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1 **The effect of varying proportion and chop length of lucerne silage in a maize**
2 **silage-based total mixed ration on diet digestibility and milk yield in dairy**
3 **cattle**

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10

11 Short title: Inclusion rate and chop length of lucerne silage

12

13 **Abstract**

14 The objective was to assess the effects of inclusion rate and chop length of lucerne

15 silage, when fed in a total mixed ration (TMR), on milk yield, dry matter (DM) intake

16 (DMI) and digestion in dairy cows. Diets were formulated to contain a 50:50

17 forage:concentrate ratio (DM basis) and to be isonitrogenous (170 g/kg_{CP}). The

18 forage portion of the offered diets was comprised of maize and lucerne silage in

19 proportions (DM basis) of either 25:75 (HL) or 75:25 (LL). Lucerne was harvested

20 and conserved as silage at either a long (L) or short (S) chop length. These variables

21 were combined in a 2x2 factorial arrangement to give four treatments (HLL, HLS,

22 LLL, LLS) which were fed in a Latin square design study to Holstein dairy cows in

23 two separate experiments. Sixteen and 8 multiparous, mid-lactation, cows were used

24 in experiments 1 and 2, respectively. To ensure sufficient silage for both

25 experiments, different cuts of lucerne silage (taken from the same sward) were used

26 for each experiment: first cut for experiment 1 ([which was of poorer quality](#)) and
27 second cut for experiment 2. Dry matter intake, milk yield and milk composition
28 were measured in both experiments, and total tract digestibility and nitrogen (N)
29 balance were assessed using four cows in experiment 2. In experiment 1 cows fed
30 LL had increased DMI (+3.2 kg/day), compared with those fed HL. In contrast, there
31 was no difference in DMI due to lucerne silage inclusion rate in experiment 2. A
32 reduction in milk yield was observed with the HL treatment in both experiment 1 and
33 2 (-3.0 and -2.9 kg/day, respectively). The HL diet had reduced digestibility of DM
34 and organic matter (OM) (-3 and -4%, respectively), and also reduced the efficiency
35 of intake N conversion into milk N (-4%). The S chop length increased total tract
36 digestibility of DM and OM (both +4%), regardless of inclusion rate. Inclusion of
37 lucerne silage at 25% of forage dry matter increased milk yield relative to 75%
38 inclusion, but a S chop length partially mitigated adverse effects of HL on DMI and
39 milk yield in experiment 1 and on DM digestibility in experiment 2.

40

41 **Keywords:** lucerne, chop length, intake, milk yield, digestibility

42

43 **Implications**

44 A high inclusion rate of lucerne at 75% of forage dry matter (DM) within a total mixed
45 ration (TMR) negatively affected diet digestibility and milk yield relative to a low
46 inclusion rate. However, a short chop length increased diet digestibility at both
47 lucerne inclusion rates, and therefore could be used to partly mitigate the negative
48 effects of high lucerne silage inclusion in diets.

49

50 **Introduction**

51 Lucerne (*medicago sativa*) is widely utilised as a forage legume in dairy cow diets
52 in semi-arid environments including parts of the US, Eastern Europe and Australia.
53 Reduced requirement for inorganic N fertilisation may make it more economical to
54 grow than well fertilised grasses depending on fertiliser price (Phelan *et al.*, 2015)
55 and therefore shows potential for greater use in intensive Northern European dairy
56 systems. Establishing guidelines for the feeding of lucerne in such systems is critical
57 for efficient utilisation.

58 Lucerne and maize silages in the diet are complementary to each other with
59 the former providing rumen degradable protein and the latter providing fermentable
60 energy from starch to drive microbial protein synthesis using ammonia and amino
61 acids from lucerne protein degradation. Previous work has shown that the milk yield
62 obtained from lucerne-maize forage combinations can equal that of grass-maize
63 combinations (Sinclair *et al.*, 2015). However, the optimum inclusion rate of each is
64 not certain. In one study where inclusion rates of chopped lucerne hay to maize
65 silage were varied between 25% and 75% lucerne inclusion within forage dry matter
66 (DM), milk production decreased by 3.3 kg/d with the high rate of lucerne hay
67 inclusion (Akbari-Afjani *et al.*, 2014).

68 Lucerne is also a source of physically effective neutral detergent fibre
69 (peNDF) in diets for lactating dairy cows as it has a highly lignified stem that can
70 encourage rumination. Physically effective NDF has been defined as the NDF
71 present in longer particles within a feed (Mertens, 1997), typically considered to be
72 particles greater than 4 mm using the Penn State Particle Separator (PSPS) system
73 (Maulfair and Heinrichs, 2012). Previous research has shown short chop lengths (5
74 mm theoretical length) of lucerne haylage (Kononoff and Heinrichs, 2003) and silage
75 (Beauchemin *et al.*, 1994) can increase DM Intake (DMI) and improve energy

76 balance relative to long chop lengths of 22 and 10 mm respectively. Therefore, the
77 objective of our study was to examine the effects of lucerne silage chop length on
78 diet DMI and milk yield. A second objective was to investigate how chop length may
79 interact with the inclusion rate of lucerne silage when substituted for maize silage in
80 a TMR. We hypothesised that a lower inclusion rate of lucerne silage and a shorter
81 chop length will increase intake and milk yield in line with previous studies discussed
82 above and that these effects will relate to increased digestibility.

83

84 **Material and methods**

85 *Forage harvesting and clamp sampling*

86 This study involved two separate experiments carried out simultaneously at the
87 Centre for Dairy Research (CEDAR), University of Reading, between June and
88 September 2015. The lucerne silage for both experiments was made on-site in the
89 year prior to the start of the trial and conserved in concrete-walled clamps sheeted
90 with a layer of oxygen-barrier film, two layers of plastic sheeting and a weighted top
91 sheet. Experiment 1 utilised a first cut, which was ensiled on 31 May 2014
92 (estimated 10% bloom). The harvested material was windrowed and wilted for 24 h.
93 Alternate swaths originating from the same field area were used to create the two
94 chop lengths, long (L) and short (S), by altering the knife arrangement of the
95 precision chop forage harvester (Claas Jaguar 840 model, Claas Group,
96 Harsewinkel, Germany) from a theoretical chop length of 14 mm (shortest setting) to
97 19 mm (longest setting). The long chopped material was collected from the field first
98 followed by short chopped material and each were placed in identical adjacent
99 clamps. The resulting silage was ensiled using Axphast Gold additive containing
100 *Lactobacillus Plantarum* (Biotal, Cardiff) for low DM silages. The silage produced for

101 Experiment 2 was created on 11 July 2014 in the same way, from the same sward,
102 at second cut (also at an estimated 10% bloom). A longer wilting period of 48 h was
103 allowed, and Axcool Gold additive containing *Lactobacillus Buchneri* (Biotal, Cardiff,
104 UK) for high DM silages was applied. Following fermentation, core samples for all
105 cuts were taken for chemical composition analyses (Sciantec Analytical Services,
106 Cawood, UK). Maize silage for the study was taken from a commercial crop of mixed
107 varieties harvested in autumn 2014 and ensiled in a concrete-walled clamp with no
108 additive and sheeted as described for the lucerne clamps. The average particle size
109 for the maize silage was determined to be 10mm using a PSPS.

110

111 *Diets*

112 A TMR with 50:50 ratio of forage:concentrate on a DM basis was fed. The forage
113 was comprised of maize and lucerne silage in proportions (DM basis) of either 25:75
114 (high lucerne; HL) or 75:25 (low lucerne; LL), respectively. The two inclusion rates
115 and the two chop lengths (L or S) were combined in a 2x2 factorial design to give
116 four treatments (HLL, HLS, LLL, LLS). Diets were formulated to be isonitrogenous
117 (170g CP/kg DM) and contain similar levels of NDF (330 and 320 g/kg DM for
118 Experiments 1 and 2 respectively) based on an analysis of core samples from the
119 silage clamps used. Maize meal was included at higher rates in the HL diet to partly
120 offset the reduction in maize starch associated with lower maize silage inclusion in
121 these diets (Table 1), however there was still a significant difference between starch
122 concentration in the resulting TMRs (Table 2).

123

124 *Table 1*

125 *Table 2*

126

127 *Animals*

128 For Experiment 1, 16 multiparous Holstein-Friesian dairy cows in mid lactation (144
129 d in milk, s.e.m. \pm 4.3) weighing 701 kg and in fourth parity on average, were blocked
130 (4 cows per block) according to milk yield and randomly assigned to one of four initial
131 treatments within each block in a replicated 4x4 Latin square design experiment with
132 three week periods. Cows were housed in a cubicle yard, bedded on sand and
133 individually fed using CALAN gates (American Calan, Northwood, NH, USA).
134 Continual access to water was given. Fresh feed was offered for *ad libitum* intakes
135 (10% refusals per day) once daily at 1000 h. Refusals were removed on Mondays,
136 Wednesday and Fridays.

137 For Experiment 2, eight multiparous Holstein-Friesian dairy cows in mid
138 lactation (141 d in milk, s.e.m. \pm 13.4) weighing 704 kg and in fourth parity on
139 average, in two blocks (of which one block contained four cows fitted in a previous
140 lactation with Bar Diamond rumen cannula (Parma, Idaho, USA)) were randomly
141 assigned within each block to one of four initial treatments according to a 4x4 Latin
142 square design with three week periods as in experiment 1. The block of four non-
143 fistulated cows were used for measurements of total tract diet digestion. All
144 procedures carried out in experiment 2 were licensed and monitored by the UK
145 government Home Office under the Animal (Scientific Procedures) Act 1986.
146 Animals were housed in a cubicle yard and individually fed once daily for *ad libitum*
147 intake through Insentec RIC feeders (Insentec B.V., Marknesse, The Netherlands)
148 during weeks one and two of each period. Cubicles were bedded with wood
149 shavings and continuous access to water was provided. In the final week of each
150 period animals were housed and milked in individual tie stalls situated adjacent to

151 the cubicle yard to facilitate sampling. Animals were given two days to acclimatise to
152 the stalls before sampling began. While in tie stalls, animals were fed twice daily at
153 1000 and 1600 hours for *ad libitum* intake (10% refusals). Refusals were taken daily
154 at 0930 h.

155

156 *Experimental routine*

157 *Intake and diet analysis.* Weights of feed offered and refused were taken during the
158 final week of each period. For the four animals used for digestibility measurements
159 (experiment 2) only measurements from five days were statistically analysed. The
160 DM of the feed offered and refused was measured in a forced air oven at 100°C for
161 24 hours. Bulked daily grab samples of the TMR and diet components were also
162 taken and frozen at -20°C until analysed. Samples of the constituents of the TMR
163 were analysed for DM, N (using the macro kjeldahl method), ash (by combustion at
164 500°C for 16 hours), NDF (assayed with heat-stable amylase, inclusive of residual
165 ash), ADF (inclusive of residual ash), starch, and water soluble carbohydrates
166 (WSC) as described previously (Reynolds *et al.*, 2014). Starch was converted to
167 glucose by treatment of the hot water extract with amyloglucosidase followed by acid
168 hydrolysis (Macrae and Armstrong, 1968). Total reducing sugars were measured
169 calorimetrically and the result was corrected for cold water soluble reducing sugars
170 (Fuller, 1967). Crude protein (CP) concentration was calculated by multiplying N
171 (g/kg DM) by 6.25. Concentrations (g/kg DM) of CP, NDF, ADF, ash, starch and
172 WSC in each TMR were calculated based on constituent inclusion rates.

173 Furthermore, TMR and diet components were analysed in triplicate for particle size
174 distribution using a PSPS with holes measuring 4 mm, 8 mm and 19 mm in diameter
175 and a bottom pan. Material from each sieve was collected and dried (at 60°C for 72

176 h) to give a DM correction. Average particle size of the sample was calculated as
177 described previously (Heinrichs, 2013).

178 Degradability of DM and N in each forage was measured using an *in situ* method
179 with rumen cannulated lactating Holstein dairy cattle (Ørskov and McDonald, 1979).
180 These cattle were housed in cubicles, in a dedicated metabolism unit, fed a
181 commercial grass-maize based TMR diet once daily. Samples (not dried or further
182 chopped) of each silage were placed in polyester bags (40 µm pore size) that were
183 then incubated sequentially in the rumen of three different animals for six time
184 intervals (3, 6, 12, 24, 48, and 72h). Three replicate '0' hour bags were soaked in a
185 tub of cold tap water with agitation for 5 minutes before being refrozen alongside the
186 bags that were incubated in the rumen. Residue was subsequently analysed for DM
187 and N concentration as described above.

188

189 *Milk yield and composition.* Cows were milked twice daily at 0630h and 1630h. In
190 experiment 1 separate milk samples were taken during each of the last four
191 consecutive milkings in each period and analysed for fat, crude protein, casein,
192 lactose, urea, and somatic cell count (SCC) by mid infra-red spectroscopy on a
193 CombiFoss machine (National Milk Laboratories, Chippenham, Wiltshire, UK). In
194 Experiment 2 milk samples obtained throughout the third week of each period were
195 analysed as for experiment 1.

196

197 *Diet apparent digestibility and N balance.* Beginning at 1000 h (prior to morning
198 feeding) on day 17 of each period cows used for digestion trials were fitted with a
199 harness and chute allowing total collection of faeces and urine for five consecutive
200 24 h periods (Reynolds *et al.*, 2014). Urine was collected into containers containing 1

201 L of 5 molar sulphuric acid. In addition, 200ml spot urine samples were collected
202 twice daily in each of the five consecutive 24h periods, immediately acidified using
203 10ml of 5 molar sulphuric acid, and bulked. At the end of the collection period a
204 representative subsample of the bulked spot samples was obtained and stored
205 frozen until analysed for N. The bulked spot samples were used to determine urinary
206 N concentration to account for any volatilised N losses. At the end of each 24 h
207 period the total faeces and urine collected were weighed. Faeces were mixed, and
208 subsampled as a fixed proportion of total volume to produce a representative bulk
209 sample and stored at -20°C for subsequent analysis. Faecal and feed samples were
210 analysed for DM, N, OM, Starch, NDF, and ADF concentration and urine samples
211 analysed for N concentration as described above for feed samples (Reynolds *et al.*,
212 2014).

213

214 *Statistical analysis*

215 For silage degradability, an exponential curve fitted to percentage degradation at
216 each time point was used to obtain fractions termed 'a', 'b' and 'c' as described
217 previously (Ørskov and McDonald, 1979). Rumen outflow rate (k) was assumed to
218 be 0.05 hr⁻¹. Feed efficiency was calculated as estimated milk energy yield (Tyrrell
219 and Reid, 1965) divided by DMI. Data from each experiment were analysed
220 separately. Experiment 1 was analysed as four simultaneous Latin Squares.
221 Averages for each cow and treatment combination were analysed to determine fixed
222 effects of square, period, lucerne inclusion rate (IR), chop length (CL), and their
223 interaction (IRxCL) and random effects of cow within square using mixed models
224 procedures of SAS (version 9.1). For experiment 2, data obtained within two
225 simultaneous Latin Squares were analysed in the same way. For each variable the

226 covariance structure giving the best fit was selected. Data from one cow (not one
227 used for the digestion trial) in experiment 2 in period four was removed as her DMI
228 and milk yield did not fully recover following mastitis that occurred during the
229 adaptation period.

230

231 **Results**

232

233 *Forage quality*

234 The first cut silage used for experiment 1 had lower DM (-354 g/kg), and a higher pH
235 (+1.1), than the second cut silage used for experiment 2 (Table 3). Higher DM
236 (second vs first cut) and shorter chop length were associated with lower pH and
237 greater lactic acid concentration but reduced acetic, butyric and propionic acid
238 concentrations. Crude protein concentrations were similar for the first and second cut
239 silages (174 g/kg DM). Of particular note, NDF and ADF were higher in the first cut
240 silages than the second cut, suggesting greater maturity in the first cut silages.

241 The degradability fraction a was smaller in the experiment 1 lucerne silages
242 than in the experiment 2 silages (-13%) and there was also reduced effective
243 degradability and total degradation of DM (fractions a + b = 64% vs 75% for
244 experiment 1 and experiment 2 silages, respectively). Degradation profiles for N
245 showed that the lucerne silages had a higher EPD than that of maize. The rate of
246 degradation of N (c) in the rumen was faster for the short chopped silages for both
247 cuts but the difference was greater within the experiment 2 silages (0.04/h for L and
248 0.09/h for S; $P < 0.001$).

249

250 ***Table 3***

251

252 *Forage and diet particle size*

253 The average silage particle length was 12.6 mm and 9.4 mm in first cut silages ($P <$
254 0.006), and 14.3 mm and 9 mm in second cut silages ($P = 0.001$) for the long and
255 short chop silages respectively. For both experiments the long chop increased
256 particles retained on the 8 mm sieve and reduced particles on the 4 mm sieve and
257 the bottom pan relative to the short chop silages ($P < 0.01$; Figure 1). The long chop
258 length increased the proportion of particles on the 19 mm sieve for the lucerne silage
259 used in experiment 2 ($P < 0.001$), but not the lucerne silage used for experiment 1
260 (Figure 1).

261

262 **Figure 1**

263

264 In both experiments, average particle size of the diets fed (Table 4) increased with
265 both greater lucerne inclusion ($P < 0.001$) and chop length ($P < 0.05$, < 0.001 in
266 experiments 1 and 2 respectively). In experiment 1, the proportion of particles
267 retained on the 19 mm screen increased ($P < 0.02$) with increased chop length. The
268 proportion of particles retained on the 4 mm screen in experiment 1 was decreased
269 ($P < 0.03$) by increased chop length for the HL, but not the LL diet (inclusion rate by
270 chop length interaction, $P = 0.03$). In experiment 2, there were greater effects of
271 chop length on particle distribution on the 19 and 4 mm screens for the HL than the
272 LL diets (inclusion rate by chop length interaction, $P < 0.01$) and a greater difference
273 on the 8 mm screen for the LL than the HL diet (inclusion rate by chop length
274 interaction, $P < 0.05$).

275

276 *Table 4*

277

278 *Intake, milk yield and milk composition*

279 The effect of lucerne silage inclusion rate on DMI varied between experiments with a
280 DMI reduction of 3.2 kg/d where HL diets were fed in experiment 1 ($P < 0.001$),
281 whereas there was no difference in DMI between treatments in experiment 2 ($P >$
282 0.22). In both experiments feeding the HL diets decreased milk yield (-3.0 and -2.9
283 kg/d in Experiments 1 and 2 respectively; $P < 0.02$; Table 4). In experiment 1, a
284 longer chop length decreased milk yield relative to using a shorter chop length by -
285 1.6 kg/d ($P < 0.001$), although this effect was not observed in experiment 2. As a
286 result, the estimated conversion efficiency of feed DM into milk energy also differed
287 between experiments, with HL diets tending to produce greater conversion efficiency
288 in experiment 1 ($P < 0.08$) and LL diets increasing feed efficiency in experiment 2 (P
289 $= 0.001$).

290

291 *Table 5*

292

293 Milk fat concentration was not affected by treatment in either experiment (Table 5),
294 however, in experiment 1, milk fat yield was greater ($P < 0.017$) when LL diets were
295 fed. In experiment 1, milk protein concentration was increased by 0.7 g/kg ($P < 0.001$)
296 when HL diets were fed, although, due to increased milk yield, milk protein yield was
297 highest ($P < 0.001$) when LL diets were fed. In experiment 2, feeding HL diets led to a
298 decrease in milk protein concentration of 1.0 g/kg ($P < 0.04$) although there were no
299 differences in total protein yield between treatments. Milk protein yield in experiment
300 1 was reduced by chop length, where a 45 g/d reduction with longer chop length ($P <$

301 0.003) was observed. Milk urea concentration was higher ($P < 0.001$) when HL diets
302 were fed in experiment 1.

303

304 *Apparent digestibility and N balance*

305 Increasing lucerne inclusion rate decreased DM and starch intake and increased
306 ADF intake of cows used for measurements of digestibility and N-balance ($P < 0.04$,
307 < 0.003 , and < 0.006 respectively, Table 6). Digestibility of DM was lower for the HL
308 diets by 3.6% relative to the LL diets ($P < 0.05$). Increasing chop length also reduced
309 DM digestibility by 4.3% ($P < 0.02$). Greater inclusion rate of lucerne and longer chop
310 length both decreased the digestibility of organic matter by 3.7% and 3.2% ($P < 0.03$
311 and $P < 0.006$, respectively). There were no differences in the digestibility of starch,
312 NDF or ADF between HL and LL diets, although NDF digestibility tended ($P < 0.10$)
313 to be lower for longer lucerne chop length diets.

314

315 **Table 6**

316

317 Intakes of N were greater for LL diets ($P < 0.01$) as a result of higher DMI (Table 7).

318 There ~~was a tendency for~~ increased ~~ds in~~ faecal N concentration ($P < 0.064$) ~~and~~
319 ~~total manure (faecal plus urine) N excretion ($P < 0.03$)~~ when HL diets were fed.

320 Faecal N also ~~tended to~~ increased ~~d~~ when the chop length was increased ($P <$

321 ~~0.0709~~). There was greater partitioning of intake N into the milk for the LL diets with

322 an increase in N use efficiency of 3.3% ($P < 0.009$) ~~and N digestibility was also~~

323 ~~greater ($P < 0.02$). Shortening the chop length of the lucerne reduced faecal N~~

324 ~~excretion by 57 g/d ($P < 0.01$).~~

325

326 *Table 7*

327

328 Discussion

329 *Forage quality and particle size*

330 The nutritive value of the four lucerne silages used in the study was variable.

331 Although crude protein levels were similar at 172 g/kg, the second cut (experiment 2)

332 silages were lower in NDF and ADF than the first cut (experiment 1) silages,

333 suggesting increased maturity in the first cut relative to the second cut forage. [In the](#)

334 [silage fed in experiment 1, high acetic acid and low lactic acid concentrations](#)

335 [indicated poor fermentation, although pH reduction was adequate.](#) High levels of

336 WSC in the experiment 2 silage may indicate that increased time spent wilting this

337 crop (48h vs. 24h for the first cut) resulting in a higher DM reduced fermentation

338 activity, or that the original concentration of sugar in this crop was higher than for the

339 first cut crop. These results collectively indicate increased silage quality in the

340 second cut silage with higher DM concentration. The effective degradation of DM

341 and protein in the lucerne silages ranged from 37.8-56.7% and 72.6-78.6%

342 respectively which are similar to previously published figures (56% for EDMD and

343 72% EPD for mid-bloom fresh lucerne (Hoffman *et al.*, 1993)).

344 Variation between the long and short chop silages within each experiment was

345 observed despite care being taken at harvest to control variables other than chop

346 length. Notably, pH and acetic, propionic and butyric acids were reduced for both

347 short cut silages relative to long cut silages while lactic acid was increased. This may

348 be explained by increased silage density through better compaction achieved with

349 the short chop which helps to create the necessary anaerobic environment in the

350 silo. Short cut silage was also collected from the field after long chopped silage

351 leading to a small increase in wilting time which may also have increased the
352 concentration of sugars available for fermentation.

353 The differences in physical structure achieved by varying chop length of the
354 silages were similar for both experiments. The theoretical difference between
355 average particle size according to the settings of the forage harvester was 5 mm
356 which was relatively close to the achieved differences of 3.6 mm and 5.3 mm for
357 experiments 1 and 2 respectively. The differences in mean particle lengths achieved
358 by varying chop length in this study are similar to those used in previous research
359 (e.g. 5mm, Beauchemin *et al.* (1994); and 7mm, Bhandari *et al.*, (2007)). Although
360 the difference in mean particle length is small, there were larger differences in the
361 relative quantities of particle size fractions measured using a PSPS.

362

363 *Intake, milk yield and nutrient digestibility*

364 *Effects of lucerne inclusion rate.* The effects of a higher dietary inclusion rate of
365 lucerne on DMI differed in the two experiments. In experiment 1, feeding the first cut
366 silage at the higher inclusion rate decreased DMI, and milk yield. In contrast,
367 feeding the second cut silage at the same rate to a smaller number of cows had no
368 effect on DMI, but a reduction in milk yield was still observed. In a similar UK study
369 where grass silage was replaced with lucerne silage in the diet a reduction in intake
370 was also seen when 60% of forage DM was comprised of lucerne silage (Sinclair *et al.*
371 *et al.*, 2015). Rumen fill can be limiting factor on DMI depending on the the extent to
372 which the diet is comprised of forage (Beauchemin *et al.*, 2003). In this case the
373 second cut silages showed 11% greater total DM degradability (a+b) over 72 hours
374 than the first cut silage indicating that the first cut silage would have contained a
375 greater mass of forage dry matter within the rumen during this time. The differences

376 in forage DM degradability might explain conflicting effects on DMI seen between
377 experiments. Furthermore, the silage used in experiment 1 had a high concentration
378 of acetic acid which has been linked with reduced intake in some studies where
379 dietary concentrations were 25-50 g/kgDM (Daniel *et al.*, 2013), and this may have
380 also contributed to lower intakes on HL diets in this instance.

381 Feeding HL diets led to a reduction in the digestibility of ~~both~~ DM, ~~and~~ OM and N.
382 This indicates that the lucerne silages used in this study were less digestible than the
383 maize silage, reflecting greater ADF concentration. Decreases in milk yield observed
384 for the HL diets in both experiments could be related to this reduction in DM and OM
385 digestibility, and therefore ME, and also the lower starch concentration of the HL
386 diets (Table 2). Furthermore, there would have been a greater imbalance between
387 supply of metabolisable protein to ME in HL diets which would contribute to the
388 reduction in milk yield observed. These findings align with previous studies which
389 show that lucerne typically has a lower ME content than many other forage legumes
390 or grasses (Steinshamn, 2010). Efficiency of N utilisation was also reduced when HL
391 diets were fed, which is partly attributable to lower milk yield seen on HL diets. Also,
392 since diets were not balanced for rumen degradable N supply in this study, there
393 was a surplus of rapidly degradable N for the HL diets which would have contributed
394 to reduced N utilisation and high milk urea values. There was greater partitioning of
395 N into faeces than urine in this study, which may in part reflect low N digestibility,
396 particularly on HL diets. In addition, the spot sampling method adopted in this study
397 may not have fully accounted for diurnal changes in urine concentration.

398

399 *Effects of lucerne chop length.* In experiment 1, DMI increased when a short chop
400 silage was fed which was also observed with lucerne haylage (Kononoff and

401 Heinrichs, 2003) but was contrary to the results of experiment 2 and a numerous
402 previous studies in which reducing lucerne chop length did not affect DMI
403 (Beauchemin *et al.*, 1994 and 2003; Bhandari *et al.*, 2007). Increased DMI with
404 shorter chop length might suggest increased speed of particle breakdown and/or
405 rumen outflow and lower rumen fill allowing higher rates of intake relative to the
406 longer chop length. Some studies found this to be the case where short and long
407 chop lucerne lengths were compared (using mean particle lengths of 1 and 8 mm;
408 Yansari *et al.*, 2004) although others noted no change in passage rate even when an
409 effect on DMI was observed (Kononoff and Heinrichs, 2003). The short chop length
410 also increased DM digestibility, and the magnitude of the effect was greater than that
411 of inclusion rate. This may be explained by smaller particles exhibiting a greater
412 surface area for microbial attachment, although this is only one of many factors that
413 govern the rate of cellulolysis in the rumen (Mason and Stuckey, 2016). The increase
414 in digestibility might also explain why the short chop mitigated some of the negative
415 effect of the HL diet on milk yield (+1.6 kg/d milk produced on LL diets; Table 6).

416 Using shorter chop lengths with high lucerne silage diets shows potential as a
417 strategy to partly mitigate reduced nutrient use efficiency when lucerne is included at
418 higher rates in the diet. Further research into lucerne agronomy and variety
419 development for delayed plant lignification to increase the acceptable harvest
420 window is an approach which may improve prospects for feeding lucerne in the
421 future.

422

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430

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486

487 **Table 1** *Ingredients used to create experimental total mixed rations in two separate*
 488 *experiments.*

Item	Diet			
	Experiment 1		Experiment 2	
	LL	HL	LL	HL
Ingredients, g/kg DM				
Lucerne silage	125	375	125	375
Maize silage	375	125	375	125
Concentrate blend				
Cracked Wheat	80	80	80	80
Maize Meal	61	70	54	97
Unmolassed Sugar Beet Feed	40	40	40	40
Soy Hulls	79	88	82	108
Soybean Meal	98	89	100	65
Rapeseed Meal	98	89	100	65
Molasses	10	10	10	10
Dicalcium phosphate	5	5	5	5
Salt	5	5	5	5
Dairy Mineral	10	10	10	10
Megalac ¹	15	15	15	15

489 LL = low lucerne diet; HL = high lucerne diet;

490 ¹ Megalac rumen protected fat supplement (Volac International Ltd., Royston, UK)

491

492 **Table 2** *The chemical composition of four total mixed rations containing a high (HL)*
 493 *or low (LL) concentration of lucerne silage at a long (L) or short (S) chop length fed*
 494 *in two separate experiments.*

Item ¹	Diet				SEM	P value		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Experiment 1, g/kg DM								
DM, g/kg	467	424	358	334	8.8	0.001	0.011	0.366
Ash	65	66	86	86	0.8	0.001	0.844	0.844
CP	181	179	163	171	3.4	0.138	0.367	0.343
NDF	334	337	329	348	6.0	0.689	0.164	0.280
ADF	269	244	227	222	4.4	0.096	0.163	0.283
Starch	256	256	165	155	7.9	0.044	0.589	0.609
WSC	37	36	27	27	2.5	0.009	0.993	0.896
<i>n</i>	4	4	4	4				
Experiment 2, g/kg DM								
DM, g/kg	553	572	611	635	3.3	0.001	0.002	0.386
Ash	61	62	77	78	0.4	0.001	0.350	0.946
CP	170	170	171	174	2.5	0.115	0.435	0.535
NDF	318	321	327	338	2.8	0.002	0.026	0.206
ADF	204 ^a	208 ^a	234 ^b	236 ^c	1.7	0.001	0.004	0.042
Starch	242	242	162	164	2.1	0.001	0.195	0.597
WSC	37	36	34	32	0.4	0.001	0.001	0.105
<i>n</i>	7	8	8	8				

495 IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL; DM = dry matter; OM =
 496 organic matter; WSC = water soluble carbohydrate.

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

499 **Table 3** Analysis of the chemical composition and degradability characteristics of
 500 four lucerne silages harvested at first cut (used in experiment 1) or second cut (used
 501 in experiment 2) at either a long (L) or short (S) chop length.

Item	Maize silage	Lucerne silage				SEM	p value
		Exp. 1		Exp. 2			
		L	S	L	S		
Chemical composition ¹ , g/kgDM							
DM, g/kg	384 ^a	218 ^b	225 ^b	587 ^c	559 ^c	10.0	0.001
CP	73 ^a	176 ^b	175 ^b	170 ^b	175 ^b	6.4	0.001
OM	965 ^a	874 ^b	875 ^b	892 ^c	893 ^c	2.1	0.001
NDF	368 ^a	513 ^b	498 ^c	408 ^d	390 ^{ad}	7.6	0.001
ADF	215 ^a	441 ^b	418 ^c	355 ^d	328 ^e	4.7	0.001
Starch	376	-	-	-	-	-	-
WSC	3 ^a	1 ^a	1 ^a	10 ^b	16 ^b	1.1	0.001
<i>n</i>	4	4	4	4	4		
Fermentation characteristics ²							
pH	-	6.2	5.6	4.9	4.7	-	-
Ethanol, g/kgDM	-	22.8	6.13	0.05	1.36	-	-
Lactic acid, g/kgDM	-	<5.0	7.29	27.1	43.1	-	-
Acetic acid, g/kgDM	-	56.9	40.7	7.77	0.97	-	-
Propionic acid, g/kgDM	-	8.07	4.80	0.51	0.39	-	-
Butyric acid, g/kgDM	-	41.3	15.6	1.02	0.72	-	-
Degradability parameters ³							
DM degradability							
a, %	44.1 ^a	26.5 ^b	16.5 ^c	32.7 ^d	34.6 ^d	0.43	0.001
b, %	37.6 ^a	38.2 ^a	46.5 ^b	42.8 ^{ab}	40.4 ^a	1.76	0.037
c, %/h	3.26 ^a	4.34 ^a	4.24 ^a	3.82 ^a	6.02 ^b	0.495	0.037
EDMD, %	58.6 ^a	44.2 ^b	37.7 ^c	51.3 ^d	56.5 ^e	0.72	0.001
Protein degradability							
a, %	62.3 ^a	67.2 ^b	63.0 ^a	59.0 ^c	61.9 ^{ac}	0.91	0.003
b, %	24.2	24.2	21.2	30.6	26.4	4.04	0.593
c, %/h	1.95 ^a	2.95 ^{ab}	4.19 ^b	3.97 ^b	8.63 ^c	0.614	0.001
EPD, %	67.4 ^a	74.8 ^b	72.6 ^c	72.5 ^c	78.6 ^d	0.76	0.001
<i>n</i>	3	3	3	3	3		

502 DM = dry matter; OM = organic matter; WSC = water soluble carbohydrates; ME = metabolisable
 503 energy; EDMD = effective dry matter degradability; EPD = effective protein degradability.

504 ¹ Average chemical composition from analyses of bulk samples taken in each period of the study
 505 analysed using mixed models with fixed effect of silage and period.

506 ² The analysis from clamp core samples taken at 3 separate points in the clamp and bulked.

507 ³ Degradability parameters determined by *in sacco* incubation in the rumen, using the model of
 508 (Ørskov and McDonald, 1979) where a = rapidly soluble material; b = non-soluble but degradable

509 material; c = rate of degradation of b ; effective degradability = $a+b[c/(c+k)]$ (Ørskov and McDonald,
510 1979) where k = an assumed outflow rate of 0.05/hr. Mean values from each of 3 cows were analysed
511 using mixed models with fixed effects of silage and random effects of cow.

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

514

515 **Table 4** *The distribution of particle size (DM basis) in four total mixed rations*
 516 *containing a high (HL) or low (LL) concentration of lucerne silage at a long (L) or*
 517 *short (S) chop length in two separate experiments.*

Item ¹	Diet				SEM	P value			
	LLS	LLL	HLS	HLL		IR	CL	IRxCL	
Experiment 1									
Material retained, %DM									
19mm	8.4	9.6	21.7	30.0	1.67	0.001	0.018	0.104	
8mm	39.9	32.8	41.3	39.9	0.60	0.001	0.421	0.226	
4mm	17.3 ^a	17.2 ^a	21.4 ^b	16.2 ^a	0.86	0.148	0.025	0.030	
Bottom pan	35.4	31.1	24.7	20.6	1.40	0.074	0.189	0.965	
Mean particle size, cm ¹	0.62	0.67	0.82	0.97	0.415	0.001	0.046	0.230	
<i>n</i>	4	4	4	4					
Experiment 2									
Material retained, %DM									
19mm	3.2 ^a	5.0 ^a	5.3 ^a	12.1 ^b	0.75	0.001	0.001	0.007	
8mm	36.4 ^a	41.9 ^b	37.4 ^{ac}	39.1 ^c	0.50	0.129	0.012	0.026	
4mm	16.5 ^a	13.5 ^b	18.7 ^c	12.6 ^b	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, cm	0.50	0.56	0.54	0.65	0.014	0.001	0.001	0.099	
<i>n</i>	3	4	4	4					

518 IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL; DM = dry matter

519 ¹ Mean particle size was determined using the recommended equation of Penn State University
 520 (Heinrichs, 2013).

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

523

524

525 **Table 5** Dry matter intake, milk yield, milk composition and feed conversion
 526 efficiency of lactating dairy cows fed a total mixed ration containing a high (HL) or
 527 low (LL) concentration of lucerne silage at a long (L) or short (S) chop length in two
 528 separate experiments.

Item ¹	Diet				SEM	P value		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Experiment 1								
DMI, kg/d	26.4	26.0	23.7	22.3	0.74	0.001	0.017	0.172
Milk yield, kg/d	35.2	33.9	32.5	30.6	1.04	0.001	0.001	0.449
Est. Milk energy, MJ/d ¹	101.4	100.0	93.6	89.7	4.12	0.001	0.073	0.379
Energy efficiency, MJ/kg ²	3.84	3.85	3.99	4.00	0.119	0.079	0.926	0.970
Milk composition								
Milk fat, g/kg	36.9	37.7	37.6	38.3	1.37	0.263	0.242	0.705
Milk protein, g/kg	30.2	30.5	31.2	30.9	0.68	0.001	0.962	0.066
Milk urea, mg/kg	292	311	424	432	14.4	0.001	0.088	0.469
Fat yield, kg/d	1.29	1.28	1.21	1.21	0.065	0.017	0.844	0.954
Protein yield, kg/d	1.10	1.06	1.00	0.95	0.036	0.001	0.003	0.706
<i>n</i>	16	16	16	16				
Experiment 2								
DMI, kg/d	23.0	23.0	23.8	23.7	0.75	0.227	0.994	0.916
Milk yield, kg/d	31.5 ^{ab}	33.7 ^b	30.8 ^a	28.7 ^a	2.21	0.013	0.953	0.043
Est. Milk energy, MJ/d ¹	85.5 ^{ab}	91.9 ^a	86.1 ^{ab}	82.1 ^b	5.95	0.074	0.636	0.047
Energy efficiency, MJ/kg ²	3.73	3.95	3.59	3.54	0.249	0.002	0.409	0.068
Milk composition								
Milk fat, g/kg	35.0	33.9	35.1	35.9	1.53	0.357	0.907	0.378
Milk protein, g/kg	30.1	30.6	29.7	29.0	0.62	0.034	0.768	0.146
Milk urea, mg/kg	291	306	324	333	23.0	0.105	0.508	0.862
Fat yield, kg/d	1.03	1.08	1.09	1.12	0.831	0.104	0.216	0.795
Protein yield, kg/d	0.92	0.95	0.92	0.90	0.667	0.261	0.842	0.387
<i>n</i>	7	8	8	8				

529 IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL; DMI = dry matter
 530 intake.

531 ¹ Estimated milk energy = Milk yield, kg*((fat concentration, g/kg *0.0384+protein concentration, g/kg
 532 *0.0223+lactose concentration, g/kg *0.0199)-0.108)

533 ² Energy Efficiency calculated as Estimated milk energy in MJ/d divided by DMI in kg

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

536

537

538 **Table 6** *The apparent DM, OM, NDF, ADF and starch digestibility of four total mixed*
 539 *rations containing a high (HL) or low (LL) concentration of lucerne silage at a long (L)*
 540 *or short (S) chop length when fed to lactating dairy cows (in experiment 2).*

Item ¹	Diet				SEM	P value		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
Dry matter								
DMI, kg/d	23.8	25.0	23.1	22.1	0.57	0.040	0.862	0.116
Faecal DM, kg/d	6.70	7.90	7.70	8.89	0.445	0.022	0.010	0.992
DM digestibility, %	70.4	67.2	67.9	62.5	1.40	0.043	0.015	0.424
Organic Matter								
OM intake, kg /d	20.9	22.7	22.0	22.0	0.91	0.846	0.376	0.368
Faecal OM, kg/d	5.66	6.69	6.38	7.42	0.378	0.029	0.007	0.984
OM digestibility,%	73.1	70.5	71.0	66.2	1.22	0.021	0.006	0.292
Starch								
Starch intake, kg/d	5.28	5.73	3.98	4.00	0.257	0.002	0.407	0.438
Faecal starch, kg/d	0.16	0.24	0.14	0.16	0.034	0.015	0.019	0.081
Starch digestibility, %	96.7	95.8	96.9	96.3	0.78	0.668	0.250	0.858
Fibre								
NDF intake, kg/d	6.99	7.69	7.85	8.07	0.313	0.107	0.208	0.492
Faecal NDF, kg/d	3.05	3.56	3.43	3.77	0.200	0.095	0.032	0.564
NDF digestibility, %	56.0	52.8	56.9	53.8	2.04	0.572	0.095	1.000
ADF Intake, kg/d	4.51	5.01	5.59	5.86	0.207	0.005	0.135	0.628
Faecal ADF, kg/d	2.30	2.69	2.56	2.93	0.355	0.616	0.490	0.985
ADF digestsibility, %	50.4	47.3	52.3	50.0	2.67	0.381	0.309	0.863
<i>n</i>	4	4	4	4				

541 IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL; DM = dry matter; DMI
 542 = dry matter intake; OM = organic matter.

543 **Table 7** *The apparent digestibility of N and N balance in lactating dairy cows fed total*
 544 *mixed rations containing a high (HL) or low (LL) concentration of lucerne silage at a*
 545 *long (L) or short (S) chop length (in experiment 2).*

Item ¹	Diet				SEM	P value		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
N intake, g/d	674	692	654	635	10.7	0.010	0.953	0.067
Faecal N, g/d (a)	<u>209</u> <u>197</u>	<u>241</u> <u>151</u>	<u>242</u> <u>33</u>	<u>278</u> <u>92</u>	<u>19.2</u> <u>7.6</u>	0.0 <u>54</u> <u>39</u>	0.0 <u>61</u> <u>09</u>	0. <u>921</u> <u>877</u>
N digested, g/d	<u>389</u> ^{ab} <u>430</u>	<u>453</u> ^{ab} <u>28</u>	<u>465</u> ^b <u>47</u>	<u>380</u> ^a <u>77</u>	<u>10.4</u> <u>22.3</u>	0. <u>820</u> <u>481</u>	0. <u>263</u> <u>164</u>	0. <u>041</u> <u>185</u>
N digestibility, %	70. <u>32</u>	6 <u>5.64</u> <u>.5</u>	6 <u>2.34</u> <u>.0</u>	5 <u>6.45</u> <u>.4</u>	<u>2.374</u> <u>.26</u>	0. <u>019</u> <u>286</u>	0. <u>053</u> <u>302</u>	0.7 <u>06</u> <u>92</u>
Urinary N, g/d (b)	157	168	187	166	12.5	0.171	0.551	0.127
Excreted N, g/d (a+b)	<u>397</u> <u>49</u>	<u>407</u> <u>13</u>	<u>416</u> <u>28</u>	<u>427</u> <u>60</u>	<u>19.3</u> <u>24.6</u>	0. <u>270</u> <u>029</u>	0. <u>497</u> <u>067</u>	0. <u>988</u> <u>489</u>
Milk N, g/d ¹	160	170	150	144	5.8	0.028	0.773	0.247
N use efficiency, % ²	25.7	25.1	22.3	21.9	0.78	0.008	0.572	0.886
<i>n</i>	4	4	4	4				

546 IR = Inclusion rate; CL = chop length; IRxCL = interaction between IR and CL; N = Nitrogen.

547 ¹ Milk N = milk protein yield / 6.25

548 ² N use efficiency calculated as the percentage of ingested N found as milk protein N.

549

550

551 **Figure captions**

552

553 **Figure 1** The effect of Short (S) or Long (L) chop length of lucerne silage on the
554 distribution of particles (dry matter corrected) across the sieves of a Penn State
555 Particle Separator for first cut silage (experiment 1) and second cut lucerne silage
556 (experiment 2). Values are the means of measurements taken in each period (n=4).

557

558