of period on time spent at pH <6.2. This is likely due methodological differences, as Dohme et al. (2008) induced challenges 14 d apart, as opposed to 21 d in the present study, and the effect of the challenges imposed were greater (using 4 kg of barley grain consumed within 1 h to induce acidosis) in their study, with nadir pH in the range of 5.13 to 5.53 versus 5.41 to 6.22 observed on recovery d 1 in our study. Furthermore, Dohme et al. (2008) also noted increased severity of subsequent acidosis challenges when cows were in early lactation as opposed to mid-lactation.

Evidence from jaw movement monitors in the present study confirmed that the long chop length increased rumination activity as would be expected; however, animals fed diets containing L chop alfalfa silage had lower ruminal pH on average on recovery d 1 than animals fed S, with those fed LAL diets having the greatest and most prolonged reduction in ruminal pH in comparison to the other diets. In this regard, our findings contrast with previously published work that suggested a positive correlation between rumen pH and peNDF concentration (Zebeli et al., 2006), which was attributed to increased rumination supplying more saliva to the rumen, although these relationships were generated from studies where no feed withholding and refeeding challenge was applied. Lengthening chop length can negatively affect diet uniformity and allow increased sorting against longer particles, which would contain the most peNDF (Leonardi and Armentano, 2003); however, this is unlikely to explain the lower rumen pH of cows on L diets on recovery d 1, as animals have previously been shown to increase selection of longer particles in response to a rumen challenge (DeVries et al., 2008). The beneficial effect of peNDF is thought to be the result of increased stimulation of rumination producing saliva to buffer the rumen, and, in line with this, HAL diets did increase rumination; however, we did not observe the same effect in the other diets, including LAL, where the concentration of peNDF was lower. Longer particles would have required rumination to aid digestion after ingestion; however, in our study rumination was reduced during the refeeding event while eating was prioritized, an effect that has

also been observed in previous refeeding work (Chilibroste et al., 2007), meanwhile smaller forage particles and concentrates can be broken down without the need for further rumination chewing. This delay in rumination due to overeating may have reduced the ability of animals fed LAL to digest the forage portion of the diet. It is also possible that fiber digestion was impaired as a result of the low-pH conditions affecting microbial populations (Grant and Mertens, 1992). Reductions in DMI in animals fed the long CL diets on both recovery d 1 and 2 relative to those fed short CL diets (a difference that was not observed at baseline) also supports this explanation, as reducing fiber digestibility of dietary alfalfa has previously been linked to reduced appetite (Getachew et al., 2011; Fustini et al., 2017), likely due to increased feeling of satiety. However, if fiber digestion was reduced, the lack of an effect on milk composition suggests the effect was short-lived. Based on the negative effect of increasing peNDF provision through increased chop length, it is likely the mitigation effect of high alfalfa IR was attributable to the buffering capacity of alfalfa, increased rumen fill during the feed withholding phase, and reduced diet starch concentration rather than any effect of peNDF per se.

In the LAL diet, effects of the challenge continued throughout recovery d 1 despite a return to baseline feeding patterns, with DMI also reduced for this diet on recovery d 2. The timeline is similar to that observed previously in the literature (Oetzel, 2007), where a cow faced with a 12-h fast followed by a refeeding challenge took 60 h for rumen pH to return to prefast levels. The extended number of days over which significant effects were seen despite no further challenges being applied highlights the need for rumen pH to be observed over several days when investigating induced SARA experimentally.

CONCLUSIONS

We concluded that a relatively short fast (6 h) followed by a refeeding event, in which a day's allocation of feed equal to the prefast level was offered ad libitum, was sufficient to induce SARA in 4 out of 8 observations where low-alfalfa diets were fed. However, a high rate of alfalfa inclusion within the diet combined with a lower dietary starch concentration mitigated the acidosis risk, and was particularly effective when the alfalfa silage was chopped to a shorter length. We attribute this mitigation effect to (1) buffering capacity provided by the alfalfa, (2) less degradable alfalfa fractions providing rumen substrate during the fast, and (3) reduced dietary starch concentration rather than increased effective fiber provision, as a longer particle length led to greater reductions in rumen pH after refeeding. Milk

lost from cows fed diets with lower inclusion rates of alfalfa would represent a significant financial loss if such a refeeding challenge were to occur regularly, highlighting the need to ensure uniformity of feeding routines in ad libitum TMR feeding systems for dairy cows on a day-to-day basis.

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