

# Digestibility and protein utilization in wethers fed whole-crop barley or grass silages harvested at different maturity stages, with or without protein supplementation<sup>1</sup>

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**ABSTRACT:** Effects of whole-crop barley and grass silages harvested at different maturity stages, with or without protein supplementation, on intake, in vivo digestibility, feces characteristics, and protein utilization in wethers were evaluated. Whole-crop barley silage harvested at heading stage (**BH**) and at medium milk stage (**BM**), grass silage (**GE**) taken at the flag leaf-early heading stage, and grass silage (**GL**) taken at medium-late heading stage were fed to eight wethers in two 4 × 4 Latin squares. Wethers in one square were fed supplementary rapeseed meal. Experimental periods lasted for 4 wk and wethers were fed ad libitum during the first 3 wk, with intake recorded during the third week. During the fourth week, wethers were fed 80% of ad libitum, and feces and urine were collected during the last 4 d. The GE and BH diets had greater ( $P < 0.05$ ) in vivo apparent digestibility of DM and its nutrients, lower proportion of fecal particle DM (**PDM**) with a greater proportion of small particles compared with GL and BM diets, respectively. The GE diet had greater ( $P < 0.001$ ) in vitro OM digestibility and in vivo digestibility of OM and fibre, resulting in a smaller ( $P < 0.001$ ) proportion of PDM with a greater ( $P < 0.001$ ) proportion of small particles

compared with the other diets. In vivo NDF digestibility was negatively related to fecal PDM across forage types ( $R^2 = 0.91$ , RMSE = 2.55). The GE silage had greater CP concentration, and animals fed the GE diet had greater intake of CP ( $P < 0.001$ ) and sum of the degradable CP fractions A, B<sub>1</sub>, and B<sub>2</sub> ( $P < 0.01$ ), resulting in greater ( $P < 0.05$ ) urinary nitrogen (N) excretion than when fed any of the other diets and a lower ( $P < 0.05$ ) N retention compared with BH and BM diets. Microbial N supply tended to increase when animals were fed the BH diet ( $P = 0.10$ ) and when rapeseed meal was added to the forages ( $P = 0.08$ ). Increased N intake ( $P = 0.008$ ) by rapeseed meal supplementation increased urinary N excretion in gram per day ( $P = 0.05$ ). The strong relationship between in vivo NDF digestibility and fecal PDM indicates potentials for using PDM as a cheap method to predict NDF digestibility. Early harvest of the forages improved in vivo digestibility of nutrients, resulting in less fecal PDM with a greater proportion of small particles compared with late harvest within forage type. However, wethers fed the GE diet had greater urinary N losses compared with wethers fed the GL diet but this effect of maturity was absent when fed whole-crop barley silage.

**Key words:** fecal characteristics, forage, in vivo digestibility, nitrogen utilization, sheep, silage

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## INTRODUCTION

Harvesting grasses for silage at early maturity stages are known to increase OM digestibility (OMD), intake, and performance of ewes (Nadeau et al., 2016a). Because of the accumulation of digestible starch during grain filling post-heading, the OMD of whole-crop cereals remains relatively constant after milk stage of maturity (Rustas et al., 2011). Fecal particle size distribution reflects the size distribution of particles leaving the rumen (Ulyatt et al., 1986), which is affected by lignification of forage cell walls (Rustas et al., 2010; Jalali et al., 2015). Furthermore, proportions of undigested particle DM (PDM) in feces from ruminants increase by increased lignification during maturation of grass (Jalali et al., 2015). There is, however, a lack of studies comparing in vivo digestibility and fecal characteristics in ruminants of grass and whole-crop barley silages harvested at different maturity stages.

About 75% of forage CP is RDP (Merchen and Bourquin 1994) of which NPN comprises 50% to 60% of the CP in silage (Muck and Hintz 2003). The NPN is lost as urea in the urine when rapidly fermented carbohydrates are not available for microbial protein synthesis (Jardstedt et al., 2017). The efficiency of nitrogen (N) utilization by ruminants sharply decreases and a significant amount is excreted when protein is overfed (Broderick 2003; Agle et al., 2010). Only limited information is available on differences in N utilization between forage types harvested at various maturity stages using wethers as model animals for ruminants. The aim of this study was to evaluate the effects of whole-crop barley and grass silages, harvested at different maturity stages, on intake, digestibility, fecal characteristics, and N excretion in wethers fed the silages with or without a rapeseed meal supplement. Our hypothesis was that digestibility decreased with delayed harvest of both forage types but that the effect of harvest date on protein utilization differed in magnitude.

## MATERIALS AND METHODS

The trial was conducted at the SLU Götala Beef and Lamb Research, Skara, southwest Sweden (58°23'N, 13°29'E), between January 24

and May 16, 2014. Experimental procedures were approved by Gothenburg Research Animal Ethics Committee (case numbers 63-2012, 182-2013).

### Forage Harvest and Ensiling

The grass sward contained timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* Huds.) and was fertilized with 25 tonnes of liquid manure, containing 0.15% N/tonne, on April 10 and 81 kg N/ha of an artificial fertilizer on April 22, 2013. The whole-crop barley (*Hordeum vulgare* L. cv. Rosalina) was fertilized with 38 kg N, 9 kg P, and 11 kg K per ha prior to sowing on May 1 and an additional 73 kg N of an artificial fertilizer on May 22, 2013. The grass sward was harvested at an early maturity stage (flag leaf-early heading) on May 31 (GE) and at a late maturity stage (medium-late heading) on June 17 (GL). Whole-crop barley was harvested at heading (growth stage 59, emergence of inflorescence completed; Zadoks et al., 1974) on June 30 (BH) and at medium milk stage (growth stage 75; Zadoks et al., 1974) on July 18 (BM). All mown forages were wilted and the wilting period differed between the forages to achieve similar DM contents at baling. The wilting period was 2 h for BM and GL, 6 h for BH, and 20 h for GE. Despite the longer wilting time for GE, this forage had a lower DM content than the other forages. Forages were ensiled in round bales using chemical additives produced by Addcon Europe GmbH, Parsevalstrasse 6, 06749 Bitterfeld-Wolfen, Germany; Kofasil Lp (sodium nitrite, hexamine, and sodium benzoate) for the grass silage and Kofasil Ultra K (sodium nitrite, hexamine, potassium sorbate, sodium benzoate, and sodium propionate) for the whole-crop barley silage, both at an application rate of 2 L/tonne of forage. The bales were wrapped with eight layers of plastic film and stored for at least 4 mo before the trial started. All baled silages were chopped to 40 mm length using a Dunker TVS 120 mixer (Storti, the Netherlands), to avoid differences in particle size between the treatments. The silages were then immediately stored in a freezer at -20 °C in small packages to ensure the quality of the silage at feeding. The silages were thawed thoroughly before feeding.

## Experimental Design

The four silages (i.e., GE, GL, BH, and BM) were fed to wethers in two  $4 \times 4$  Latin squares, with animals in one square receiving silage supplemented with rapeseed meal and animals in the other square receiving silage without rapeseed meal. The same amount of rapeseed meal was fed to the four wethers prior to feeding the silage in the morning (150 g rapeseed meal/d during ad libitum feeding; 120 g rapeseed meal/d during restricted feeding (80% of ad libitum)). The chemical composition of the rapeseed meal is presented in [Table 1](#).

Each experimental period lasted for 4 wk (29 d), starting with 2 wk in which the wethers were fed the experimental feed ad libitum to allow them to adapt. During the third week, feed intake was recorded and 15% of orts were allowed. During the 4th week, the wethers were fed 80% of ad libitum and were allowed to adapt to the restricted intake for the first 3 d, followed by total collection of feces and urine during the last 4 d of that week.

## Animals and Housing

Eight 20-mo old cross-bred (Texel  $\times$  Swedish Finewool/Dorset) wethers were used in the study. The wethers were divided randomly into two groups, with four wethers per group. Initial BW; mean  $\pm$  SD was  $86.7 \pm 7.1$  kg for the first group and  $85.6 \pm 6.8$  kg for the second group, while BCS was  $3.3 \pm 0.2$  and  $3.3 \pm 0.2$ , respectively. Body condition was scored by one trained technician according to [Jefferies \(1961\)](#) and [Russell et al., \(1969\)](#), using a

**Table 1.** Mean ( $\pm$ SD) of chemical composition of rapeseed meal used as a protein supplement for wethers fed silage-based diets

DM, %	88.4 $\pm$ 0.14
Starch, g/kg DM	76.0 $\pm$ 6.70
NDF, g/kg DM	292 $\pm$ 7.2
ADF, g/kg DM	237 $\pm$ 7.5
Ash, g/kg DM	72.0 $\pm$ 0.30
CP, g/kg DM	379 $\pm$ 14.1
Protein fractions, % of CP	
A	8.1 $\pm$ 2.83
B <sub>1</sub>	16.9 $\pm$ 0.82
B <sub>2</sub>	62.8 $\pm$ 1.68
B <sub>3</sub>	6.0 $\pm$ 0.27
C	6.3 $\pm$ 0.06

Values are mean of  $n = 2$  samples.

A = NPN, B<sub>1</sub> = buffer-soluble protein, B<sub>2</sub> = neutral detergent-soluble protein, B<sub>3</sub> = acid detergent-soluble protein, C = ADIN.

five-point scale with quarter grade steps, where 1 is emaciated and 5 severely obese.

During the first 3 wk (days 1 to 21) of each period, the wethers were housed in individual 6 m<sup>2</sup> pens with deep straw bedding. During the 4th week (days 22 to 29), the wethers were kept in individual metabolic cages measuring 1.5 m  $\times$  0.8 m to enable total collection of feces and urine. The metabolic cages had mesh floors, with rubber mats at the front for better comfort. The wethers were fed the treatment diets individually once a day in both the pens and the metabolic cages. All wethers received 20 g of minerals daily, except during the last 4 d of each period when urine and feces were collected. The wethers had free access to water and salt block during the trial.

## Sample Collection and Chemical Analysis

Silage and orts were sampled daily during the third week, when the wethers were fed ad libitum, and during the last 4 d of the fourth week, when the wethers were fed at 80% of ad libitum. Rapeseed meal was sampled once every period and pooled to a total of two samples. Samples of feed and orts were stored at  $-20$  °C until preparation for analysis. Feces were collected in plastic containers placed on the floor under the metabolic cages. Feces from the floor of the cages were brushed down to the container, wool fragments were removed, and the feces were weighed and frozen at  $-20$  °C. Urine was collected through funnels into stainless steel bowls placed on the floor under the metabolic cages. To decrease pH below 3 and to inhibit microbial activity, 300 mL of 10% sulfuric acid were added to each urine collection bowl. The urine was stirred and passed through a strainer to remove feed particles and wool, urine volume was recorded, and a 200-mL sample was taken and immediately frozen at  $-20$  °C.

The DM concentration of silage and orts during collection week was determined by drying daily 150 g samples or all orts when sample size was small, in a drying cabinet at 60 °C for 20 h. The DM concentration of feces was determined on daily individual samples by drying 150 g of feces at 60 °C for 48 h. Silage samples were pooled per feed and week, while samples of orts, feces, and urine were pooled per wether and week and mixed thoroughly, and then subsamples were taken for chemical analysis.

A subsample of 200 g of feed, orts, and feces was sent to LKS mbH, Lichtenwalde, Germany for analysis of CP, CP fractions, NDF, ADF, ADL, and ash. Starch content of the rapeseed

meal was analyzed enzymatically, according to the Boehringer–Mannheim test.

Another subsample of 200 g of each silage was sent to the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, for analysis of *in vitro* organic matter digestibility (IVOMD), water-soluble carbohydrates (WSC), and fermentation parameters (acids, alcohols, pH, and ammonia-N). Urine samples were analyzed for total nitrogen, urea, allantoin, uric acid, and creatinine at the LKS mbH laboratory.

Samples of feed, orts, and feces were milled to pass through a 1-mm screen before laboratory analysis. Ash was determined for feed, orts, and feces by combustion of dried and milled sample at 525 °C for 16 h. The fiber technology method of (Van Soest *et al.*, 1991) was used to determine NDF, ADF, and ADL concentrations in dried, milled samples. The NDF analysis was modified by adding heat-stable  $\alpha$ -amylase (Novozymes, Bagsvaerd, and Denmark) and omitting sodium sulfite. Reported concentrations of NDF, ADF, and ADL were corrected for residual ash after ADL treatment.

Concentrations of N were determined with the Kjeldahl method on fresh, pooled samples of feed and orts, and on freeze-dried samples of feces (AOAC 2012). The CP concentration was calculated as total N  $\times$  6.25. CP fractions (A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, and C), based on degradability characteristics according to the Cornell Net Carbohydrate and Protein System (Sniffen *et al.*, 1992), were determined according to Licitra *et al.* (1996). The A fraction is NPN, which is the N recovered in the filtrate after precipitation with tungstic acid. The B fraction is degradable true protein, which is further divided into fraction B<sub>1</sub>, which is soluble in borate-phosphate buffer at rumen pH and rapidly degraded in the rumen; fraction B<sub>2</sub>, which is insoluble in borate-phosphate buffer, but soluble in neutral detergent solution and has variable degradation; and fraction B<sub>3</sub>, which is insoluble in neutral detergent solution, but soluble in acid detergent solution. Fraction B<sub>3</sub> is digestible but slowly degradable, with most degradation occurring postruminally (Licitra *et al.*, 1996). Fraction C is considered to be indigestible and is insoluble in acid detergent solution. True protein is the CP concentration minus the A fraction.

The N concentration of urine was analyzed with a Kjeldahl procedure. Urine concentrations of creatinine, allantoin, and uric acid (samples diluted 50 fold) were analyzed with HPLC as described by Shingfield and Offer (1999), but with the modification of using a second mobile phase

containing methanol, acetonitrile, and distilled water (45/45/10) and a Kinetex XB-C18 column (150  $\times$  4.6 mm, 5  $\mu$ m). Analysis of urea concentration (samples diluted 50 fold) was performed by spectrophotometry according to LKS (2006).

The IVOMD of silages was analyzed by incubation at 38 °C for 96 h of 0.5 g dried, milled sample in 49 mL buffer, and 1 mL rumen fluid (Lindgren, 1979, 1983). The content of WSC was assessed by a simplified enzymatic method according to Larsson and Bengtsson (1983). Concentrations of lactic acid, acetic acid, propionic acid, butyric acid, and ethanol were assessed with HPLC according to Ericson and André (2010). The pH was determined in a water extract of the silage using a pH meter (Metrohm 654, Herisau, Schweiz).

### *Intake, Sorting, BW, and Condition Score*

Offered feed was weighed individually in the third and fourth weeks of each period. Orts were weighed individually in the third week, and when present during the last 4 d of the fourth week, for feed intake determination. Sorting was calculated as the difference between NDF concentration of the feed and NDF concentration of the orts during *ad libitum* feeding of the wethers in the 3rd week. Wethers were weighed and scored for body condition once on the first day of each 4-wk period. In addition, the wethers were weighed once at the start and at the end of the third week and body conditioned once at the start of the third week.

### *In Vivo Digestibility and Feces Characteristics*

The *in vivo* digestibility of DM, OM, CP, NDF, and ADF was calculated as the difference between intake and feces output of each, divided by the intake. As endogenous N in feces was not considered in the calculations, the value obtained was apparent *in vivo* digestibility.

For feces particle size evaluation, a 200-g subsample of the individual pooled feces sample from each period was sent to the University of Copenhagen. Subsamples of 4 g feces were placed in nylon bags with pore size of 0.01 mm and 4 mL of a commercial detergent (BIOTEX Color, Blumøller, Denmark) were added per bag, with triplicate bags per sample. The bags were gently massaged to dissolve the detergent into the feces, washed at 40 °C for 2 h, and centrifuged at 700 rpm/min in a regular washing machine. The residual contents from the triplicate bags were transferred to an aluminum tray using distilled water. The trays were kept in a

freezer for 1 h and then freeze-dried (HETOSICC CD8 Freeze dryer) for 48 h. Residual PDM was defined as the proportion of fecal DM left after washing and freeze-drying.

The PDM was sorted into six sieve fractions, 2.36, 1.0, 0.5, 0.212, 0.106 mm, and the bottom bowl (0.0 mm), using a Retsch AS200 sieve shaker as described by Nørgaard et al. (2004) and Jalali et al. (2012). Residues from each sieve were collected and weighed, and the proportion of particles retained on each sieve was estimated. The arithmetic mean particle size (APS) and the geometric mean particle size (GPS) were calculated according to Waldo et al. (1971). The most frequent particle size, i.e., median particle size (MPS), and 95th percentile particle size were estimated from the proportions of particles in the individual sieve fractions, as described by Nørgaard and Kornfelt (2006).

### Protein Utilization

To evaluate total excretion of nitrogenous compounds, concentrations of N compounds in the urine were multiplied by urine volume. The difference between N intake and loss of N through urine and feces was used to calculate the nitrogen balance from the feeds. Excretion of the purine derivatives allantoin and uric acid was determined and intestinal flow of microbial N was calculated according to Chen and Gomes (1992). The quantitative relationship between absorption of microbial purines ( $PD_{Abs}$  mmol/d) from the intestines and excretion of PD in urine ( $PD_{Ex}$  mmol/d) were computed with the following equation:  $PD_{Ex} = 0.84PD_{Abs} + (0.150W^{0.75} e^{-0.25PD_{Abs}})$ . The slope of 0.84 represents the proportion of absorbed purines recovered as PD in urine, the component within parenthesis denotes the endogenous contribution of PD per day where  $W^{0.75}$  is metabolic BW (kg). The calculation of  $PD_{Abs}$  from  $PD_{Ex}$  was performed by using the Newton–Raphson iteration process:

$$PD_{Abs(n+1)} = PD_{Abs} - f(PD_{Abs}) / f'(PD_{Abs})$$

where  $f(PD_{Abs}) = 0.84PD_{Abs} + 0.150W^{0.75} e^{-0.25PD_{Abs}} - PD_{Ex}$   
and  $f'(PD_{Abs}) = 0.84 - 0.0380W^{0.75} e^{-0.25PD_{Abs}}$

The initial value of  $PD_{Abs} = PD_{Ex}/0.84$  and the iteration process were performed until the  $PD_{Abs}$  reached a constant value, which was used in the equation for calculation of microbial N (g/d) =  $(PD_{Abs} \times 70)/(0.116 \times 0.83 \times 1,000)$ , where 70 mg/mmol is the N content of the purines, 11.6:100 is the ratio of purine N to total N in mixed rumen microbes and 0.83 is the digestibility

of microbial purines in the small intestine (Chen and Gomes 1992).

### Statistical Analysis

Silage quality data were analyzed by analysis of variance for a randomized block design using the GLM procedure of SAS version 9.3 software (SAS Inst. Inc., Cary, NC), where period ( $n = 4$ ) in the Latin square design was treated as block and forage as treatment. Data on feed intake, in vivo digestibility, feces particle size distribution, and protein utilization were analyzed for two  $4 \times 4$  Latin squares using the MIXED procedure of SAS. The statistical model was:

$$Y_{ijkl} = \mu + F_i + S_j + (FS)_{ij} + P_k + W_{l(j)} + C_{m(ijkl)} + e_{ijkl}$$

where  $Y_{ijkl}$  = observed response,  $\mu$  = overall mean,  $F_i$  = effect of forage ( $i = 1$  to 4),  $S_j$  = effect of supplementation of protein (equals effect of square;  $j = 1$  to 2),  $(FS)_{ij}$  = interaction between forage and supplementation of protein,  $P_k$  = effect of period ( $k = 1$  to 4),  $W_{l(j)}$  = random effect of wether nested within protein supplementation (equals square;  $l = 1$  to 8),  $C_{m(ijkl)}$  = carry-over effect between periods for the combination of  $ijkl$  ( $m = 4$ ), and  $e_{ijkl}$  = residual error.

As no interactions between forage and protein supplementation were found and the carry-over effect was not significant,  $(FS)_{ij}$  and  $C_{m(ijkl)}$  were excluded from the model. Pair-wise comparisons were made between least square means (LS-means) with Tukey–Kramer adjustment when effects were significant at  $P \leq 0.05$  in the  $F$ -test. Pair-wise differences were considered significant at  $P \leq 0.05$  and as a tendency to significance at  $0.05 < P \leq 0.10$ . Relationships between in vivo NDF digestibility and fecal PDM were analyzed using the fit model procedure of JMP (version 13.1, SAS).

## RESULTS

### Chemical Composition and Fermentation Parameters of the Silages

The early-harvested grass (GE) silage had a lower DM concentration ( $P < 0.001$ ), but a greater ash concentration ( $P < 0.001$ ), than the other silages, which did not differ (Table 2). Forages differed in IVOMD, with GE having the greatest IVOMD ( $P < 0.001$ ), followed by BH, BM, and GL. The late-harvested grass (GL) silage had the greatest

ADF concentration ( $P < 0.001$ ), followed by GE, BH, and BM. The GL silage also had a greater ADL concentration ( $P < 0.001$ ) than the other forages, which did not differ. The CP concentration was greatest for GE ( $P < 0.001$ ), followed by GL, BH, and BM. The only CP fractions that differed between the forages were A and B<sub>3</sub> ( $P < 0.05$ ), with BH silage having a larger A fraction than GL ( $P = 0.02$ ) and with GL having a larger B<sub>3</sub> fraction than the other forages ( $P < 0.01$ ), which did not differ.

Whole-crop barley silage harvested at heading (BH) had the greatest WSC content ( $P < 0.001$ ), followed by BM, GL, and GE, but all silages had similarly low pH values ( $P = 0.87$ ; Table 2). The GE silage showed a greater extent of fermentation, having greater concentrations of lactic acid and acetic acid than the other silages. The other silages did not differ in lactic acid concentration, but the BM silage had the lowest acetic acid concentration ( $P < 0.01$ ). Concentrations of propionic acid and butyric acid in the silages were low (range 0.4 to 0.9 g/kg DM and 0.4 to 0.7 g/kg DM, respectively). Furthermore, the BM silage had greater ethanol concentration ( $P < 0.01$ ) than the other silages,

which did not differ. The GE silage had the greatest concentration of NH<sub>3</sub>-N ( $P < 0.01$ ), followed by GL, which had a greater NH<sub>3</sub>-N concentration than the BM.

### Feed Intake and Sorting

Overall, intake of all evaluated nutrients ( $P < 0.001$ ) differed among forage diets, when averaged over protein supplementation (Table 3). Animals receiving the BH diet showed greater intakes (kg/d) of DM and OM than animals receiving the GE and GL diets, which did not differ. When expressed as a percentage of BW, animals fed the BH diet had greater DMI than animals fed all the other forage diets, which did not differ. The BH and GL diets resulted in the greatest NDF intake as percentage of BW. Wethers fed the GE diet had the greatest CP intake ( $P < 0.001$ ) and wethers fed the BM diet had the lowest ( $P < 0.05$ ), with no difference between the BH and GL diets. Intake of the sum of CP fractions A, B<sub>1</sub>, and B<sub>2</sub> was greatest for animals fed the GE diet ( $P < 0.01$ ). Negative values were obtained for feed sorting for wethers fed the BH, BM, or GL diets, showing selection in favor

**Table 2.** Chemical composition and fermentation characteristics of whole-crop barley and grass silages harvested at different maturity stages

Item	Forages				SEM	P-value
	BH	BM	GE	GL		
DM, g/kg	335 <sup>a</sup>	374 <sup>a</sup>	261 <sup>b</sup>	356 <sup>a</sup>	9.8	<0.001
Ash, g/kg DM	57.3 <sup>b</sup>	51.1 <sup>b</sup>	108 <sup>a</sup>	58.6 <sup>b</sup>	2.83	<0.001
IVOMD, % of OM	83.7 <sup>b</sup>	81.1 <sup>c</sup>	87.7 <sup>a</sup>	77.3 <sup>d</sup>	0.34	<0.001
NDF, g/kg DM	471	480	472	556	23.2	0.08
ADF, g/kg DM	259 <sup>c</sup>	240 <sup>d</sup>	302 <sup>b</sup>	341 <sup>a</sup>	2.65	<0.001
ADL, g/kg DM	22.4 <sup>b</sup>	26.5 <sup>b</sup>	26.0 <sup>b</sup>	42.3 <sup>a</sup>	1.73	<0.001
CP, g/kg DM	105 <sup>c</sup>	83.3 <sup>d</sup>	190 <sup>a</sup>	116 <sup>b</sup>	1.45	<0.001
Protein fractions, % of CP						
A	74.8 <sup>a</sup>	68.7 <sup>ab</sup>	59.4 <sup>ab</sup>	53.6 <sup>b</sup>	4.00	0.02
B <sub>1</sub>	1.24	2.41	3.37	2.32	0.82	0.39
B <sub>2</sub>	18.4	21.9	29.0	31.5	3.45	0.08
B <sub>3</sub>	2.69 <sup>b</sup>	3.85 <sup>b</sup>	4.41 <sup>b</sup>	8.99 <sup>a</sup>	0.75	0.001
C	2.84	3.10	3.87	3.61	0.40	0.31
WSC, g/kg DM	155 <sup>a</sup>	126 <sup>b</sup>	7.06 <sup>d</sup>	56.0 <sup>c</sup>	5.04	<0.001
pH	4.21	4.23	4.20	4.23	0.03	0.87
Lactic acid, g/kg DM	51.9 <sup>b</sup>	39.4 <sup>b</sup>	88.2 <sup>a</sup>	49.7 <sup>b</sup>	4.10	<0.001
Acetic acid, g/kg DM	17.2 <sup>b</sup>	6.30 <sup>d</sup>	21.8 <sup>a</sup>	12.0 <sup>c</sup>	0.90	<0.001
Ethanol, g/kg DM	1.70 <sup>b</sup>	8.00 <sup>a</sup>	4.40 <sup>b</sup>	3.60 <sup>b</sup>	0.60	<0.001
NH <sub>3</sub> -N <sup>1</sup> , g/kg total N	69.6 <sup>bc</sup>	59.3 <sup>c</sup>	111 <sup>a</sup>	83.1 <sup>b</sup>	4.30	<0.001

<sup>a-d</sup>LS means within a row with different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values include N from the additives used.

BH = whole-crop barley silage harvested at heading stage; BM = whole-crop barley silage harvested at medium milk stage; GE = grass silage harvested at flag leaf – early heading stage; GL = grass silage harvested at medium – late heading stage of maturity.

IVOMD = in vitro OM digestibility; A = NPN; B<sub>1</sub> = buffer-soluble protein; B<sub>2</sub> = neutral detergent-soluble protein; B<sub>3</sub> = acid detergent-soluble protein; C = ADIN; WSC = water-soluble carbohydrates.

**Table 3.** Effects of diet and rapeseed meal supplementation on intake, feed sorting, BCS, and BW of wethers fed ad libitum

Item	Diet				SEM	P-value	Rapeseed meal		SEM	P-value
	BH	BM	GE	GL			Without	With		
Intake										
FDM, kg/d	2.48 <sup>a</sup>	2.25 <sup>ab</sup>	2.02 <sup>b</sup>	2.15 <sup>b</sup>	0.080	<0.001	2.16	2.29	0.080	0.29
DM, kg/d	2.55 <sup>a</sup>	2.32 <sup>ab</sup>	2.08 <sup>b</sup>	2.21 <sup>b</sup>	0.076	<0.001	2.16	2.42	0.082	0.06
OM, kg/d	2.40 <sup>a</sup>	2.20 <sup>ab</sup>	1.86 <sup>c</sup>	2.08 <sup>bc</sup>	0.073	<0.001	2.01	2.26	0.076	0.06
NDF, kg/d	1.19 <sup>ab</sup>	1.07 <sup>bc</sup>	0.96 <sup>c</sup>	1.28 <sup>a</sup>	0.043	<0.001	1.07	1.18	0.044	0.12
DM, % of BW	2.57 <sup>a</sup>	2.32 <sup>b</sup>	2.13 <sup>b</sup>	2.21 <sup>b</sup>	0.059	<0.001	2.21	2.41	0.041	0.002
NDF, % of BW	1.21 <sup>a</sup>	1.07 <sup>b</sup>	0.98 <sup>b</sup>	1.27 <sup>a</sup>	0.031	<0.001	1.09	1.17	0.022	0.02
CP, g/d	313 <sup>b</sup>	225 <sup>c</sup>	410 <sup>a</sup>	288 <sup>b</sup>	15.0	<0.001	277	341	11.9	0.009
AB <sub>1</sub> B <sub>2</sub> , g/d	294 <sup>b</sup>	208 <sup>c</sup>	375 <sup>a</sup>	251 <sup>bc</sup>	13.8	<0.001	253	310	10.2	0.008
Selection <sup>1</sup> , g/kg DM	-9.74 <sup>b</sup>	-9.68 <sup>b</sup>	17.8 <sup>a</sup>	-11.6 <sup>b</sup>	5.03	<0.001	2.52	-9.15	3.93	0.08
Mean BCS	3.91	3.97	3.87	3.97	0.06	0.21	3.87	3.98	0.079	0.37
Mean BW, kg	100.1 <sup>a</sup>	100.2 <sup>a</sup>	97.6 <sup>b</sup>	99.8 <sup>ab</sup>	2.56	0.01	97.9	101.0	3.55	0.55

<sup>a-c</sup>LS means within rows with different superscripts differ significantly ( $P < 0.05$ ).

BH = whole-crop barley silage harvested at heading stage; BM = whole-crop barley silage harvested at medium milk stage; GE = grass silage harvested at flag leaf-early heading stage; GL = grass silage harvested at medium-late heading stage.

FDM = forage DM; AB<sub>1</sub>B<sub>2</sub> = sum of CP fractions A, B<sub>1</sub> and B<sub>2</sub>, which are considered rumen degradable. A = NPN, B<sub>1</sub> = buffer-soluble protein, B<sub>2</sub> = neutral detergent-soluble protein.

<sup>1</sup>Difference between NDF concentration of the diet and NDF concentration of theorts. Negative value shows sorting against NDF.

of less fibrous portions of the diet, whereas wethers fed the GE diet showed no feed sorting. Mean BCS of the wethers was not affected by forage treatment. Animals fed the GE diet had lower mean BW than wethers fed the BH or BM diets ( $P < 0.05$ ).

Supplementation with rapeseed meal increased DMI as a percentage of BW, tended to increase intakes of DM and OM in kg/d, and increased intakes of CP and the AB<sub>1</sub>B<sub>2</sub> fraction, but did not affect mean BW and BCS of wethers fed silage-based diets (Table 3). A tendency ( $P = 0.08$ ) for feed sorting was observed, with animals receiving the protein supplement selecting in favor of less fibrous portions of the diet.

### *In Vivo Digestibility and Feces Characteristics*

Overall, the in vivo apparent digestibility of all nutrients in wethers fed 80% of the ad libitum intake differed among forages ( $P < 0.001$ ), when averaged over protein supplementation (Table 4). The DM digestibility was greater ( $P < 0.05$ ) when animals were fed the BH and GE diets compared with the BM and GL diets, indicating that DM digestibility was positively affected by early maturity for both forage types. In vivo OMD was greatest ( $P < 0.001$ ) for the GE diet, followed by the BH, BM, and GL diets. Furthermore, in vivo digestibility of CP, NDF, and ADF was similarly affected by forage type, with greater ( $P < 0.05$ ) digestibility

being observed when wethers received the GE diet, followed by the BH and GL diets, which did not differ, and the lowest digestibility when fed the BM diet ( $P < 0.05$ ). The CP digestibility increased when the wethers were fed rapeseed meal as a protein supplement, but in vivo digestibility of DM, OM, NDF, and ADF was not affected by protein supplementation, when averaged over forage diets.

Fecal DM concentration was greater ( $P < 0.001$ ) for wethers fed the GE diet, followed by the GL, BH, and BM diets, with no difference between the last two diets (Table 5). Fecal PDM was greatest for the BM diet ( $P < 0.001$ ), followed by GL and BH, which did not differ, and lowest for the GE diet ( $P < 0.001$ ). The GE diet had a greater percentage of feces particles smaller than 0.2 mm ( $P < 0.001$ ), followed by GL, BH, and BM. The GE and GL diets had the greatest proportion of feces particles smaller than 0.5 mm ( $P < 0.001$ ), followed by the BH and BM diets. Proportion of feces particles smaller than 1 mm was greater for the GL diet than for the BM diet ( $P = 0.010$ ). The BM diet had a greater proportion of feces particles between 1.0 and 2.36 mm ( $P = 0.016$ ) compared with the GL diet. There was no effect of forage diet on feces particles larger than 2.36 mm, GPS, APS, MPS, or 95 percentile PS. Supplementation of rapeseed meal to the forage diets significantly decreased the 95 percentile PS and tended to decrease GPS, APS, and MPS (Table 5).

**Table 4.** Effects of diet and rapeseed meal supplementation on in vivo digestibility in wethers fed at 80% of ad libitum DMI

Item	Diet				SEM	<i>P</i> -value	Rapeseed meal		SEM	<i>P</i> -value
	BH	BM	GE	GL			Without	With		
DM, %	68.3 <sup>a</sup>	65.2 <sup>b</sup>	70.0 <sup>a</sup>	63.3 <sup>b</sup>	0.76	<0.001	67.1	66.3	0.73	0.46
OM, %	69.6 <sup>b</sup>	66.8 <sup>c</sup>	73.6 <sup>a</sup>	64.5 <sup>d</sup>	0.71	<0.001	68.9	68.4	0.70	0.58
CP, %	86.0 <sup>b</sup>	84.1 <sup>c</sup>	88.2 <sup>a</sup>	86.3 <sup>b</sup>	0.44	<0.001	85.4	86.9	0.33	0.02
NDF, %	57.7 <sup>b</sup>	47.0 <sup>c</sup>	72.1 <sup>a</sup>	58.5 <sup>b</sup>	1.01	<0.001	59.5	58.1	0.97	0.34
ADF, %	58.2 <sup>b</sup>	45.4 <sup>c</sup>	73.5 <sup>a</sup>	57.7 <sup>b</sup>	1.14	<0.001	60.2	57.2	1.17	0.12

<sup>a-d</sup>LS means within rows with different superscripts differ significantly ( $P < 0.05$ ).

BH = whole-crop barley silage harvested at heading stage; BM = whole-crop barley silage harvested at medium milk stage; GE = grass silage harvested at flag leaf-early heading stage; GL = grass silage harvested at medium-late heading stage.

**Table 5.** Effects of diet and rapeseed supplementation on feces DM content and particle size characteristics of wethers fed at 80% of ad libitum DMI

Item	Diet				SEM	<i>P</i> -value	Rapeseed meal		SEM	<i>P</i> -value
	BH	BM	GE	GL			Without	With		
DM, %	36.0 <sup>c</sup>	35.2 <sup>c</sup>	47.3 <sup>a</sup>	41.6 <sup>b</sup>	1.32	<0.001	41.6	38.4	1.15	0.21
PDM, % of DM	64.4 <sup>b</sup>	72.3 <sup>a</sup>	51.7 <sup>c</sup>	66.5 <sup>b</sup>	0.94	<0.001	63.7	63.7	0.79	0.98
PS <0.2 mm, %	53.3 <sup>c</sup>	43.8 <sup>d</sup>	77.5 <sup>a</sup>	66.9 <sup>b</sup>	1.07	<0.001	59.9	60.8	0.96	0.55
PS <0.5 mm, %	91.0 <sup>b</sup>	87.1 <sup>c</sup>	96.0 <sup>a</sup>	94.9 <sup>a</sup>	0.66	<0.001	92.3	92.2	0.71	0.85
PS <1 mm, %	99.1 <sup>ab</sup>	98.7 <sup>b</sup>	99.2 <sup>ab</sup>	99.5 <sup>a</sup>	0.17	0.01	99.0	99.2	0.18	0.51
1 < PS <2.36 mm, %	0.77 <sup>ab</sup>	0.93 <sup>a</sup>	0.67 <sup>ab</sup>	0.43 <sup>b</sup>	0.12	0.02	0.75	0.65	0.12	0.55
PS >2.36 mm, %	0.16	0.34	0.11	0.09	0.09	0.14	0.21	0.14	0.08	0.53
GPS, mm	0.28	0.27	0.28	0.29	0.02	0.94	0.30	0.27	0.016	0.09
APS, mm	0.41	0.39	0.41	0.43	0.03	0.88	0.44	0.38	0.022	0.06
MPS, mm	0.41	0.39	0.41	0.43	0.03	0.88	0.44	0.38	0.022	0.07
95 percentile PS, mm	0.45	0.42	0.45	0.48	0.05	0.82	0.49	0.40	0.032	0.04

<sup>a-d</sup>LS means within rows with different superscripts differ significantly ( $P < 0.05$ ).

BH = whole-crop barley silage harvested at heading stage; BM = whole-crop barley silage harvested at medium milk stage; GE = grass silage harvested at flag leaf-early heading stage; GL = grass silage harvested at medium-late heading stage.

PDM = proportion of fecal particles left after washing and freeze-drying relative to total fecal DM; PS = particle size; GPS = geometric mean particle size; APS = arithmetic mean particle size; MPS = median particle size.

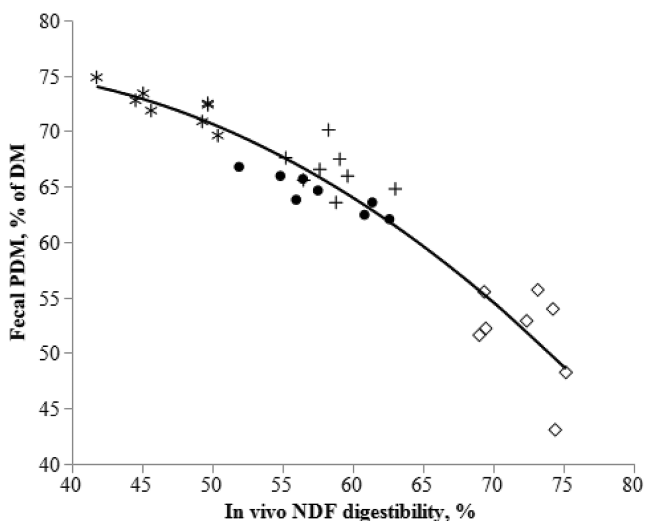
There was a negative relationship ( $R^2 = 0.91$ ) between in vivo NDF digestibility and fecal PDM, with NDF digestibility increasing with decreasing PDM (Figure 1). Using a quadratic relationship for best fit, a one-unit increase in NDF digestibility was accompanied by a 0.77% decrease in fecal PDM, and the response varied in magnitude depending on the in vivo NDF digestibility of the forage diet. When the forage diet had an NDF digestibility >58.8%, fecal PDM decreased by 0.79 percentage units per unit increase in NDF digestibility, whereas when the forage diet had an NDF digestibility lower than 58.8%, fecal PDM decreased by 0.76 percentage units per unit increase in NDF digestibility.

### Protein Utilization

Wethers fed the GE diet had the greatest N intake ( $P < 0.001$ ), followed by wethers fed the BH

and GL diets, which did not differ but had greater intake than wethers fed the BM diet ( $P < 0.05$ ; Table 6). Furthermore, wethers fed barley silage (BH and BM) diets had greater intake of digestible OM ( $P < 0.05$ ) than wethers fed grass silage diets (GE and GL). Animals fed the GE diet had a greater urine volume ( $P < 0.001$ ) than wethers fed the other diets. Wethers fed the GE diet showed the greatest N excretion in urine (g/d), followed by those fed the GL and BM diets, which both were similar to those fed the BH diet. When N excretion in urine was expressed as a percentage of N intake, wethers fed the GE diet had greater excretion ( $P < 0.05$ ) than those fed the other diets, which did not differ. Greater N excretion in feces ( $P < 0.01$ ; g/d) was observed for animals receiving the GE diet compared with animals fed the other diets, which did not differ. However, when excretion of N in feces was expressed as a percentage of N intake, wethers fed





**Figure 1.** Relationship between in vivo NDF digestibility and fecal particle DM (PDM) content in wethers fed whole-crop barley or grass silages harvested at different maturity stages.  $Y = 110.3 + (-0.77X) + (-0.014 \times (X - 58.8)^2)$   $R^2 = 0.91$  RMSE = 2.55. ● = whole-crop barley silage harvested at heading stage (BH); \* = whole-crop barley silage harvested at medium milk stage (BM); ◇ = grass silage harvested at flag leaf-early heading stage (GE); + = grass silage harvested at medium-late heading stage (GL).

the BM diet had the greatest excretion ( $P < 0.05$ ), followed by those fed BH and GL, which did not differ but had greater ( $P < 0.05$ ) N excretion than wethers fed the GE diet. Retention of N (g/d) was not affected by forage treatment. However, when N retention was expressed as a percentage of N intake, there was a difference between the forage diets, with BH and BM having a greater ( $P < 0.05$ ) retention than the GE diet.

Wethers fed the GE diet showed greater urea-N excretion in urine ( $P < 0.001$ ) than those fed the other diets (Table 6). There was a tendency for significant effects of forage diet on urinary excretion of allantoin ( $P = 0.08$ ), with greater excretion in wethers that received the BH diet compared with the BM diet. Likewise, ruminal microbial N flow tended to be greater for the BH diet than for the BM diet ( $P = 0.10$ ). However, when urinary allantoin excretion and microbial N flow from the rumen were expressed per unit of digestible OM intake, no differences were found among the forage diets. Greater urinary excretion of hippuric acid ( $P < 0.05$ ) was observed in the urine of animals fed the GE diet compared with the BM and GL diets. There was no effect of forage diet on urinary excretion of creatinine and uric acid.

Protein supplementation with rapeseed meal increased N intake and excretion of N in urine but no differences were found in urinary N excretion when expressed in proportion to N intake (Table 6). In addition, protein supplementation increased N excretion in faeces when expressed in gram per

day but this was reversed to a decrease when expressed in proportion to N intake. Retention of N expressed in gram per day tended to increase in wethers fed rapeseed meal in the diet but this difference disappeared when expressed as a percentage of N intake.

There was no effect of protein supplementation on urinary excretion of urea-N, creatinine, uric acid, or hippuric acid (Table 6). There was a tendency for greater urinary excretion of allantoin ( $P = 0.08$ ) and microbial N flow ( $P = 0.07$ ) expressed in mmol/d when animals were fed silage-based diets with protein supplementation, but no difference between treatments was found when allantoin excretion and ruminal microbial N flow were expressed per kilogram digestible OM intake.

## DISCUSSION

Grass maturity is one of the most important factors affecting forage quality (Nelson and Moser, 1994; Jalali et al., 2012). According to Van Soest (1994), advanced stage of maturity at harvest increases the contents of NDF and lignin, and the ADL:NDF ratio in forage cell walls, decreasing the digestibility (Allen, 2000; Rustas et al., 2011). Harvesting grasses at early maturity stages increases the forage energy value for ruminants by decreasing the fiber concentration and increasing fiber digestibility and the CP concentration (Nadeau et al., 2016a). In the present study, grass and barley silage at an early maturity stage showed greater IVOMD than its counterpart harvested at late maturity stage, and also greater in vivo digestibility of DM and of all nutrients investigated.

Various in vitro methods for predicting the digestibility of forages are frequently used. However, the results do not correspond exactly to digestibility determined in vivo (Huhtanen et al., 2006), since the in vitro methods do not consider many of the rumen digestive processes, such as rates of particle breakdown and passage. Sheep fed at maintenance level represent a model of ruminant digestion commonly used as the basis for estimation of digestibility in feed evaluation systems and can be considered the best estimate of the intrinsic digestibility value of feed ingredients for ruminants (Stefanska et al., 2008). In vivo digestibility of nutrients is currently determined at either ad libitum or restricted feeding level (Andueza et al., 2013). At ad libitum feed intake digestibility can decrease, as increased feeding level increases the rate of digesta passage from the rumen (Bourquin et al., 1990). Alternatively, it can increase if animals select the highly digestible parts of the plants (Mero and Udén, 1998). During the ad

**Table 6.** Effects of diet and rapeseed supplementation on intake of nitrogen (N) and digestible organic matter, urine volume, N excretion in urine and feces, N retention, and urinary excretion of purine derivatives, microbial N yield, hippuric acid, and creatinine in wethers fed at 80% of DMI

Item	Diet						Rapeseed meal				P-value
	BH	BM	GE	GL	SEM	P-value	Without	With	SEM		
N intake, g/d	34.7 <sup>b</sup>	27.9 <sup>c</sup>	51.0 <sup>a</sup>	34.0 <sup>b</sup>	1.54	<0.001	32.8	41.0	1.49	0.008	
DOM intake, kg/d	1.26 <sup>a</sup>	1.20 <sup>a</sup>	1.07 <sup>b</sup>	1.04 <sup>b</sup>	0.042	<0.001	1.08	1.20	0.04	0.100	
Urine, L/d	1.96 <sup>b</sup>	1.87 <sup>b</sup>	3.54 <sup>a</sup>	2.02 <sup>b</sup>	0.24	<0.001	2.09	2.60	0.27	0.24	
Urinary N, g/d	16.4 <sup>bc</sup>	12.4 <sup>c</sup>	31.7 <sup>a</sup>	17.6 <sup>b</sup>	1.40	<0.001	17.3	21.7	1.27	0.05	
Fecal N, g/d	4.84 <sup>b</sup>	4.38 <sup>b</sup>	5.96 <sup>a</sup>	4.57 <sup>b</sup>	0.18	<0.001	4.65	5.23	0.17	0.05	
Urinary N, % of N intake	47.6 <sup>b</sup>	44.3 <sup>b</sup>	61.9 <sup>a</sup>	51.2 <sup>b</sup>	2.64	<0.001	50.4	52.1	2.04	0.57	
Fecal N, % of N intake	14.0 <sup>b</sup>	15.9 <sup>a</sup>	11.8 <sup>c</sup>	13.6 <sup>b</sup>	0.44	<0.001	14.6	13.1	0.40	0.02	
N retention, g/d	13.4	11.0	13.3	11.8	1.08	0.25	10.8	14.0	1.00	0.06	
N retention, % of N intake	38.4 <sup>a</sup>	39.7 <sup>a</sup>	26.3 <sup>b</sup>	35.1 <sup>ab</sup>	2.82	0.010	35.0	34.8	2.25	0.96	
Urinary urea-N, g/d	11.4 <sup>b</sup>	8.40 <sup>b</sup>	24.2 <sup>a</sup>	12.7 <sup>b</sup>	1.55	<0.001	12.7	15.7	1.17	0.13	
Uric acid, mmol/d	0.53	0.49	0.46	0.48	0.11	0.97	0.42	0.56	0.08	0.27	
Allantoin, mmol/d	15.9	9.01	10.2	11.2	1.90	0.08	9.86	13.3	1.34	0.08	
Microbial N, g/d	14.1	8.10	9.00	9.78	1.74	0.10	8.63	11.88	1.23	0.08	
Allantoin, mmol/kg DOMI	12.6	7.50	9.76	10.8	1.84	0.29	9.16	11.1	1.30	0.29	
Microbial N, g/kg DOMI	11.2	6.74	8.58	9.40	1.69	0.33	8.00	9.96	1.20	0.26	
Hippuric acid, mmol/d	28.4 <sup>ab</sup>	11.0 <sup>b</sup>	43.5 <sup>a</sup>	14.9 <sup>b</sup>	7.45	0.01	29.6	19.3	6.78	0.32	
Creatinine, mmol/d	11.6	7.08	8.27	11.0	2.19	0.38	7.73	11.3	1.78	0.21	
Creatinine, mg/kg BW	13.3	8.26	9.87	12.6	2.51	0.43	9.11	12.9	2.03	0.24	

<sup>a-c</sup>LS means within rows with different superscripts differ significantly ( $P < 0.05$ ).

BH = whole-crop barley silage harvested at heading stage; BM = whole-crop barley silage harvested at medium milk stage; GE = grass silage harvested at flag leaf-early heading stage; GL = grass silage harvested at medium-late heading stage.

DOM = digestible OM.

libitum period in the present study, there was selection by the wethers in favor of less fibrous feed particles for all the forage diets except for the GE diet. At restricted feed intake, the opportunity for diet selection or overfeeding is diminished, decreasing the variation in retention time. This in turn affects digestibility, indicating that the *in vivo* digestibility of nutrients should best be measured at restricted feed intake (Cherney et al., 1990).

According to Van Soest (1994), fiber degradation by rumen microorganisms is enhanced when animals are fed forages with high NDF digestibility. Rumen fill is the main factor inhibiting voluntary feed intake when high-fiber diets are fed or when animals are in high energy demand (Mertens, 1994). In this regard, increased fiber particle breakage during rumination, allowing faster NDF turnover in the rumen, might benefit intake and performance. It is well known that no further particle size reduction occurs after the rumen (Poppi et al., 1981). Consequently, fecal particle size distribution reflects the size distribution of particles leaving the rumen (Ulyatt et al., 1986; Kennedy et al., 1992) and is affected by forage maturity (Jalali et al., 2012). Nørgaard and Kornfelt (2006) observed a higher proportion of small particles in the feces of cattle when early-cut grass silage with greater digestibility was fed compared with barley straw with lower fiber quality. Similarly, in a study in which dairy steers were fed whole-crop barley silage, Rustas et al. (2010) observed that mean fecal particle size increased with advanced barley maturity from heading to dough stage. Advanced forage maturity at harvest increases the resistance to particle breakdown in the rumen (De Boever et al., 1990), and less lignified forage NDF particles are degraded into smaller and thinner entities compared with more lignified forage NDF particles (Jalali et al., 2012). Similar results were obtained in the present study, where animals fed the GE or BH diets produced greater proportions of small feces particles (PS < 0.2 mm) than animals fed their more advanced maturity counterparts GL and BM, respectively. This is likely due to the more digestible NDF in early-harvested forage being more extensively digested in the rumen. Likewise, a smaller proportion of fecal PDM was found in wethers fed early-harvested grass and barley silages than their late-harvested counterparts.

Proportion of fecal PDM was strongly correlated with *in vivo* NDF digestibility across forage types in this and in our previous study (Nadeau et al., 2016b), with the proportion of fecal PDM decreasing as *in vivo* NDF digestibility increased.

Forage NDF digestibility is an important quality parameter that is highly variable among forages and has consistent effects on intake and productivity (Oba and Allen, 2005). Thus, it should be considered when formulating diets for ruminants (Mertens, 2006). On the other hand, NDF digestibility analyses are laborious, expensive, and time-consuming, so to obtain practical results faster and more cheaply the proportion of fecal PDM could be used to estimate the NDF digestibility of diets. However, more data from a variety of forages at various maturity stages, and fed to various types of ruminants at different physiological stages and intake levels, are needed to enable development of a mathematical model that is capable of predicting NDF digestibility by using the proportion of fecal PDM.

Early harvesting improved the quality of both grass and barley silages, but to different degrees. Besides greater ADF and similar NDF and ADL contents, the GE silage had a greater IVOMD and *in vivo* digestibility of OM, NDF, and ADF, resulting in a smaller proportion of fecal PDM and a greater distribution of small particles in feces compared with the BH silage. Van Amburgh et al. (2015) observed that corn silages showing identical lignin concentrations varied widely in NDF digestibility, whereas Raffrenato et al. (2017) concluded that cross-linking of phenolic compounds within cell walls has a much greater effect on NDF digestibility than the lignin content of corn and perennial grasses. Plants containing lower or similar lignin concentrations might have more ferulate cross-linkages, decreasing the digestibility, so using lignin content as the sole reference for fiber quality might be questionable from a nutritional perspective (Chabannes et al., 2001).

High-quality grasses are an important protein source for ruminants fed forage-based diets (Nadeau et al., 2016a). However, when grasses are ensiled, a large proportion of the true protein is converted to NPN, which impairs utilization of forage protein by ruminants as NPN can easily be converted to ammonia and lost as urea-N in the urine (Givens and Rulquin, 2004). In the present study, the GE silage had a greater CP concentration, and intakes of CP and RDP, estimated as the CP fractions AB<sub>1</sub>B<sub>2</sub>, were greater for animals fed the GE diet compared with the other diets. This resulted in more total N and urea-N being excreted in urine by animals fed the GE diet than when fed any of the other diets, both in gram per day and as percentage of N intake. Intake of N has been identified as the key driver of N excretion (Dijkstra et al., 2013),

mainly in urine (Dong et al., 2014). Likewise, increased N intake by rapeseed meal supplementation to the forages in the present study increased the total daily N excretion in urine and feces with a larger increase in the urine. The absence of this increase in urinary N excretion when expressed as percentage of N intake confirms that intake of N is the key driver for urinary N excretion.

Urine plays an important role in livestock waste, mostly through urea-N being rapidly hydrolyzed to ammonia upon deposition on feces, barn floor, or soil (Bernier et al., 2014; Jardstedt et al., 2017). However, apart from the high susceptibility to gaseous losses, urine can be useful in evaluating diets, as it is known that urine excretion of the purine derivative allantoin is highly related to rumen microbial protein synthesis (Moorby et al., 2006), and that hippuric acid is related to dietary concentration of degradable phenolic acids (Dijkstra et al., 2013). Jardstedt et al. (2018) found that purine derivative excretion in urine was mainly related to intake of digestible OM. Similar observations were made in the present study, where animals fed the BH diet had greater intakes of DM and digestible OM than those fed the grass silage diets (GE and GL) and tended to excrete more allantoin in the urine, which was closely related to the tendency for increased microbial N synthesis in animals fed the BH diet. The higher WSC content of the BH silage compared with the grass silages also contributed readily available fermentable substrates for microbial protein synthesis in wethers fed the BH diet. Likewise, rapeseed meal supplementation to the forages tended to increase intakes of DM and digestible OM as well as urinary excretion of allantoin and the microbial N supply. The lack of significant differences between diets and between rapeseed meal supplementations when allantoin excretion and microbial N supply were expressed per kilogram of digestible OM intake indicate similar efficiency of microbial protein synthesis per kilogram of digestible OM intake across diets and across rapeseed supplementations.

Most feed purines are broken down by rumen microbes (McAllan and Smith, 1973), and the majority of purines absorbed, digested, and excreted in the urine are from microbial rumen flora (McAllan, 1980). Hippuric acid is formed by conjugation of benzoic acid with glycine, with the main dietary precursors of benzoic acid being phenolic compounds (Martin, 1982). With advancing stage of grass maturity, rumen solubilization and degradation of plant phenolic compounds decrease as lignin concentration increases, reducing excretion

of hippuric acid in urine (Martin, 1970; Dijkstra et al., 2013). Greater hippuric acid concentration observed in the urine of wethers fed the early-maturity grass silage and to a lesser extent in wethers fed the early-maturity barley silage probably was due to the greater in vivo digestibility of DM, OM, NDF, and ADF observed in early compared with the respective late-maturity forages.

## CONCLUSIONS

In vivo digestibility of DM and nutrients in forage diets was positively affected by harvesting at early maturity stages, resulting in greater proportions of small feces particles and a lower proportion of fecal PDM compared with late-harvested material within forage type. The GE diet had greater in vitro and in vivo digestibility of OM, NDF, and ADF, resulting in a lower proportion of fecal PDM with a greater proportion of small particles in feces compared with the other diets. The proportion of fecal PDM decreased with increasing NDF digestibility in vivo and the strong relationship between them indicates the potentials for using the proportion of PDM as a cheap indirect method to predict NDF digestibility. Wethers fed the early-maturity grass silage, which had the highest CP concentration of the forages tested, showed greater intakes of CP and of the sum of the degradable CP fractions A, B<sub>1</sub>, and B<sub>2</sub>, resulting in more N excreted, mainly in the urine, than when fed any of the other silage diets. However, there was no effect of maturity on N excretion from animals fed whole-crop barley silage diets. Wethers fed the BH diet had a tendency for an improved rumen microbial protein synthesis that was most likely related to the greater WSC content of the BH silage and the greater intakes of DM and digestible OM by animals fed the BH diet than when fed the grass silage diets. Increased N intake by rapeseed meal supplementation to the forages increased urinary N excretion indicating an oversupply of RDP.

*Conflict of interest statement.* None declared.

## LITERATURE CITED

- Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V. K. Vaddella. 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. *J. Dairy Sci.* 93(4):1625–1637. doi: 10.3168/jds.2009–2579
- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2

- Andueza, D., F. Picard, M. Jestin, and J. Aufrere. 2013. The effect of feeding animals ad libitum vs. at maintenance level on the in vivo digestibility of mown herbage from two permanent grasslands of different botanical composition. *Grass Forage Sci.* 68(3):418–426. doi: 10.1111/gfs.12004
- AOAC. 2012. Official methods of analysis. 19th ed. Gaithersburg, MD: Association of Official Analytical Chemists.
- Bernier, J. N., M. Undi, K. H. Ominski, G. Donohoe, M. Tenuta, D. Platen, J. C. Plaizier, and K. M. Wittenberg. 2014. Nitrogen and phosphorus utilization and excretion by beef cows fed a low quality forage diet supplemented with dried distillers grains with solubles under thermal neutral and prolonged cold conditions. *Anim. Feed Sci. Technol.* 193:9–20. doi: 10.1016/j.anifeedsci.2014.03.010
- Bourquin, L. D., K. A. Garleb, N. R. Merchen, and G. C. Fahey, Jr. 1990. Effects of intake and forage level on site and extent of digestion of plant cell wall monomeric components by sheep. *J. Anim. Sci.* 68:2479–2495. doi:10.2527/1990.6882479x
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381. doi:10.3168/jds.S0022-0302(03)73721-7
- Chabannes, M., A. Barakate, C. Lapiere, J. M. Marita, J. Ralph, M. Pean, S. Danoun, C. Halpin, J. Grima-Pettenati, and A. M. Boudet. 2001. Strong decrease in lignin content without significant alteration of plant development is induced by simultaneous down-regulation of cinnamoyl CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD) in tobacco plants. *Plant J.* 28:257–270. doi:10.1046/j.1365-313X.2001.01140.x
- Chen, X. B., and Gomes, M. J. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of the technical details. Aberdeen, UK: Rowett Research Institute; p. 19.
- Cherney, D. J., D. R. Mertens, and J. E. Moore. 1990. Intake and digestibility by wethers as influenced by forage morphology at three levels of forage offering. *J. Anim. Sci.* 68:4387–4399. doi:10.2527/1990.68124387x
- De Boever, J. L., J. I. Andries, D. L. Debrabander, B. G. Cottyn, and F. X. Buysse. 1990. Chewing activity of ruminants as a measure of physical structure - a review of factors affecting it. *Anim. Feed Sci. Technol.* 27(4):281–291. doi: 10.1016/0377-8401(90)90143-v
- Dijkstra, J., O. Oenema, J. W. van Groenigen, J. W. Spek, A. M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N<sub>2</sub>O emissions. *Animal.* 7(Suppl. 2):292–302. doi:10.1017/S1751731113000578
- Dong, R. L., G. Y. Zhao, L. L. Chai, and K. A. Beauchemin. 2014. Prediction of urinary and fecal nitrogen excretion by beef cattle. *J. Anim. Sci.* 92:4669–4681. doi:10.2527/jas.2014-8000
- Ericson, B., and J. André. 2010. HPLC – applications for agricultural and animal science. In: Proceedings of 1st Nordic Feed Science Conference Uppsala, Sweden. Report 274. Swedish University of Agricultural Sciences, Department of Animal Nutrition Management; p. 23–26.
- Givens, D. I., and H. Rulquin. 2004. Utilisation by ruminants of nitrogen compounds in silage-based diets. *Anim. Feed Sci. Technol.* 114(1–4):1–18. doi: 10.1016/j.anifeedsci.2003.09.005
- Huhtanen, P., J. Nousiainen, and M. Rinne. 2006. Recent developments in forage evaluation with special reference to practical applications. *Agric. Food Sci.* 15(3):293–323. doi: 10.2137/145960606779216317
- Jalali, A. R., P. Nørgaard, M. R. Weisbjerg, and E. Nadeau. 2012. Effect of stage of maturity of grass at harvest on intake, chewing activity and distribution of particle size in faeces from pregnant ewes. *Animal.* 6(11):1774–1783. doi: 10.1017/s1751731112000493
- Jalali, A. R., M. R. Weisbjerg, E. Nadeau, A. T. Randby, B. O. Rustas, M. Eknæs, and P. Nørgaard. 2015. Effects of forage type, animal characteristics and feed intake on faecal particle size in goat, sheep, llama and cattle. *Anim. Feed Sci. Technol.* 208:53–65. doi: 10.1016/j.anifeedsci.2015.07.003
- Jardstedt, M., A. Hessle, P. Nørgaard, L. Frendberg, and E. Nadeau. 2018. Intake and feed utilization in two breeds of pregnant beef cows fed forages with high-fiber concentrations. *J. Anim. Sci.* 96:3398–3411. doi:10.1093/jas/sky199
- Jardstedt, M., A. Hessle, P. Nørgaard, W. Richardt, and E. Nadeau. 2017. Feed intake and urinary excretion of nitrogen and purine derivatives in pregnant suckler cows fed alternative roughage-based diets. *Livest. Sci.* 202:82–88. doi: 10.1016/j.livsci.2017.05.026
- Jefferies, B. C. 1961. Body condition scoring and its use in management. *Tasm. J. Agric.* 32:19–21.
- Kennedy, P. M., C. S. McSweeney, and J. G. Welch. 1992. Influence of dietary particle-size on intake, digestion, and passage rate of digesta in goats and sheep fed wheat (Triticum-Aestivum) hay. *Small Rumin. Res.* 9(2):125–138. doi: 10.1016/0921-4488(92)90191-6
- Larsson, K., and S. Bengtsson. 1983. Bestämning av lat-tillgangliga kolhydrater i vaxtmateriel. Method no 22. Metodbeskrivning – Statens Lantbrukskemiska Laboratorium, Uppsala, Sweden.
- Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57(4):347–358. doi: 10.1016/0377-8401(95)00837-3
- Lindgren, E. 1979. Vallfodrets näringsvärde bestämt in vivo och med olika laboriemetoder. Report 45. Department of animal nutrition and management. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- Lindgren, E. 1983. Nykalibrering av VOS metoden för bestämning av energivärde hos vallfoder. Working paper. Department of animal nutrition and management. Uppsala, Sweden: Swedish University of Agricultural Sciences.
- LKS – Landwirtschaftliche Kommunikation Sund Servicegesellschaft mbH. 2006. Bestimmung des Harnstoff Gehaltes. Qualitätsmanagementhandbuch, Arbeitsanweisung no. 123; p. 5.
- Martin, A. K. 1970. The urinary aromatic acids excreted by sheep given S24 perennial ryegrass cut at six stages of maturity. *Br. J. Nutr.* 24:943–959. doi: 10.1079/bjn19700098
- Martin, A. K. 1982. The origin of urinary aromatic compounds excreted by ruminants. 2. The metabolism of phenolic cinnamic acids to benzoic acid. *Br. J. Nutr.* 47:155–164. doi: 10.1079/bjn19820020
- McAllan, A. B. 1980. The degradation of nucleic acids in, and the removal of breakdown products from the small intestines of steers. *Br. J. Nutr.* 44:99–112. doi: 10.1079/bjn19800014

- McAllan, A. B., and R. H. Smith. 1973. Degradation of nucleic acid derivatives by rumen bacteria in vitro. *Br. J. Nutr.* 29:467–474. doi: 10.1079/bjn19730122
- Merchen, N. R., L. D. Bourquin. 1994. Processes of digestion and factors influencing digestion of forage-based diets by ruminants. In: *Forage, quality, evaluation, and utilization*. Lincoln, USA; p. 564–612.
- Mero, R. N., and P. Udén. 1998. Promising tropical grasses and legumes as feed resources in Central Tanzania IV. Effect of feeding level on digestibility and voluntary intake of four herbaceous legumes by sheep. *Anim. Feed Sci. Technol.* 70(1–2):97–110. doi: 10.1016/s0377-8401(97)00074-6
- Mertens, D. R. 1994. Regulation of forage intake. In: G. C. Fahey Jr., editor, *Forage Quality, Evaluation and Utilization*. p. 450–493. Madison, WI: Soil Science Society of America Inc., American Society of Agronomy Inc., Crop Science Society of America Inc.
- Mertens, D. R. 2006. Do we need to consider NDF digestibility in the formulation of ruminant diets?. In *27th Western Nutrition Conference*. Winnipeg, Canada; p. 19–20.
- Moorby, J. M., R. J. Dewhurst, R. T. Evans, and J. L. Danelón. 2006. Effects of dairy cow diet forage proportion on duodenal nutrient supply and urinary purine derivative excretion. *J. Dairy Sci.* 89:3552–3562. doi:10.3168/jds.S0022-0302(06)72395-5
- Muck, R. E., and R. W. Hintz. 2003. Effects of breeding for quality on alfalfa ensilability. *Trans. ASAE.* 46(5):1305–1309.
- Nadeau, E., A. Arnesson, and C. Helander. 2016a. Effects of grass silage feed value on feed intake and performance of pregnant and lactating ewes and their lambs. *Grass Forage Sci.* 71(3):448–457. doi: 10.1111/gfs.12197
- Nadeau, E., W. Richardt, and P. Nørgaard. 2016b. Fibre and protein quality of silages and their effects on ruminant performance. In: *Proceedings of 17th International Conference For. Cons. Horný Smokovec, Slovak Republic.* 27–29 September 2016; p. 39–53.
- Nelson, C. J., and L. E. Moser. 1994. Plant factors affecting forage quality. In: *Forage quality, evaluation, and utilization*. Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc.; p. 115–154.
- Nørgaard, P., S. Husted, and H. Ranvig. 2004. Effect of supplementation with whole wheat or whole oat grains on the dimensions of faeces particles from lambs. *J. Anim. Feed Sci.* 13:175–178. doi: 10.22358/jafs/73770/2004
- Nørgaard, P., and L. F. Kornfelt. 2006. Particle size distribution in rumen contents and faeces from cows fed grass silages in different physical form or barley straw supplemented with grass pellets. *J. Anim. Sci.* 84:262–262 (Abstract).
- Oba, M., and M. Allen. 2005. In vitro digestibility of forages. In: *Proceedings of Tri-State Dairy Nutrition Conference Fort Wayne, Indiana, USA.*
- Poppi, D. P., D. J. Minson, and J. H. Ternouth. 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses .1. The voluntary intake, digestibility and retention time in the reticulo-rumen. *Aust. J. Agric. Res.* 32(1):99–108. doi: 10.1071/ar9810099
- Raffrenato, E., R. Fievisohn, K. W. Cotanch, R. J. Grant, L. E. Chase, and M. E. Van Amburgh. 2017. Effect of lignin linkages with other plant cell wall components on in vitro and in vivo neutral detergent fiber digestibility and rate of digestion of grass forages. *J. Dairy Sci.* 100:8119–8131. doi:10.3168/jds.2016-12364
- Russell, A. J. F., J. M. Doney, and R. J. Gunn. 1969. Subjective assessment of body fat in live sheep. *J. Agric. Sci.* 72:451–454. doi:10.1017/S0021859600024874
- Rustas, B. O., J. Bertilsson, K. Martinsson, T. Elverstedt, and E. Nadeau. 2011. Intake and digestion of whole-crop barley and wheat silages by dairy heifers. *J. Anim. Sci.* 89:4134–4141. doi:10.2527/jas.2010-3585
- Rustas, B. O., P. Nørgaard, A. R. Jalali, and E. Nadeau. 2010. Effects of physical form and stage of maturity at harvest of whole-crop barley silage on intake, chewing activity, diet selection and faecal particle size of dairy steers. *Animal.* 4:67–75. doi:10.1017/S1751731109990887
- Shingfield, K. J., and N. W. Offer. 1999. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed-phase high-performance liquid chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 723(1–2):81–94. doi: 10.1016/s0378-4347(98)00549-0
- Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562–3577. doi:10.2527/1992.70113562x
- Stefanska, J., M. Rinne, T. Heikkilä, and S. Ahvenjärvi. 2008. In vivo digestibility of different types of forages using sheep as a model of ruminants. *Maataloustieteen Päivät [www.smts.fi](http://www.smts.fi)*. – [accessed 28 June 2018].
- Ulyatt, M. J., D. W. Dellow, A. John, C. S. W. Reid, and G. C. Waghorn. 1986. Contribution of chewing during eating and rumination to the clearance of digesta from the ruminoreticulum. In: L. P. Milligan, W. L. Grovum, and A. Dobson, editors, *Ruminant physiology* pp. 498–515. Reston, VA: Reston Publishers.
- Van Amburgh, M. E., R. Grant, K. Cotanch, A. Zontini, D. Ross, and A. Foskolos. 2015. NDF–Making something old, new again. *Herd Health and Nutrition Conference*, Syracuse, NY. Ithaca, NY: Cornell University Press. <http://hdl.handle.net/1813/39211>.
- Van Soest, P. J. 1994. *Nutritional ecology of the ruminant*, 2nd ed. Ithaca, NY: Cornell University Press.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2
- Waldo, D. R., L. W. Smith, E. L. Cox, B. T. Weinland, and H. L. Lucas. 1971. Logarithmic normal distribution for description of sieved forage materials. *J. Dairy Sci.* 54(10):1465. doi: 10.3168/jds.S0022-0302(71)86048-4
- Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Research.* 14:415–421. doi:10.1111/j.1365-3180.1974.tb01084.x