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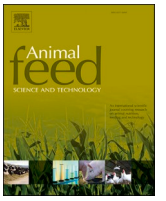

Intake, in vivo digestibility and protein utilization of wethers fed timothy or tall fescue when harvested at different dates in the first regrowth cycle

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ABSTRACT

The objective of this study was to evaluate the effects of harvest date in the first regrowth cycle and grass species on intake, in vivo digestibility and its relation to protein utilization in wethers fed timothy or tall fescue silages. Timothy and tall fescue were harvested at regular (RTI and RTF, respectively) or late date (LTI and LTF, respectively) in the first regrowth, creating four experimental silages. Eight wethers were used in a duplicated 4 × 4 Latin square. 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. Wethers receiving RTI showed the greatest DM intake when expressed as kg/d or as percentage of body weight (BW) ($P \leq 0.05$). The intake of neutral detergent fiber (aNDFom) was affected by forage species only, where animals fed timothy silages had greater aNDFom intake than animals fed tall fescue silages ($P<0.001$). Intakes of CP and sum of the protein fractions A, B_1 and B_2 (AB_1B_2) were affected by the interaction between harvest date and forage species, where wethers fed RTI showed the greatest intakes of CP ($P=0.001$) and AB_1B_2 ($P=0.02$). Harvesting the forages at late date decreased the in vivo digestibility in wethers but only for timothy, where animals fed LTI silage showed the lowest DM ($P<0.001$), organic matter (OM) ($P<0.001$), aNDFom ($P=0.02$) and acid detergent fiber ($P=0.004$) digestibility, and a tendency for lower CP digestibility ($P=0.07$) compared with the other silages. Wethers fed RTI silage showed greater intake of nitrogen (N) ($P=0.001$) and digestible OM ($P=0.003$), greater allantoin ($P=0.03$) and hippuric acid ($P=0.05$) excretions, greater microbial N flow ($P=0.03$), and a tendency for greater excretion of fecal N ($P=0.09$) compared with the other silage-fed animals. In conclusion, delayed harvest decreased in vivo digestibility only in timothy, but even with lower in vivo digestibility wethers fed timothy silages showed a greater intake than wethers fed tall

Abbreviations: DM, dry matter; DMI, DM intake; OM, organic matter; OMI, OM intake; DOM, digestible OM; ANDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; ADL, acid detergent lignin; CW, cell wall; FA, ferulic acid; *p*CA, *p*-coumaric acid; CP, crude protein; N, nitrogen; NPN, non-protein nitrogen; A, NPN; B_1, buffer-soluble protein; B_2, neutral detergent-soluble protein; AB_1B_2, sum of fractions A, B_1 and B_2; B_3, acid detergent-soluble protein; C, indigestible true protein; RUP_5, rumen undegradable protein at a passage rate of 0.05 h^{-1}; BW, body weight; RTF, tall fescue harvested at regular date; RTI, timothy harvested at regular date; LTF, tall fescue harvested at late date; LTI, timothy harvested at late date.

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fescue silages, likely due to lower concentration of hydroxycinnamic acids observed in timothy compared with tall fescue as published previously.

1. Introduction

Timothy (*Phleum pratense* L.) is a perennial winter-hardy cool-season grass that has reliable productivity under moist conditions and has a relatively slow regrowth rate. Due to its shallow root system and that new tillers have to develop their own root system, timothy is sensitive to droughty conditions (McElroy and Kunelius, 1995). Consequently, the climate change with frequent warmer and drier summers the regrowth of timothy can be compromised, reducing its productivity and nutritive value (Bertrand et al., 2008; Qian et al., 2013). Contradictory, tall fescue (*Festuca arundinacea* Schreb.) is a perennial bunchgrass that spreads primarily through vertical tillers and its root system develops much deeper than other cool-season grasses contributing to a superior adaptability to a wide range of climates and its tolerance for cold, heat and drought (Fisher et al., 1993). Thus, tall fescue appears to be a possible alternative to timothy for feeding ruminants (Richard et al., 2020).

Our previous study (Sousa et al., 2021) compared the effects of timothy and tall fescue harvested for silage at regular or late date in the first regrowth on cell wall (CW) composition and their relationship to dairy cow performance; and even with increased concentration of fiber components observed in timothy harvested at late date, the concentration of most of the hydroxycinnamic acids in the CW were similar within grass species but greater in tall fescue silages than in timothy silages, regardless of the harvest date. Similarly, milk production was affected only by forage species, where cows receiving timothy silages showed a greater energy corrected milk production than cows receiving tall fescue silages (36.2 vs. 33.8 kg/d, respectively), suggesting that the concentration of hydroxycinnamic acids was the main factor regulating the milk production of cows and lower hydroxycinnamic acid concentrations in timothy silage were responsible for greater milk yield.

Hydroxycinnamic acids, such as ferulic acid (FA) and *p*-coumaric acid (pCA) appear to prevent potentially digestible cell wall polysaccharides from being extensively digested in the rumen (Novo-Uzal et al., 2011). Ferulic acid cross-links lignin to hemicellulosic carbohydrates through covalent ester and ether bonds and pCA acts as terminal pendant by attaching to syringyl lignin through covalent ester linkage, contributing to the reduction of CW degradability (Jung et al., 2012; Hatfield et al., 2017). Additionally, these cross-linkages between CW carbohydrates and lignin and the direct linkage to lignin through hydroxycinnamic acids can reduce fiber digestibility by limiting rumen bacteria growth (Akin et al., 1993) and reducing the attachment of these bacteria to the cell wall (Iiyama and Lam, 2001). The various linkages of cell wall components is an important mechanism of plants that increases firmness of cell walls and resistance against pathogens (Tan et al., 1992; Ikegawa et al., 1996; Waldron et al., 1997). However, these characteristics that are favorable to the plant result in significant deleterious effects on fiber digestibility (Novo-Uzal et al., 2011).

To our knowledge, Sousa et al. (2021) is the only study evaluating the effects of forage species and harvest date of tall fescue and timothy on concentrations of hydroxycinnamic acids and ruminant performance, but in vivo digestibility of grasses could not be evaluated in the dairy cows. Thus, in order to further evaluate the comparison between timothy and tall fescue harvested at regular or late date in the first regrowth, the present study was conducted using wethers, where in vivo digestibility was precisely assessed. We hypothesized that the delay in harvesting timothy and tall fescue for silage would increase the concentration of fiber components, reducing intake. However, in vivo digestibility would not be affected due to the similar concentration of most of the hydroxycinnamic acids between regular and late harvest date within grass species (Sousa et al., 2021). The aim of this study was to evaluate the effects of harvest date and grass species on intake, in vivo digestibility, and its relation to protein utilization in wethers fed timothy or tall fescue silages.

2. Materials and methods

The experiment was conducted at the SLU Götala Beef and Lamb Research Center, Skara, southwest Sweden (58°23′N, 13°29′E). Experimental procedures were approved by Gothenburg Research Animal Ethics Committee (case number 63–2012, 90–2015).

2.1. Forage harvest and ensiling

Two studies using the same experimental silages were conducted to evaluate the effects of harvest date and forage species on ruminant performance, digestibility, and protein utilization. The first study was performed with dairy cows and detailed information regarding the cultivation, harvest, and ensiling, can be accessed in our previous publication (Sousa et al., 2021).

Tall fescue cv. Swaj (SW Lantmännen, Malmö, Sweden) and timothy cv. Switch (SW Lantmännen, Malmö, Sweden) were harvested at two different harvest dates (regular and late) during the first regrowth cycle of 2016. Tall fescue seeds contained no endophytes, when being analysed. As the grass species differ in regrowth rates, tall fescue and timothy were harvested at different dates to aim at similar contents of neutral detergent fiber (aNDFom) and crude protein (CP) between the species, which was determined by near infrared spectroscopy scanning of dried and ground samples of the grasses before harvest. Half of the tall fescue sward was harvested at regular harvest time (RTF) on June 27, which occurred 32 d after the first cut, and the second half was harvested late (LTF) on July 8, which occurred 43 d after the first cut. Similarly, half of the timothy sward was harvested on July 8 (regular harvest, RTI) and the second half was harvested 60 d after the first cut on July 25 (late harvest, LTI).

As tall fescue is a nonjointing grass whereas timothy is a jointing grass, the developmental stages differed between the grass species

within regular and late harvest times. At the regular harvest time, tall fescue was at the leaf (94 %)-to-stem elongation-to-heading (6 %) stage and timothy was at the leaf (30 %)-to-stem elongation (33 %)-to flag leaf (23 %)-to-heading (14 %) stage. At the late harvest time, tall fescue was at the leaf (94 %)-to-stem elongation-to-heading (6 %) stage and timothy was at the stem elongation (67 %)-to-flag leaf (17 %)-to-heading (16 %) stage. The developmental stages of the grasses were evaluated according to (Gustavsson, 2011).

All forages were mowed on the day before chopping and wilted to similar dry-matter (DM) concentrations at harvest. Wilted forages were chopped to 20 mm theoretical length of cut and ensiled in hard-pressed round bales wrapped with 8 layers of 0.025-mm plastic film and stored for 16 months before opening of the bales. Forages were treated with the chemical additive Xtrasil Lp (sodium nitrite, hexamethylene tetramine, sodium benzoate; Konsil Scandinavia, Tvåker, Sweden) at 2.0 L/t of forage at the time of chopping. Silages were well preserved and showed similar fermentation characteristics among treatments (Sousa et al., 2021). The silage bales used in the present study were opened and immediately stored in small packages in a freezer at −20 °C to ensure high quality of the silage at feeding. Silages were thawed thoroughly before being fed to the wethers. The Chemical composition of the experimental silages is presented in Table 1.

2.2. Experimental design

The four experimental silages (RTF, RTI, LTF and LTI) were fed to wethers in a duplicated 4 × 4 Latin square. 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, ad libitum feed intake was recorded and 15% orts were allowed. During the 4th week, the wethers were fed 80% of ad libitum intake 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

2.3. Animals and housing

Eight 33-mo old cross-bred Texel × Swedish Finewool wethers were used in the study. The wethers were divided according to their body weights into two groups, with four wethers per group. Initial body weight (BW); mean ± SD was 84.4 ± 2.43 kg for the first group and 90.0 ± 2.16 kg for the second group.

During the first 3 wk (days 1–21) of each period, the wethers were housed in individual 6 m^2 pens with deep straw bedding. During the 4th week (days 22–29), the wethers were kept in individual metabolic cages measuring 1.5 m × 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 silages 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 whole trial. No concentrate was fed to the wethers throughout the study.

2.4. 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. 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 all the feces from each wether for each of the 4 d 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

Table 1
Chemical composition of the experimental silages.

	Silage[a]				SEM
Item	RTF	RTI	LTF	LTI	
DM, g/kg	309	287	256	261	6.4
Ash, g/kg DM	81.2	74.7	84.5	69.0	0.92
aNDFom, g/kg DM	532	486	538	572	5.4
ADFom, g/kg DM	308	284	320	341	3.3
ADL, g/kg DM	26.1	25.5	24.5	41.9	4.66
CP, g/kg DM	234	209	203	149	2.7
Protein fractions, % of CP[b]					
A	69.9	55.2	68.3	50.0	0.84
B_1	1.67	1.69	1.70	2.18	0.630
B_2	23.0	29.0	23.7	28.7	0.70
B_3	3.42	11.2	3.85	13.7	0.604
C	2.05	2.91	2.45	5.44	0.174
RUP_5	1.67	12.8	7.64	25.4	0.619

[a] RTF = tall fescue harvested at regular date on June 27; RTI = timothy harvested at regular date on July 8; LTF = tall fescue harvested at late date on July 8; LTI = timothy harvested at late date on July 25.

[b] A = NPN; B_1 = buffer-soluble protein; B_2 = neutral detergent-soluble protein; B_3 = acid detergent-soluble protein; C = ADIN; RUP_5 = RUP at a passage rate of 0.05 h^{-1} (Kirchhof et al., 2010).

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 daily for the last 4 days of each period.

The DM concentration of silage and orts during collection week was determined by drying daily 150-g subsample 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 collection week, while samples of orts, and feces were pooled per wether and collection week and mixed thoroughly, and then subsamples were taken for chemical analysis.

A fresh-frozen subsample of 200 g of feed, orts, and feces was sent to LKS mbH, Lichtenwalde, Germany for analysis of CP, CP fractions, aNDFom, acid detergent fiber (ADFom), acid detergent lignin (ADL), and ash. Concentrations of nitrogen (N) were determined with the Kjeldahl method on fresh, pooled samples of feed and orts, and on freeze-dried samples of feces (AOAC, 2012). Frozen daily urine samples were sent to LKS mbH laboratory, where the samples were thawn, swirled and composited per wether and collection week in relation to the daily urine volume excreted by each wether. The composited Urine samples were analyzed for total nitrogen, urea, allantoin, uric acid, and creatinine.

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 aNDFom, ADFom, and ADL concentrations in dried, milled samples. The aNDFom analysis was modified by adding heat-stable α-amylase (Novozymes, Bagsvaerd, and Denmark) and omitting sodium sulfite. Reported concentrations of aNDFom, ADFom, and ADL were corrected for residual ash.

The CP concentration was calculated as total N × 6.25. Crude protein fractions (A, B_1, B_2, B_3, 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 non-protein nitrogen (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_1, which is soluble in borate-phosphate buffer at rumen pH and rapidly degraded in the rumen; fraction B_2, which is insoluble in borate-phosphate buffer, but soluble in neutral detergent solution and has variable degradation; and fraction B_3, which is insoluble in neutral detergent solution, but soluble in acid detergent solution. Fraction B_3 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. Analysis methods and results of fermentation characteristics and cell wall content of acetyl bromide lignin and hydroxycinnamic acids of the experimental silages can be found in our previous publication (Sousa et al., 2021).

The N concentration of urine was analyzed with a Kjeldahl procedure. Urine concentrations of creatinine, allantoin, uric acid and hippuric 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 × 4.6 mm, 5 μm). Analysis of urea concentration (samples diluted 50-fold) was performed by spectrophotometry according to LKS (2006).

2.5. Feed intake, body weight, sorting, and in vivo digestibility

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. Intake of each nutrient was adjusted based on the chemical composition of its respective orts. Digestible organic matter (OM) intake was obtained by multiplying OM intake by in vivo OM digestibility. Wethers were weighed 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.

Sorting was calculated as the difference between aNDFom concentration of the feed and aNDFom concentration of the orts during ad libitum feeding of the wethers in the third week. The apparent in vivo digestibility of DM, OM and CP, and the true in vivo digestibility of aNDFom, and ADFom were 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 of in vivo digestibility of DM, OM and CP, the value obtained was apparent in vivo digestibility.

2.6. 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 retention 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: $PD_{Ex} = 0.84PD_{Abs} + (0.150\ W^{0.75}\ e^{-0.25PDAbs})$. 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:

$$PD_{Abs(n+1)} = PD_{Abs} - f\,(PDA_{bs\ n})\ /f'\ (PD_{Abs\ n})$$

where $f\,(PD_{Abs}) = 0.84PD_{Abs} + 0.150W^{0.75}e^{-0.25PDAbs} - PD_{Ex}$

and $f'(PD_{Abs}) = 0.84 - 0.0380W^{0.75}e^{-0.25PDAbs}$

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) = $(PD_{Abs} \times 70)/(0.116 \times 0.83 \times 1000)$, 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).

2.7. Statistical analysis

Data on feed intake, in vivo digestibility, and protein utilization were analyzed for a duplicated 4 × 4 Latin square using the MIXED procedure of SAS. The statistical model was:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + p_k + s_l + w_{m(l)} + e_{ijklm}$$

where Y_{ijklm} = observed response, μ = overall mean, α_i = fixed effect of forage species (i = 1–2), β_j = fixed effect of harvest date (j = 1–2), $(\alpha\beta)_{ij}$ = fixed effect of interaction between forage species and harvest date, p_k = fixed effect of period (k = 1–4), s_l = random effect of square (l = 1–2), $w_{m(l)}$ = random effect of wether nested within square (m = 1–8), and e_{ijklm} = residual error. Carry-over effects were evaluated initially but were removed from the model because of non-significance (P>0.10). To determine the denominator degrees of freedom in the test, Satterthwaite's method was used.

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 $0.05 \leq P \leq 0.10$.

3. Results

3.1. Feed intake, body weight, sorting, and in vivo digestibility

Wethers receiving RTI showed the greatest dry matter intake (DMI) when expressed as kg/d (P=0.05) or as percentage of BW (P=0.05), and a tendency for greater OM intake (P=0.06) compared to the other silages (Table 2). Intakes of DM (P=0.03 as kg/d and P=0.02 as percentage of BW) and OM (P=0.03) decreased with delayed harvest date for timothy but not for tall fescue (P>0.99). The intake of aNDFom was affected by forage species only, where animals fed timothy silages had greater aNDFom intake than animals fed tall fescue silages (1.34 vs. 1.07 kg/d or % of BW; P<0.001). Intakes of CP and AB_1B_2 were affected by the interaction between harvest date and forage species, where wethers fed RTI showed the greatest intakes of CP (P=0.001) and AB_1B_2 (P=0.02), which decreased with delayed harvest of timothy (P<0.001 and P=0.006, respectively) but not with delayed harvest of tall fescue (P>0.1).

Feed sorting was affected by grass species (P<0.001), where wethers fed timothy silages sorted the diets in favor of less fibrous portions compared with wethers fed tall fescue silages that sorted for fibrous particles of the diets. Body weight was not affected by the experimental silages (P>0.1). Harvesting the forages at late date decreased the in vivo digestibility in wethers but only for timothy

Table 2
Effects of the experimental silages on intake and BW of wethers fed ad libitum.

	Silage[d]					P-value[e]		
Item	RTF	RTI	LTF	LTI	SEM	H	S	H x S
Intake								
DM, kg/d	2.00[c]	2.74[a]	1.99[c]	2.34[b]	0.143	0.03	<0.001	0.05
OM, kg/d	1.84[(c)]	2.53[(a)]	1.82[(c)]	2.18[(b)]	0.132	0.03	<0.001	0.06
aNDFom, kg/d	1.07	1.33	1.07	1.34	0.081	0.92	<0.001	0.92
DM, % of BW	2.02[c]	2.73[a]	1.99[c]	2.36[b]	0.122	0.03	<0.001	0.05
aNDFom, % of BW	1.08	1.33	1.07	1.35	0.071	0.82	<0.001	0.76
CP, g/d	477[b]	574[a]	408[bc]	346[c]	30.1	<0.001	0.42	0.001
AB_1B_2, g/d[f]	190[b]	235[a]	186[b]	189[b]	13.2	0.009	0.02	0.02
Feed sorting, g/kg[g]	14.1	–8.6	10.2	–19.6	5.92	0.23	<0.001	0.56
BW, kg	98.9	100	99.5	98.6	1.91	0.60	0.82	0.17

[a] Least squares means within rows with different superscripts differ significantly ($P \leq 0.05$) or trend toward significance if in parentheses ($0.05 < P \leq 0.10$).

[b] Least squares means within rows with different superscripts differ significantly ($P \leq 0.05$) or trend toward significance if in parentheses ($0.05 < P \leq 0.10$).

[c] Least squares means within rows with different superscripts differ significantly ($P \leq 0.05$) or trend toward significance if in parentheses ($0.05 < P \leq 0.10$).

[d] RTF = tall fescue harvested at regular date; RTI = timothy harvested at regular date; LTF = tall fescue harvested at late date; LTI = timothy harvested at late date.

[e] H = main effect of harvest date; S = main effect of forage species; H × S = H × S interaction.

[f] AB_1B_2 = sum of CP fractions A, B1, and B2, which are considered rumen degradable.

[g] Difference between aNDFom concentration of the diet and aNDFom concentration of the orts. Negative value shows sorting against aNDFom.

(Table 3), where animals fed LTI silage showed the lowest DM ($P<0.001$), OM ($P<0.001$), aNDFom ($P=0.02$) and ADFom ($P=0.004$) digestibility, and a tendency for lower CP digestibility ($P=0.07$) compared with the other silages.

3.2. Protein utilization and excretion

Wethers fed RTI silage showed greater intake of N ($P=0.001$; Table 4) and digestible OM (DOM; $P=0.003$), greater allantoin ($P=0.03$) and hippuric acid ($P=0.05$) excretions, greater microbial N flow ($P=0.03$), and a tendency for greater excretion of fecal N ($P=0.09$) compared with the other silage-fed animals. Furthermore, the above-mentioned parameters decreased with advanced harvest date of timothy but not of tall fescue. The excretion of urinary N was lower for animals fed forages harvested at late date, where LTF and LTI showed lower excretion compared with RTF ($P=0.01$) and RTI ($P<0.001$), respectively. There was a tendency for greater excretion of N in the feces expressed as percentage of N intake for wethers fed LTI compared with wethers fed RTF and LTF ($P=0.06$). The excretion of urinary N expressed as percentage of N intake decreased with advanced harvest date ($P=0.02$) and was greater for animals receiving tall fescue silages ($P=0.007$). Wethers fed silages harvested at regular date showed greater excretion of urinary urea-N (28.0 vs. 17.5 g/d; $P<0.001$) and uric acid (5.98 vs. 4.79 mmol/d; $P=0.004$) than wethers receiving late harvested silages. Wethers fed silages harvested at regular date showed greater excretion of creatinine expressed as mmol/d (34.8 vs. 31.6 mmol/d; $P=0.02$) and as mg/kg of BW (39.2 vs. 35.8 mg/kg of BW; $P=0.02$) compared with wethers fed late harvested silages. Furthermore, there was a tendency for greater creatinine excretion for wethers receiving timothy silages when expressed as mmol/d ($P=0.1$) and as mg/kg of BW ($P=0.05$) compared with wethers fed tall fescue silages.

4. Discussion

The greatest DMI and organic matter intake (OMI) observed in wethers fed RTI can be related to the lowest concentrations of aNDFom and ADFom compared to the other silages, where lower dietary fiber concentration likely led to a faster ruminal digesta clearance affecting intake positively (Allen, 1996). However, LTI showed greater DMI compared to both tall fescue silages but the concentrations of aNDFom and ADFom in LTI were greater than observed in RTF and LTF, suggesting that the concentration of fiber components per se does not completely explain the intake regulation in wethers fed different grass species.

As stated in our previous study (Sousa et al., 2021), the concentration of fiber components is not the only factor controlling intake when comparing different grass species but also the CW composition. Cell wall composition of the experimental silages was evaluated, and tall fescue silages showed greater concentrations of hydroxycinnamic acids than timothy silages, resulting in less digestible CW per unit of lignin, which was related to decreased rate of CW degradation of the tall fescue silages compared with timothy silages (5.2 vs. 6.1 %/h, respectively; Sousa et al., 2021).

Hydroxycinnamic acids, such as ferulic acid, *p*-coumaric acid and their respective cis isomerizations, are elements that can reduce the digestion rate of potentially digestible fiber. In the CW, ferulic acid cross-links soluble carbohydrates to lignin (Hatfield et al., 2017), while *p*-coumaric acid attaches to syringyl lignin units that modifies the CW structure (Jung et al., 2012), reducing the degradability of potentially digestible fiber. More specifically, hydroxycinnamic acids are the main factors limiting fiber digestibility in the rumen (Adesogan et al., 2019).

The effect of CW composition was clear when aNDFom intake was evaluated as kg/d and as % of BW in the present study, where even with greater feed sorting in favor of less fibrous portions of the diet, wethers receiving timothy silages showed greater aNDFom intakes compared with wethers fed tall fescue silages, regardless of harvest date, despite the lower in vivo aNDFom digestibility of LTI compared to the other silages. However, even with similar CW composition of the hydroxycinnamic acids (Sousa et al., 2021), LTI showed generally lower in vivo digestibility than RTI in the present study. Despite the fact that in vivo digestibility was not evaluated in the dairy cow study, there was a similar milk production for the cows fed RTI and LTI, suggesting that the lower in vivo digestibility

Table 3
Effects of the experimental silages on in vivo digestibility in wethers fed at 80 % of ad libitum DMI.

	Silage[d]					*P*-value[e]		
Item	RTF	RTI	LTF	LTI	SEM	H	S	H x S
DM, g/kg	689[a]	695[a]	680[a]	634[b]	9.5	<0.001	0.003	<0.001
OM, g/kg	702[a]	703[a]	693[a]	642[b]	8.6	<0.001	<0.001	<0.001
CP, g/kg	772[(a)]	735[(b)]	749[(ab)]	684[c]	7.6	<0.001	<0.001	0.07
aNDFom, g/kg	685[a]	674[a]	668[a]	611[b]	9.2	<0.001	<0.001	0.020
ADFom, g/kg	691[a]	669[a]	689[a]	605[b]	14.3	0.003	<0.001	0.004

[a] Least squares means within rows with different superscripts differ significantly ($P\leq0.05$) or trend toward significance if in parentheses ($0.05<P\leq0.10$).

[b] Least squares means within rows with different superscripts differ significantly ($P\leq0.05$) or trend toward significance if in parentheses ($0.05<P\leq0.10$).

[c] Least squares means within rows with different superscripts differ significantly ($P\leq0.05$) or trend toward significance if in parentheses ($0.05<P\leq0.10$).

[d] RTF = tall fescue harvested at regular date; RTI = timothy harvested at regular date; LTF = tall fescue harvested at late date; LTI = timothy harvested at late date.

[e] H = main effect of harvest date; S = main effect of forage species; H × S = H × S interaction.

Table 4
Effects of the experimental silages 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.

	Silage[d]					*P*-value[e]		
Item	RTF	RTI	LTF	LTI	SEM	H	S	H x S
N intake, g/d	59.9[b]	72.2[a]	51.1[bc]	43.9[c]	3.12	<0.001	0.27	0.001
DOM intake, kg/d	1.04[b]	1.41[a]	1.04[b]	1.11[b]	0.068	0.004	<0.001	0.003
Urine, L/d	4.23	4.71	4.80	4.30	0.476	0.71	0.97	0.03
Urinary N, g/d	39.3[a]	41.3[a]	29.2[bc]	21.5[c]	2.36	<0.001	0.18	0.03
Fecal N, g/d	13.8[(b)]	19.0[(a)]	11.3[(b)]	13.8[(b)]	0.93	<0.001	<0.001	0.09
Urinary N, % of N intake	66.2	57.2	58.4	49.2	3.10	0.02	0.007	0.97
Fecal N, % of N intake	22.8[(b)]	26.5[(ab)]	22.4[(b)]	31.6[(a)]	1.61	0.11	<0.001	0.06
N retention, g/d	8.4	12.6	10.5	9.93	2.095	0.87	0.37	0.24
N retention, % of N intake	14.2	17.4	20.4	22.0	4.09	0.20	0.57	0.84
Urinary urea-N, g/d	27.7	28.4	20.0	14.9	2.06	<0.001	0.28	0.15
Uric acid, mmol/d	5.71	6.25	5.03	4.54	0.549	0.004	0.95	0.18
Allantoin, mmol/d	42.5[b]	57.5[a]	40.9[b]	42.0[b]	3.60	0.009	0.01	0.03
Microbial N, g/d	41.7[b]	55.2[a]	39.8[b]	40.3[b]	3.50	0.006	0.02	0.03
Allantoin, mmol/kg DOMI	11.3	9.65	11.0	9.63	1.578	0.86	0.18	0.88
Microbial N, g/kg DOMI	41.4	39.5	40.4	37.4	3.02	0.60	0.41	0.83
Hippuric acid, mmol/d	176[(b)]	258[(a)]	163[(b)]	160[(b)]	23.7	0.01	0.07	0.05
Creatinine, mmol/d	33.1	36.4	31.1	32.1	1.64	0.02	0.10	0.35
Creatinine, mg/kg BW	37.3	41.0	34.9	36.6	1.63	0.02	0.05	0.46

[a] Least squares means within rows with different superscripts differ significantly ($P \leq 0.05$) or trend toward significance if in parentheses ($0.05 < P \leq 0.10$).
[b] Least squares means within rows with different superscripts differ significantly ($P \leq 0.05$) or trend toward significance if in parentheses ($0.05 < P \leq 0.10$).
[c] Least squares means within rows with different superscripts differ significantly ($P \leq 0.05$) or trend toward significance if in parentheses ($0.05 < P \leq 0.10$).
[d] RTF = tall fescue harvested at regular date; RTI = timothy harvested at regular date; LTF = tall fescue harvested at late date; LTI = timothy harvested at late date.
[e] H = main effect of harvest date; S = main effect of forage species; H × S = H × S interaction.

of LTI showed by the wethers does not explain the similar lactation performance observed in the dairy cows (Sousa et al., 2021). Additionally, both tall fescue silages showed greater in vivo digestibility compared with LTI but it did not reflect on intake of wethers in the present study or in the milk production of dairy cows in our previous study, where cows receiving tall fescue silages showed a lower milk yield than cows receiving timothy silages, regardless of the harvest date (Sousa et al., 2021). Thus, the difference in intake of wethers in the present study could be related to the higher concentrations of hydroxycinnamic acids in tall fescue silage compared to timothy silage (Sousa et al., 2021).

In the present study, the analysis of in vivo digestibility was performed under intake restriction, where wethers received only 80 % of the ad libitum DMI, and rumen passage rate was considered similar across treatments. With limited intake the passage rate of digesta from the rumen is reduced, increasing the retention time and consequently the extent of digestion (Krizsan et al., 2010). It is well established that both ruminal and total mean retention time of particles increase as intake decreases (Dufreneix et al., 2019). Thus, under intake restriction the extent of digestion is comprehensive but limited to the content of iNDF; and as LTI showed the greatest iNDF concentration (245 for LTI vs. 123, 167 and 138 g/kg of aNDFom for RTF, RTI and LTF, respectively; Sousa et al., 2021), its extent of digestion was likely lower than the other silages, leading to the lowest in vivo digestibility observed in the present study. However, in our previous study with dairy cows, intake was not restricted and forages were likely digested under a regular retention time in the rumen, where timothy-based diets that showed lower concentration of hydroxycinnamic acids than tall fescue-based diets resulted in greater milk yield (Sousa et al., 2021), likely due to the greater digestion rate of potentially digestible fiber that delivered more energy for milk production.

Daily N excretion in urine and feces was generally greater when wethers received RTI compared with the other silages, likely due to the greater N intake observed. Even though the concentrations of CP or protein fractions A, B_1 and B_2 were not the highest in RTI, the intake of CP and the sum of fractions A, B_1 and B_2 (AB_1B_2) of wethers fed RTI was the highest, leading to the greatest N intake observed among the experimental silages. As stated by Dijkstra et al. (2013), the N excretion is primarily related to the intake of N. However, when N excretion was evaluated as % of N intake there was no difference between wethers fed RTI and wethers fed the other silages. Similar N excretion as % of N intake suggests that there was a similar efficiency of N utilization among treatments.

In the present study, wethers fed RTI showed the greatest intake of DOM that likely resulted in greater excretion of allantoin and synthesis of microbial N compared to wethers receiving the other silages. According to Jardstedt et al. (2018) intake of DOM is positively related to purine derivative excretion; and the last is highly related to rumen microbial protein synthesis (Moorby et al., 2006). Furthermore, there was no difference among silages when allantoin excretion or synthesis of microbial N was evaluated based on intake of DOM, indicating there was a similar efficiency of microbial protein synthesis per kg of DOM consumed across silages, regardless of the level of intake.

Hippuric acid is known to be a product of the degradation of phenolic compounds in the rumen (Dijkstra et al., 2013). Thus, with

advanced maturity stage it is expected to reduce the degradation of phenolic compounds and consequently the excretion of hippuric acids as lignification increases. Nadeau et al. (2019) compared grass silages containing a mixture of timothy and meadow fescue that were harvested at early or late stages and observed that wethers fed the early-maturity grass silage showed a greater hippuric acid excretion compared with wethers fed the late-maturity grass silage. However, in the present study it was true only for timothy, where the excretion of hippuric acid was greater for wethers fed RTI compared with LTI. Maturity stage did not affect the hippuric acid excretion of wethers fed tall fescue as there was no difference between RTF and LTF. Additionally, when the same silages were fed to dairy cows there was no effect of maturity stage within the same grass species, but cows eating timothy-based diets showed a greater hippuric acid excretion compared with cows receiving tall fescue-based diets, likely due to the lower concentration of hydroxycinnamic acids that increased ruminal fiber digestibility (Sousa et al., 2021).

5. Conclusions

Delayed harvest increased fiber components and decreased in vivo digestibility only in timothy. However, even with greater concentration of fiber components and lower in vivo digestibility, wethers fed timothy silages showed a greater intake than wethers fed tall fescue silages, likely due to lower concentration of hydroxycinnamic acids observed in timothy compared with tall fescue.

Harvesting timothy at regular date resulted in a combination of two important factors such as lower concentrations of fiber components and hydroxycinnamic acids that led to wethers showing the greatest intake of all nutrients, which resulted in greater daily N excretion and synthesis of microbial N when RTI was fed, but the efficiencies of N utilization as percentage of N intake was similar among silages.

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Wolfram Richardt: Methodology, Writing – review & editing. **Elisabet Nadeau:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. **Ronald Hatfield:** Methodology, Writing – review & editing. **Dannylo Sousa:** Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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