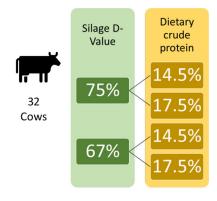
Dairy cow performance and nutrient utilization when offered high or low digestibility grass silages at 2 levels of total diet crude protein

A. Craig, ¹ • A. W. Gordon, ² • and C. P. Ferris ¹ * •

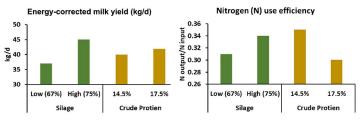
Graphical Abstract

Does silage digestibility affect cow performance response to crude protein level?

Four treatments organized in a 2 x 2 factorial change-over design study



No interactions found between silage digestibility and total diet crude protein level



Improving silage digestibility improved performance and nitrogen use efficiency (NUE). Reducing dietary crude protein content also improved NUE, but reduced cow performance. Therefore, the key to mitigate loss of performance while improving NUE is the production of high-quality grass silage.

Summary

This 2-period change-over design study (n = 32 cows) investigated whether there was an interaction between grass silage digestibility and total diet crude protein (CP). The hypothesis was that total dietary CP could be lowered to a greater extent with higher digestibility grass silages. Four treatments comprised of 2 grass silages differing in digestible organic matter in the dry matter (D-value) were organized in a 2 (silage digestibility; high-D or low-D) \times 2 (total dietary CP; high-CP or low-CP) factorial design. Aside from milk urea nitrogen, no interactions were observed between silage digestibility and total diet CP. Therefore, total dietary CP level cannot be reduced to a greater extent with higher digestibility silages. Improving silage digestibility improved performance (+8 kg/day energy-corrected milk) and nitrogen use efficiency. Reducing dietary CP content also improved nitrogen use efficiency (NUE), but cow performance was reduced (-1.9 kg/day energy-corrected milk). Therefore, the key to maintaining performance while improving NUE is the production of high-quality grass silage.

Highlights

- No interactions were observed between grass silage digestibility and total diet CP.
- Higher silage digestibility increased cow performance and NUE.
- Lower CP improved NUE but reduced cow performance.



¹Agri-Food and Biosciences Institute, Sustainable Agri-Food Sciences Division, Large Park, Hillsborough, County Down BT26 6DR, Northern Ireland, ²Agri-Food and Biosciences Institute, Sustainable Agri-Food Sciences Division, Belfast BT9 5PX, Northern Ireland. *Corresponding author: conrad.ferris@afbini .gov.uk. © 2025, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association*. This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/). Received May 13, 2024. Accepted July 18, 2024.



Dairy cow performance and nutrient utilization when offered high or low digestibility grass silages at 2 levels of total diet crude protein

A. Craig, ¹ • A. W. Gordon, ² • and C. P. Ferris ¹* •

Abstract: The hypothesis of this study was that grass silage digestibility would influence the response of dairy cows offered diets differing in CP content. Thirty-two mid-lactation Holstein dairy cows were used in a 2-period (21-d adaption phase, 7-d measurement phase), partially balanced change-over experiment. Four treatments were organized in a 2 × 2 factorial arrangement, comprising 2 grass silages differing in digestible OM in the DM (D-value; 748 and 668 g/kg DM, high-D and low-D, respectively) and 2 total diet CP levels (target 145 and 175 g/kg DM, high CP and low CP, respectively). The latter were achieved using 2 iso-energetic concentrates that differed in CP level (173 and 228 g/kg DM). Silages and concentrates were mixed and offered as a TMR in a 50:50 DM ratio. At the end of the feeding study, nutrient utilization was measured using 4 cows per treatment. Except for milk urea nitrogen there were no interactions between silage digestibility and total diet CP for cow performance and nutrient utilization. The high-D silage improved cow performance and increased nitrogen use efficiency (NUE). Reducing total diet CP also increased NUE, but there were negative impacts on cow performance. While there were benefits of offering grass silage with a high digestibility, responses to total diet CP were similar at both levels of silage digestibility.

The efficiency with which dairy cows convert dietary N into milk N, defined as nitrogen use efficiency (NUE), is typically between 25% and 30% (Huhtanen and Hristov, 2009). This poor NUE is related to the low efficiency of N utilization within the rumen. Part of the N (in the form of ammonia) released through the breakdown of diet RDP is not incorporated into microbial protein, but rather moves across the rumen wall into the bloodstream and is carried to the liver and converted to urea, and then excreted in urine. Increasing the availability of energy to rumen microbes has potential to improve protein utilization within the rumen (Aldrich et al., 1993), and as such reduce N excretion to the environment.

Given that the CP concentration of the diet is the most important dietary factor influencing NUE (Huhtanen and Hristov, 2009), the impact of reducing diet CP levels on cow performance and efficiency has been extensively examined. While the response to diet CP level is influenced by a wide range of factors, the impact of forage quality on the response to dietary CP level has received relatively little recent attention. Mayne (1989) examined the concentrate sparing effects associated with changing silage digestibility and concentrate CP content, whereas Rinne et al. (1999) compared a range of grass silage digestibilities at 2 concentrate feeding and protein levels. The objective of this study was to determine if there is an interaction between grass silage digestibility and total diet CP content. We hypothesized that grass silage digestibility would influence the response of dairy cows offered diets differing in CP content, and that the impact of reducing total diet CP content would be less with a high-quality silage compared with a lower quality silage.

This study was conducted at the Agri-Food and Biosciences Institute, Northern Ireland, with all experimental procedures conducted under an experimental license (no. 2877) granted by the Department of Health, Social Services and Public Safety for Northern Ireland in accordance with the Animals (Scientific Procedures) Act 1986. Cows were housed in a freestall house and had access to individual cubicles. Thirty-two mid-lactation (120 \pm 51.1 DIM) multiparous (mean lactation number, 2.9 ± 0.83) Holstein dairy cows were used in a 2-period partially balanced restricted change-over design experiment. Each period consisted of a 21-d feed adaption period, and a 7-d measurement period. Cows were blocked according to pre-experimental milk fat plus protein yield (8 blocks of 4 cows), and cows within each block randomly allocated to one of 4 treatments. Treatments, in a 2 × 2 factorial arrangement, comprised 2 grass silages differing in digestible OM in the DM (D-value), namely high-D and low-D (748 and 668 g/ kg DM, respectively), and 2 levels of total diet CP, namely high-CP or low-CP (target 145 and 175 g/kg DM, respectively). The high-D and low-D silages (Lolium perenne L.) were produced from the same swards. Herbage was mown on May 14 (primary growth: early ear emergence) and July 9 (secondary growth: postear emergence), respectively, tedded and allowed to wilt for 24 h, and harvested using a precision-chop forage harvester. Grass was treated at harvest with a bacterial inoculant (Super MV-50, Biotal, Worcestershire, UK) at approximately 20 mL per tonne of fresh herbage, before being ensiled in separate bunker silos. The 2 total diet CP levels were achieved by mixing 2 concentrates (differing in CP levels, 228 and 173 g/kg DM), with the silages. The concen-

¹Agri-Food and Biosciences Institute, Sustainable Agri-Food Sciences Division, Large Park, Hillsborough, County Down BT26 6DR, Northern Ireland, ²Agri-Food and Biosciences Institute, Sustainable Agri-Food Sciences Division, Belfast BT9 5PX, Northern Ireland. *Corresponding author: conrad.ferris@afbini .gov.uk. © 2025, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association*. This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/). Received May 13, 2024. Accepted July 18, 2024.

Table 1. Chemical composition of the high- and low-CP concentrates, and high- and low-D value silages offered during the experiment

	Conce	Silage			
Item	High-CP	Low-CP	High-D	Low-D	
DM¹ (g/kg)	894	894	297	230	
CP (g/kg DM)	228	173	120	130	
ADF (g/kg DM)	148	144	271	346	
NDF (g/kg DM)	237	235	442	529	
Ash (g/kg DM)			82	94	
Gross energy (MJ/kg DM)	18.6	18.1	18.5	18.2	
Starch (g/kg DM)	228	301			
$ME^2 (MJ/d)$	13.1	13.1	12.0	10.7	
ERDP supply ³ (g/kg DM)	120	108			
DUP supply ³ (g/kg DM)	96	72			
pH			3.7	3.8	
Lactic acid (g/kg DM)			106	78	
Acetic acid (g/kg DM)			15	12	
Ammonia (g/kg total N)			52	81	
D-value (g/kg DM)			748	668	

¹Concentrates, oven DM basis; silages, volatile corrected oven DM basis.

trates were iso-energetic, had similar fiber contents, but differed in starch content by 73 g/kg DM. Individual cows were restricted to the same silage type, whereas total diet CP level changed between periods. The chemical composition of the feedstuffs offered is presented in Table 1.

Grass silage and concentrates where offered as a TMR in a 50:50 DM ratio. The rations were prepared daily using a feeder wagon (Vari-Cut 12, Redrock, Armagh, Northern Ireland) and offered ad libitum at 1.07% of the previous day's intake. The total quantity of silage required for the high-D treatments was initially mixed for 5 min and then deposited on a clean silo floor. The quantity of silage required for each individual treatment was then removed from this "pile" in turn, placed back in the feeder wagon, and the appropriate quantities of concentrate added to the mix and mixed for a further 5 min. Following mixing the rations were transferred from the mixer wagon to a series of feed boxes mounted on weigh scales, with cows accessing food in these boxes via an electronic identification system, enabling individual cow intakes to be recorded daily (Controlling and Recording Feed Intake, Bio-Control, Rakkestad, Norway). Uneaten ration was removed the following day. This process was repeated for the low-D treatments. The ingredient composition (g/kg, fresh basis) for the high-CP concentrate was as follows: maize meal, 197; barley, 72; wheat, 38; soybean meal, 80; rapeseed meal, 174; maize distillers, 110; soy hulls, 57; sugar beet pulp, 125; Sopralin (Trouw Nutrition, Belfast, UK), 75; Megalac (Volac Wilmar Feed Ingredients Ltd., Hertfordshire, UK), 23; mineral and vitamin mix, 30; molasses, 20. The ingredient composition (g/kg, fresh basis) for the low-CP concentrate was as follows: maize meal, 301; barley, 164; soybean meal, 107; rapeseed meal, 92; maize distillers, 25; soy hulls, 108; sugar beet pulp, 125; Megalac, 23; mineral and vitamin mix, 36; molasses, 20. Cows had free access to fresh water at all times.

A sample of each of the grass silages offered was taken daily throughout the experiment and dried at 60°C for 48 h to determine oven DM content. Twice weekly dried samples were retained,

bulked for each 14-d period, with the bulked sample milled through a sieve with 0.8 mm aperture, and analyzed for NDF, ADF, and ash concentrations. A further fresh silage sample was taken weekly and analyzed for gross energy (GE), N, pH, ammonia-N and volatile components, and for D-value, the latter using near infrared reflectance spectroscopy according to Park et al. (1998). Grass silage ME content was calculated as D-value × 0.016. A sample of each concentrate was taken weekly, and a subsample dried at 60°C for 48 h to determine oven DM content, and the dried sample bulked for each 14-d period, milled through a 0.8-mm sieve, and subsequently analyzed for N, NDF, ADF, ash, starch, and GE. Analysis of feedstuffs was as described by Craig et al. (2024).

Cows were milked twice daily using a 50-point rotary milking parlor (Boumatic, Madison, WI). Milk yields were automatically recorded at each milking, and a total daily milk yield for each cow for each 24-h period calculated. Milk samples were taken during 6 consecutive milkings at the end of each measurement period, treated with a preservative tablet (Broad Spectrum Microtabs II, Advanced Instruments, Norwood, MA), and stored at 4°C until analyzed (normally within 48 h). Milk samples were analyzed for fat, protein, and MUN concentrations using an infrared milk analyzer (Milkoscan Combifoss 7; Foss Electric, Hillerød, Denmark), and a weighted concentration of each constituent determined for each 24-h sampling period. A mean composition over the 3-d sampling period was subsequently calculated for each cow and ECM calculated as described by Craig et al. (2024). A further milk sample was taken, in proportion to milk yield, during 2 successive milkings at the end of each measurement period, and analyzed for milk fatty acids (FA), as described by Craig et al. (2020).

Body weight was recorded twice daily during the measurement week of each experimental period (immediately after each milking) using an automated weighbridge, and a mean BW for each cow determined. The BCS of each cow was assessed during the measurement week of each experimental period by a trained technician, as described by Edmonson et al. (1989). Two blood samples

²Concentrates predicted using book values (DietCheck version 10.1, DietCheck Ltd., Lancaster, UK); silages predicted using near-infrared spectroscopy.

 $^{^{3}}$ Predicted using book values. ERDP = effective rumen degradable protein; DUP = digestible undegradable protein.

were collected during the final week of each measurement period, one in a heparin-coated tube (BHB and urea) and one in a fluoride oxalate tube (nonesterified fatty acids; **NEFA**), and centrifuged $(1,509 \times g \text{ at } 4^{\circ}\text{C} \text{ for } 15 \text{ min})$. Plasma BHB, NEFA, and urea concentrations were determined as described in Craig et al. (2020).

On completion of the feeding study, 4 cows from each treatment (n = 16), balanced for daily milk yield and BW, were selected for use in a nutrient utilization study using a total collection method. Cows were tied by the neck in individual stalls within a nutrient utilization facility, with stalls fitted with a rubber mat. Cows accessed experimental rations from boxes at the front of each stall. Experimental rations were offered ad libitum daily. Uneaten food was removed the following day, sampled, and analyzed for oven DM content. Cows had access to fresh water at all times via a drinker located within each stall. Measurement of nutrient utilization commenced 24 h after cows were placed in the nutrient utilization facility, with feces and urine measured and sampled daily over a 6-d period. Details of feces, urine, and milk sampling, and their subsequent analysis, were as described by Craig et al. (2020). During the nutrient digestibility study, feedstuffs were analyzed for the same chemical components as during the main study.

Animal data recorded during the final week of each experimental period (DMI, milk yield, milk composition, BW and blood metabolites) were analyzed using linear mixed model methodology according to the 2-period change-over design, with constant + grass silage digestibility + total diet CP + silage digestibility × total diet CP as the fixed model, and block + block × cow + block × period as the random model. In all cases REML was used as the estimation method. Data from the nutrient utilization study were analyzed using linear mixed model methodology with the REML estimation method and a factorial arrangement of silage digestibility and total diet CP fitted as fixed effects. If any of the fixed effects were significant (P < 0.05) then Fisher's least significant difference test was used to compare individual levels of the effects. All data were analyzed using GenStat (21st edition; VSN International Limited, Oxford, UK).

Over 20 years ago, Rinne et al. (1999) examined the relationship between grass silage digestibility and dietary protein levels and found no interactions between digestibility and concentrate CP level. However, the silages used had a wide range of CP concentrations and the cows had lower yields compared with the current study and as such the concept was re-examined. The hypothesis of this study was that grass silage digestibility would influence the response of higher yielding dairy cows offered diets differing in CP content. It was expected that when cows were offered silage with a high digestibility, a reduction in total diet CP content would result in a smaller loss of performance than when offered silage with a lower digestibility. Total ration composition for high-D low-CP, high-D high-CP, low-D low-CP, and low-D high-CP was as follows: CP, 149, 172, 153, and 176 g/kg DM; NDF, 340, 341, 383, and 381 g/kg DM; starch, 145, 114, 147, and 120 g/kg DM, respectively. The CP content of the low-CP concentrate was higher than formulated, resulting in the low-CP diets having a higher than planned CP content.

Except for MUN there were no interactions between silage digestibility and total dietary CP level, indicating that the response to a reduction in diet CP was the same irrespective of silage quality. It is possible that the small increase in total diet starch levels (approximately 30 g/kg DM) with low-protein diets may have contributed

to a small increase in performance. Nevertheless, any such effect would have been expected to have been small, and to have been similar with both silage types. Therefore, the main effects of grass silage digestibility and total ration CP are presented and discussed.

Silage DMI was 2.0 kg/d greater with the high- compared with the low-D grass silage (P < 0.001; Table 2), aligning with the expected increase in silage DMI associated with the 80 g/kg difference in D-value (0.22 kg of DMI per 10 g increase in D-value; Keady et al., 2013). However, diets in this study were offered in the form of a TMR; therefore, high-D increased total DMI by 3.6 kg/d (P < 0.001), much more than expected. It is likely that the greater DM content of the high-D silage (297 vs. 230 g/kg), and lower ammonia N content, contributed to the greater than expected increase in total DMI. The greater total DMI and greater ME concentration of the high-D silage increased ME intake by an additional 58 MJ/d, resulting in a significant milk yield (+6.3 kg/d; P < 0.001) and ECM yield (+8.1 kg/d; P < 0.001) response. Based on the assumption that the production of 1 kg ECM requires approximately 5.3 MJ of ME, approximately 72% of the additional ME consumed by the cows offered the high-D silage was reflected in the ECM yield response. The remaining energy was likely diverted to body reserves, with this reflected in the greater BCS (P = 0.018) and BW (+15.6 kg; P < 0.001) of cows offered high-D. Furthermore, the increased concentration of plasma NEFA (± 0.05 mmol/L; P =0.001; Table 2) with low-D suggests some mobilization of body tissue with this treatment.

The milk protein content response (± 2.2 g/kg; P < 0.001) with high-D was substantially greater than expected based on the increase in silage digestibility alone (between 0.09 and 0.14 g/kg milk, per 10 g/kg increase in D-value; Rinne et al., 1999; Keady et al., 2013). This likely reflects the greater energy intake with high-D contributing to an increased synthesis of microbial CP (Aldrich et al., 1993). Although the fat content of milk was not significantly affected by silage type, the FA composition of the milk differed, high-D increasing total saturated (P < 0.001), total PUFA (P = 0.001), and the ratio of saturated to unsaturated FA (P < 0.001) compared with the low-D silage. The greater total FA content, and the increased concentration of PUFA, is likely due to the high-D silage being harvesting when less mature (Dewhurst et al., 2001).

Both MUN (-35.6 mg/kg; P < 0.001) and blood urea (-0.89 mmol/L; P < 0.001) concentrations were lower in cows offered the high-D compared with the low-D silage. It is possible that by providing additional energy to the rumen microbes, the high-D silage facilitated a greater utilization of rumen ammonia (Nousiainen et al., 2004). This is supported by the fact that the reduction in MUN with high-D silage was greater when the low-CP diet was offered (P = 0.042). These lower MUN and blood urea values were also reflected in an increased NUE (0.34 vs. 0.31; P < 0.001) with high-D. The increase in NUE was driven by the higher output of N in milk, as N digestibility and N output in manure and proportion of N excreted in feces and urine was similar between the high-D and low-D grass silages.

Reducing total diet CP reduced total DMI by 1.4 kg/d (P = 0.002; Table 2), highlighting the relationship between DMI and dietary CP content (Sinclair et al., 2014). The lower DMI was associated with a reduced milk yield (-1.9 kg/d; P < 0.001), fat plus protein yield (0.14 kg/d; P = 0.035), and ECM (-1.9 kg/d; P = 0.029) yield. Whereas cow BW and BCS were unaffected by CP level, there was a trend (P = 0.052) for elevated NEFA with the low-CP

Table 2. Effects of silage digestibility and total diet CP content on feed intake, milk production and composition, BW, and blood metabolites

	High-D silage		Low-D silage			<i>P</i> -value		
Item ¹	High-CP	Low-CP	High-CP	Low-CP	SED ²	Silage	СР	Silage × CP
Silage DMI (kg/d)	13.5	12.7	11.3	10.9	0.30	<0.001	0.014	0.282
Total DMI (kg/d)	26.8	25.1	22.8	21.8	0.57	< 0.001	0.002	0.384
Milk yield (kg/d)	42.1	39.5	35.1	33.8	0.72	< 0.001	< 0.001	0.200
Milk fat (g/kg)	46.6	46.4	44.4	46.4	1.24	0.198	0.304	0.222
Milk FA concentration								
(mg of FA/mL of milk)								
Total SFA	28.9	30.1	26.0	26.5	1.27	< 0.001	0.335	0.689
Total MUFA	7.2	7.1	7.6	7.1	0.35	0.324	0.215	0.458
Total PUFA	0.95	0.95	0.87	0.84	0.038	0.001	0.651	0.489
Saturated/unsaturated ratio	3.57	3.75	3.70	3.42	0.956	< 0.001	0.003	0.669
Milk protein (g/kg)	34.9	35.3	32.9	32.8	0.31	< 0.001	0.361	0.300
MUN (mg/kg)	155	105	183	148	5.1	< 0.001	< 0.001	0.042
Fat plus protein yield (kg/d)	3.44	3.21	2.73	2.67	0.089	< 0.001	0.035	0.184
ECM yield (kg/d)	46.6	43.4	37.3	36.6	1.15	< 0.001	0.029	0.143
BW (kg)	653	647	634	634	3.8	< 0.001	0.234	0.227
Blood metabolite (mmol/L)								
Plasma BHB	0.44	0.36	0.40	0.39	0.044	0.821	0.170	0.337
Plasma NEFA	0.11	0.11	0.13	0.19	0.021	0.001	0.052	0.059
Plasma urea	3.06	1.97	3.89	2.92	0.221	< 0.001	< 0.001	0.704
Nitrogen use efficiency	0.31	0.37	0.28	0.33	0.006	<0.001	<0.001	0.292

¹FA = fatty acid; NEFA = nonesterified fatty acid.

diets, which may indicate that body tissue mobilization may have contributed to supporting milk yield with this treatment. The trend for an interaction between silage digestibility and total diet CP for NEFA (P=0.059) suggests that a combination of a lower CP diet and lower digestibility silage resulted in greater tissue mobilization. That this was not reflected in BCS data (P=0.881) may be due to the short duration of the experimental periods.

Milk fat is generally unaffected by total diet CP level (Barros et al., 2017; Yang et al., 2022), whereas milk protein concentration responses to dietary CP are variable (Law et al., 2009; Barros et al., 2017). In the current study there was no effect of total diet CP on milk fat or milk protein concentration. Dietary CP had little effect on the milk FA profile, aside from a greater saturated/unsaturated

FA ratio with the high-CP diet, which may reflect differences in concentrate ingredients. Cows offered the low-CP diet had a lower MUN concentration (-43 mg/kg; P < 0.001) and blood urea concentration (-1.03 mmol/L; P < 0.001). However, MUN values were within the range (80 to 140 mg/kg, with values below 80 mg/kg indicating RDP deficiency) suggested by Ishler (2016), suggesting that the low CP diet was able to supply the N requirements of rumen microbes, whereas the high CP diet supplied excess RDP. As observed in many previous studies, reducing total diet CP levels resulted in a greater NUE (0.30 vs. 0.35; P < 0.001), although in this study, the gain in NUE was at the expense of cow performance.

Reducing total diet CP content reduced digestibility of protein, energy, and DM in the study by Yang et al. (2022). Whereas DM,

Table 3. Effects of silage digestibility and total diet CP content on total ration digestibility coefficients, and nitrogen (N) intake, output, and utilization efficiency of dairy cows

Item	High-D silage		Low-D silage			<i>P</i> -value		
	High-CP	Low-CP	High-CP	Low-CP	SED ¹	Silage	СР	Silage × CP
Digestibility coefficient (g/g)								
OM	0.78	0.79	0.76	0.77	0.014	0.027	0.506	0.880
Nitrogen	0.68	0.63	0.70	0.65	0.024	0.235	0.004	0.867
N intake and output (g/d)								
Total N intake	653	506	605	482	33.9	0.163	< 0.001	0.615
Fecal N	207	186	183	168	19.5	0.159	0.228	0.844
Urine N	149	143	171	171	32.5	0.295	0.895	0.896
Milk N	192	186	161	148	11.1	0.001	0.251	0.659
N utilization (g/g)								
Fecal N/N intake	0.32	0.37	0.30	0.35	0.024	0.258	0.014	0.869
Urine N/N intake	0.23	0.29	0.28	0.36	0.057	0.158	0.106	0.900
Manure N/N intake	0.54	0.66	0.58	0.70	0.058	0.349	0.013	0.957
Fecal N/manure N	0.59	0.57	0.53	0.50	0.057	0.126	0.559	0.933
Urine N/manure N	0.41	0.43	0.47	0.50	0.057	0.101	0.548	0.931

¹SED = standard error of the difference.

²SED = standard error of the difference.

GE, and fiber digestibility were unaffected by diet CP level in the current study, N digestibility was lower with the low-compared with the high-CP diet (-0.05 g/g; P = 0.004; Table 3). With lower CP diets, proportionally greater endogenous protein excretion can contribute to a lower apparent N digestibility (Giallongo et al., 2015). Although diet had no effect on total N excretion in manure (g/d), the low-CP diet increased manure N as a proportion of N intake (+0.12 g/g; P = 0.013), with this driven by the increase in fecal N as a proportion of intake (+0.05 g/g; P = 0.014). The release of ammonia from fecal N is lower compared with urinary N (Dijkstra et al., 2013), so reducing the amount of N excreted in urine can still reduce the environmental impact of dairy farming. However, decreasing dietary CP level did not change the proportion of feces N or urine N within manure, in contrast to previous studies that found an increased proportion of N excreted in feces compared with urine when CP level was decreased (Huhtanen et al., 2008; Yang et al., 2022).

In conclusion, improving grass silage digestibility resulted in a substantial increase in both intake and milk production, and an overall improvement in NUE which highlights the benefits of improving silage digestibility. While reducing dietary CP content also improved NUE, this was associated with a reduction in both intakes and milk production. However, grass silage digestibility had no impact on cow performance response to total dietary CP level, which suggests that total diet CP cannot be reduced to a greater extent with a high compared with a low digestibility silage.

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Notes

A. Craig, https://orcid.org/0000-0002-5142-0836
A. W. Gordon, https://orcid.org/0000-0002-9806-6424
C. P. Ferris https://orcid.org/0000-0001-8623-5983

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Nonstandard abbreviations used: DUP = digestible undegradable protein; D-value = digestible OM in the DM; ERDP = effective rumen degradable protein; FA = fatty acid; GE = gross energy; high-CP = high total diet crude protein; high-D = high digestibility grass silage; low-CP = low total diet crude protein; low-D = low digestibility grass silage; NEFA = nonesterified fatty acids; NUE = nitrogen use efficiency; SED = standard error of the difference.