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

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Short title: Inclusion rate and chop length of lucerne silage

Abstract

The objective was to assess the effects of inclusion rate and chop length of lucerne silage, when fed in a total mixed ration (TMR), on milk yield, dry matter (DM) intake (DMI) and digestion in dairy cows. Diets were formulated to contain a 50:50 forage:concentrate ratio (DM basis) and to be isonitrogenous (170 g/kg CP). The forage portion of the offered diets was comprised of maize and lucerne silage in proportions (DM basis) of either 25:75 (HL) or 75:25 (LL). Lucerne was harvested and conserved as silage at either a long (L) or short (S) chop length. These variables were combined in a 2x2 factorial arrangement to give four treatments (HLL, HLS, LLL, LLS) which were fed in a Latin square design study to Holstein dairy cows in two separate experiments. Sixteen and 8 multiparous, mid-lactation, cows were used in experiments 1 and 2, respectively. To ensure sufficient silage for both experiments, different cuts of lucerne silage (taken from the same sward) were used

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

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

Implications

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

Introduction

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

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

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

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

Material and methods

Forage harvesting and clamp sampling

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

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

Diets

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

Table 1

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. ± 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. ± 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 500ºC 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 60ºC for 72

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

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

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

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

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

Statistical analysis

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

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

Results

Forage quality

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

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

Table 3

Forage and diet particle size

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

Figure 1

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

Table 4

Intake, milk yield and milk composition

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

Table 5

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

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

Apparent digestibility and N balance

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

Table 6

Intakes of N were greater for LL diets ($P < 0.01$) as a result of higher DMI (Table 7). There was a tendency forere increaseds in faecal N concentration ($P < 0.064$) and total manure (faecal plus urine) N excretion ($P < 0.03$) when HL diets were fed. Faecal N also tended to increased when the chop length was increased ($P < 0.0709$). There was greater partitioning of intake N into the milk for the LL diets with an increase in N use efficiency of 3.3% ($P < 0.009$) and N digestibility was also greater ($P < 0.02$). Shortening the chop length of the lucerne reduced faecal N excretion by 57 g/d ($P < 0.01$).

Table 7

Discussion

Forage quality and particle size

The nutritive value of the four lucerne silages used in the study was variable. Although crude protein levels were similar at 172 g/kg, the second cut (experiment 2) silages were lower in NDF and ADF than the first cut (experiment 1) silages, suggesting increased maturity in the first cut relative to the second cut forage. In the silage fed in experiment 1, high acetic acid and low lactic acid concentrations indicated poor fermentation, although pH reduction was adequate. High levels of WSC in the experiment 2 silage may indicate that increased time spent wilting this crop (48h vs. 24h for the first cut) resulting in a higher DM reduced fermentation activity, or that the original concentration of sugar in this crop was higher than for the first cut crop. These results collectively indicate increased silage quality in the second cut silage with higher DM concentration. The effective degradation of DM and protein in the lucerne silages ranged from 37.8-56.7% and 72.6-78.6% respectively which are similar to previously published figures (56% for EDMD and 72% EPD for mid-bloom fresh lucerne (Hoffman *et al*., 1993)).

Variation between the long and short chop silages within each experiment was observed despite care being taken at harvest to control variables other than chop length. Notably, pH and acetic, propionic and butyric acids were reduced for both short cut silages relative to long cut silages while lactic acid was increased. This may be explained by increased silage density through better compaction achieved with the short chop which helps to create the necessary anaerobic environment in the silo. Short cut silage was also collected from the field after long chopped silage

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

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

Intake, milk yield and nutrient digestibility

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

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

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

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

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

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

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

	Diet			
	Experiment 1		Experiment 2	
Item	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

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

[1] Megalac rumen protected fat supplement (Volac International ltd., Royston, UK)

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

	Diet					*P* value		
Item[1]	LLS	LLL	HLS	HLL	SEM	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
n	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
n	7	8	8	8				

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

[a,b] Where there is a significant interaction, values within a row with different superscripts differ significantly at $P<0.05$.

Table 3 *Analysis of the chemical composition and degradability characteristics of four lucerne silages harvested at first cut (used in experiment 1) or second cut (used 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
n	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
n	3	3	3	3	3		

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

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

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

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

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

[a,b] Where there is a significant interaction, values within a row with different superscripts differ significantly at $P<0.05$.

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

	Diet					*P* value		
Item[1]	LLS	LLL	HLS	HLL	SEM	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
n	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
n	3	4	4	4				

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

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

[a,b] Where there is a significant interaction, values within a row with different superscripts differ significantly at $P<0.05$.

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

	Diet					*P* value		
Item[1]	LLS	LLL	HLS	HLL	SEM	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
n	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
n	7	8	8	8				

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

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

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

[a,b] Where there is a significant interaction, values within a row with different superscripts differ significantly at $P<0.05$.

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

	Diet					*P* value		
Item[1]	LLS	LLL	HLS	HLL	SEM	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 digetsibility, %	50.4	47.3	52.3	50.0	2.67	0.381	0.309	0.863
n	4	4	4	4				

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

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

	Diet					*P* value		
Item[1]	LLS	LLL	HLS	HLL	SEM	IR	CL	IRxCL
N intake, g/d	674	692	654	635	10.7	0.010	0.953	0.067
Faecal N, g/d (a)	209197	24151	24233	27892	19.27.6	0.05439	0.06109	0.921877
N digested, g/d	389[ab]430	453[ab]28	465[b]47	380[a]77	10.422.3	0.820481	0.263164	0.041185
N digestibility, %	70.32	65.64.5	62.34.0	56.45.4	2.374.26	0.019286	0.053302	0.70692
Urinary N, g/d (b)	157	168	187	166	12.5	0.171	0.551	0.127
Excreted N, g/d (a+b)	39749	40713	41628	42760	19.324.6	0.270029	0.497067	0.988489
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
n	4	4	4	4				

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

[1] Milk N = milk protein yield / 6.25

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

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).

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