Effects of replacing maize silage with lucerne silage and lucerne silage chop length on rumen function and milk fatty acid composition

A. L. Thomson, D. J. Humphries, K. E. Kliem, M. T. Dittmann, and C. K. Reynolds¹

Centre for Dairy Research, School of Agriculture, Policy and Development, University of Reading, PO Box 237, Earley Gate, Reading, RG6 6AR, United Kingdom

ABSTRACT

The objective of this study was to investigate whether higher lucerne (*Medicago sativa*; alfalfa) silage inclusion rate and longer lucerne chop length improves rumen function through increased provision of physically effective fiber, when included in a maize and lucerne silage-based total mixed ration. Diets were formulated to contain a 50:50 forage:concentrate ratio [dry matter (DM) basis] and be isonitrogenous and contain equal levels of neutral detergent fiber (320 g/kg). The forage portion of the offered diets was composed of maize and lucerne silage DM in proportions (wt/wt) of either 25:75 (high lucerne; HL) or 75:25 (low lucerne; LL). Second-cut lucerne was harvested and conserved as silage at either a long (L) or a short (S) chop length (geometric mean particle lengths of 9.0 and 14.3 mm, respectively). These variables were combined in a 2×2 factorial arrangement to give 4 treatments (HLL, HLS, LLL, LLS), which were fed in a 4×4 Latin square design study to 4 rumen-cannulated, multiparous, Holstein dairy cows in mid lactation. Effects on DM intake, chewing behavior, rumen volatile fatty acid concentration, rumen pH, rumen and fecal particle size, milk production, and milk fatty acid profile were measured. Longer chop length increased rumination times per kilogram of DM intake (+2.8 min/kg) relative to the S chop length, with HLL diets resulting in the most rumination chews. Rumen concentrations of total volatile fatty acids, acetate, and n-valerate were higher for the HLS diet than the other 3 diets, whereas rumen propionate concentration was lowest for the HLL diet. Physically effective fiber (particles >4 mm) percentage in the rumen mat was increased when L chop length was fed regardless of lucerne inclusion rate. No effect of treatment was observed for milk yield, although milk protein concentration was increased by L for the LL diet (+1.6 g/kg) and decreased by L for the HLL diet

(−1.4 g/kg). Milk fat concentrations of total *cis*-18:1 (+3.7 g/100 g of fatty acids) and 18:3 n-3 (+0.2 g/100 g of fatty acids) were greater with HL. In conclusion, longer lucerne silage chop length increased time spent ruminating per kilogram of DM intake, but had no effect on rumen pH in the present study. Increasing dietary lucerne inclusion rate had no effects on rumination activity or rumen pH, but decreased the ratio of n-6:n-3 polyunsaturated fatty acid concentrations in milk fat.

Key words: lucerne, silage, rumination, rumen health, milk fatty acid, effective fiber

INTRODUCTION

The physical form of a TMR can affect rumen function and the efficiency of digestion in lactating dairy cows (Allen, 1997). Lucerne silage is thought to promote rumen health as it contains high NDF and ADF concentrations as well as having a higher natural buffering capacity (based on cation exchange capacity) than silages such as maize or ryegrass (McBurney et al., 1983). Factors that are considered markers of rumen health include pH, VFA profile, time spent ruminating (increasing saliva production), and consistency of the rumen mat (Weidner and Grant, 1994; Plaizier et al., 2008; Zebeli et al., 2012). For optimal rumen health, highly fermentable concentrate feedstuffs must be adequately balanced by forage physically effective fiber (**peNDF**) in TMR.

Physically effective fiber is defined as the NDF present within the long forage particles (Mertens, 1997) and can be increased by lengthening forage particle size. However, relationships between particle size and the rumen environment are complex and different particle sizes can play different roles, such as rumen mat formation and stimulation of rumination, although there are conflicting views within the literature on the relative effectiveness of different particle sizes. For example, Zebeli et al. (2012) suggested that all particles greater than 1.18 mm are effective at stimulating rumination but only particles greater than 8 mm form the structure

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¹Corresponding author: c.k.reynolds@reading.ac.uk

of the rumen mat, whereas Heinrichs (2013) suggested that only particles greater than 4 mm should be considered physically effective. Furthermore, an oversupply of long particles has been shown, in some instances, to reduce DMI and milk yield, possibly through excessive rumen fill (Kononoff and Heinrichs, 2003) and reduced surface area for bacterial attachment and thus digestibility (Zebeli et al., 2008). Therefore, the optimum dietary inclusion rate (**IR**) of individual forages may vary depending on their chop lengths (**CL**). To this end, the main objective of this study was to evaluate the effect of 2 IR of lucerne silage within a maize and lucerne silage-based TMR with 2 different lucerne **CL** on parameters associated with rumen health and function. A secondary objective was to examine whether any changes in diet composition and rumen fermentation were associated with changes in milk yield and composition.

MATERIALS AND METHODS

Forage Harvesting and Clamp Sampling

The present study formed part of a larger trial reported previously (Thomson et al., 2017) utilizing the same dietary treatments and a larger cohort of cows. In brief, the lucerne silage used was a second cut crop, harvested in the calendar year before the present study at an estimated 10% bloom, windrowed, and wilted for 48 h to produce a high DM concentration (576 g/kg) silage. Alternate swaths originating from the same field area were used to create 2 silages with differing **CL**, long (**L**) and short (**S**), by altering the knife arrangement of a precision chop forage harvester (Claas Jaguar, Claas Group, Harzewinkel, Germany) from a theoretical **CL** of 14 mm (shortest setting) to 19 mm (longest setting), which produced silages of 9.0 and 14.3 mm geometric mean particle length, respectively, assessed using a Penn State Particle Separator (**PSPS**; Heinrichs, 2013). The **L** and **S** chopped material was ensiled separately in identical adjacent clamps. Maize silage for the study was taken from a crop of mixed varieties harvested in the year before the present study and ensiled in a concrete-walled clamp with no additive. The geometric mean particle length for the maize silage was determined to be 10 mm.

Diets

A TMR with 50:50 ratio of forage:concentrate (DM basis) was fed. The forage was composed of maize and lucerne silage at **IR** (DM basis) of either 25:75 (high lucerne; **HL**) or 75:25 (low lucerne; **LL**), respectively. The 2 **IR** (**LL** or **HL**) and the 2 **CL** (**L** or **S**) were

Table 1. Ingredients used to create experimental TMR

Ingredient (g/kg of DM)	Diet ¹	
	LL	HL
Lucerne silage	125	375
Maize silage	375	125
Concentrate blend		
Cracked wheat	80	80
Maize 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	10	10
Megalac ²	15	15

¹LL = low lucerne diet; HL = high lucerne diet.
²Megalac rumen-protected fat supplement (Volac International Ltd., Royston, UK).

combined in a 2 × 2 factorial arrangement to give 4 treatments (**HLL**, **HLS**, **LLL**, **LLS**). Diets were formulated (Thomas, 2004) to be isonitrogenous (170 g of CP/kg of DM) and contain similar levels of NDF (320 g/kg of DM) through variation in the **IR** of soy hulls and rapeseed meal, based on preliminary analysis of core silage samples and reference values for other components. Maize meal was included at higher rates in the **HL** diet to offset the reduction in maize silage starch inclusion (Table 1); however, starch concentration was still greater in **LL** diets than **HL** diets (Table 2) and predicted ME concentration was lower in **HL** than **LL** diets (11.5 and 12.0 MJ/kg of DM, respectively).

Animals

Four multiparous Holstein-Friesian dairy cows in mid lactation (161 DIM, SEM ±23.1), producing 39.7 L/d milk yield (SEM ±6.2 L), and in sixth parity (SEM ± 0.3) on average at the start of the study were used. Cows weighed 741 kg (SEM ±13.9) at the start of the study and gained 25 kg (SEM ±34.6) on average over the study duration. Cows were fitted in a previous lactation with rumen cannulas (Bar Diamond, Parma, ID). Animals 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 regulated animal procedures used were licensed and monitored by the UK Government Home Office under the Animal (Scientific Procedures) Act 1986. Animals were housed in a cubicle yard and individually fed once daily for ad libitum intake through Insentec RIC feeders (Insentec B.V., Marknesse, the Netherlands) during wk 1 and 2 of each period. Cubicles were bedded with wood

Table 2. The chemical and physical composition of 4 TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length

Item	Diet				SEM	<i>P</i> -value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IR × CL
Chemical composition (g/kg of DM, unless otherwise indicated)								
DM (g/kg)	555	571	610	632	5.0	0.022	0.065	0.364
OM	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
Fatty acid profile (g/kg of DM)								
16:0	8.82 ^a	7.13 ^b	7.21 ^b	7.51 ^b	0.391	0.089	0.061	0.014
18:0	1.03	1.03	0.98	0.86	0.091	0.247	0.453	0.494
18:1 <i>cis</i> -9	8.43	8.75	6.76	4.82	0.828	0.023	0.302	0.201
18:2n-6	10.9	10.6	8.9	7.0	0.74	0.003	0.077	0.182
18:3n-3	1.51 ^c	1.73 ^c	2.40 ^a	2.07 ^b	0.034	0.012	0.336	0.027
Total fatty acids	33.3	33.3	29.4	23.5	2.42	0.038	0.218	0.248
Particle size distribution ³								
Material retained (% of DM)								
19 mm	3.2 ^b	5.0 ^b	5.3 ^b	12.1 ^a	0.75	0.001	0.001	0.007
8 mm	36.4 ^c	41.9 ^a	37.4 ^{bc}	39.1 ^b	0.50	0.129	0.012	0.026
4 mm	16.5 ^b	13.5 ^c	18.7 ^a	12.6 ^c	0.24	0.033	0.001	0.004
Bottom pan	43.8	39.8	37.9	36.3	0.50	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 (% of DM)								
peNDF _{>19mm}	1.03 ^b	1.64 ^b	1.74	4.04 ^a	0.268	0.001	0.001	0.009
peNDF _{>8mm}	12.3	14.8	13.8	18.2	0.27	0.056	0.030	0.137
peNDF _{>4mm}	17.2	19.9	20.5	21.3	0.38	0.003	0.004	0.051
n ⁶	3	4	4	4				

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

¹IR = inclusion rate; CL = chop length.

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 as described by Heinrichs (2013).

⁵Physically effective fiber determined as the proportion of particles (DM basis) greater than the threshold length (specified in subscript) multiplied by NDF concentration.

⁶Number of observations.

shavings and continuous access to water was provided. In the final week of each period, animals were housed and milked in individual tie stalls situated adjacent to the cubicle yard to facilitate sampling. Animals were given 3 d to acclimatize to the stalls before sampling began. While in tie stalls, animals were fed twice daily at 1000 and 1600 h for ad libitum intake (10% refusals). Refusals were taken daily at 0930 h.

Experimental Routine

Intake and Diet Analysis. Weights of feed offered and refused were taken during d 14 to 17 of each period and the DM of both was determined by oven drying at 100°C for 24 h. Bulk daily grab samples of the TMR and diet components fed were frozen at −20°C until analysis. Diet components were analyzed for DM,

N, ash, NDF, ADF, starch, and water-soluble carbohydrates as described previously (Kliem et al., 2008) and concentrations for each TMR were calculated based on constituent IR. Crude protein concentration was calculated by multiplying N (g/kg of DM) by 6.25. The fatty acid (FA) profile of the TMR was determined using dried and ground (1-mm screen; Cyclotec Mill, Foss Systems, Hillerød, Denmark) TMR samples from each cow in each period. A 1-step extraction-transesterification procedure was performed as described previously (Kliem et al., 2008) using methyl heneicosanoate in toluene as the internal standard. A sample of each TMR was analyzed in triplicate for particle size distribution using a PSPS (fresh-weight basis). The PSPS used had sieves with holes measuring 4, 8, and 19 mm in diameter and a bottom pan. Material from each sieve was collected, bulked over each of the triplicate sub-samples,

and oven-dried at 60°C for 72 h to give a DM correction. Average particle size of the sample was calculated as described previously (Heinrichs, 2013). During the sample week of each period, each cow was fitted with a chew-monitoring headcollar and supporting analytical software (Rumiwatch, ITIN+HOCH GmbH, Fütterungstechnik, Liestal, Switzerland) capable of detecting jaw movements through pressure on the noseband and categorizing them as eating, ruminating, or drinking (Ruuska et al., 2016).

Milk Yield and Composition. Cows were milked twice daily at 0630 and 1630 h. Representative 30-mL milk samples, preserved using potassium dichromate, were taken at 6 consecutive milkings between d 15 and 18 of each period and analyzed for fat, protein, casein, lactose, urea, and SCC by mid-infrared spectroscopy on a CombiFoss machine (National Milk Laboratories, Chippenham, Wiltshire, UK). On d 18 a further sample was taken at each milking and stored at -20°C before being thawed, pooled according to milk yield, and analyzed for FA profile. Lipid was extracted from 1 mL of milk using ethanol, hexane, and diethylether and transesterified to FAME using methanolic sodium methoxide with subsequent FAME separation using GC (3400, Varian Inc., Palo Alto, CA) equipped with a flame-ionization detector as described previously (Kliem et al., 2008). Concentrations of FA are presented as g/100 g of total FA. Apparent recovery rates for 18:3n-3 and 18:2n-6 were calculated as the daily yield of these FA in milk as a percentage of the daily amount ingested in feed based on mean DMI, milk yield, and milk composition data for each cow in each period.

Rumen and Fecal Sampling. On d 15 of the treatment period 100-mL samples of rumen liquor were taken at just before feeding and then at 0.5, 1.0, 1.5, 2.0 h postfeeding and at each subsequent hour until 2200 h, making a total of 15 samples. Rumen samples were collected by aspiration using a 50-mL catheter tip syringe through a coarse filtered sample tube as described previously (Dittmann et al., 2016). The fluid was mixed immediately and the pH was measured (Hanna Instruments, Woonsocket, RI) after which a subsample was acidified to pH <2 using concentrated H₂SO₄ and stored at -20°C before analysis for NH₃ using a segmented flow analyzer as described previously (Sutton et al., 2000). A further nonacidified subsample was immediately placed in a freezer (-20°C) until analyzed for VFA concentration using GC (3400, Varian Inc.) procedures as described previously (Erwin et al., 1961).

On d 15 and 16 of each period spot samples (approximately 500 g) of feces voided were collected and bulked, until sufficient material (approximately 3 kg daily) was obtained. Up to 6 samples were obtained

per day provided that the feces were uncontaminated by bedding. Furthermore, on d 17, a grab sample of the rumen mat (approximately 3 kg) was obtained at 4 h postfeeding. The sample was taken by vertically removing handfuls of material until the liquid phase in the ventral rumen was reached, with each handful immediately placed into a collection bucket. Bulk samples of rumen and fecal material were mixed and a subsample of each was oven-dried at 60°C for 72 h. Subsamples were then sieved using an adaptation of the wet sieving procedure described by Kononoff and Heinrichs (2003) using 3 sieves of 1, 2, and 4 mm diameter. Sieves were manually shaken while held under a cold water tap at a fixed flow rate for 30 s. Any material passing through the 1-mm sieve could not be retained and was assumed to be very small or soluble—a value for this was obtained as the difference between the starting dry weight and the combined dry weight of the other 3 fractions. A minimum of 4 replicates per sample were sieved with resulting material on each sieve being collected and bulked across replicates. Material from each sieve was analyzed for DM and NDF concentration by oven drying each fraction at 60°C for 72 h followed by subsequent determination of NDF concentration as described for feed samples.

Rumen pH. A weighted (300 g) indwelling pH meter (Sentix 41-3 probe, WTW Trifhof, Weilheim, Upper Bavaria) inserted through the bung of the rumen cannula and anchored to 50 cm of nylon cord was placed within the rumen of each animal for 24 h beginning just before feeding on d 15 of each period until refusals were removed at 0930 h on d 16. The probe was calibrated before every insertion and checked for drift after use through immersion in standard solutions of pH 4 and 7. The pH probe was attached to a datalogger (ph340i, WTW Trifhof) with readings recorded every 10 min. Readings were further averaged over each hour for analysis.

Data Analysis

Feed conversion efficiency was calculated as estimated milk energy yield (Tyrrell and Reid, 1965) divided by DMI. Dietary peNDF was calculated as the percentage of particles greater than 4, 8, or 19 mm (measured using a PSPS) multiplied by total dietary NDF for each TMR in each period on a DM basis. All measured daily mean variables were averaged for each cow and treatment combination and analyzed using mixed models procedures of SAS (version 9.4, SAS Institute Inc., Cary, NC) to determine fixed effects of period, lucerne IR, lucerne CL, and IR and CL interaction, and random effects of cow, with period included as a repeated effect. For rumen VFA, NH₃, and pH measurements,

the effect of time (**T**) was also included in the model as a repeated effect, with cow (period) as the subject, and tested for interactions with IR and CL ($T \times IR$, $T \times CL$, $T \times IR \times CL$) with the SLICE option used to test for treatment effects at each time point. For each variable, the covariance structure giving the best fit for repeated effects of period or of time were selected according to the structure giving the smallest Bayesian information criterion (from compound symmetry, heterogeneous compound symmetry, auto-regressive, heterogeneous autoregressive, unstructured, or spatial power covariance; spatial power was always used for time-based data where spacing was unequal between spot measurements). Data from 1 cow in period 4 were removed as her DMI and milk yield did not fully recover following mastitis that occurred during the adaptation period.

RESULTS

Diet Composition

Concentrations of DM, OM, and ADF (Table 2) were higher in HL diets than LL diets (all $P < 0.03$), whereas starch and water-soluble carbohydrate concentrations were lower (both $P < 0.04$) despite inclusion of maize meal in the HL diets. The HL diets also had lower concentrations of *cis*-9 18:1 ($P < 0.03$) and 18:2 n-6 FA ($P < 0.01$), whereas the concentration of 18:3n-3 was higher ($P < 0.02$). Furthermore, for HL diets, CL had a significant effect on 18:3n-3 concentration, with a greater concentration measured in HLS diets than HLL diets, whereas CL had no effect in LL diets ($IR \times CL$ interaction, $P < 0.03$). Overall, the HL diets contained less total FA than the LL diets ($P < 0.04$). The HLL

diet contained more than double the concentration of very long particles (>19 mm) relative to the other diets, an effect mirrored by concentrations of $peNDF_{>19mm}$ ($IR \times CL$ interaction, $P < 0.009$). The concentration of $peNDF_{>8mm}$ was mainly influenced by CL, with L diets containing 3.5% more $peNDF_{>8mm}$ than S diets ($P < 0.03$), whereas HL and L both increased $peNDF_{>4mm}$ ($P < 0.004$). A longer lucerne CL also increased concentration of ADF ($P < 0.007$) and decreased water-soluble carbohydrate concentration ($P < 0.02$) relative to a shorter CL.

Rumination Patterns and Rumen Parameters

Cows fed the L diets spent more time ruminating per unit of DMI ($P < 0.04$) and also tended to chew a greater number of times during rumination ($P < 0.09$) than when fed S diets (Table 3). The greatest number of rumination chews per day was observed for the HLL diet ($IR \times CL$ interaction, $P < 0.05$), whereas the greatest eating chews per day was observed for the LLS diet ($IR \times CL$ interaction, $P < 0.05$), which contained the least physically effectively fiber.

Over 12 h postfeeding, HL diets increased the concentration of rumen NH_3 relative to LL diets ($P < 0.001$; Table 4). Additionally, both CL and IR affected the rumen VFA profile in this time period, with LL diets increasing concentration of propionate ($P < 0.01$) and i-butyrate ($P < 0.007$) and reducing acetate:propionate ratio ($P < 0.001$), whereas total VFA and n-butyrate concentration were greater in S diets than L diets ($P < 0.03$). The HLS diet resulted in higher concentrations of total VFA, acetate, and n-valerate concentrations than the other 3 diets as indicated by $IR \times CL$ interactions (all $P < 0.009$; Table 4). In the case of propionate, LLS,

Table 3. Rumination activity and eating patterns of lactating dairy cows fed TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length

Item	Diet				SEM	<i>P</i> -value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IR $\times$ CL
Time spent								
Ruminating (min/d)	447	453	445	566	41.9	0.106	0.077	0.097
Eating (min/d)	290	223	239	221	34.8	0.362	0.205	0.441
Ruminating (min/kg of DMI)	19.4	20.7	19.0	23.4	1.18	0.341	0.035	0.140
Eating (min/kg of DMI)	12.9	10.2	10.0	9.5	1.89	0.132	0.208	0.375
Number of chews								
Ruminating ($\times 1,000$ /d)	27.9 ^a	26.9 ^b	25.6 ^c	34.9 ^a	2.69	0.192	0.081	0.043
Eating ($\times 1,000$ /d)	19.0 ^a	12.3 ^b	12.6 ^b	13.1 ^{ab}	2.37	0.093	0.081	0.050
Ruminating ($\times 1,000$ /kg of DMI)	1.27	1.24	1.09	1.40	0.090	0.882	0.147	0.097
Eating ($\times 1,000$ /kg of DMI)	0.84 ^a	0.55 ^b	0.54 ^b	0.58 ^b	0.123	0.052	0.087	0.042
n ²	3	4	4	4				

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

¹IR = inclusion rate; CL = chop length.

²Number of observations.

Table 4. Rumen pH, VFA profile, and NH_3 concentration of lactating dairy cows fed TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length

Item	Diet				SEM	<i>P</i> -value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IR $\times$ CL
Manual sample ²								
NH_3 (mg/L)	90	96	156	141	14.1	0.001	0.664	0.331
Rumen pH	6.25	6.24	6.22	6.38	0.141	0.186	0.103	0.058
VFA profile (mM)								
Total VFA	118 ^b	121 ^b	130 ^a	114 ^b	7.11	0.296	0.030	0.002
Acetate	72.3 ^b	74.4 ^b	81.6 ^a	74.2 ^b	3.39	0.013	0.132	0.009
Propionate	24.1 ^a	25.9 ^a	24.5 ^a	20.2 ^b	2.58	0.009	0.181	0.004
n-Butyrate	16.4	15.1	17.8	15.0	1.52	0.253	0.002	0.185
i-Butyrate	0.70	0.77	1.03	0.87	0.064	0.006	0.526	0.097
n-Valerate	1.82 ^b	2.07 ^b	2.55 ^a	1.91 ^b	0.201	0.009	0.062	0.001
i-Valerate	1.35	1.41	1.65	1.19	0.143	0.737	0.136	0.054
Caproate	0.71	1.00	0.95	0.74	0.161	0.927	0.760	0.067
Acetate:propionate	3.10	3.05	3.37	3.74	0.257	0.001	0.155	0.079
n	3	4	4	4				
24-h pH measurement ³								
Average pH	6.52	6.38	6.31	6.43	0.281	0.764	0.973	0.622
Maximum pH	6.84	6.70	6.68	6.81	0.148	0.702	0.916	0.142
Minimum pH	5.97	5.88	6.02	6.14	0.127	0.254	0.378	0.686
n ⁴	3	4	4	4				

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

¹IR = inclusion rate; CL = chop length.

²The least squares means of measurements taken at -0.5, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h after morning feeding in each cow in each period.

³pH measurements were taken every 10 min over a 24-h period with an indwelling pH meter with data averaged every hour for analysis.

⁴Number of observations.

LLL, and HLS diets showed similar concentrations with an average of 25 mM, whereas feeding the HLL diet resulted in a lower propionate concentration of 20 mM (IR $\times$ CL interaction, $P < 0.004$).

Rumen propionate concentration was consistently lower throughout the 12-h time period with the HLL diet compared with the other diets with significant differences recorded at 1300, 2000, 2100, and 2200 h ($P < 0.05$, Figure 1a). The HLS diet resulted in a higher rumen concentration of both acetate and total VFA at certain time points, but the effect was inconsistent (Figure 1b and 1c). Despite these effects on VFA profile, treatments had no significant effect on average, minimum, or maximum rumen pH measured over the same 24-h period, although the mean pH during 12 h postfeeding did show a tendency for HLL diets to have an elevated pH in comparison to the means of the other 3 diets (IR $\times$ CL interaction, $P < 0.06$; Figure 1d).

For samples of rumen mat, feeding L increased the DM percentage of large particles (>4 mm) by 14 percentage points ($P < 0.002$; Table 5) and decreased the DM percentage of medium particles (2–4 mm) by 3.8 percentage points ($P < 0.002$). The percentage of medium-length particles in the mat was greatest when the HLS diet was fed (IR $\times$ CL interaction, $P < 0.008$ on a DM basis and < 0.001 on an NDF basis). On an

NDF basis, feeding HL diets led to more small particles (1–2 mm) retained within the rumen mat than LL diets ($P < 0.05$). Fecal particle structure was largely unaffected by treatment diets, except for an increase in NDF retained on the 1-mm sieve when HL versus LL diets were fed ($P < 0.03$). Cows fed HL diets also had a tendency to void feces with a higher DM concentration ($P < 0.06$).

Intake, Milk Yield, and Composition

No consistent effect was observed of CL or IR on DMI, although LLL resulted in a lower DMI relative to LLS (IR $\times$ CL interaction, $P < 0.03$; Table 6). Milk yield and the yield of milk solids was not affected by diet, although milk protein yield tended to be greater for LL versus HL diets ($P < 0.063$). Milk protein concentration was increased by longer CL with the LL diets and decreased by longer CL with the HL diets (CL $\times$ IR interaction, $P < 0.001$), whereas overall milk protein concentration was higher for LL than HL diets ($P < 0.033$). Inclusion rate affected concentrations of some milk FA (Table 7). Milk concentrations of total *cis*-18:1 isomers (mainly composed of *cis*-9 18:1) and 18:3n-3 were both higher for diets containing a higher IR (both $P < 0.006$). Cows fed HL diets also produced milk

Figure 1. The rumen concentrations of (a) propionate, (b) acetate, (c) total VFA, and (d) pH of lactating dairy cows just before, and until 12 h after morning feeding when fed a TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length. *Asterisks indicate significant effects of time ($P < 0.05$).

with higher 4:0 and lower 10:0 concentration relative to cows fed LL (both $P < 0.04$). The apparent recovery of 18:2n-6 was increased by 3.8% when HL diets were fed ($P < 0.04$). Total MUFA concentration was higher in the HLL diet than in the LLL or HLS diet (IR $\times$ CL interaction, $P < 0.04$). A longer CL of lucerne tended to increase 18:1 and decrease 18:3n-3 concentrations in comparison to the shorter CL (both $P < 0.07$). In addition, longer CL increased n-6:n-3 PUFA concentration ratio in milk fat ($P < 0.018$).

DISCUSSION

Diet Physical Properties and Rumen Function

Particle size distribution in the diet was affected by both lucerne IR and CL (Table 2). Heinrichs (2013) suggested that all dietary particles greater than 4 mm contribute to formation of the rumen mat; however, the model of Zebeli et al. (2012) proposes that only particles greater than 8 mm in length promote increased rumination. In this study, both HL and L increased diet peNDF measured in all particle sizes. The longer lucerne CL, but not lucerne IR, tended to increase both rumination chews per unit of DMI and the concentration of particles >4 mm within the rumen mat, which suggests agreement with the proposed model of Zebeli et al. (2012) and is consistent with findings of several studies that have examined the effect of CL of the diet (Beauchemin et al., 1994; Clark and Armentano, 2002; Teimouri Yansari et al., 2004). From these data it could be concluded that although HL inclusion did increase effective fiber concentration in the diet relative to LL, the effect of CL on rumination was greater than the effect of IR. This may be due in part to the lack of effect of IR on particles >4 mm in the rumen mat. In the case of rumination, numbers of chews and time spent ruminating were highest for the HLL diet (although the effect did not always reach statistical significance). However, rumination activity did not always correlate with peNDF concentration as might be expected. For example, cows fed the LLS diet chewed more when eating than cows fed the other diets and also chewed more when ruminating relative to both LLL and HLS diets. Differences might be partly attributed to increased uniformity of the diet altering particle prehension or preventing cows sorting against the longer particles as has been observed previously in CL studies (Kmicikewycz and Heinrichs, 2015). Regardless of the cause, the higher chewing activity in the LLS diet could have led to a higher saliva production, which could explain why the daily mean pH of cows fed this diet were comparatively high.

Table 5. Rumen and fecal particle size distribution of lactating dairy cows fed TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length

Item	Diet				SEM	P-value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IR × CL
Rumen particle profile								
Total DM (g/kg)	182	149	166	159	10.3	0.789	0.116	0.250
Total NDF (g/kg of DM)	583	579	584	550	14.2	0.385	0.183	0.259
Material retained (% of DM)								
<1 mm or soluble ²	36.1	28.6	30.0	28.5	3.45	0.297	0.148	0.311
1–2 mm	14.5	15.0	19.3	15.7	2.02	0.400	0.593	0.500
2–4 mm	14.8 ^b	12.3 ^c	17.1 ^a	12.0 ^c	0.46	0.021	0.002	0.008
>4 mm	34.6	44.8	33.8	43.3	2.27	0.482	0.002	0.840
Material retained (% of NDF)								
<1 mm or soluble ²	19.9	9.2	10.5	7.7	3.63	0.129	0.072	0.259
1–2 mm	19.2	17.8	24.3	21.8	1.95	0.045	0.280	0.764
2–4 mm	17.7 ^b	15.5 ^c	22.0 ^a	14.9 ^c	0.61	0.003	0.001	0.001
>4 mm	43.4	57.4	42.7	56.2	2.80	0.662	0.001	0.901
Fecal particle profile								
Total DM (g/kg)	147	145	156	157	0.68	0.060	0.834	0.752
Total NDF (g/kg of DM)	474	488	442	433	1.57	0.168	0.855	0.486
Material retained (% of DM)								
<1 mm or soluble ²	51.3	51.7	54.1	53.1	2.51	0.323	0.878	0.760
1–2 mm	18.5	21.4	21.6	22.7	1.45	0.075	0.118	0.425
2–4 mm	11.5	13.3	14.1	11.4	1.72	0.845	0.733	0.216
>4 mm	14.6	13.1	12.7	12.9	0.85	0.143	0.213	0.111
Material retained (% of NDF)								
<1 mm or soluble ²	31.7	33.6	22.6	25.8	5.09	0.152	0.637	0.893
1–2 mm	30.7	33.7	39.9	40.4	2.76	0.024	0.484	0.624
2–4 mm	18.8	17.6	22.4	20.1	1.37	0.058	0.187	0.587
>4 mm	14.8	13.4	16.0	15.2	6.96	0.344	0.416	0.817
n ³	3	4	4	4				

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

¹IR = inclusion rate; CL = chop length.

²<1 mm or soluble material calculated as the starting amount minus material retained on each of the 3 sieves.

³Number of observations.

Table 6. Intake, milk yield, milk composition, and feed conversion efficiency of lactating dairy cows fed TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length

Item	Diet				SEM	P-value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IR × CL
DMI (kg/d)	25.1 ^a	22.4 ^b	23.1 ^{ab}	24.4 ^{ab}	1.08	0.991	0.152	0.027
Milk yield (kg/d)	29.3	29.4	29.1	28.4	3.93	0.519	0.704	0.646
Milk composition								
Fat (g/kg)	34.4	34.9	35.7	34.6	1.35	0.648	0.798	0.472
Protein (g/kg)	30.0 ^c	31.6 ^a	31.2 ^b	29.8 ^c	0.64	0.033	0.560	0.001
Lactose (g/kg)	45.0	44.6	45.5	44.6	0.60	0.572	0.190	0.642
Casein (g/kg)	22.8 ^b	24.4 ^a	23.9 ^a	22.2 ^b	0.88	0.051	0.763	0.019
Urea (mg/kg)	276	257	270	281	40.6	0.591	0.799	0.393
Component yield (kg/d)								
Fat	1.02	1.02	1.00	0.98	0.139	0.215	0.519	0.745
Protein	0.92	0.93	0.86	0.83	0.120	0.063	0.850	0.520
Lactose	1.32	1.37	1.29	1.22	0.184	0.172	0.859	0.401
Casein	0.70	0.71	0.66	0.64	0.095	0.096	0.773	0.570
n ²	3	4	4	4				

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

¹IR = inclusion rate; CL = chop length.

²Number of observations.

Rumen propionate concentrations in cows fed the HLL diet were consistently lower than in cows fed the other diets over a 12-h period after morning feeding. The reduction in propionate concentration might be attributed to reduced starch intake combined with longer CL reducing the rate of production or that the increased rumination seen in cows fed HLL diets led to higher levels of saliva production thus increasing the provision of bicarbonate, which is involved in the removal of VFA from the rumen by epithelial absorption (Dijkstra et al., 2012). In contrast, cows fed the HLS diet had the highest total VFA concentration and also the highest acetate concentration. The HLS diet,

with a higher concentration of short particles, may have facilitated a greater rate of fermentation in the rumen leading to a more rapid supply of VFA (Allen, 1997). The observation that the rumen mat of cows fed HLS diets had a greater proportion of 2 to 4 mm particles and a lower concentration of particles >4 mm than other diets at 4 h after feeding may support this explanation. Particles >4 mm within the rumen mat are thought to play a role in trapping smaller particles within the rumen longer, allowing increased digestion of nutrients (Zebeli et al., 2012).

Positive changes in rumination, peNDF concentration, and rumen mat structure are often associated

Table 7. Milk fatty acid profile of lactating dairy cows fed TMR in which the forage portion comprised lucerne silage and maize silage DM in proportions (wt/wt) of either 75:25 (high lucerne; HL) or 25:75 (low lucerne; LL) where lucerne silage was chopped to either a long (19 mm; L) or a short (14 mm; S) chop length

Item	Diet				SEM	P-value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IR × CL
Fatty acid profile (g/100 g of FA)								
4:0	2.49	2.49	2.75	2.76	0.134	0.001	0.894	0.871
6:0	1.71	1.69	1.73	1.69	0.054	0.670	0.894	0.765
8:0	1.11	1.07	1.02	0.99	0.054	0.168	0.426	0.987
10:0	2.73	2.63	2.44	2.27	0.194	0.034	0.300	0.750
12:0	3.36	3.28	2.19	2.70	0.656	0.159	0.700	0.603
14:0	11.3	11.2	10.4	10.7	0.30	0.084	0.631	0.307
16:0	32.0	32.9	31.7	32.4	1.17	0.461	0.161	0.807
18:0	8.88	8.19	9.05	9.11	0.628	0.266	0.501	0.427
18:1 <i>cis</i> -9	17.1	17.6	20.3	21.6	0.51	0.008	0.099	0.253
18:1 total <i>cis</i>	18.5	19.0	21.8	23.0	0.47	0.006	0.065	0.172
18:1 total <i>trans</i>	3.49	3.36	3.43	3.61	0.258	0.621	0.884	0.405
18:2n-6	2.46	2.40	2.67	2.69	0.176	0.141	0.869	0.780
18:2 total excluding CLA	2.94	2.87	3.25	3.17	0.203	0.127	0.681	0.967
18:2 total CLA	0.77	0.76	0.74	0.78	0.077	0.957	0.838	0.577
18:3n-3	0.37	0.35	0.57	0.47	0.018	0.002	0.066	0.197
20:0	0.14	0.14	0.14	0.16	0.018	0.400	0.649	0.560
20:1 total <i>cis</i>	0.08	0.09	0.10	0.13	0.027	0.238	0.392	0.773
20:2n-6	0.04	0.03	0.04	0.04	0.006	0.425	0.519	0.276
20:3n-6	0.07	0.07	0.06	0.10	0.030	0.794	0.392	0.373
20:4n-6	0.14	0.15	0.22	0.13	0.029	0.338	0.126	0.096
20:5n-3	0.03	0.03	0.05	0.04	0.003	0.012	0.349	0.630
22:0	0.14	0.13	0.46	0.11	0.030	0.885	0.177	0.497
22:4n-6	0.06	0.06	0.05	0.05	0.008	0.332	0.743	1.000
22:5n-3	0.07	0.08	0.08	0.06	0.015	0.675	0.759	0.380
Summary (g/100 g of fatty acid)								
Total SFA	66.5	67.5	67.5	65.2	0.83	0.385	0.377	0.170
Total MUFA	28.7 ^{ab}	28.0 ^b	27.7 ^b	29.8 ^a	0.62	0.250	0.103	0.036
Total <i>cis</i> MUFA	23.8	23.9	25.3	24.7	0.98	0.196	0.780	0.653
Total <i>trans</i> MUFA	4.07	3.93	4.06	4.18	0.277	0.568	0.967	0.509
Total PUFA	4.53	4.53	5.06	4.96	0.303	0.093	0.826	0.833
Total n-3 PUFA	0.65	0.65	0.86	0.76	0.090	0.106	0.464	0.560
Total n-6 PUFA	2.89	2.84	3.11	3.12	0.195	0.177	0.901	0.854
Ratio n-6:n-3 PUFA	4.72	5.00	3.31	3.77	0.413	0.002	0.018	0.438
Total unsaturates	33.6	32.6	32.4	34.8	0.82	0.409	0.326	0.145
Total <i>trans</i> -fats excluding CLA	4.54	4.41	4.64	4.67	0.297	0.434	0.801	0.717
Recovery rate (%)								
Apparent recovery 18:2n-6	10.3	10.4	12.3	16.0	1.63	0.034	0.212	0.250
Apparent recovery 18:3n-3	10.3	10.2	10.1	9.3	1.33	0.576	0.682	0.725
n ²	3	4	4	4				

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

¹IR = inclusion rate; CL = chop length.

²Number of observations.

with a rise in rumen pH (Zebeli et al., 2006). However, in the present study, no effects of lucerne silage IR or CL on daily mean rumen pH were observed, although there was a tendency for increased pH over the first 12 h postfeeding in the HLL diet. This contrasts with numerous studies investigating CL that have reported decreased rumen pH when average particle size is decreased (Kononoff and Heinrichs, 2003; Bhandari et al., 2008), although the effect is not always seen (Beauchemin and Yang, 2005; Suarez-Mena et al., 2013), perhaps indicating that a plateau can be reached above which greater inclusion of peNDF in the diet ceases to affect rumen pH. This is in agreement with Zebeli et al. (2006) who also observed a plateau in the relationship between peNDF and rumen pH at 30% peNDF in diet DM; however, the threshold length for peNDF measured using PSPS has since been increased from 1.18 to 4 mm, preventing a fair comparison with the present study. Similar to our study, altered ratios of maize silage to lucerne hay in the diet had no effect on rumen pH in lactating dairy cows in a study by Akbari-Afjani et al. (2014). Differences in the response of rumen pH to lucerne silage IR and CL may be influenced by the extent to which the basal diet represents a rumen pH challenge. For instance, in this study daily mean rumen pH was above 6.3 and minimum values were above 5.9, indicating that the threshold for SARA was never reached, even though the LLS, LLL, and HLS diets contained less than 18% peNDF_{>8mm}, which is proposed to be the threshold below which SARA risk increases (Zebeli et al., 2010). Zebeli et al. (2010) proposes that SARA risk can be avoided even in high-starch diets by balancing rumen degradable starch concentration with an equal or greater concentration of peNDF. In this study, the ratio of peNDF_{>4mm} to estimated total rumen degradable starch supply was 0.96, 1.11, 1.54, and 1.60 for the LLS, LLL, HLS, and HLL diets, respectively, which may explain why treatment diets had little effect on rumen pH. However, the range from minimum to maximum pH was greatest in the LLS diet (0.87 pH units) with a smaller range observed in HL diets (0.67 pH units on average), which could be a consequence of the higher buffering capacity of the lucerne silage.

Effect on Milk Yield and Composition

Diets used in this study had little effect on milk or milk constituent yield. The decrease in milk protein concentration for the HL versus LL diets was observed when these diets were fed to a larger group of cows (Thomson et al., 2017) and reflected the lower starch concentration provided by diets with a high concentration of lucerne silage. Milk yield and milk composition can also be negatively affected by lower digestibility

of lucerne-based diets, leading to a reduction of fermentable energy to drive microbial protein synthesis (Sinclair et al., 2015).

Substituting legumes for other forages such as grass has been shown to alter the FA profile of milk in ways that can be advantageous to milk quality (Dewhurst et al., 2006). Lucerne silage contains a higher concentration of 18:3n-3 than maize silage (Onetti et al., 2002; Benchaar et al., 2007), which was consistent with the present study. The higher concentration of 18:3n-3 FA in HL diets was probably the main reason for the higher concentration of 18:3n-3 in milk fat from cows fed HL diets (Khiaosa-Ard et al., 2015), which led to a reduced n-6:n-3 FA ratio. The reduction in the n-6:n-3 FA ratio in milk fat observed in the present study is consistent with previous reports of effects of increased dietary lucerne IR (Dhiman et al., 1999; Sinclair et al., 2015). For instance, in the study of Sinclair et al. (2015), the concentration of 18:3n-3 increased by 0.6 g/kg of FA when lucerne silage IR was increased from 40 to 60% of offered forage DM; which is in line with the present study where a larger increase in lucerne concentration (50% points of forage DM) resulted in a 1.6 g/kg of FA increase in 18:3n-3 concentration in milk fat. In the case of 18:2n-6, HL diets supplied a lower concentration than LL diets; however, due to a greater recovery rate in these diets, 18:2n-6 concentration was numerically greater in the milk from cows fed HL diets. Khiaosa-Ard et al. (2015) proposed that recovery rate of 18:2 n-6 is exponentially increased where a low rate of this FA (<10 g/kg of diet DM) is supplied by the diet. As HL diets were below the 10 g/kg of 18:2n-6 threshold, this might explain increased efficiency of dietary 18:2n-6 transfer to milk fat observed in the present study. High lucerne silage diets also increased milk fat *cis*-9 18:1 concentrations despite containing less *cis*-9 18:1 per kilogram of DM. Around 50% of *cis*-9 18:1 in milk fat is derived from the action of mammary Δ^9 -desaturase on 18:0 from the circulation (Enjalbert et al., 1998), which arises following complete biohydrogenation of dietary unsaturated 18-carbon FA. This is reflected in the numerically higher concentration of 18:0 in milk fat from cows fed HL diets. As HL diets contained less total unsaturated 18-carbon FA than LL diets, the difference in *cis*-9 18:1 is probably due to the rumen environment promoted by the HL diets, which likely favored complete biohydrogenation. The CL had relatively little effect on the milk FA profile. The ratio of n-6:n-3 PUFA concentrations in milk fat was decreased for S compared with L diets. In addition, tendencies were observed for lower concentrations of 18:3 n-3 and higher concentrations of 18:1 *cis*-9 when L was fed relative to S. This may suggest that L diets create a rumen environment that leads to more complete

biohydrogenation of dietary PUFA, perhaps through a reduction of rumen passage rate. Similarly, Dhiman et al. (1999) observed a small increase in total 18:1 isomer concentration (+2.1 g/kg of FA) when coarse alfalfa hay was fed in place of finely ground alfalfa hay, which is similar to the present study where total 18:1 concentration increased by 2.8 g/kg of FA when L was fed in place of S.

CONCLUSIONS

In the present study, feeding a higher lucerne silage IR and longer lucerne silage CL increased the dietary concentration of peNDF. Longer lucerne silage CL, but not greater IR, increased peNDF_{>4mm} in the rumen mat and rumination activity. However, dietary treatments had no effects on rumen pH, despite LL diets being higher in starch and lower in peNDF. Although lucerne silage IR had no effects on rumination activity or rumen pH in the present study, greater IR and shorter CL both decreased the ratio of n-6:n-3 PUFA concentrations in milk fat.

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