

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

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1 **Interpretive summary**

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

4

5 Thomson

6 Including a longer chop length lucerne silage in dairy cow diets had positive effects on
7 rumination time per unit feed intake and daily rumination time was highest when longer chop
8 lucerne silage was fed at higher inclusion rates. Longer chopped lucerne silage may be
9 beneficial for diets where low rumen pH is a concern. In addition, higher lucerne levels in cow
10 diets improved milk fatty acid profile in terms of human health, potentially increasing its value
11 for human consumption.

12 EFFECT OF LUCERNE SILAGE ON RUMEN PARAMETERS

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15

16

17 **Effects of replacing maize silage with lucerne silage and lucerne silage chop length on**

18 **rumen function and milk fatty acid composition**

19

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ABSTRACT

26
27 The objective of this study was to investigate whether higher lucerne (*medicago sativa*; alfalfa)
28 silage inclusion rate and longer lucerne chop length improves rumen function through
29 increased provision of physically effective fiber, when included in a maize and lucerne silage-
30 based total mixed ration. Diets were formulated to contain a 50:50 forage:concentrate ratio (dry
31 matter [DM] basis) and be isonitrogenous and contain equal levels of neutral detergent fiber
32 (320 g/kg). The forage portion of the offered diets was comprised of maize and lucerne silage
33 DM in proportions (w/w) of either 25:75 (high lucerne; HL) or 75:25 (low lucerne; LL). Second
34 cut lucerne was harvested and conserved as silage at either a long (L) or a short (S) chop length
35 (geometric mean particle lengths of 9.0 and 14.3 mm, respectively). These variables were
36 combined in a 2 x 2 factorial arrangement to give four treatments (HLL, HLS, LLL, LLS)
37 which were fed in a 4 x 4 Latin square design study to four rumen-cannulated, multiparous,
38 Holstein dairy cows in mid-lactation. Effects on dry matter intake (DMI), chewing behaviour,
39 rumen volatile fatty acid (VFA) concentration, rumen pH, rumen and fecal particle size, milk
40 production and milk fatty acid (FA) profile were measured. Longer chop length increased
41 rumination times/kg DMI (+2.8 min/kg) relative to the S chop length, with HLL diets resulting
42 in the most rumination chews. Rumen concentrations of total VFA, acetate, and n-valerate were
43 higher for the HLS diet than the other three diets, while rumen propionate concentration was
44 lowest for the HLL diet. Physically effective fiber (particles >4 mm) percentage in the rumen
45 mat was increased when L chop length was fed regardless of lucerne inclusion rate. No effect
46 of treatment was observed for milk yield although milk protein concentration was increased by
47 L for the LL diet (+1.6 g/kg) and decreased by L for the HLL diet (-1.4 g/kg). Milk fat
48 concentrations of total *cis*-18:1 (+3.7 g/100g FA) and 18:3 n-3 (+0.2 g/100g FA) were greater
49 with HL. In conclusion, longer lucerne silage chop length increased time spent ruminating per
50 kg DMI, but had no effect on rumen pH in the present study. Increasing dietary lucerne

51 inclusion rate had no effects on rumination activity or rumen pH, but decreased the ratio of n-
52 6:n-3 polyunsaturated fatty acid concentrations in milk fat.

53

54 **Keywords:** lucerne, silage, rumination, rumen health, milk fatty acids, effective fiber,

55

56

INTRODUCTION

57 The physical form of a total mixed ration (**TMR**) can affect rumen function and the efficiency
58 of digestion in lactating dairy cows (Allen, 1997). Lucerne silage is thought to promote rumen
59 health as it contains high NDF and ADF concentrations as well as having a higher natural
60 buffering capacity (based on cation exchange capacity) than silages such as maize or ryegrass
61 (McBurney *et al.*, 1983). Factors that are considered markers of rumen health include pH,
62 volatile fatty acid (**VFA**) profile, time spent ruminating (increasing saliva production), and
63 consistency of the rumen mat (Weidner and Grant, 1994; Plaizier *et al.*, 2008; Zebeli *et al.*,
64 2012). For optimal rumen health, highly fermentable concentrate feedstuffs must be adequately
65 balanced by forage physically effective fiber (**peNDF**) in TMR.

66 Physically effective fiber is defined as the NDF present within the long forage particles
67 (Mertens, 1997) and can be increased by lengthening forage particle size. However,
68 relationships between particle size and the rumen environment are complex and different
69 particle sizes can play different roles, such as rumen mat formation and stimulation of
70 rumination, although there are conflicting views within the literature on the relative
71 effectiveness of different particle sizes. For example, Zebeli *et al.* (2012) suggested that all
72 particles greater than 1.18 mm are effective at stimulating rumination whereas only particles
73 greater than 8 mm form the structure of the rumen mat; whereas Heinrichs (2013) suggested
74 that only particles greater than 4 mm should be considered physically effective. Furthermore,
75 an oversupply of long particles has been shown, in some instances, to reduce DMI and milk

76 yield, possibly through excessive rumen fill (Kononoff and Heinrichs, 2003) and reduced
77 surface area for bacterial attachment and thus digestibility (Zebeli *et al.*, 2008). Therefore, the
78 optimum dietary inclusion rate (**IR**) of individual forages may vary depending on their chop
79 lengths (**CL**). To this end, the main objective of this study was to evaluate the effect of two IRs
80 of lucerne silage within a maize and lucerne silage-based TMR with two different lucerne CLs
81 on parameters associated with rumen health and function. A secondary objective was to
82 examine whether any changes in diet composition and rumen fermentation were associated
83 with changes in milk yield and composition.

84

85 **MATERIALS AND METHODS**

86 ***Forage Harvesting and Clamp Sampling***

87 The present study formed part of a larger trial reported previously (Thomson *et al.*, 2017)
88 utilizing the same dietary treatments and a larger cohort of cows. In brief, the lucerne silage
89 used was a second cut crop, harvested in the calendar year before the present study at an
90 estimated 10 % bloom, windrowed, and wilted for 48 h to produce a high DM concentration
91 (576 g/kg) silage. Alternate swaths originating from the same field area were used to create
92 two silages with differing chop length (CL), long (**L**) and short (**S**) by altering the knife
93 arrangement of a precision chop forage harvester (Claas Jaguar, Claas Group, Harsewinkel,
94 Germany) from a theoretical CL of 14 mm (shortest setting) to 19 mm (longest setting) which
95 produced silages of 9.0 and 14.3 mm geometric mean particle length respectively; assessed
96 using a Penn State Particle Separator (**PSPS**) (Heinrichs, 2013). The L and S chopped material
97 was ensiled separately in identical adjacent clamps. Maize silage for the study was taken from
98 a crop of mixed varieties harvested in the year before the present study and ensiled in a
99 concrete-walled clamp with no additive. The geometric mean particle length for the maize
100 silage was determined to be 10 mm.

101

102 *Diets*

103 A TMR with 50:50 ratio of forage:concentrate (DM basis) was fed. The forage was
104 comprised of maize and lucerne silage at IRs (DM basis) of either 25:75 (high lucerne; **HL**)
105 or 75:25 (low lucerne; **LL**), respectively. The two IRs (LL or HL) and the two CL (**L** or **S**)
106 were combined in a 2 x 2 factorial arrangement to give four treatments (**HLL**, **HLS**, **LLL**,
107 **LLS**). Diets were formulated (Thomas, 2004) to be isonitrogenous (170 g CP/kg DM) and
108 contain similar levels of NDF (320 g/kg DM) through variation in the inclusion rates of soy
109 hulls and rapeseed meal, based on preliminary analysis of core silage samples and reference
110 values for other components. Maize meal was included at higher rates in the HL diet to offset
111 the reduction in maize silage starch inclusion (Table 1), however, starch concentration was
112 still greater in LL diets than HL diets (Table 2) and predicted metabolisable energy
113 concentration was lower in HL than LL diets (11.5 and 12.0 MJ/kg DM, respectively).

114

115 *Animals*

116 Four multiparous Holstein-Friesian dairy cows in mid lactation (161 d in milk, s.e.m. \pm 23.1),
117 producing 39.7 L/d milk yield (s.e.m \pm 6.2 L), in 6th parity (s.e.m \pm 0.3) on average at the start
118 of the study were used. Cows weighed 741 kg (s.e.m. \pm 13.9) at the start of the study and gained
119 25 kg (s.e.m. \pm 34.6) on average over the study duration. Cows were fitted in a previous
120 lactation with rumen cannula (Bar Diamond, Parma, Idaho, USA). Animals were randomly
121 assigned to one of four initial treatments according to a 4x4 Latin square design balanced for
122 carryover effects with 21 day periods. All regulated animal procedures used were licensed and
123 monitored by the UK Government Home Office under the Animal (Scientific Procedures) Act
124 1986. Animals were housed in a cubicle yard and individually fed once daily for *ad libitum*
125 intake through Insentec RIC feeders (Insentec B.V., Marknesse, The Netherlands) during

126 weeks 1 and 2 of each period. Cubicles were bedded with wood shavings and continuous access
127 to water was provided. In the final week of each period animals were housed and milked in
128 individual tie stalls situated adjacent to the cubicle yard to facilitate sampling. Animals were
129 given 3 days to acclimatise to the stalls before sampling began. While in tie stalls, animals were
130 fed twice daily at 1000 and 1600 h for *ad libitum* intake (10 % refusals). Refusals were taken
131 daily at 0930 h.

132

133 ***Experimental Routine***

134 ***Intake and Diet analysis.*** Weights of feed offered and refused were taken during days 14 – 17
135 of each period and the DM of both determined by oven drying at 100°C for 24 h. Bulked daily
136 grab samples of the TMR and diet components fed were frozen at -20 °C until analysed. Diet
137 components were analysed for DM, nitrogen (N), ash, NDF and ADF, starch, and water soluble
138 carbohydrates (WSC) as described previously (Kliem *et al.*, 2008) and concentrations for each
139 TMR were calculated based on constituent inclusion rates. Crude protein concentration was
140 calculated by multiplying N (g/kg DM) by 6.25. The fatty acid (FA) profile of the TMR was
141 determined using dried and ground (1 mm screen; Cyclotec Mill; Foss Systems, Hillerød,
142 Denmark). TMR samples from each cow in each period. A one step extraction-
143 transesterification procedure was performed as described previously (Kliem *et al.*, 2008) using
144 methyl heneicosanoate in toluene as the internal standard. A sample of each TMR was analysed
145 in triplicate for particle size distribution using a PSPS (fresh weight basis). The PSPS used had
146 sieves with holes measuring 4 mm, 8 mm and 19 mm in diameter and a bottom pan. Material
147 from each sieve was collected, bulked over each of the triplicate sub-samples, and oven-dried
148 at 60°C for 72 h to give a DM correction. Average particle size of the sample was calculated
149 as described previously (Heinrichs, 2013). During the sample week of each period, each cow
150 was fitted with a chew-monitoring headcollar and supporting analytical software (Rumiwatch,

151 ITIN+HOCH GmbH, Fütterungstechnik, Liestal, Switzerland) capable of detecting jaw
152 movements through pressure on the noseband and categorising them as either eating,
153 ruminating or drinking (Ruuska *et al.*, 2016).

154

155 ***Milk Yield and Composition.*** Cows were milked twice daily at 0630h and 1630h.
156 Representative 30 ml milk samples, preserved using potassium dichromate, were taken at six
157 consecutive milkings between days 15 and 18 of each period and analysed for fat, protein,
158 casein, lactose, urea, and somatic cell count (SCC) by mid infra-red spectroscopy on a
159 CombiFoss machine (National Milk Laboratories, Chippenham, Wiltshire, UK). On day 18 a
160 further sample was taken at each milking and stored at -20°C before being thawed, pooled
161 according to milk yield, and analysed for FA profile. Lipid was extracted from 1 ml of milk
162 using ethanol, hexane and diethylether and transesterified to FA methyl esters (**FAME**) using
163 methanolic sodium methoxide with subsequent FAME separation using a gas chromatograph
164 (GC; 3400, Varian Inc., Palo Alto, CA, USA) equipped with a flame-ionisation detector as
165 described previously (Kliem *et al.*, 2008). Concentrations of FA are presented as g/100g total
166 FA. Apparent recovery rates for 18:3 n-3 and 18:2 n-6 were calculated as the daily yield of
167 these FA in milk as a percentage of the daily amount ingested in feed based on mean DMI,
168 milk yield and milk composition data for each cow in each period.

169

170 ***Rumen and Fecal Sampling.*** On day 15 of the treatment period 100ml samples of rumen liquor
171 were taken at just prior to feeding and then at 0.5, 1.0, 1.5, 2.0 h post feeding and at each
172 subsequent hour until 2200 h making a total of 15 samples. Rumen samples were collected by
173 aspiration using a 50 ml catheter tip syringe through a coarse filtered sample tube as described
174 previously (Dittmann *et al.*, 2016). The fluid was mixed immediately and the pH was measured
175 (HANNA instruments, Woonsocket) after which a subsample was acidified to pH <2 using

176 concentrated H₂SO₄ and stored at -20 °C prior to analysis for ammonia (NH₃) using a
177 segmented flow analyzer as described previously (Sutton *et al.*, 2000). A further non-acidified
178 subsample was immediately placed in a freezer (-20 °C) until analysed for VFA concentration
179 using GC (3400, Varian Inc., Palo Alto, CA, USA) procedures as described previously (Erwin
180 *et al.*, 1961).

181 On day 15 and 16 of each period spot samples (approx. 500 g) of feces voided were
182 collected and bulked, until sufficient material (approx. 3 kg daily) was obtained. Up to six
183 samples were obtained per day providing that the feces were uncontaminated by bedding.
184 Furthermore, on day 17, a grab sample of the rumen mat (approx. 3 kg) was obtained at 4 h
185 post feeding. The sample was taken by vertically removing handfuls of material until the liquid
186 phase in the ventral rumen was reached, with each handful immediately placed into a collection
187 bucket. Bulked samples of rumen and fecal material were mixed and a subsample of each was
188 oven-dried at 60 °C for 72 h. Subsamples were then sieved using an adaptation of the wet
189 sieving procedure described by Kononoff and Heinrichs (2003) using three sieves of 1, 2 and
190 4 mm diameter. Sieves were manually shaken while held under a cold water tap at a fixed flow
191 rate for 30 seconds. Any material passing through the 1 mm sieve could not be retained and
192 was assumed to be very small or soluble – a value for this was obtained as the difference
193 between the starting dry weight and the combined dry weight of the other three fractions. A
194 minimum of four replicates per sample were sieved with resulting material on each sieve being
195 collected and bulked across replicates. Material from each sieve was analyzed for DM and
196 NDF concentration by oven drying each fraction at 60 °C for 72 h followed by subsequent
197 determination of NDF concentration as described for feed samples.

198

199 **Rumen pH.** A weighted (300 g) indwelling pH meter (Sentix 41-3 probe, WTW Trifthof,
200 Weilheim, Upper Bavaria) inserted through the bung of the rumen cannula and anchored to 50

201 cm of nylon cord was placed within the rumen of each animal for 24 h beginning just prior to
202 feeding on day 15 of each period until refusals were removed at 0930 h on day 16. The probe
203 was calibrated before every insertion and checked for drift after use through immersion in
204 standard solutions of pH 4 and 7. The pH probe was attached to a datalogger (ph340i, WTW,
205 Trifthof, Weilheim, Upper Bavaria) with readings recorded every 10 minutes. Readings were
206 further averaged over each hour for analysis.

207

208 *Data Analysis*

209 Feed conversion efficiency (**FCE**) was calculated as estimated milk energy yield (Tyrrell and
210 Reid, 1965) divided by DMI. Dietary peNDF was calculated as the percentage of particles
211 greater than either 4, 8 or 19 mm (measured using a PSPS) multiplied by total dietary NDF for
212 each TMR in each period on a DM basis. All measured daily mean variables were averaged for
213 each cow and treatment combination and analysed using mixed models procedures of SAS
214 (version 9.4, SAS Institute Inc., Cary, NC, USA) to determine fixed effects of period, lucerne
215 IR, lucerne CL, and IR and CL interaction, and random effects of cow, with period included as
216 a repeated effect. For rumen VFA, NH₃ and pH measurements the effect of time (T) was also
217 included in the model as a repeated effect, with cow (period) as the subject, and tested for
218 interactions with IR and CL (TxIR, TxCL, TxIRxCL) with the 'SLICE' option used to test for
219 treatment effects at each time point. For each variable the covariance structure giving the best
220 fit for repeated effects of period or of time were selected according to the structure giving the
221 smallest Bayesian Information Criterion (from compound symmetry, heterogeneous compound
222 symmetry, auto-regressive, heterogeneous autoregressive, unstructured, or spatial power
223 covariance; spatial power was always used for time-based data where there were unequal
224 spacing between spot measurements). Data from one cow in period 4 was removed as her DMI

225 and milk yield did not fully recover following mastitis that occurred during the adaptation
226 period.

227

228 **RESULTS**

229 *Diet Composition*

230 Concentrations of DM, OM and ADF (Table 2) were higher in HL diets than LL diets (all $P <$
231 0.03) while starch and water soluble carbohydrate concentrations were lower (both $P <$ 0.04)
232 despite inclusion of maize meal in the HL diets. The HL diets also had lower concentrations of
233 *cis*-9 18:1 ($P <$ 0.03) and 18:2 n-6 FA ($P <$ 0.01), while the concentration of 18:3 n-3 was
234 higher ($P <$ 0.02). Furthermore, for HL diets there was a significant effect of CL on 18:3 n-3
235 concentration, with a greater concentration measured in HLS diets than HLL diets, whereas CL
236 had no effect in LL diets (IR x CL interaction, $P <$ 0.03). Overall the HL diets contained less
237 total FA than LL diets ($P <$ 0.04). The HLL diet contained more than double the concentration
238 of very long particles (>19 mm) relative to the other diets, an effect mirrored by concentrations
239 of peNDF_{>19mm} (IR x CL interaction, $P <$ 0.009). The concentration of peNDF_{>8mm} was mainly
240 influenced by CL, with L diets containing 3.5 % more peNDF_{>8mm} than S diets ($P <$ 0.03),
241 while HL and L both increased peNDF_{>4mm} ($P <$ 0.004). A longer lucerne CL also increased
242 concentration of ADF ($P <$ 0.007) and decreased WSC concentration ($P <$ 0.02) relative to a
243 shorter CL.

244

245 *Rumination Patterns and Rumen Parameters*

246 Cows fed the L diets spent more time ruminating per unit DMI ($P <$ 0.04) and also tended to
247 chew a greater number of times during rumination ($P <$ 0.09) than when fed S diets. The
248 greatest number of rumination chews per day was observed for the HLL diet (CL x IR

249 interaction, $P < 0.05$), whereas the greatest eating chews per day was observed for the LLS diet
250 (IR x CL interaction, $P < 0.05$), which contained the least physically effectively fiber.
251 Over 12 h post feeding, HL diets increased the concentration of rumen NH_3 relative to LL diets
252 ($P < 0.001$; Table 4). Additionally, both CL and IR affected the rumen VFA profile in this time
253 period, with LL diets increasing concentration of propionate ($P < 0.01$) and i-butyrate ($P <$
254 0.007) and reducing acetate:propionate ratio ($P < 0.001$), while total VFA and n-butyrate
255 concentration were greater in S diets than L diets ($P < 0.03$). The HLS diet resulted in higher
256 concentrations of total VFA, acetate and n-valerate concentrations than the other three diets as
257 indicated by CL x IR interactions (all $P < 0.009$; Table 4). In the case of propionate, LLS, LLL
258 and HLS diets showed similar concentrations with an average of 25 mM whereas feeding the
259 HLL diet resulted in a lower propionate concentration of 20 mM (CL x IR interaction, $P <$
260 0.004).

261 Rumen propionate concentration was consistently lower throughout the 12 h time
262 period with the HLL diet compared with the other diets with significant differences recorded
263 at 1300, 2000, 2100, and 2200 h ($P < 0.05$, figure 1a). The HLS diet resulted in a higher rumen
264 concentration of both acetate and total VFA at certain time points, but the effect was
265 inconsistent (Figure 1b, 1c). Despite these effects on VFA profile, there were no significant
266 effect of treatments on average, minimum, or maximum rumen pH measured over the same 24
267 h period, although, the mean pH during 12 h post-feeding did show a tendency for HLL diets
268 to have an elevated pH in comparison to the means of the other three diets (IR x CL interaction,
269 $P < 0.06$; Figure 1d).

270 For samples of rumen mat, feeding L increased the DM percentage of large particles
271 ($>4\text{mm}$) by 14 % units ($P < 0.002$; Table 5) and decreased the DM percentage of medium
272 particles (2-4 mm) by 3.8 % units ($P < 0.002$). The percentage of medium length particles in
273 the mat was greatest when the HLS diet was fed (IR x CL interaction, $P < 0.008$ on a DM basis

274 and < 0.001 on an NDF basis). On an NDF basis, feeding HL diets led to more small particles
275 (1-2 mm) retained within the rumen mat than LL diets ($P < 0.05$). Fecal particle structure was
276 largely unaffected by treatment diets, except for an increase in NDF retained on the 1 mm sieve
277 when HL versus LL diets were fed ($P < 0.03$). There was also a tendency for cows fed HL diets
278 to void feces with a higher DM concentration ($P < 0.06$).

279

280 *Intake, Milk Yield and Composition*

281 There was no consistent effect of CL or IR on DMI although LLL resulted in a lower DMI
282 relative to LLS (IR x CL interaction, $P < 0.03$; Table 6). Milk yield and the yield of milk solids
283 was not affected by diet, although milk protein yield tended to be greater for LL versus HL
284 diets ($P < 0.063$). Milk protein concentration was increased by longer CL with the LL diets
285 and decreased by longer CL with the HL diets (CL x IR interaction, $P < 0.001$), whilst overall
286 milk protein concentration was higher for LL than HL diets ($P < 0.033$). Inclusion rate affected
287 concentrations of some milk FA (Table 7). Milk concentrations of total *cis*-18:1 isomers
288 (mainly comprised of *cis*-9 18:1) and 18:3 n-3 were both higher for diets containing a higher
289 IR (both $P < 0.006$). Cows fed HL diets also produced milk with higher 4:0 and lower 10:0
290 concentration relative to cows fed LL (both $P < 0.04$). The apparent recovery of 18:2 n-6 was
291 increased by 3.8 % where HL diets were fed ($P < 0.04$). Total MUFA concentration was higher
292 in the HLL diet than in the LLL or HLS diet (IR x CL interaction $P < 0.04$). A longer CL of
293 lucerne tended to increase 18:1 and decrease 18:3 n-3 concentrations in comparison to the
294 shorter CL (both $P < 0.07$). In addition, longer CL increased n-6:n-3 PUFA concentration ratio
295 in milk fat ($P < 0.018$).

296

297

DISCUSSION

298 *Diet Physical Properties and Rumen Function*

299 Particle size distribution in the diet was affected by both lucerne IR and CL (Table 2). Heinrichs
300 (2013) suggested that all dietary particles greater than 4 mm contribute to formation of the
301 rumen mat however the model of Zebeli *et al.* (2012) proposes that only particles greater than
302 8 mm in length promote increased rumination. In this study, both HL and L increased diet
303 peNDF measured in all particles sizes. The longer lucerne CL, but not lucerne IR, tended to
304 increase both rumination chews per unit DMI and the concentration of particles >4 mm within
305 the rumen mat which suggests agreement with the proposed model of Zebeli *et al.* (2012) and
306 is consistent with findings of a number of studies that have examined the effect of CL of the
307 diet (Beauchemin *et al.*, 1994; Clark and Armentano, 2002; Teimouri Yansari *et al.*, 2004).
308 From these data it could be concluded that although HL inclusion did increase effective fiber
309 concentration in the diet relative to LL, the effect of CL on rumination was greater than the
310 effect of IR. This may be due in part to the lack of effect of IR on particles >4 mm in the rumen
311 mat. In the case of rumination, numbers of chews and time spent ruminating were highest for
312 the HLL diet (although the effect did not always reach statistical significance). However,
313 rumination activity did not always correlate with peNDF concentration as might be expected.
314 For example, cows fed the LLS diet chewed more when eating than cows fed the other diets
315 and also chewed more when ruminating relative to both LLL and HLS diets. Differences might
316 be partly attributed to increased uniformity of the diet altering particle prehension or preventing
317 cows sorting against the longer particles as has been observed previously in CL studies
318 (Kmicikewycz and Heinrichs, 2015). Regardless of the cause, the higher chewing activity in
319 the LLS diet could have led to a higher saliva production, which could explain why the daily
320 mean pH of cows fed this diet were comparatively high.

321 Rumen propionate concentrations in cows fed the HLL diet were consistently lower
322 than in cows fed the other diets over a 12 h period after morning feeding. The reduction in
323 propionate concentration might be attributed to reduced starch intake combined with longer

324 CL reducing the rate of production or that the increased rumination seen in cows fed HLL diets
325 led to higher levels of saliva production thus increasing the provision of bicarbonate, which is
326 involved in the removal of VFAs from the rumen by epithelial absorption (Dijkstra *et al.*,
327 2012). In contrast cows fed the HLS diet had the highest total VFA concentration and also the
328 highest acetate concentration. The HLS diet, with a higher concentration of short particles, may
329 have facilitated a greater rate of fermentation in the rumen leading to a more rapid supply of
330 volatile fatty acids (Allen, 1997). The observation that the rumen mat of cows fed HLS diets
331 had a greater proportion of 2-4 mm particles and a lower concentration of particles >4 mm than
332 other diets at 4 h after feeding may support this explanation. Particles >4 mm within the rumen
333 mat are thought to play a role in trapping smaller particles within the rumen for longer, allowing
334 increased digestion of nutrients (Zebeli *et al.*, 2012).

335 Positive changes in rumination, physically effective fiber concentration, and rumen mat
336 structure are often associated with a rise in rumen pH (Zebeli *et al.*, 2006). However, in the
337 present study, no effects of lucerne silage IR or CL on daily mean rumen pH were observed
338 although there was a tendency for increased pH over the first 12 h post-feeding in the HLL
339 diet. This contrasts with numerous studies investigating CL that have reported decreased rumen
340 pH when average particle size is decreased (Kononoff and Heinrichs, 2003; Bhandari *et al.*,
341 2008) although the effect is not always seen (Beauchemin and Yang, 2005; Suarez-Mena *et al.*,
342 2013), perhaps indicating that a plateau can be reached above which greater inclusion of
343 peNDF in the diet ceases to affect rumen pH. This is in agreement with Zebeli *et al.* (2006)
344 who also observed a plateau in the relationship between peNDF and rumen pH at 30 % peNDF
345 in diet DM, however the threshold length for peNDF measured using PSPS has since been
346 increased from 1.18 mm to 4 mm preventing a fair comparison with the present study. Similar
347 to our study, altered ratios of maize silage to lucerne hay in the diet had no effect on rumen pH
348 in lactating dairy cows in a study by Akbari-Afjani *et al.* (2014). Differences in the response

349 of rumen pH to lucerne silage IR and CL may be influenced by the extent to which the basal
350 diet represents a rumen pH challenge. For instance, in this study daily mean rumen pH was
351 above 6.3 and minimum values were above 5.9, indicating that the threshold for SARA was
352 never reached, even though the LLS, LLL and HLS diets contained less than 18 % peNDF_{>8mm}
353 which is proposed to be the threshold below which SARA risk increases (Zebeli *et al.*, 2010).
354 Zebeli *et al.* (2010) proposes that SARA risk can be avoided even in high starch diets by
355 balancing rumen degradable starch (RDS) concentration with an equal or greater concentration
356 of peNDF. In this study the ratio of peNDF_{>4mm} to estimated total RDS supply was 0.96, 1.11,
357 1.54 and 1.60 for the LLS, LLL, HLS and HLL diets, respectively, which may explain why
358 there was little effect of treatment diets on rumen pH. However, the range from minimum to
359 maximum pH was greatest in the LLS diet (0.87 pH units) with a smaller range observed in
360 HL diets (0.67 pH units on average), which could be a consequence of the higher buffering
361 capacity of the lucerne silage.

362

363 ***Effect on Milk Yield and Composition***

364 Diets used in this study had little effect on milk or milk constituent yield. The decrease in milk
365 protein concentration for the HLL versus HLS diets was observed when these diets were fed to
366 a larger group of cows (Thomson *et al.*, 2017) and reflected the lower starch concentration
367 provided by diets with high concentration of lucerne silage. Milk yield and milk composition
368 can also be negatively affected by lower digestibility of lucerne-based diets leading to a
369 reduction of fermentable energy to drive microbial protein synthesis (Sinclair *et al.*, 2015).

370 Substituting legumes for other forages such as grass has been shown to alter the FA
371 profile of milk in ways which can be advantageous to milk quality (Dewhurst *et al.*, 2006).
372 Lucerne silage contains a higher concentration of 18:3 n-3 than maize silage (Onetti *et al.*,
373 2002; Benchaar *et al.*, 2007), which was consistent with the present study. The higher

374 concentration of 18:3 n-3 FA in HL diets was probably the main reason for the higher
375 concentration of 18:3 n-3 in milk fat from cows fed HL diets (Khiaosa-ard *et al.*, 2015), which
376 led to a reduced n-6:n-3 FA ratio. The reduction in the n-6:n-3 FA ratio in milk fat observed
377 in the present study is consistent with previous reports of effects of increased dietary lucerne
378 IR (Dhiman *et al.*, 1999; Sinclair *et al.*, 2015). For instance, in the study of Sinclair *et al.*
379 (2015), the concentration of 18:3 n-3 increased by 0.6 g/kg FA when lucerne silage IR was
380 increased from 40 to 60 % of offered forage DM; which is in line with the present study where
381 a larger increase in lucerne concentration (50 % units of forage DM) resulted in a 1.6 g/kg FA
382 increase in 18:3 n-3 concentration in milk fat. In the case of 18:2 n-6, HL diets supplied a lower
383 concentration than LL diets, however due to a greater recovery rate in these diets, 18:2 n-6
384 concentration was numerically greater in the milk from cows fed HL diets. Khiaosa-ard *et al.*
385 (2015) proposed that recovery rate of 18:2 n-6 is exponentially increased where a low rate of
386 this FA (<10 g/kg of diet DM) is supplied by the diet. As HL diets were below the 10g/kg 18:2
387 n-6 threshold this might explain increased efficiency of dietary 18:2 n-6 transfer to milk fat
388 observed in the present study. High lucerne silage diets also increased milk fat *cis*-9 18:1
389 concentrations despite containing less *cis*-9 18:1 per kg DM. Around 50 % of *cis*-9 18:1 in milk
390 fat is derived from the action of mammary Δ^9 desaturase on 18:0 from the circulation (Enjalbert
391 *et al.*, 1998), which arises following complete biohydrogenation of dietary unsaturated 18-
392 carbon FA. This is reflected in the numerically higher concentration of 18:0 in milk fat from
393 cows fed HL diets. As HL diets contained less total unsaturated 18-carbon FA than LL diets,
394 the difference in *cis*-9 18:1 is probably due to the rumen environment promoted by the HL
395 diets, which likely favoured complete biohydrogenation. There was relatively little effect of
396 CL on the milk fatty acid profile. The ratio of n-6:n-3 PUFA concentrations in milk fat was
397 decreased for S compared to L diets. In addition, there were tendencies for lower concentration
398 of 18:3 n-3 and higher concentration of 18:1 *c*9 when L was fed relative to S. This may suggest

399 that L diets create a rumen environment that leads to more complete biohydrogenation of
400 dietary PUFA, perhaps through a reduction of rumen passage rate. Similarly, Dhiman *et al.*
401 (1999) observed a small increase in total 18:1 isomer concentration (+2.1 g/kg FA) when coarse
402 alfalfa hay was fed in place of finely ground alfalfa hay, which is similar to the present study
403 where total 18:1 concentration increased by 2.8 g/kg FA when L was fed in place of S.

404

405

CONCLUSION

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

413

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421

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544 **Table 1** Ingredients used to create experimental total mixed rations.
 545

Item	Diet ¹	
	LL	HL
Ingredients, g/kg DM		
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

546 ¹ LL = low lucerne diet; HL = high lucerne diet;

547 ² Megalac rumen protected fat supplement (Volac International ltd., Royston, UK)

548

549 **Table 2** The chemical and physical composition of four total mixed rations containing a high
 550 (4:1 ratio with maize silage, DM basis; HL) or low (1:4 ratio with maize silage, DM basis;
 551 LL) concentration of lucerne silage at a long (19 mm; L) or short (14mm; S) chop length.

Item	Diet				SEM	<i>P</i> value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Chemical composition, g/kg DM								
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 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>c</i> 9	8.43	8.75	6.76	4.82	0.828	0.023	0.302	0.201
18:2 <i>n</i> -6	10.9	10.6	8.9	7.0	0.74	0.003	0.077	0.182
18:3 <i>n</i> -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, %DM								
19mm	3.2 ^b	5.0 ^b	5.3 ^b	12.1 ^a	0.75	0.001	0.001	0.007
8mm	36.4 ^c	41.9 ^a	37.4 ^{bc}	39.1 ^b	0.50	0.129	0.012	0.026
4mm	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 , %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
<i>n</i>	3	4	4	4				

552 ¹ IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL;

553 ² WSC = water soluble carbohydrate.

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

556 ⁴ Mean particle size was determined as described by Heinrichs (2013).

557 ⁵ peNDF determined as the proportion of particles (DM basis) greater than the threshold length (specified in
 558 subscript) multiplied by NDF concentration.

559 ^{a,b} Where there is a significant interaction, values within a row with different superscripts differ significantly at
 560 *P*<0.05.

561 **Table 3** Rumination activity and eating patterns of lactating dairy cows fed total mixed
 562 rations containing a high (4:1 ratio with maize silage, DM basis; HL) or low (1:4 ratio with
 563 maize silage, DM basis; LL) concentration of lucerne silage at a long (19 mm; L) or short
 564 (14mm; S) chop length.

Item	Diet				SEM	<i>P</i> value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Time spend								
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 DMI	19.4	20.7	19.0	23.4	1.18	0.341	0.035	0.140
Eating, min/kg DMI	12.9	10.2	10.0	9.5	1.89	0.132	0.208	0.375
Number of chews								
Ruminating, '000/d	27.9 ^a	26.9 ^b	25.6 ^c	34.9 ^a	2.69	0.192	0.081	0.043
Eating, '000/d	19.0 ^a	12.3 ^b	12.6 ^b	13.1 ^{ab}	2.37	0.093	0.081	0.050
Ruminating, '000/kgDMI	1.27	1.24	1.09	1.40	0.090	0.882	0.147	0.097
Eating, '000/kgDMI	0.84 ^a	0.55 ^b	0.54 ^b	0.58 ^b	0.123	0.052	0.087	0.042
<i>n</i>	3	4	4	4				

¹ IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL.

^{a,b} Where there is a significant interaction, values within a row with different superscripts differ significantly at *P*<0.05.

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570 **Table 4** Rumen pH, volatile fatty acid profile and ammonia concentration of lactating dairy
 571 cows fed total mixed rations containing a high (4:1 ratio with maize silage, DM basis; HL) or
 572 low (1:4 ratio with maize silage, DM basis; LL) concentration of lucerne silage at a long (19
 573 mm; L) or short (14mm; S) chop length.

Item	Diet				SEM	<i>P</i> value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Manual samples ²								
Ammonia, 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
<i>n</i>	3	4	4	4				
24h pH measurements 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
<i>n</i>	3	4	4	4				

574 ¹ IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL; VFA = volatile fatty acids.

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

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

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

580

581 **Table 5** Rumen and fecal particle size distribution of lactating dairy cows fed total mixed
582 rations containing a high (4:1 ratio with maize silage, DM basis; HL) or low (1:4 ratio with
583 maize silage, DM basis; LL) concentration of lucerne silage at a long (19 mm; L) or short
584 (14mm; S) chop length.

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

585 ¹ IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL.

586 ² >1mm or soluble material calculated as the starting amount minus material retained on each of the three sieves.

587 ^{a,b} Where there is a significant interaction, values within a row with different superscripts differ significantly at
588 *P*<0.05.

589 **Table 6** Intake, milk yield, milk composition and feed conversion efficiency of lactating
 590 dairy cows fed total mixed rations containing a high (4:1 ratio with maize silage, DM basis;
 591 HL) or low (1:4 ratio with maize silage, DM basis; LL) concentration of lucerne silage at a
 592 long (19 mm; L) or short (14mm; S) chop length.

Item	Diet				SEM	<i>P</i> value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
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, g/kg								
Fat	34.4	34.9	35.7	34.6	1.35	0.648	0.798	0.472
Protein	30.0 ^c	31.6 ^a	31.2 ^b	29.8 ^c	0.64	0.033	0.560	0.001
Lactose	45.0	44.6	45.5	44.6	0.60	0.572	0.190	0.642
Casein	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
<i>n</i>	3	4	4	4				

593 ¹IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL;

594 ²FCE = feed conversion efficiency.

595 ^{a,b} Where there is a significant interaction, values within a row with different superscripts differ significantly at
 596 *P*<0.05.

597

598

599 **Table 7** Milk fatty acid profile of lactating dairy cows fed total mixed rations containing a
600 high (4:1 ratio with maize silage, DM basis; HL) or low (1:4 ratio with maize silage, DM
601 basis; LL) concentration of lucerne silage at a long (19 mm; L) or short (14mm; S) chop
602 length.

Item	Diet				SEM	<i>P</i> value ¹		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Fatty acid profile, g/100g 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>c</i> 9	17.1	17.6	20.3	21.6	0.51	0.008	0.099	0.253
18:1 total cis	18.5	19.0	21.8	23.0	0.47	0.006	0.065	0.172
18:1 total trans	3.49	3.36	3.43	3.61	0.258	0.621	0.884	0.405
18:2 n-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
18:3 n-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 cis	0.08	0.09	0.10	0.13	0.027	0.238	0.392	0.773
20:2 n-6	0.04	0.03	0.04	0.04	0.006	0.425	0.519	0.276
20:3 n-6	0.07	0.07	0.06	0.10	0.030	0.794	0.392	0.373
20:4 n-6	0.14	0.15	0.22	0.13	0.029	0.338	0.126	0.096
20:5 n-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
22:4 n-6	0.06	0.06	0.05	0.05	0.008	0.332	0.743	1.000
22:5 n-3	0.07	0.08	0.08	0.06	0.015	0.675	0.759	0.380
Summary, g/100g FA ²								
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 cis MUFA	23.8	23.9	25.3	24.7	0.98	0.196	0.780	0.653
Total trans 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 trans-fats excluding CLA	4.54	4.41	4.64	4.67	0.297	0.434	0.801	0.717
Recovery rates, %								
Apparent recovery 18:2 n-6	10.3	10.4	12.3	16.0	1.63	0.034	0.212	0.250

	Apparent recovery 18:3 n-3	10.3	10.2	10.1	9.3	1.33	0.576	0.682	0.725
	<i>n</i>	3	4	4	4				

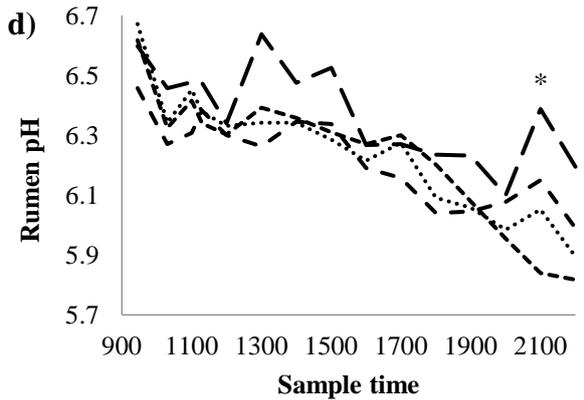
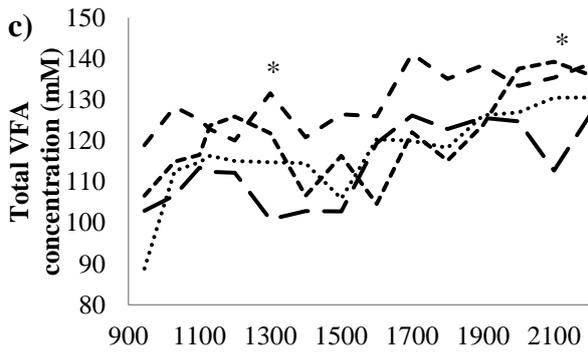
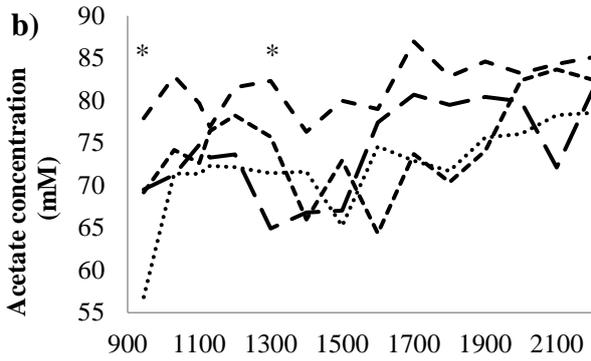
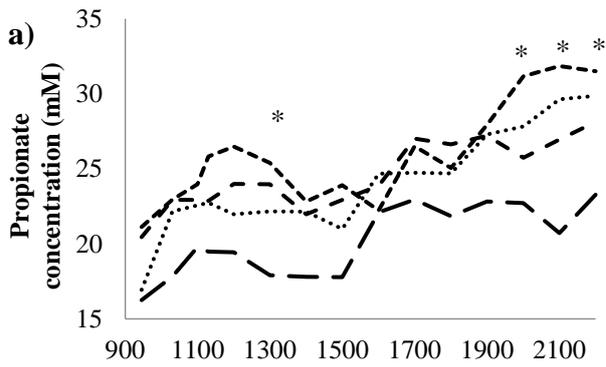
603 ¹IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL;

604 ²FA = fatty acid.

605 ^{a,b} Where there is a significant interaction, values within a row with different superscripts differ significantly at
606 *P*<0.05.

607 Thomson **Figure 1**

608



613 **Figure captions**

614

615 **Figure 1** The rumen concentrations of (a) acetate, (b) propionate (c) total volatile fatty acids
616 and (d) pH of lactating dairy cows just prior to, and until 12 h post morning feeding when fed
617 total mixed rations containing forage with a high (3:1 ratio with maize silage, DM basis; HL)
618 or low (1:3 ratio with maize silage, DM basis; LL) concentration of lucerne silage at a long
619 (19 mm; L) or short (14mm; S) chop length. Significant effects of time ($P < 0.05$) are marked
620 (*).