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Effects of grass species and harvest date on cell wall components and feed efficiency of dairy cows

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ABSTRACT

There is a balance between DM yield and feed value when choosing types of grasses on a farm depending on the acreages of farmland and types of ruminants to be fed. Therefore, optimisation of the harvest strategy for grass silage is important for profitable dairy farming. Tall fescue has high DM yield and can replace traditional grasses, such as timothy, in Northern Europe in a changing climate as it has been shown to be more drought tolerant. As differences in climate responses previously have been related to differences in cell wall structure between grass species and, consequently, in digestibility, it is highly relevant to compare these species at similar maturity stages and to investigate if a very early harvest date will diminish potential differences between the species. This study evaluated the effects of harvest date and forage species on the concentration of hydroxycinnamic acids in silages and its relationship to feed efficiency of dairy cows. Tall fescue and timothy were harvested at very early date on May 25 or at early date on May 31 in the spring growth cycle. Forty lactating dairy cows were used in a block design. Cows received 1 of 4 treatments: (1) tall fescue harvested at very early date, (2) timothy harvested at very early date, (3) tall fescue harvested at early date, and (4) timothy harvested at early date. Diets were formulated to have the same forage-to-concentrate ratio (49:51 on DM basis). Tall fescue silages showed greater concentrations of DM, ash, and CP than timothy silages. Grasses harvested at early date showed greater concentrations of NDF, ADL, and cell wall than grasses harvested at very early date. Tall fescue silages showed greater concentration of *p*-coumaric acid and lower in vitro organic matter digestibility (**IVOMD**) compared to timothy silages. Milk production and composition were not affected by treatments but cows fed tall fescue-based diets showed lower milk protein yield and greater milk urea nitrogen than when timothy-based diets were fed. Furthermore, cows receiving timothy-based diets showed greater feed efficiency compared to cows receiving tall fescue-based diets. Thus, the lower concentration of *p*-coumaric acid and the higher IVOMD was associated with greater feed efficiency of cows fed timothy-based diets compared to tall fescue-based diets.

Implications

Feed efficiency of dairy cows is compromised when feeding tall fescue-based diets and the reason remains unclear. This study evaluated the effects of tall fescue and timothy on the concentration of hydroxycinnamic acids and their relationship to feed efficiency of dairy cows. Tall fescue showed greater hydroxycinnamic acid concentration and lower in vitro organic matter digestibility compared to timothy. Feed efficiency was lower for cows fed tall fescue- than timothy-based diets. Hydroxycinnamic acid concentration can be used to better select grass species to feed dairy cows at farm level. Breeders can develop new varieties with lower concentration of hydroxycinnamic acids.

Introduction

Timothy (*Phleum pratense* L.) is one of the most cultivated perennial cool-season grasses and has a consistent productivity under favourable conditions but is highly sensitive to drought, which can result in decreased yield and nutritive value for animal feeding (Bertrand et al., 2008). On the other hand, tall fescue (*Festuca arundinacea* Schreb.) appears as an alternative to timothy due to its superior drought tolerance and adaptability to a wide range

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of climates (Richard et al., 2020). However, it is often observed in practice that when tall fescue replaces timothy as the main forage source in the diet of lactating dairy cows, milk production is compromised but intake remains similar. Thereafter, aiming to understand the effects grass species and harvest date on the performance of dairy cows, a novel project was conducted where Sousa et al. (2021) compared tall fescue and timothy harvested at regular and late maturity, Sousa et al. (2023) compared tall fescue and timothy harvested at regular maturity from first and second cut, and the present study compared tall fescue and timothy harvested at very early and early dates.

Sousa et al. (2021) observed that cows receiving timothy silages showed greater energy–corrected milk production than cows receiving tall fescue silages, which was related to the concentration of hydroxycinnamic acids, where timothy silages showed the lowest concentrations regardless of maturity stage. Sousa et al. (2023) observed similar intake, but cows receiving timothy-based diet showed greater milk yield and energy corrected milk (**ECM**) than cows receiving tall fescue-based diet, which was related to the lower concentrations of hydroxycinnamic acids, such as p-coumarate and the sum of p-coumarate and ferulate, observed in timothy silage compared to tall fescue silage.

Hydroxycinnamic acids are polyphenolic compounds that act in preventing potentially digestible cell wall (**CW**) polysaccharides from being extensively digested in the rumen (Novo-Uzal et al., 2011) and are considered to be the main forage-related factor limiting ruminal fibre digestibility (Adesogan et al., 2019). Besides the effects, which we observed when tall fescue and timothy were harvested at regular and late maturity in Sousa et al. (2021) and Sousa et al. (2023), Raffrenato et al. (2017) suggested that the effect of hydroxycinnamic acids on fibre digestion is highly variable among maturity stages. Thus, our hypothesis was that the difference we observed previously between tall fescue and timothy may be reduced if forages were harvested at earlier maturity stages. Therefore, the objective of this study was to evaluate the effects of tall fescue and timothy harvested at very early and early dates on the concentration of hydroxycinnamic acids and their relationship to the feed efficiency of dairy cows.

Material and methods

This study was conducted at the Lantmännen Dairy Research Farm Nötcenter Viken, south-west Sweden (58°17′N, 13°55′E), from August 31 to October 11, 2015. It is part of a novel project evaluating the effects of grass species and harvest date on high-producing dairy cows. Thereafter, similarities related to the material and methods between the present manuscript and our previous publication (Sousa et al., 2021) are expected.

Forage harvest and ensiling

Two monocultures of 2-year–old swards, one containing tall fescue cv. Swaj (SW Lantmännen) and another one containing timothy cv. Switch (SW Lantmännen) was used in the experiment. The grass swards were fertilised with 30 tonnes of slurry per hectare and with 100 kg N and 15 kg S per hectare as mineral fertiliser in the spring of 2015 and were not irrigated. The grass species were harvested at a very early maturity stage (leaf (35%)-to-stem elongation (65%) stage) on May 25 and at an early maturity stage (tall fescue: leaf (44%)-to-stem elongation (43%) to flag leaf (13%) stage; timothy: leaf (3%)-to-stem elongation (73%)-to flag leaf (23%) stage) on May 31 during the spring growth cycle of 2015 (Gustavsson, 2011) creating four experimental forages: (1) tall fescue harvested at very early date, (2) timothy harvested at very early date (**VTI**), (3) tall fescue harvested at early date, and (4) timothy harvested at early date (**ETI**).

All forages were mowed around 1600 h the day before baling and wilted by wide spreading to DM contents of 30–35% at harvest. Wilted forages were cut to 40 mm theoretical length of cut and pressed in square bales, treated with an acid (formic acid, propionic acid and formate; Perstorp AB, Perstorp) at 4 l tonne^{-1} forage and wrapped with eight layers of plastic film and stored for at least 2 months before the experiment started. Approximately, 23 bales of silage were used for each treatment in the present study. Chemical composition, fermentation profile, and in situ digestion kinetics of NDF and CP of the experimental silages are presented in Table 1.

Animals, experimental design, and diets

Forty lactating cows (5 primiparous and 35 multiparous) of Swedish Red (15), Holstein (14) and crossbreds of these breeds and Montbéliard (11) were used in a randomised complete block design with a 2 × 2 factorial arrangement of treatments. At the beginning of the experiment, the cows were on average (mean ± SD) at 610 ± 48 kg of BW, 110 ± 24 DIM, and 35.9 ± 4.5 kg of milk/d. Cows were housed in an insulated loose housing barn and milked twice a day, at 0600 and 1600 h. Cows had free access to water and salt blocks and were individually fed the experimental TMR *ad libitum*.

The experimental factors were two levels of forage species (tall fescue and timothy) and two levels of harvest dates (very early and early). Cows were blocked according to their lactation number, DIM and milk yield, and randomly assigned to treatments within block (n = 10). The experiment started with an adaptation period of 2 weeks, when all the cows received the same diet containing a mixture of 50% tall fescue and 50% timothy as forage source. After the adaptation period, the experiment lasted for 6 weeks, which was divided into three periods with cows receiving the assigned diet continuously. The first sampling period occurred during week 3, the second period occurred during week 5 and the third period during week 6. Originally, the present study was planned for 7 weeks with registration periods on weeks 3, 5 and 7. However, the grass yields were not sufficient for a 7-week experiment. Therefore, the study was shortened to 6 weeks but still having 3 registration weeks with the last two at the end of the experiment to achieve as stable DMI and milk yield (MY) as possible.

Diets were formulated using NRC (2001) to have the same forage–to–concentrate ratio regardless of the chemical composition of the silages. All diets had the same pelleted concentrate feed (Komplett Norm 180) that was produced by Lantmännen Lantbruk AB (Malmö, Sweden) and composed of (g/kg DM) barley (330), rapeseed meal (240), triticale (128), corn (117), oat hull (70), dried sugar beet pulp (30), vegetable fat (30) sugar beet molasses (20), limestone (10), palm expeller (11), salt (8), premix mineral (2), magnesium oxide (2), methionine bypass MetaSmart Dry and lysine LysiPearl (2). The dietary and nutrient compositions of the experimental diets are presented in Table 2. Diets were mixed using a TMR mixer (Cormall Feed Mixer-Multimix, Sønderborg, Denmark), delivered to each feed bunk twice a day by an automatic feeding wagon (GEA Free Stall Feeder M2000 XL, Düsseldorf, Germany) and fed *ad libitum* allowing 10% orts.

Data collection, feed sampling, and chemical analysis

Individual feed intake was continuously recorded throughout the experiment by an automatic system (Biocontrol, CRFI, Rakkestad, Norway). Diets were adjusted twice a week based on the DM of the silages by oven-drying at 60 °C for 16 h at the experimental farm (Sousa et al., 2021). Milk yield was recorded twice a day

Table 1
Chemical composition, fermentation profile, and in situ digestion kinetics of NDF and CP of the experimental silages (VTF, VTI, ETF, and ETI; n = 1) fed to dairy cows.

	Silage			
Item	VTF	VTI	ETF	ETI
Indigestible NDF, g/kg of NDF	99.3	89.2	112	82.4
ADL, g/kg of NDF	35.4	40.5	38.6	41.0
Potentially digestible NDF, g/kg of NDF	901	911	888	918
kdpdNDF, %/h	6.4	8.2	5.4	7.5
EFD, g/kg of NDF	592	661	563	642
iNDF/ADL	2.8	2.2	2.9	2.0
Soluble CP, g/kg of CP	718	720	707	677
kdpdCP, %/h	8.9	11.1	7.5	9.7
pH	5.65	5.06	4.57	4.29
Lactic acid, g/kg of DM	7.23	18.5	67.6	56.7
Acetic acid, g/kg of DM	2.41	4.00	7.88	9.17
Propionic acid, g/kg of DM	2.01	4.50	1.24	1.08
Butyric acid, g/kg of DM	0.40	0.50	0.41	0.54
Ammonium N, g/kg of total N	64.0	60.0	75.7	50.6

Abbreviations: VTF=tall fescue harvested at very early date; VTI=timothy harvested at very early date; ETF=tall fescue harvested at early date; ETI=timothy harvested at early date; kdpdNDF=digestion rate of potentially digestible NDF; EFD=effective fibre degradability; iNDF=indigestible NDF; kdpdCP=digestion rate of potentially digestible CP; N=nitrogen.

Table 2
Ingredients and nutrient composition of the experimental diets (VTF, VTI, ETF, and ETI) fed to dairy cows.

	Diet			
Item	VTF	VTI	ETF	ETI
Dietary ingredient, % of DM				
Grass silage	49	49	49	49
Concentrate	49	49	49	49
Salt/vitaminised minerals	2	2	2	2
Nutrient composition, g/kg of DM				
DM, g/kg	613	582	611	576
OM	549	523	546	517
NDF	300	288	314	322
Forage NDF	197	185	211	219
CP	173	170	171	166
Starch	164	164	164	164
Crude fat	40	40	40	40
MP	92.9	92.2	92.5	91.6
ME, Mcal/kg	2.78	2.79	2.76	2.75

Abbreviations: VTF=tall fescue harvested at very early date; VTI=timothy harvested at very early date; ETF=tall fescue harvested at early date; ETI=timothy harvested at early date; OM=organic matter; MP=metabolisable protein; ME=metabolisable energy. MP and ME allowable milk were 36.06 and 39.05 (VTF); 36.77 and 39.25 (VTI); 36.77 and 38.67 (ETF); and 36.26 and 38.42 (ETI), respectively.

during the whole experiment and milk samples were collected at each milking during 3 consecutive days in the sampling week of each period for analysis of contents of fat, true protein, lactose and urea (MilkoScan FT, Foss, Hilleroed, Denmark) by Eurofins Steins Laboratory Inc. (Jonkoping, Sweden). Feed intake and milk yield were recorded during 7 days in the sampling week. Energy-corrected milk was calculated as defined by Sjaunja et al. (1990) from the same 3 consecutive days as milk samples were collected.

Silages and total mixed rations were sampled once daily over 5 d, and the concentrates were sampled once during the collection week of each period and stored at − 20 °C until preparation for analysis. Silages and total mixed rations were pooled by period, and 200-g subsamples were analysed for DM content by drying at 60 °C for 24 h at the Swedish University of Agricultural Sciences (**SLU**), Skara. Another pooled subsample of 800 g of silage was sent to the Department of Animal Nutrition and Management, SLU, Uppsala, for analysis of crude ash, CP, NDF, ADF, ADL, and in vitro organic matter digestibility (**IVOMD**). The concentrate was analysed for DM by drying at 105 °C for 24 h at SLU, Skara and another subsample of 800 g, which was pooled over the three periods, was analysed for crude ash, CP, NDF, crude fat and starch at the Department of Animal Nutrition and Management, SLU, Uppsala.

Feed samples were milled to pass through a 1-mm screen before laboratory analysis. Crude ash was determined by combustion of dried and milled sample at 525 °C for 16 h. Neutral detergent fibre, ADF, and ADL are determined sequentially according to Van Soest et al. (1991). Heat-stable α-amylase (Novozymes, Bagsvaerd, and Denmark) was added and sodium sulfite was omitted for NDF analysis. Silage NDF, ADF, and ADL concentrations were adjusted for residual ash after ADL treatment. In vitro OMD of silages were analysed according to Lindgren (1979; 1983) by incubation at 38 °C for 96 h of 0.5 g dried, milled sample in 49 mL buffer, and 1 mL rumen fluid. Crude fat was determined based on the EU Council Directive 64/1998/EC (1998).

Cell wall composition of the silages was analysed at the U.S. Dairy Forage Research Center, Madison, WI, USA. Samples of approximately 1.6 g were weighed into 40-mL Oakridge tubes on a DM basis (55 °C). All samples were extracted as outlined in Wallsten and Hatfield (2016). Final CW concentration was determined after drying at 55 °C for at least 24 h and was expressed on an ash-free basis. Cell wall components were determined upon the isolated CW residue. Cell wall residues were oven-dried at 55 °C for 24 h before weighing subsamples for the different CW analytical procedures (Sousa et al., 2021). Acetyl bromide lignin was measured following the procedure of Morrison (1972) as modified by Hatfield et al. (1999). *p*-Coumaric acid and ferulic acid were determined by sequential method proposed by Grabber et al. (1995). Detailed information regarding the analysis of lignin and cell-wall constituents can be found in our previous publication (Sousa et al., 2021).

A subsample of the silages was pooled over the 3 registration weeks for analysis of indigestible NDF (**iNDF**), in situ analysis of NDF and CP digestion kinetics, and fermentation characteristics (acids, pH, and ammonia N), at the Department of Animal Nutrition and Management, SLU, Uppsala. The in situ method for iNDF and rumen degradation of NDF and CP of the silages was analysed on samples dried at 45° for 20 h and milled to pass a screen size of 1.5 mm according to NorFor (Åkerlind et al., 2011). Samples for analysis of iNDF were incubated in polyester bags with a pore size of 12 μm for 288 h in 2 non−lactating rumen-fistulated dairy cows fed a standard maintenance diet.

Samples for analysis of digestion kinetics of NDF and CP were incubated in polyester bags with a pore size of 38 μm in 3 non−lactating rumen-fistulated dairy cows fed the same standard maintenance diet as for analysis of iNDF (Åkerlind et al., 2011). Digestion rate of potentially digestible NDF was calculated by using the incubation times 2, 4, 8, 16, 24, 48, and 96 h, and digestion rate of potentially digestible CP was calculated by using the same incubation times except omitting the 96-h incubation in the NorFor method based on Ørskov and McDonald (1979). Soluble CP of dried and milled sample was determined following extraction of the sample in borate-phosphate buffer at 39 °C for 1 h and analysis of the supernatant for total N (Åkerlind et al., 2011).

The effective NDF degradability and the effective protein degradability were calculated at 3 and 8% passage rate, respectively, using both an exponential function to accommodate net disappearance between time points (Kristensen et al., 1982; Lindgren, 1991) and non−linear curve fitting of the same data (Ørskov and McDonald, 1979). Concentrations of lactic acid, acetic acid, propionic acid, and butyric acid 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). Ammonia N was determined with an autoanalyser system (Broderick and Kang, 1980).

Statistical analysis

Silage quality data were analysed by ANOVA for a randomised block design using the MIXED procedure of SAS version 9.3 software (SAS Inst. Inc., Cary, NC) with a 2 × 2 factorial arrangement of treatments with two levels of forage species (tall fescue vs timothy) and two levels of harvest date (very early vs early). Silages were sampled during each of the 3 sampling weeks and composited to one sample per week, which was used as experimental unit. Sampling period was treated as block (n = 3) and each block was treated independently as the cows in each treatment were fed a new bale of silage every 2 days. Kenward-Roger's method was used to compute the denominator df. The statistical model was

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + b_k + e_{ijk}$$

where μ is the population mean, α_i is the fixed effect of forage species (i = 1–2), β_j is the fixed effect of harvest date (j = 1–2), $\alpha\beta_{ij}$ is the interaction between forage species and harvest date, b_k is the random effect of block and e_{ijk} is the error term.

Data on feed intake, milk production and composition were analysed as a randomised block design using the MIXED procedure of SAS as described above but with a different block design, where cows were blocked according to their lactation number, DIM and ECM yield, and randomly assigned to treatments within block. Blocks were tested at the start of the experiment, and no difference was observed. Sampling period (n = 3) was treated as repeated measure. The statistical model was

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + w_k + b_l + c_m(b_l) + \alpha\beta_{ij} + \alpha w_{ik} + \beta w_{jk} + \alpha\beta w_{ijk} + e_{ijklm}$$

where μ is the population mean, α_i is the fixed effect of forage species (i = 1–2), β_j is the fixed effect of harvest date (j = 1–2), w_k is the fixed effect of period (k = 1–3), b_l is the random effect of block (l = 1–10), $c_m(b_l)$ is the random effect of cow nested within block, $\alpha\beta_{ij}$ is the fixed effect of interaction between forage species and harvest date, αw_{ik} is the fixed effect of the interaction between forage species and period, βw_{jk} is the fixed effect of the interaction between harvest date and period, $\alpha\beta w_{ijk}$ is the fixed effect of the interaction between forage species, harvest date and period, and e_{ijklm} is the error term. When the interactions were significant at $P \leq 0.05$ in the F-test, pair-wise comparisons were made between least square means (LS-means) with Tukey adjustment. Pair-wise differences were considered significant at $P \leq 0.05$.

Sample size was determined by a power analysis test using JMP Pro (version 16, SAS), considering a level of significance in the F-test of 0.05, a SD for MY of the cows within the group of 4.5 kg, and an expected difference to detect the significance of 2.25 kg MY (Supplementary Material S1). The minimum sample size required for an 80% power test was thirty-four cows. We used forty (10 cows per group), which showed a power of 87.5%.

Results

Tall fescue silages showed greater concentrations of DM (P = 0.004), ash (P = 0.007), and CP (P = 0.050) than timothy silages (Table 3). Silages harvested at an early date showed greater concentrations of NDF (P = 0.003), ADL (P = 0.024), and CW (P = 0.012) than silages harvested at very early date. The concentration of ADF was affected by the interaction between harvest date and species (P = 0.047), where ETI showed the greatest concentration compared to the other treatments. *p*-Coumaric acid and IVOMD were affected by harvested date and species, where grasses harvested at an early date showed the greater concentration of *p*-coumaric acid (P = 0.014) and lower IVOMD (P < 0.001) compared to grasses harvested at very early date. Additionally, tall fescue silages showed greater concentration of *p*-coumaric acid (P = 0.002) and lower IVOMD (P < 0.001) compared to timothy silages.

Table 4 shows the least squares means of the interaction between harvest date and forage species and the *P*-values of the main effects of species, harvest date, period, and their three-way interaction. *P*-values of two-way interactions were omitted from the table as there were significant three-way interactions between harvest date, forage species and period (Fig. 1). DM intake, milk protein yield and feed efficiency were affected by the three-way interaction (Table 4; Fig. 1). Cows fed VTI showed the greatest DMI but only in period 3 (P < 0.001). Cows fed ETI showed the greatest feed efficiency (ECM/DMI) but only in period 3 (P < 0.001). Milk production and composition were not affected by treatments (P > 0.05). However, when averaged over periods cows receiving timothy-based diets showed greater milk protein yield (P = 0.034) and feed efficiency (P = 0.045) compared to cows receiving tall fescue-based diets. Cows fed tall fescue-based diets showed greater milk urea nitrogen (**MUN**) than when timothy-based diets were fed (P < 0.001).

Table 3
Chemical composition and digestibility of the experimental silages (VTF, VTI, ETF, and ETI; n = 3) fed to dairy cows.

	Silage					*P*- value		
Item	VTF	VTI	ETF	ETI	SEM	H	S	H×S
DM, %	37.4	30.9	36.8	29.7	1.62	0.56	0.004	0.84
Ash, g/kg of DM	70.1	57.8	69.5	57.4	3.68	0.87	0.007	0.97
CP, g/kg of DM	182	175	176	167	5.39	0.079	0.050	0.67
NDF, g/kg of DM	393	369	421	437	11.2	0.003	0.74	0.11
ADF, g/kg of DM	218^{bc}	213^{c}	231^{b}	251^{a}	5.47	0.002	0.22	0.047
ADL, g/kg of DM	14.1	14.8	16.1	18.4	0.95	0.024	0.15	0.42
CW, g/g of DM	0.46	0.43	0.50	0.50	0.02	0.012	0.43	0.24
Acetyl bromide lignin, g/kg of CW	183	192	212	208	18.0	0.14	0.86	0.62
Ester linkages, g/kg of CW								
cis-*p*-Coumaric acid	0.14	0.24	0.20	0.14	0.06	0.72	0.72	0.24
cis-Ferulic acid	0.10	0.26	0.05	0.21	0.08	0.51	0.06	0.99
p-Coumaric acid	2.49	1.52	3.12	2.19	0.19	0.014	0.002	0.91
Ferulic acid	4.76	4.88	5.64	4.85	0.44	0.33	0.44	0.30
Total monomers	7.49	6.90	9.01	7.40	0.69	0.17	0.14	0.46
Ether linkages, g/kg of CW								
cis-*p*-Coumaric acid	0.34	0.00	0.03	0.07	0.61	0.80	0.68	0.73
cis-Ferulic acid	0.09	0.06	0.10	0.09	0.02	0.54	0.42	0.63
p-Coumaric acid	0.46	0.61	0.49	0.41	0.12	0.51	0.78	0.40
Ferulic acid	2.83	2.02	2.54	2.18	0.37	0.86	0.15	0.55
Total monomers	3.72	2.63	3.11	2.81	0.34	0.48	0.09	0.32
In vitro OM digestibility, %	90.0	94.3	88.5	92.4	0.34	<0.001	<0.001	0.53

Abbreviations: VTF=tall fescue harvested at very early date; VTI=timothy harvested at very early date; ETF=tall fescue harvested at early date; ETI=timothy harvested at early date; H=main effect of harvest date; S=main effect of grass species, H×S=interaction between H and S; CW=cell wall; OM=organic matter.

$^{a-c}$Least square means within a row with different superscripts differ significantly at $P \leq 0.05$.

Table 4
Intake, milk yield and composition, and feed efficiency of dairy cows (n = 10) fed the experimental diets (VTF, VTI, ETF, and ETI).

	Diet					*P*-value						
Item	VTF	VTI	ETF	ETI	SEM	H	S	P	H×S	H×P	S×P	H×S×P
DMI, kg/d	24.5	25.0	24.2	23.9	0.94	0.39	0.93	0.48	0.66	0.66	0.23	<0.001
Milk yield, kg/d	35.2	36.1	34.3	35.9	1.56	0.66	0.34	<0.001	0.80	0.15	0.13	0.13
ECM, kg/d	33.5	36.3	33.8	34.8	1.33	0.61	0.11	<0.001	0.44	0.40	0.09	0.28
Composition, g/kg												
Fat	3.82	4.05	4.08	3.86	0.16	0.83	0.98	0.006	0.15	0.92	0.82	0.30
Protein	3.45	3.66	3.59	3.61	0.07	0.51	0.13	<0.001	0.18	0.50	0.53	0.07
Lactose	4.78	4.79	4.71	4.64	0.06	0.06	0.63	0.31	0.53	0.54	0.76	0.20
Yield, kg/d												
Fat	1.34	1.46	1.37	1.38	0.06	0.62	0.25	0.38	0.32	0.51	0.26	0.89
Protein	1.21	1.32	1.22	1.29	0.05	0.88	0.034	0.20	0.62	0.10	0.11	0.040
Lactose	1.68	1.73	1.62	1.67	0.08	0.39	0.49	<0.001	0.94	0.13	0.13	0.09
ECM/DMI, kg/kg	1.39	1.54	1.42	1.48	0.05	0.84	0.045	0.007	0.39	0.002	0.67	<0.001
MUN, mg/dL	12.0	10.4	11.8	10.6	0.32	0.97	<0.001	<0.001	0.59	0.07	<0.001	0.60

Abbreviations: VTF=tall fescue harvested at very early date; VTI=timothy harvested at very early date; ETF=tall fescue harvested at early date; ETI=timothy harvested at early date; H=main effect of harvest date; S=main effect of grass species; P=main effect of period; H×S×P=interaction between H, S and P; DMI=DM intake; ECM=energy corrected milk; MUN=milk urea nitrogen.

Discussion

Feed efficiency in lactating dairy cows is the cow's effectiveness in converting consumed feedstuff to milk, which has a major influence on profitability and environmental constraints in the dairy industry (VandeHaar et al., 2016). Feed efficiency can be maximised when the digestion of nutrients in the rumen is comprehensive, increasing the amount of metabolic fuels delivered for milk synthesis, but the digestion rate decreases as fibre components increase in the rumen (Allen, 1996). However, in the present study, we observed that even with greater concentrations of fibre components in silages harvested at early date compared to very early date, feed efficiency was not decreased. However, greater feed efficiency was observed in cows fed timothy-based diets compared to cows fed tall fescue-based diets, regardless of harvest date.

The greater feed efficiency observed in cows receiving timothy-based diets is correlated with the lower concentration of *p*-coumaric acid and, consequently, greater IVOMD of timothy silages compared to tall fescue silages. *p*-Coumaric acid is a hydroxycinnamic acid that in its ester form acts as terminal pendant group by attaching to lignin monomers (Jung et al., 2012), reducing the digestibility of CW components (Raffrenato et al., 2017). Similar results were observed in our previous study (Sousa et al., 2021), where timothy silages had lower concentrations of hydroxycinnamic acids compared to tall fescue silages, resulting in more digestible CW per unit of lignin, which was associated with an increased rate of CW degradation, increasing the availability of energy for milk production in cows fed timothy-based diets compared to cows fed tall fescue-based diets.

Besides the effect of harvest date on increasing the concentration of fibre components on advanced maturity grasses, the content of hydroxycinnamic acids was mainly affected by grass species, which reflected directly on the IVOMD and the performance of dairy cows as observed in our studies. Harvesting tall fescue and

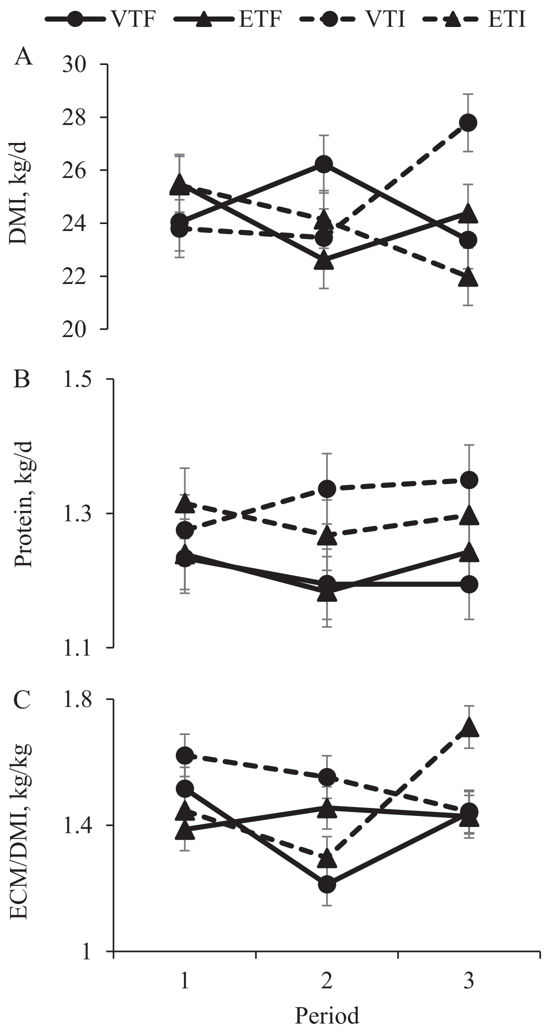

Fig. 1. Effects of treatments on (A) DM intake (DMI), (B) milk protein yield, and (C) energy-corrected milk/DM intake (ECM/DMI) of dairy cows over time during each period. Treatments were tall fescue harvested at very early date (VTF), timothy harvested at very early date (VTI), tall fescue harvested at early date (ETF), and timothy harvested at early date (ETI). Error bars indicate SEM. Error bars not overlapping indicate average means are different at $P \leq 0.05$. Error bars overlapping indicate average means are not different at $P > 0.05$.

timothy at regular and late dates resulted in an extensive effect of grass species on the composition of CW, where tall fescue showed greater concentration of all ester-linked hydroxycinnamic acids evaluated in our previous study (Sousa et al., 2021).

However, even though *p*-coumaric acid was the only hydroxycinnamic acid affected in the present study, the effect of grass species on IVOMD remained consistent. Likewise, Raffrenato et al. (2017) stated that hydroxycinnamic acids and their linkages are negatively related to fibre digestion, where the highest in vivo and in vitro fibre digestibility were observed in whole-plant corn silage containing the lowest concentration of *p*-coumaric acid. *p*-Coumaric acid modifies the structure of lignin by stopping the formation of large lignin polymers, creating numerous indigestible small fragments. Thereafter, it might increase their distribution over a wider extent of the CW, slowing the overall digestion of fibre in the rumen.

Cows fed timothy-based diets showed greater milk protein yield and lower MUN compared to cows fed tall fescue-based diets, suggesting that cows fed timothy-based diets had a better N utilisation than cows fed tall fescue-based diets. Although experimental diets were formulated to have similar CP concentrations, tall fescue silage showed greater CP concentration compared to timothy silage, which is mainly composed of rumen-degradable protein (fraction AB_1B_2; Sousa et al., 2021) that is highly and rapidly degraded in the rumen. Furthermore, a dietary association of high rumen-degradable protein and low-digestibility forage likely results in low efficiency of N utilisation and high N excretion by dairy cows due to the lack of energy to metabolise the available N in the rumen (Phuong et al., 2013). In addition, timothy silages showed greater digestibility that might result in additional energy, enhancing rumen microbial protein production and nitrogen utilisation while decreasing rumen ammonia and MUN (Kendall et al., 2009).

Conclusion

Feed efficiency of the dairy cows was related to differences in concentrations of *p*-coumaric acid and IVOMD between forage species and not to increased fibre concentrations with delayed harvest date. Consequently, *p*-coumaric acid was negatively associated to the feed efficiency of cows fed tall fescue and timothy silages harvested at very early and early date, where lower *p*-coumaric acid concentration was related to greater feed efficiency. However, more research is needed to comprehend the role of CW components in affecting the performance of high-producing dairy cows.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101256.

Ethics approval

All experimental procedures were approved by the Gothenburg Research Animal Ethics Committee (case number 120-2016) and complied with the Swedish Animal Welfare Ordinance (SFS 1988:539) and Swedish Board of Agriculture regulations and general recommendations on laboratory animals (SJVFS 2012:26).

Data and model availability statement

None of the data were deposited in an official repository. Data that support the findings of this study are available upon request from the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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