



Tall fescue as an alternative to timothy fed with or without alfalfa to dairy cows

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ABSTRACT

Tall fescue might be an alternative to timothy in northeastern North America because of its tolerance of recurring drought periods and its good summer regrowth, but is not always considered as an option in dairy rations because of its possible lack of palatability. The objective of this study was to evaluate the effects on the performance of lactating dairy cows of (1) replacing timothy silage by tall fescue silage, offered as sole forage in the diet or in combination with alfalfa silage, and (2) feeding tall fescue as silage (35% dry matter, DM) or haylage (55% DM). Experimental diets with a forage-to-concentrate ratio of 70:30 were (1) 100% timothy silage (TS); (2) 100% tall fescue silage (TFS); (3) 55:45 timothy:alfalfa silages (TS + AS); (4) 55:45 tall fescue:alfalfa silages (TFS + AS); and (5) 100% tall fescue haylage (TFH). Fifteen Holstein cows in mid-lactation (5 fitted with a rumen fistula) were randomly assigned to treatments in a triple 5 × 5 Latin square design with treatment periods of 21 d. Preplanned contrasts were timothy versus tall fescue silages, sole grass species versus grass-alfalfa, interaction between sole grass species and grass-alfalfa, and TFS versus TFH. Grass species did not affect dry matter intake (DMI) or milk yield and fat concentration. Milk protein concentration was not affected by grass species when offered in combination with alfalfa, but it was higher with the TS diet than the TFS diet when offered as sole forages. Adding alfalfa to either tall fescue or timothy silage resulted in greater DMI and milk yield, but lower milk fat concentration, than when the grass silages were the sole forage in the diet. The molar proportion of propionate in the rumen was greater when cows were fed diets with tall fescue silage compared with timothy silage, which resulted in a lower acetate-to-propionate ratio.

Milk fat concentrations of fatty acids from microbial origin, namely branched-chain fatty acids, were greater when grass silage, and especially timothy silage, were fed as sole forages rather than with alfalfa silage. Feeding TFH rather than TFS caused a decrease in DMI and tended to lower milk protein concentration, but did not affect milk yield. A more fibrolytic fermentation profile was observed in rumen of cows fed TFH compared with TFS, as indicated by the increase in the molar proportion of acetate and the higher acetate-to-propionate ratio in rumen fluid, and a concomitant increase in branched-chain fatty acid concentration in milk fat. Tall fescue as silage or haylage is a valuable alternative to timothy silage for lactating dairy cows.

Key words: dairy cows, tall fescue, timothy, silage, haylage

INTRODUCTION

Forages in dairy cow diets of eastern Canadian farms are often a mixture of a grass species with alfalfa (*Medicago sativa* L.). Alfalfa-grass mixtures combine the readily available energy from grass species with the higher protein content of forage legumes, thus improving the efficiency of N utilization by dairy cows (Brito et al., 2008). Moreover, growing alfalfa with grasses is beneficial for both yield and nutritive value of forage crops (Bélanger et al., 2014). Timothy (*Phleum pratense* L.) is the main perennial forage grass species grown in mixture with alfalfa in the cool and humid region of northeastern North America. Timothy is known for its superior winter survival (Bélanger et al., 2006) and its good yield potential under cool conditions. Its regrowth, however, is poor under the warm and dry summer conditions that often occur in northeastern North America. Air temperature and water stress are predicted to increase in the near future, which could further reduce timothy regrowth (Qian et al., 2013). An air temperature increase of 2 to 3°C could negatively affect the productivity and the nutritive value of timothy (Bertrand et al., 2008). These changes may reduce the advantages of timothy over other forage grass species.

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Tall fescue (*Schedonorus arundinacea* Schreb.) is a possible alternative to timothy because of its tolerance to recurring drought periods (Peratoner et al., 2011), its good summer regrowth (Drapeau et al., 2005), and its good performance in eastern Canada when grown in mixture with alfalfa (Pomerleau-Lacasse et al., 2019). Cherney et al. (2004) compared tall fescue silage (25% DM) to orchardgrass and alfalfa silages in dairy cow rations and found no significant difference in DMI when fed in TMR. By contrast, Johansen et al. (2017b) observed a decrease in DMI, which resulted in lower ECM yield when cows received tall fescue silage compared with other grass species, namely early perennial ryegrass and festulolium. Moreover, concerns exist about the possible lack of palatability of tall fescue in dairy rations, mainly because of its leaf roughness (Peratoner et al., 2011). Forage conservation processes have been found to decrease leaf toughness, being highest in pasture, intermediate in hay, and lowest in silage (Peratoner et al., 2011). Silages may differ in DM concentration, and information is lacking about the effects of feeding tall fescue as silage with 35% DM and as haylage (55% DM), both being commonly used on dairy farms.

Our objective was to evaluate the effect on DMI, milk production, and milk composition of lactating Holstein cows of (1) replacing timothy silage by tall fescue silage, offered as the sole forage in the diet or in combination with alfalfa silage, and (2) feeding tall fescue as silage (35% DM) or haylage (55% DM). We hypothesized that substituting timothy silage with tall fescue silage, fed either as sole forage or in combination with alfalfa silage, would result in similar performance of dairy cows in established lactation. We also hypothesized that the forage conservation type would not affect DMI and milk production.

MATERIALS AND METHODS

Forage Production

Tall fescue (cv. Courtenay), timothy (cv. AC Alliance), and alfalfa (cv. Genoa), established without cover crop, were grown in similar but different fields at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, Canada; 46°40'N, 71°55'W). In the first year after their establishment, tall fescue and timothy were cut on Jun. 6, 2015, at the early heading stage of development, using a disk mower equipped with a roller-type conditioner and adjusted to a residual height of 10 cm (Discbine, model 1432, New Holland Agriculture, New Holland, PA). Timothy was wilted for 24 h in the field until it reached approximately 35% DM, whereas tall fescue was wilted for 24 or 30 h in the field until alternate rows reached respectively

35% DM or 55% DM. Then, both timothy at 35% DM and tall fescue at 35% DM were finely chopped and stored in plastic bag silos (Bag All, Klerk's Plastic Products Manufacturing Inc., Richburg, SC); they are hereafter referred to as timothy and tall fescue silages. Tall fescue at 55% DM was harvested as large square bales with a baler (New Holland Agriculture square baler, model BB9060) and wrapped using a bale wrapper (Anderson Group single bale wrapper, model 780, Chesterville, QC, Canada); it is hereafter referred to as tall fescue haylage. Each large square bale of haylage was chopped before mixing of the diets using a TMR vertical mixer (V-Mix 575, Valmetal, St-Germain-de-Grantham, QC, Canada) for 20 min before the mixing of the diet. Alfalfa was cut at the early bloom stage of development on Jun. 13, 2015, wilted in the field until it reached approximately 35% DM, finely chopped, and conserved in a plastic bag silo using the same procedure as for tall fescue and timothy wilted to 35% DM.

Cows, Experimental Design, and Diets

The experiment was carried out at the Centre de Recherche en Sciences Animales de Deschambault. All procedures with cows were approved by the Animal Care Committee of Université Laval (Québec, QC, Canada) in accordance with the guidelines of the Canadian Council on Animal Care (Olfert et al., 1993).

Fifteen multiparous (3.1 ± 1.3 parities; mean \pm SD) Holstein cows (128 ± 33 DIM and 692 ± 69 kg of BW at the beginning of the experiment), 5 of them fitted with a rumen fistula (internal diameter 10 cm; Bar Diamond Inc., Parma, ID), were housed in a tiestall barn and fed once daily at 1000 h. Cows were randomly assigned to treatments in a triple 5×5 Latin square design with 21-d periods, the last 3 d being used for data and sample collections. Five experimental diets (70:30 forage-to-concentrate ratio) were compared: (1) 100% timothy silage (**TS**); (2) 100% tall fescue silage (**TFS**); (3) 55% timothy silage with 45% alfalfa silage (**TS + AS**); (4) 55% tall fescue silage with 45% alfalfa silage (**TFS + AS**); and (5) 100% tall fescue chopped haylage (**TFH**; Table 1). The 5 diets were adjusted based on the initial feed ingredient composition (Table 2) to ensure that CP concentration was similar between pure grass diets and their respective mixed diets (Table 3) and that all diets met or exceeded NRC (2001) recommendations. Diets were offered as TMR for ad libitum intake, allowing for 10%orts on an as-fed basis according to the intake of the previous day, and TMR were regularly pushed back toward the cows during the day. During the experiment, the silages and haylage were sampled weekly. The samples were dried for 72 h at 55°C to determine DM concentration, and the com-

Table 1. Ingredients of the experimental diets

Item, % of DM	Diet ¹				
	TS	TFS	TS + AS	TFS + AS	TFH
Timothy silage	70.7	—	38.1	—	—
Tall fescue silage or haylage	—	70.7	—	38.1	70.7
Alfalfa silage	—	—	31.3	31.3	—
Ground corn	23.4	23.4	26.9	26.9	23.4
Corn gluten meal	2.7	2.7	2.2	2.2	2.7
Soybean meal	1.8	1.8	—	—	1.8
Mineral-vitamin mix ²	1.4	1.4	1.5	1.5	1.4

¹TS = 100% timothy silage; TFS = 100% tall fescue silage; TS + AS = 55% timothy silage plus 45% alfalfa silage; TFS + AS = 55% tall fescue silage plus 45% alfalfa silage; TFH = 100% tall fescue haylage.

²Mineral-vitamin mix = 18-5, La Coop, St-Romuald, QC, Canada: 18% Ca, 5% P, 6% Mg, 9.5% Na, 15% Cl, 0.06% K, 1% S, 3,620 mg/kg Fe, 600 mg/kg Cu, 2,000 mg/kg Mn, 3,000 mg/kg Zn, 45 mg/kg I, 25 mg/kg Se, 20 mg/kg Co, 1,000 IU/kg vitamin A, 1,000 IU/kg vitamin D, 1,500 IU/kg vitamin E.

position of the diets on an as-fed basis was adjusted accordingly. Water was added to TMR to target a 35% DM concentration. Cows had free access to water at all times during the experiment.

Measurements and Sampling

Amounts of feed offered and refused were recorded and diets were sampled on d 19, 20, and 21 of the experimental periods. During this 3-d collection period, 1 sample of each silage used in the diets was also taken. All samples were stored at -20°C for further analysis. Samples of diets were thawed and pooled by period to

evaluate their respective particle size distribution using the Penn State Particle Separator (Kononoff and Heinrichs, 2003a). At 1300 h, on d -3 , -2 , and -1 of the first period, cows were weighed on a platform mounted on 4 load cells connected to an indicator (CI 2001 AS series, Kilotech, Lachine, QC, Canada).

For the 5 cows of one of the Latin squares, rumen fluid was collected from the ventral sac through a fistula on d 18 of each period at 0, 1, 2, 4, and 6 h relative to feeding time. A 10-mL rumen fluid sample was collected as described by Baumann et al. (2016) and the pH immediately recorded (pHTestr 30, Oakton Instruments, Vernon Hills, IL). Rumen fluid samples

Table 2. Chemical composition and digestibility of the forages (mean \pm SD) used in the experimental diets (n = 5)

Item, % of DM (unless otherwise stated)	Timothy silage	Tall fescue silage	Tall fescue haylage	Alfalfa silage
DM, % as fed	31.3 \pm 3.2	34.1 \pm 4.5	50.6 \pm 7.7	28.8 \pm 2.1
OM	91.6 \pm 0.6	90.3 \pm 0.9	90.3 \pm 0.9	91.3 \pm 1.4
CP	15.4 \pm 0.2	13.6 \pm 0.7	14.1 \pm 0.2	18.3 \pm 0.3
aNDF ¹	58.3 \pm 2.2	54.3 \pm 1.5	58.2 \pm 0.4	40.4 \pm 3.9
ADF	38.5 \pm 1.2	35.3 \pm 1.7	37.0 \pm 0.8	34.8 \pm 2.1
NE _L , ² Mcal/kg of DM	1.23	1.26	1.14	1.30
Total FA, ³ % of DM	1.99 \pm 0.07	2.12 \pm 0.05	1.91 \pm 0.07	2.04 \pm 0.15
FA, % of FA				
16:0	18.29 \pm 1.06	17.05 \pm 0.48	18.17 \pm 0.80	21.54 \pm 2.08
16:1	0.28 \pm 0.01	0.28 \pm 0.02	0.25 \pm 0.01	0.30 \pm 0.03
17:0	0.39 \pm 0.02	0.38 \pm 0.01	0.41 \pm 0.02	0.51 \pm 0.05
18:0	1.80 \pm 0.23	1.68 \pm 0.24	1.70 \pm 0.18	2.89 \pm 0.28
18:1	4.53 \pm 0.23	4.18 \pm 0.19	3.85 \pm 0.37	3.92 \pm 0.53
18:2	20.73 \pm 0.74	16.58 \pm 0.52	16.09 \pm 2.20	21.89 \pm 0.63
18:3	49.80 \pm 1.71	56.55 \pm 1.08	57.59 \pm 3.34	45.02 \pm 2.76
Other	4.19 \pm 1.57	3.30 \pm 0.70	1.93 \pm 0.37	3.93 \pm 0.67
IVTD, ⁴ % of DM	86.3 \pm 1.0	86.1 \pm 0.8	87.1 \pm 1.0	82.3 \pm 2.5
NDFd, ⁵ % of aNDF	76.4 \pm 2.1	74.4 \pm 1.0	77.8 \pm 1.7	57.3 \pm 2.2

¹Neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite.

²Calculated based on the average chemical composition of each forage, according to NRC (2001).

³Fatty acid.

⁴In vitro true digestibility of DM.

⁵In vitro NDF digestibility.

Table 3. Chemical composition, digestibility, and particle length (mean \pm SD) of the experimental diets (n = 5)

Item, % of DM (unless otherwise stated)	Diet ¹				
	TS	TFS	TS + AS	TFS + AS	TFH
Chemical composition					
DM, % as fed	33.8 \pm 2.2	34.1 \pm 4.1	34.2 \pm 2.2	34.7 \pm 2.0	34.0 \pm 4.0
OM	92.4 \pm 0.2	91.7 \pm 0.4	92.8 \pm 0.2	92.6 \pm 0.5	91.2 \pm 0.6
CP	14.9 \pm 0.5	13.8 \pm 0.4	14.9 \pm 0.4	13.9 \pm 0.6	13.9 \pm 0.6
aNDF	43.8 \pm 1.5	39.8 \pm 1.4	37.7 \pm 0.9	34.7 \pm 1.0	45.2 \pm 1.6
ADF	28.0 \pm 1.3	25.2 \pm 0.7	26.5 \pm 1.0	24.1 \pm 1.3	28.4 \pm 1.0
NE _L , ² Mcal/kg of DM	1.54	1.55	1.55	1.56	1.49
Total FA, ³ % of DM	2.47 \pm 0.06	2.44 \pm 0.08	2.51 \pm 0.11	2.48 \pm 0.11	2.02 \pm 0.04
FA, % of FA					
16:0	17.32 \pm 0.21	16.54 \pm 0.18	18.12 \pm 0.38	17.54 \pm 0.37	18.59 \pm 0.71
16:1	0.24 \pm 0.04	0.24 \pm 0.04	0.25 \pm 0.03	0.22 \pm 0.03	0.28 \pm 0.10
18:0	2.04 \pm 0.05	1.97 \pm 0.07	2.33 \pm 0.15	2.31 \pm 0.07	2.20 \pm 0.08
18:1	12.98 \pm 0.40	13.03 \pm 0.54	13.36 \pm 0.68	13.97 \pm 0.97	13.57 \pm 0.89
18:2	35.52 \pm 0.59	33.35 \pm 0.83	35.94 \pm 1.03	35.95 \pm 1.55	32.55 \pm 1.20
18:3	29.24 \pm 0.98	32.39 \pm 1.48	27.03 \pm 1.64	27.52 \pm 2.31	31.08 \pm 2.61
Other	2.66 \pm 0.21	2.49 \pm 0.39	2.97 \pm 0.75	2.50 \pm 0.59	1.73 \pm 0.28
Digestibility					
IVTD, ⁴ % of DM	89.4 \pm 0.61	90.4 \pm 0.66	88.3 \pm 0.71	89.0 \pm 0.32	90.1 \pm 0.86
NDFd, ⁵ % of aNDF	75.7 \pm 1.12	75.9 \pm 1.57	69.0 \pm 2.00	68.2 \pm 1.54	78.1 \pm 1.80
Particle length determinations, % of DM retained on sieves					
19.0 mm	73.2 \pm 5.2	70.4 \pm 7.5	67.2 \pm 4.9	56.8 \pm 8.1	83.2 \pm 3.9
8.0 mm	16.3 \pm 4.4	19.1 \pm 4.7	22.1 \pm 5.7	27.0 \pm 8.9	7.5 \pm 3.6
4.0 mm	7.4 \pm 3.1	8.1 \pm 2.0	8.1 \pm 1.6	12.3 \pm 2.8	4.5 \pm 1.4
Pan	3.0 \pm 1.4	2.5 \pm 1.0	2.9 \pm 0.7	3.9 \pm 1.7	4.8 \pm 3.1

¹TS = timothy silage (100%); TFS = tall fescue silage (100%); TS + AS = timothy silage (55%) and alfalfa silage (45%); TFS + AS = tall fescue silage (55%) and alfalfa silage (45%); TFH = tall fescue haylage (100%).

²Calculated based on the average chemical composition of each experimental diet, according to NRC (2001).

³Fatty acid.

⁴In vitro true digestibility of DM.

⁵In vitro NDF digestibility.

were then stabilized with 0.2 mL of H₂SO₄ (50%, vol/vol) and stored at -20°C until determination of VFA and NH₃-N concentrations.

Cows were milked at 0700 and 1700 h daily. Milk production was measured using calibrated milk meters (Flomaster Pro, DeLaval, Tumba, Sweden), and milk samples were collected at each milking from d 19 to 21. Milk samples from the evening and morning milking were composited daily, proportionally to their respective milk yields. A composite milk sample was stored at 4°C with bronopol (2-bromo-2-nitropropane-1,3-diol) until further determination of fat, protein, lactose, and urea nitrogen. A second milk sample was collected and stored at -20°C without preservative and further pooled by cow and period for subsequent fatty acid (FA) analysis.

Chemical Analyses

Before analysis, samples of the diets and silages were thawed at 4°C , pooled by period (silages), by period and treatment (offers), or by period and cow (refusals), dried in a forced-air oven for 72 h at 55°C to determine

DM concentration, and ground to 1 mm using a Wiley mill (model 4, Arthur M. Thomas Co., Philadelphia, PA). All silage and diet samples were analyzed for residual moisture (AOAC International, 2005), OM (method 942.05, AOAC International, 2005), ADF (Ankom 2017a; AOAC International, 2005), neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite and not corrected for residual ash (aNDF; Ankom 2017b; AOAC International, 2005), and CP ($N \times 6.25$; AOAC International, 2005). Dietary FA were directly transesterified and FAME extracted as described by Villeneuve et al. (2013). Determination of dietary FA composition was carried out by gas chromatography, according to the method described by Fauteux et al. (2016). Both the in vitro true digestibility of DM (IVTD) and the in vitro NDF digestibility (NDFd) in feed samples were measured based on a 48-h incubation with buffered rumen fluid, followed by aNDF determination of the post-digestion residues (Goering and Van Soest, 1970). The rumen fluid incubation was performed with Ankom F57 filter bags and an Ankom Daisy II incubator, using the bath incubation procedures outlined by Ankom Technology

(Ankom Technology, Macedon, NY). Rumen fluid was obtained from a ruminally fistulated dairy cow offered a diet of 37% grass silage, 15% corn silage, 8% hay, 30% corn grain, and 10% concentrate mix (DM basis) formulated to meet the nutritional requirements of a lactating dairy cow expected to produce 10,200 kg of milk/yr. The IVTD (g/kg of DM) and the in vitro NDFd (g/kg of aNDF) were calculated as follows:

IVTD

$$= \left(1 - \frac{\text{post-digestion dry weight following aNDF wash}}{\text{pre-digestion dry weight}} \right)$$

$\times 1,000$;

NDFd

$$= \left(1 - \frac{\text{post-digestion dry weight following aNDF wash}}{\text{pre-digestion dry weight of aNDF}} \right)$$

$\times 1,000$.

Before the time of analysis, acidified samples of rumen fluid were thawed and centrifuged at $16,000 \times g$ for 15 min at 4°C . The supernatant was analyzed for $\text{NH}_3\text{-N}$ as in Weatherburn (1967), using a Varioskan spectrophotometer (type 3001, cat. no. 5250010, Thermo Electron Corporation, Vantaa, Finland) at 625 nm. Ruminant VFA concentrations were determined according to Weatherburn (1967) using a gas chromatograph (Clarus 680; Perkin Elmer, Waltham, MA) equipped with a polar capillary column (HP-Innowax 30-m length, 0.320-mm internal diameter, 0.25- μm film thickness; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a flame ionization detector. The split ratio was 25:1. At the time of sample injection, the column temperature was 80°C , maintained for 0.5 min, followed by a first increase to 180°C at $10^{\circ}\text{C}/\text{min}$ and a second increase to 220°C at $30^{\circ}\text{C}/\text{min}$. The temperature was then maintained at 220°C for 2 min.

Milk fat, protein, lactose, and MUN concentrations were determined according to AOAC method 972.160 (AOAC International, 2012), using an infrared procedure with a Foss MilkoScan FT 6000 by Valacta (Ste-Anne-de-Bellevue, QC, Canada). Fat- and energy-corrected milk yields were calculated based on milk yield and composition using the formulas of Gaines (1928) and Madsen et al. (2008), respectively. Milk lipid extraction and methylation of FA were performed according to procedures described by Chouinard et al. (1997). Milk FA profile was determined according to Boivin et al. (2013) using a gas chromatograph (Agilent 7890A, Agilent Technologies) equipped with a 100-m CP-Sil-88 capillary column (0.25-mm internal diameter, 0.20- μm

film thickness; Agilent Technologies) and a flame ionization detector.

Statistical Analyses

Data on DMI and milk production and composition were analyzed using the MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) as a triple 5×5 Latin square design, according to the following model:

$$Y_{ijkl} = \mu + T_i + S_j + P(S)_{kj} + C(S)_{lj} + \varepsilon_{ijkl},$$

where Y_{ijkl} = individual observation ($n = 75$), μ = overall mean, T_i = fixed effect of treatment ($i = 1$ to 5), S_j = fixed effect of square ($j = 1$ to 3), $P(S)_{kj}$ = fixed effect of period ($k = 1$ to 5) within square, $C(S)_{lj}$ = random effect of cow ($l = 1$ to 5 and $j = 1$ to 3) within square, and ε_{ijkl} = residual error term. Rumen fermentation parameters were analyzed for 1 Latin square with 5 fistulated animals, using the REPEATED statement in the MIXED procedure of SAS, using the following model:

$$Y_{ijkl} = \mu + T_i + P_j + H_k + (T \times H)_{ik} + C_l + \varepsilon_{ijkl},$$

where Y_{ijkl} = individual observation ($n = 75$), μ = overall mean, T_i = fixed effect of treatment ($i = 1$ to 5), P_j = fixed effect of period ($j = 1$ to 5), H_k = fixed effect of sampling time ($k = 0, 1, 2, 4,$ and 6 h postprandial), $(T \times H)_{ik}$ = fixed effect of the interaction, C = random effect of cow ($l = 1$ to 5), and ε_{ijkl} = residual error. Cow within period \times treatment was the subject of the repeated statement. Because no treatment \times sampling time interaction was observed, values for rumen parameters were averaged by day and analyzed according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + \varepsilon_{ijk},$$

where Y_{ijk} = individual observation ($n = 25$), μ = overall mean, T_i = fixed effect of treatment ($i = 1$ to 5), P_j = fixed effect of period ($j = 1$ to 5), C = random effect of cow ($k = 1$ to 5), and ε_{ijk} = residual error. For all models, the best covariance structure was selected based on the Akaike information criterion. Four pre-planned contrasts were used to test the effects of the diets: (1) timothy versus tall fescue silage [TS + (TS + A) vs. TFS + (TFS + A); hereafter called "Species"]; (2) grass silage offered as sole forage or in combination with alfalfa silage [TS + TFS vs. (TS + A) + (TFS + A); hereafter called "Mixture"]; (3) interaction between grass silage species and grass:alfalfa silage mixtures;

and (4) TFS versus TFH. Differences were declared significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

DMI

Grass species, offered either as sole silage or in combination with alfalfa silage, did not affect DMI (Table 4). This result is consistent with other studies, which found that DMI was similar for tall fescue and other grass species, provided that the grass species were harvested at the same stage of development (Fisher et al., 1993; Cherney et al., 2004). In another study, when comparing different grass species, Johansen et al. (2017b) established that DMI was related to silage OM digestibility. Accordingly, grass silages used in the current study had very similar IVTD and NDFd (Table 2). Regardless of the grass species, feeding grass silage in combination with alfalfa silage increased DMI by 7.5% compared with feeding only grass silage (Table 4). It is well established that DMI is greater when cows are fed forage legumes compared with forage grasses (Hoffman et al., 1998; Steinshamm, 2010; Johansen et al., 2018). According to Mertens (1994) and Hoffman et al. (1998), the difference in DMI is mainly explained by the lower NDF concentration in forage legumes than in forage grasses, the dietary NDF concentration being negatively correlated with DMI. In line with these observations, the aNDF concentration in the current experiment was greater in diets containing only grass silages (TS and TFS) than in diets with both grass and alfalfa silages (TS + A and TFS + A; Table 3), leading to a respective 9% increase in aNDF intake (Table 4). However, despite a lower aNDF concentration, IVTD of diets with both grass and alfalfa silages was numerically lower than grass silage diets, which could be explained by the lower NDFd of alfalfa compared with grass silages. Although it was not measured in this experiment, alfalfa is reported to have a greater rate of degradation (Hoffman et al., 1993) and rate of passage (Cherney et al., 2004) in the rumen than forage grasses. Substantial differences in the gross morphology and cellular structure between alfalfa and grasses can affect patterns of ingestion, mastication, rumination, and particle size reduction (Wilson and Kennedy, 1996; Dewhurst et al., 2003a,b), which can modify the passage rate and affect DMI.

Dry matter intake was also affected by the conservation method of tall fescue. Cows fed TFS consumed 8.7% more DM than did cows fed TFH. The DM concentration of the tall fescue haylage and silage averaged 50.6 and 34.1%, respectively (Table 2). In the current experiment, dietary DM was adjusted using water to

obtain values ranging from 33.8 to 34.7% and standard deviations from 2.0 to 4.2% (Table 3). Thus, the variation in DMI between TFS and TFH could not be attributed to differences in DM concentration of the TMR. Huhtanen et al. (2007) considered multiple factors to predict forage consumption by dairy cows from forage characteristics. When comparing fresh-cut and wilted forages, they found a quadratic increase of forage consumption with increasing forage DM concentration, and, when considered alone, optimal values were obtained with a forage DM of 41.9%. Fermentation characteristics and final chemical composition of silages can be affected by forage DM concentration and conservation type (Hristov and Sandev, 1998; McEniry et al., 2008), and when the former were included in the model, Huhtanen et al. (2007) found that the optimal forage DM concentration varied between 33.7 and 37.0%. In contrast, when comparing grass-clover silages with varying DM concentration obtained through different wilting times, Johansen et al. (2017a) found that DMI was not affected by DM concentration of wilted silages. The differences in particle size of the diets (Table 3) could also explain the greater DMI observed with TFS compared with TFH. The proportion of particles retained on the 19-mm sieve of the Penn State Particle Separator was approximately 13 percentage units greater in TFH than in TFS. Both TFS and TFH were chopped before either ensiling (silage) or TMR mixing (haylage). The differences in particle size are representative of the forages produced using these 2 conservation methods. Shorter particle size in silages and diets are reported to increase feed intake (Kononoff and Heinrichs, 2003b).

Rumen Fermentation

The rumen pH was similar between cows fed diets with timothy and tall fescue (Table 5). The relatively high values for rumen pH encountered in the current study (>6.19) are in accordance with the high aNDF concentrations ($>34.7\%$) of the experimental diets. The molar proportion of propionate in the rumen was greater when cows were fed diets with tall fescue silage compared with timothy silage (Table 5), which resulted in a lower acetate-to-propionate ratio. This effect could be linked to the lower aNDF concentration of tall fescue silage compared with timothy silage (54.3 vs. 58.3%; Table 2).

Cows fed diets containing both grass and alfalfa silages had lower rumen pH compared with cows fed grass silage-based diets (6.23 vs. 6.38; Table 5). The greater proportion of ground corn (26.9 vs. 23.4%; Table 1) and lower aNDF concentration (36.2 vs. 41.8%; Table 3) of grass and alfalfa mixture diets compared with

Table 4. DMI, milk yield, and milk composition from cows fed diets with timothy silage (TS, 100%), tall fescue silage (TFS, 100%), timothy silage and alfalfa silage (TS + AS, 55:45%), tall fescue silage and alfalfa silage (TFS + AS, 55:45%), and tall fescue haylage (TFH, 100%)

Item	Diet						Contrast, <i>P</i> -value ¹			
	TS	TFS	TS + AS	TFS + AS	TFH	SEM	Species	Mixture	Species × Mixture	TFS vs. TFH
DMI, kg/d	22.6	22.6	24.7	23.9	20.8	0.6	0.36	<0.01	0.28	<0.01
aNDF intake, kg/d	9.89	9.01	9.32	8.26	9.40	0.24	<0.01	<0.01	0.64	0.13
Digestible aNDF intake, kg/d	7.49	6.84	6.43	5.63	7.34	0.18	<0.01	<0.01	0.62	0.02
Milk yield, kg/d										
Actual	26.1	27.2	28.6	28.0	26.8	1.01	0.62	<0.01	0.11	0.58
4% FCM ²	27.2	28.2	29.2	28.5	27.7	1.25	0.70	0.02	0.08	0.48
ECM ³	26.8	27.7	28.8	28.2	27.2	1.25	0.85	0.01	0.11	0.48
Milk fat										
Concentration, %	4.28	4.27	4.17	4.13	4.23	0.08	0.55	<0.01	0.65	0.53
Yield, g/d	1,116	1,158	1,185	1,155	1,135	56	0.78	0.12	0.10	0.46
Milk protein										
Concentration, %	3.53	3.40	3.49	3.46	3.44	0.06	<0.01	0.52	<0.01	0.06
Yield, g/d	913	917	986	961	916	38	0.60	<0.01	0.45	0.98
Milk lactose										
Concentration, %	4.41	4.49	4.48	4.48	4.42	0.06	0.01	0.09	<0.01	<0.01
Yield, g/d	1,165	1,231	1,290	1,262	1,190	60	0.38	<0.01	0.03	0.19
MUN, mg/dL	9.0	6.5	8.7	7.1	7.6	0.4	<0.01	0.45	0.06	<0.01

¹Four preplanned contrasts: Species [TS + (TS + AS) vs. TFS + (TFS + AS)]; Mixture [TS + TFS vs. (TS + AS) + (TFS + AS)]; interaction between Species and Mixture; and TFS vs. TFH.

²4% FCM = $[0.4 \times \text{milk yield (kg/d)}] + [15 \times \text{fat yield (kg/d)}]$.

³ECM = $\{[23.8 \times \text{fat yield (kg/d)}] + [38.9 \times \text{protein yield (kg/d)}] + [16.3 \times \text{lactose yield (kg/d)}]\}/3.14$.

Table 5. Ruminant $\text{NH}_3\text{-N}$ and VFA concentrations and pH from cows fed diets with timothy silage (TS, 100%), tall fescue silage (TFS, 100%), timothy silage and alfalfa silage (TS + AS, 55:45%), tall fescue silage and alfalfa silage (TFS + AS, 55:45%), and tall fescue haylage (TFH, 100%)

Item	Diet							Contrast, <i>P</i> -value ¹		
	TS	TFS	TS + AS	TFS + AS	TFH	SEM	Species	Mixture	Species × Mixture	TFS vs. TFH
pH	6.34	6.41	6.26	6.19	6.35	0.04	0.98	<0.01	0.13	0.34
$\text{NH}_3\text{-N}$, mg/dL	10.06	9.09	11.29	8.67	7.77	0.98	0.07	0.66	0.37	0.32
VFA, mol/100 mol										
Acetate	70.1	68.3	67.8	67.7	72.8	0.7	0.22	0.06	0.26	<0.01
Propionate	17.5	19.7	18.3	19.5	16.3	0.6	0.01	0.61	0.40	<0.01
Butyrate	9.3	9.3	10.6	9.7	7.9	0.4	0.24	0.05	0.23	0.02
Acetate:propionate	4.1	3.5	3.7	3.5	4.5	0.2	0.03	0.32	0.38	<0.01

¹Preplanned contrasts: Species [TS + (TS + AS) vs. TFS + (TFS + AS)]; Mixture [TS + TFS vs. (TS + AS) + (TFS + AS)]; interaction between Species and Mixture; and TFS vs. TFH.

grass silage diets could explain this difference. It has long been recognized that increasing readily fermentable carbohydrates and decreasing fiber concentration of a diet can decrease rumen pH in dairy cows (Krause et al., 2002).

The rumen pH of cows fed diets with tall fescue as either silage or haylage did not differ (Table 5). However, feeding cows with TFH rather than TFS increased molar proportion of acetate and decreased molar proportion of propionate, leading to a higher acetate-to-propionate ratio in rumen fluid (Table 5). In a previous study, Johansen et al. (2017a) found that DM concentration of grass-clover silage had no effect on rumen pH or concentrations of acetate or propionate in rumen fluid. However, as previously discussed, the TFH diet had a greater particle size than the THS diet, which could have reduced the rumen passage rate. A decrease in passage rate combined with the higher aNDF digestibility of TFH (Table 2) could have allowed for a more intense fibrolytic fermentation, leading to the observed changes in VFA profile with the absence of effect on rumen pH.

Milk Yield and Composition

Grass species in silage-based diets did not affect the actual, fat-corrected, or energy-corrected milk yields, milk fat concentration and yield, or milk lactose yield (Table 4). Similarly, Cherney et al. (2004) did not detect any difference in milk yield and milk fat concentration for cows fed either tall fescue or orchardgrass silages when those 2 forage grasses were harvested at the recommended fiber concentration. This is consistent with a recent meta-analysis in which milk production in cows fed different grass species was found to be similar, provided that forage OM digestibility was similar (Johansen et al., 2018). In the current experiment, timothy and tall fescue silages had similar IVTD (86.2% of DM; Table 2). Concentration of MUN was greater and that of rumen $\text{NH}_3\text{-N}$ tended to be greater ($P = 0.07$) in diets with timothy silage (TS and TS + A) than in diets with tall fescue silage (TFS and TFS + A; Tables 4 and 5), which could be due to the difference in CP concentration between the 2 silages (15.4 vs. 13.6%; Table 2). This difference in CP concentration was reflected in the diets (14.9% of CP for TS and TS + AS, and 13.9% of CP for TFS and TFS + AS; Table 3) even though diets were formulated to have a similar CP concentration between pure grass diets and their respective mixed-forage diets. Similarly, milk protein concentration was greater when cows were fed the TS diet than the TFS diet, but similar when alfalfa silage was added to the diets (Table 4). This interaction suggests a shortage of N when tall fescue silage was the

sole forage in the diet. Adding alfalfa silage might have improved the N balance, whereas this addition was not as beneficial when timothy silage was fed. Milk lactose concentration did not differ when cows were fed diets with timothy and tall fescue silages in combination with alfalfa silage, but it was lower with timothy silage than with tall fescue silage when those 2 silages were the only source of forage in the diet (Table 4).

Diets with tall fescue or timothy silages in combination with alfalfa silage resulted in lower milk fat concentration but greater milk yield than when only grass silages were included in the diet (Table 4). Therefore, fat-corrected and energy-corrected milk yields were also greater when AS complemented timothy or tall fescue silages in the forage proportion of the diets, despite a significant decrease in milk fat concentration. Moorby et al. (2009) also reported a decrease in milk fat concentration when the legume proportion was increased in the diet of dairy cows. Feeding grass silage in combination with alfalfa silage increased milk protein yield compared with the grass-only diets (Table 4). This difference might be attributed to an enhanced N use efficiency. Indeed, it has been shown that mixing legumes with grass silages improves microbial N flow to the duodenum in relation to the amount of dietary N consumed, resulting in an improved N use efficiency (Merry et al., 2006). Milk lactose yield was higher when cows were fed diets that included both grass and alfalfa silages compared with a diet with only a grass silage. This result could be linked with the higher milk production of cows fed grass silage in combination with alfalfa silage compared with diets with only a grass silage.

The lower DMI when cows were fed TFH rather than TFS did not affect the actual, fat-corrected, or energy-corrected milk yields, milk fat concentration and yield, milk protein yield, or milk lactose yield (Table 4). Similar milk yield with a lower DMI resulted from a higher milk efficiency when cows were fed TFH compared with TFS. Similarly, TFH seems to have allowed for a greater metabolizable protein supply, as indicated by a higher MUN concentration and a tendency ($P = 0.06$) for greater milk protein concentration when cows were fed TFH compared with TFS (Table 4). A decrease in MUN concentration can be associated with a decrease in dietary CP (Broderick and Clayton, 1997) or an improved N use efficiency (Fadul-Pacheco et al., 2017). In the current experiment, where high-forage diets with similar concentrations of CP were fed to cows in mid-lactation (13.8 vs. 13.9% for the TFS and TFH diets, respectively), our results point to an improved forage N utilization when cows were fed TFH compared with TFS. Accordingly, Johansen et al. (2017a) observed that pre-wilting to a higher DM concentration increased metabolizable protein of grass-clover silage. They at-

tributed this difference to a reduced degradation of feed protein, an increase in rumen microbial synthesis, and an increase in intestinal digestibility of amino acids when silage DM concentration was increased.

Apart from greater concentrations of FA originating from mammary de novo synthesis and consequent decreases in concentrations of preformed FA in milk from cows fed the TS + AS diet, diets had a limited effect on major milk FA concentrations (Table 6). This decrease in concentrations of preformed FA does not seem to originate from dietary FA, as diets had similar FA concentrations (% of DM) and compositions (% of FA; Table 3). However, feeding diets with grass species as sole forages increased the concentration of total milk branched-chain FA, and the amplitude of this increase was greater when cows were fed timothy silage than tall fescue silage. As previously mentioned, an increase was detected in the molar proportion of acetate-to-propionate ratio in the rumen for cows fed the timothy silage diets compared with the tall fescue diets, a greater pH in the rumen of cows fed the solely grass silage diets, and a concomitant tendency for an increase in the molar proportion of acetate (Table 5). These observations could be related to the potential effects of the respective aNDF concentration of the diets and its potential influence on microbial population in the rumen. A greater dietary aNDF concentration fosters the growth of cellulolytic bacteria in the rumen and is expected to increase the acetate proportion or, inversely, decrease the propionate proportion in the rumen (Beauchemin et al., 2008; Fievez et al., 2012). The dietary NDF concentration as well as the acetate-to-propionate ratio in rumen fluid have been positively associated with most milk branched-chain FA (Vlaeminck et al., 2006). Accordingly, in the current experiment, milk fat concentrations of *iso* 14:0, *anteiso* 15:0, *iso* 16:0, *iso* 17:0, and *anteiso* 17:0 were greater when grass silage, and especially timothy silage, were fed as sole forages rather than with AS. Milk *iso* 15:0 concentration was also greater when timothy silage, and especially tall fescue silage, were fed as sole forages rather than in combination with AS. Milk fat concentration of branched-chain FA also increased when cows were fed TFH compared with TFS, which is in accordance with the increase in the molar proportion of acetate and the higher acetate-to-propionate ratio in rumen fluid. These results support the relationship between milk branched-chain FA and fermentation patterns occurring in the rumen.

CONCLUSIONS

Feeding established-lactation cows with tall fescue or timothy silages, offered either as the sole forage source

Table 6. Fatty acid composition of milk from cows fed diets with timothy silage (TS, 100%), tall fescue silage (TFS, 100%), tall fescue silage and alfalfa silage (TS + AS, 55:45%), tall fescue silage and alfalfa silage (TFS + AS, 55:45%), and tall fescue haylage (TFH, 100%); a more detailed fatty acid composition is available in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-18120>)

Item, % of total fatty acids	Diet						Contrast, <i>P</i> -value ¹			
	TS	TFS	TS + AS	TFS + AS	TFH	SEM	Species	Mixture	Species × Mixture	TFS vs. TFH
4:0	3.53	3.53	3.69	3.51	3.65	0.11	0.41	0.52	0.42	0.43
6:0	2.20	2.22	2.36	2.25	2.24	0.06	0.48	0.12	0.29	0.85
8:0	1.29	1.30	1.40	1.33	1.28	0.04	0.24	0.02	0.19	0.70
10:0	3.10	3.14	3.41	3.20	3.02	0.10	0.21	<0.01	0.07	0.19
12:0	3.81	3.83	4.16	3.94	3.66	0.12	0.15	<0.01	0.08	0.08
<i>iso</i> 13:0	0.027	0.029	0.025	0.026	0.029	0.001	0.14	<0.01	0.81	0.86
<i>anteiso</i> 13:0	0.016	0.018	0.018	0.020	0.018	0.001	0.06	0.04	>0.99	0.95
<i>iso</i> 14:0	0.162	0.137	0.138	0.124	0.147	0.006	<0.01	<0.01	0.21	0.13
14:0	12.79	12.69	12.98	12.60	12.70	0.20	0.02	0.61	0.20	0.92
<i>cis</i> -9 14:1	1.09	1.05	1.07	1.06	1.09	0.05	0.14	0.99	0.40	0.08
<i>iso</i> 15:0	0.273	0.279	0.233	0.233	0.301	0.008	0.61	<0.01	0.62	0.02
<i>anteiso</i> 15:0	0.482	0.474	0.445	0.451	0.569	0.013	0.92	<0.01	0.46	<0.01
15:0	1.21	1.26	1.36	1.37	1.27	0.04	0.34	<0.01	0.61	0.72
<i>iso</i> 16:0	0.306	0.261	0.255	0.243	0.280	0.011	<0.01	<0.01	0.06	0.11
16:0	32.34	32.80	32.64	33.30	32.39	0.66	<0.01	0.05	0.62	0.16
<i>cis</i> -9 16:1	1.32	1.31	1.29	1.39	1.32	0.09	0.25	0.45	0.14	0.76
<i>iso</i> 17:0 ²	0.259	0.238	0.226	0.227	0.278	0.009	0.02	<0.01	0.01	<0.01
<i>anteiso</i> 17:0 ³	0.388	0.379	0.361	0.373	0.452	0.009	0.88	<0.01	0.08	<0.01
17:0	0.455	0.478	0.498	0.517	0.494	0.011	<0.01	<0.01	0.77	0.16
<i>cis</i> -9 17:1	0.157	0.160	0.165	0.177	0.167	0.007	0.10	<0.01	0.28	0.26
<i>iso</i> 18:0	0.044	0.032	0.036	0.030	0.035	0.002	<0.01	0.01	0.11	0.24
18:0	9.76	9.69	8.91	8.88	9.77	0.33	0.33	<0.01	0.90	0.77
<i>trans</i> -6-8 18:1	0.301	0.322	0.298	0.306	0.300	0.013	0.27	0.48	0.62	0.24
<i>trans</i> -9 18:1	0.590	0.600	0.550	0.549	0.585	0.030	0.87	0.14	0.86	0.72
<i>trans</i> -10 18:1	0.309	0.367	0.342	0.370	0.322	0.016	<0.01	0.20	0.29	0.03
<i>trans</i> -11 18:1	0.960	1.041	0.897	0.877	0.942	0.045	0.38	<0.01	0.14	0.04
<i>trans</i> -12 18:1	0.152	0.173	0.188	0.189	0.174	0.013	0.43	0.06	0.46	0.98
<i>trans</i> -15 18:1	0.368	0.372	0.400	0.396	0.372	0.014	1.00	0.03	0.72	0.97
<i>trans</i> -16 18:1	0.248	0.262	0.312	0.301	0.282	0.014	0.87	<0.01	0.18	0.17
<i>cis</i> -9-10 18:1 ⁴	15.15	14.73	14.06	14.61	15.03	0.34	0.77	0.01	0.04	0.36
<i>cis</i> -11 18:1	0.534	0.530	0.535	0.519	0.517	0.021	0.59	0.80	0.75	0.63
<i>cis</i> -12 18:1	0.298	0.284	0.340	0.316	0.294	0.018	0.25	0.02	0.77	0.66
<i>cis</i> -13 18:1	0.103	0.083	0.100	0.081	0.089	0.010	0.06	0.83	0.93	0.64
<i>cis</i> -9, 12 18:2	1.09	1.02	1.10	1.13	1.04	0.04	0.42	0.03	0.06	0.62
<i>cis</i> -9, <i>trans</i> -11, 18:2	0.353	0.378	0.324	0.339	0.329	0.017	0.11	<0.01	0.67	<0.01
<i>cis</i> -9, 12, 15 18:3	0.374	0.350	0.434	0.429	0.392	0.011	<0.01	<0.01	0.08	<0.01
Sum										
de novo fatty acids ⁵	28.29	28.21	29.59	28.37	28.10	0.51	0.08	0.04	0.11	0.81
C16	33.94	34.39	34.22	34.97	33.98	0.72	<0.01	0.06	0.50	0.20
Preformed fatty acids ⁶	37.77	37.40	36.19	36.66	37.93	0.69	0.90	<0.01	0.29	0.34
Odd-chain fatty acids	1.98	2.09	2.23	2.28	2.09	0.06	0.07	<0.01	0.53	0.94
Branched-chain fatty acids	1.96	1.85	1.74	1.73	2.11	0.04	0.02	<0.01	0.04	<0.01

¹Preplanned contrasts: Species [TS + (TFS + AS) vs. TFS + (TFS + AS)]; Mixture [TS + TFS vs. (TS + AS) + (TFS + AS)]; interaction between Species and Mixture; and TFS vs. TFH.

²Coelution with minor concentration of *trans*-10 16:1.

³Coelution with minor concentration of *cis*-10 16:1.

⁴Coelution with *trans*-13/14 18:1.

⁵Sum of straight even-chain fatty acids from C6 to C14.

⁶Sum of branched-chain fatty acids (*iso* 13:0, *anteiso* 13:0, *iso* 14:0, *iso* 15:0, *anteiso* 15:0, *iso* 16:0, *iso* 17:0, and *anteiso* 17:0), odd-chain fatty acids (13:0 and 15:0), and all fatty acids with a chain length of 17 C or more.

in the diet or in combination with alfalfa silage, did not affect DMI, milk yield, or milk fat concentration. Feeding tall fescue or timothy grass silage in combination with alfalfa silage resulted in higher DMI and milk yield but lower milk fat concentration than feeding these silages as the sole forage source in the diet. Milk protein concentration was not affected by grass species when the grass silage was offered in combination with alfalfa silage, but was higher with timothy than tall fescue silage when they were offered as the forage source in the diet. Diets with tall fescue haylage rather than tall fescue silage resulted in lower DMI but similar milk yield and milk fat concentration, which suggests a greater feed use efficiency for cows fed tall fescue haylage. Our results confirm the potential of using tall fescue silage or haylage to replace timothy silage in dairy cow diets.

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