

Fatty acids in forages. I. Factors affecting concentrations

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Boufaïed, H., Chouinard, P. Y., Tremblay, G. F., Petit, H. V., Michaud, R. and Bélanger, G. 2003. **Fatty acids in forages. I. Factors affecting concentrations.** Can. J. Anim. Sci. **83**: 501–511. When forages represent a high proportion of ruminant diets they provide a significant quantity of fatty acids (FA). Effects of growth stage, fertilization, conservation method, growth period, species, and cultivar on forage FA were determined in four experiments. Concentrations of C16:0, C18:2, C18:3, and total FA (TFA) in timothy (*Phleum pratense* L.) decreased ($P < 0.01$), respectively, by 15, 16, 31, and 23% between stem elongation and early flowering. Nitrogen fertilization (120 vs. 0 kg N ha⁻¹) caused an increase ($P < 0.01$) of 18% of C16:0, 12% of C18:2, 40% of C18:3, and 26% of TFA concentrations. Phosphorus was not deficient and P fertilization (45 vs. 0 kg P ha⁻¹) had no significant effect on timothy FA concentrations. Wilting and drying decreased ($P < 0.01$) timothy C18:2, C18:3, and TFA concentrations. Concentrations of C18:2, C18:3, and TFA were higher in summer regrowth than in spring growth, primarily in orchardgrass (*Dactylis glomerata* L.) and timothy ($P < 0.01$). Significant variation for all FA concentrations was observed among 12 species ($P < 0.05$); on average, the C18:3, C18:2, and C16:0 accounted for 88% of TFA in studied species. Timothy was the only species in which the difference among cultivars was simultaneously significant ($P < 0.05$) for concentrations of C18:2, C18:3, and TFA. Among the grasses, an annual ryegrass (*Lolium multiflorum* Lam.) cultivar had the highest C18:3 concentration (20.6 mg g⁻¹ DM) whereas a timothy cultivar had the lowest (7.3 mg g⁻¹ DM) ($P < 0.05$). Among legumes, a white clover (*Trifolium repens* L.) cultivar had the highest C18:3 concentration (16.5 mg g⁻¹ DM) whereas an alfalfa (*Medicago sativa* L.) cultivar had the lowest (6.0 mg g⁻¹ DM) ($P < 0.05$). Polyunsaturated FA concentrations in forages can be increased by harvesting timothy at an early stage of development and as fresh grass, by increasing N fertilization of timothy, and by choosing species with higher FA concentrations such as white clover and annual ryegrass.

Key words: Fatty acids, forages, species, cultivar, growth stage, conservation methods

Boufaïed, H., Chouinard, P. Y., Tremblay, G. F., Petit, H. V., Michaud, R. et Bélanger, G. 2003. **Acides gras des fourrages. I. Facteurs affectant les concentrations.** Can. J. Anim. Sci. **83**: 501-511. Les plantes fourragères représentent une source importante d'acides gras (AG) dans les rations des ruminants. Les effets du stade de croissance, de la fertilisation, du mode de conservation, de la coupe, de l'espèce et du cultivar sur la concentration en AG des fourrages ont été mesurés dans quatre expériences. Les concentrations en C16:0, C18:2, C18:3 et acides gras totaux (AGT) ont diminué ($P < 0,01$) respectivement de 15, 16, 31 et 23% entre le début montaison et le début floraison chez la fléole (*Phleum pratense* L.). La fertilisation azotée (120 vs. 0 N kg ha⁻¹) a causé une augmentation ($P < 0,01$) de 18% du C16:0, 12% du C18:2, 40% du C18:3 et 26% des AGT. Le P n'étant pas déficitaire, la fertilisation en P (45 vs. 0 kg P ha⁻¹) n'a pas eu d'effet sur les concentrations en AG de la fléole. Les concentrations en C18:2, C18:3 et AGT de la fléole ont diminué ($P < 0,01$) avec le préfanage et le séchage. Les concentrations en C18:2, C18:3 et AGT étaient plus élevées en croissance d'été qu'en croissance de printemps et ce, surtout chez le dactyle (*Dactylis glomerata* L.) et la fléole ($P < 0,01$). Une variation significative entre 12 espèces a été observée pour tous les AG ($P < 0,05$); en moyenne, les C18:3, C18:2 et C16:0 représentaient 88% des AGT chez les espèces étudiées. La fléole était la seule espèce où la variation entre cultivars était significative à la fois pour le C18:2, le C18:3 et les AGT ($P < 0,05$). Chez les graminées, un cultivar de ray-grass annuel (*Lolium multiflorum* Lam.) avait la plus forte (20,6 mg g⁻¹ DM), alors qu'un cultivar de fléole avait la plus faible (7,3 mg g⁻¹ DM) concentration en C18:3 ($P < 0,05$). Chez les légumineuses, un cultivar de trèfle blanc (*Trifolium repens* L.) et un cultivar de luzerne (*Medicago sativa* L.) avait respectivement la plus élevée (16,5 mg g⁻¹ DM) et la plus faible (6,0 mg g⁻¹ DM) concentration en C18:3 ($P < 0,05$). La concentration en AG des fourrages peut être augmentée en récoltant la fléole à un stade précoce et sous forme d'herbe fraîche, en augmentant la fertilisation azotée des graminées, et en choisissant des espèces plus riches en acides gras comme le trèfle blanc et le ray-grass annuel.

Mots clés: acides gras, plantes fourragères, espèce, cultivar, stade de croissance, méthodes de conservation

Intake of dietary omega-3 FA, including α -linolenic acid (C18:3), is associated with a decreased risk of cardiovascular diseases and hyperlipidemia (Hebeisen et al. 1993). Therefore, maximizing C18:3 in milk and dairy products would benefit human health and nutrition. The C18:3 con-

centration in milk fat in dairy cows is influenced by C18:3 concentration in forage (Hebeisen et al. 1993). Consequently,

Abbreviations: CFU, colony forming units; DM, dry matter; FA, fatty acids; FM, fresh matter; LAB, lactic acid bacteria; LWR, leaf to weight ratio; NDF, neutral detergent fibers; TFA, total fatty acids

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Table 1. Fatty acid concentrations of timothy at four growth stages and two N fertilization applications (exp. 1)^z

Growth stage	N (kg ha ⁻¹)	Fatty acid (mg g ⁻¹ of DM)								
		C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TFA ^y
Stem elongation	0	0.07	0.13	3.27	0.35	0.41	1.11	3.97	8.71	18.01
	120	0.06	0.13	3.67	0.44	0.39	1.21	4.26	11.43	21.58
Early heading	0	0.07	0.13	2.96	0.30	0.35	0.92	3.51	6.86	15.09
	120	0.07	0.14	3.51	0.46	0.37	1.10	3.85	10.32	19.82
Late heading	0	0.06	0.12	2.82	0.30	0.33	1.05	3.39	6.37	14.42
	120	0.06	0.17	3.47	0.42	0.43	1.12	3.99	9.35	19.02
Early flowering	0	0.07	0.13	2.70	0.27	0.33	1.06	3.19	5.96	13.72
	120	0.07	0.16	3.17	0.36	0.34	1.09	3.71	7.90	16.80
SEM ^x		0.002	0.013	0.076	0.014	0.026	0.047	0.093	0.255	0.447
Source of variation					<i>Probabilities</i>					
Growth stage (GS)		†	NS	**	**	NS	NS	**	**	**
Linear effect (GS _L)		NS	NS	**	**	†	NS	**	**	**
Quadratic effect (GS _Q)		NS	NS	NS	*	NS	NS	NS	†	NS
P fertilization (0 vs. 45 kg P ha ⁻¹)		NS	NS	†	NS	NS	*	NS	NS	NS
Growth stage × P fertilization		NS	NS	NS	NS	NS	†	NS	NS	NS
N fertilization		NS	**	**	**	NS	**	**	**	**
Growth stage × N fertilization		*	NS	NS	*	NS	NS	NS	*	NS
N × P fertilization		NS	NS	NS	NS	NS	NS	†	†	†
N × GS × P		NS	NS	NS	NS	NS	NS	*	NS	NS

^zMeans for the two P fertilization applications are not presented because the effect of P fertilization was significant ($P < 0.05$) only for one FA as indicated in the lower part of this table.

^yTFA = total fatty acids.

^xStandard error of the mean.

† Non-significant at $0.10 > P \geq 0.05$, and *, ** significant at $P < 0.05$, $P < 0.01$, respectively; NS, non-significant at $P \geq 0.10$.

agronomic practices affecting the FA concentration of forages could impact the C18:3 content of milk.

Forages provide substantial lipids and FA in ruminant diets (Harfoot and Hazlewood 1988). Lipids represent up to 8% of the leaf DM in forage plants (Harfoot 1981). They are often localized in leaf chloroplasts (Jarrige et al. 1995), which contain 22 to 25% lipids on a DM basis. Complex lipids constitute most leaf tissues, mainly as glycolipids and phospholipids (Harwood 1980; Harfoot and Hazlewood 1988). The esterified lipids in forages represent two-thirds of the total lipids (5% of DM); their composition is 33% simple lipids (diglycerides, free FA, waxes, and sterol esters), 50% galactolipids (mono- and digalactosyldiglycerides), and 17% phospholipids (Bauchart et al. 1985). The FA composition of forage lipids is dominated by high proportions of polyunsaturated linolenic and linoleic acids (C18:2) (Harfoot and Hazlewood 1988), but also small amounts of oleic acid (C18:1) (Harfoot 1981). In fresh grasses, C18:3 represents between 50 and 75% of TFA (Hawke 1973); C18:2 and palmitic acid (C16:0) are the next most abundant (McDonald et al. 1988). In ryegrass, C18:3 represents 55 to 66% of TFA but only 40% in alfalfa (Bauchart et al. 1985). Lough and Anderson (1973) found that C18:3 was the major component of esterified FA (simple and complex lipids) in mixed pasture grasses including ryegrass (*Lolium multiflorum* Lam.), timothy (*Phleum pratense* L.), and meadow fescue (*Festuca pratensis* Huds.). In ensiled grasses, however, C18:3 was present in small proportions as esterified FA, but absent from complex lipids.

The FA concentration in forages depends on many factors, including species and senescence (Harwood 1980; Harfoot and Hazlewood 1988), growth stage (Bauchart et al. 1984), conservation method (Lough and Anderson 1973;

Yang and Fujita 1997), as well as wilting, shading, and silage additives (Dewhurst and King 1998). In grasses, FA concentration is increased by N fertilization (Jarrige et al. 1995). Timothy is the most important perennial forage grass species in eastern Canada but little is known about the factors affecting its FA concentration.

The overall objective of this research was to study the variability in FA concentrations of forage crops. We measured the FA concentrations of: (1) different species and cultivars in spring and summer, (2) timothy grown under different N and P fertilizer applications, (3) timothy harvested at different growth stages, and (4) timothy conserved using different methods.

MATERIALS AND METHODS

Four experiments were conducted to determine the FA concentrations of several forages under different conditions.

Experiment 1: Growth Stage with N and P Fertilization

The objective of this experiment was to measure FA concentrations in timothy at several growth stages, and how they were affected by N and P fertilization (Table 1). Sixty-four plots (3.25 m²) of timothy (cv. Champ), sown in 1998 at Saint-Joseph-de-la-Pointe-de-Lévy (Lat. 46°48'N, Long. 71°05'W), received two levels of N fertilizer (0 and 120 kg N ha⁻¹; calcic ammonium nitrate) in combination with two levels of P fertilizer (0 and 45 kg P ha⁻¹; triple superphosphate) prior to the start of growth in spring 1999. Plots were harvested at a 5-cm height with a forage plot harvester at four growth stages: stem elongation of timothy on 2 June, early heading on 9 June, late heading on 16 June, and early flowering on 23 June 1999. The harvested material had ini-

Table 2. Fatty acid concentrations of timothy harvested following different methods (exp. 2)

Treatment	Fatty acid (mg g ⁻¹ of DM)								
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TFA ^z
1. Fresh grass (230 g DM kg ⁻¹)	0.06	0.11	3.42	0.42	0.37	1.11	4.54	9.26	19.29
2. Wilted grass (400 g DM kg ⁻¹)	0.04	0.10	3.11	0.37	0.30	0.71	3.85	8.11	16.60
3. Grass hay (850 g DM kg ⁻¹)	0.11	0.10	3.14	0.37	0.32	0.84	3.77	8.25	16.91
4. Haylage (400 g DM kg ⁻¹)	0.05	0.12	3.41	0.39	0.36	0.78	4.47	9.28	18.87
5. Haylage with LAB ^y inoculant (10 ⁵ CFU ^x g ⁻¹ of FM)	0.04	0.11	3.37	0.39	0.33	0.75	4.46	8.98	18.44
6. Haylage with LAB inoculant (10 ⁶ CFU ^x g ⁻¹ of FM)	0.05	0.11	3.25	0.39	0.34	0.74	4.30	8.60	17.78
7. Haylage with formic acid (2 L Mg ⁻¹ of FM)	0.05	0.11	3.29	0.40	0.33	0.71	4.34	8.85	18.07
8. Haylage with formic acid (6 L Mg ⁻¹ of FM)	0.05	0.11	3.19	0.38	0.31	0.71	4.12	8.38	17.24
9. Silage (230 g DM kg ⁻¹)	0.05	0.11	3.64	0.44	0.34	0.82	4.98	10.31	20.70
10. Silage with LAB inoculant (10 ⁵ CFU ^x g ⁻¹ of FM)	0.05	0.11	3.62	0.43	0.33	0.85	4.82	9.69	19.90
11. Silage with LAB inoculant (10 ⁶ CFU ^x g ⁻¹ of FM)	0.05	0.11	3.60	0.44	0.34	0.82	4.69	9.69	19.75
12. Silage with formic acid (2 L Mg ⁻¹ of FM)	0.05	0.11	3.52	0.43	0.33	0.77	4.78	10.03	20.02
13. Silage with formic acid (6 L Mg ⁻¹ of FM)	0.05	0.11	3.55	0.44	0.38	0.90	4.87	10.12	20.42
SEM ^w	0.007	0.003	0.059	0.014	0.017	0.037	0.078	0.236	0.397
Source of variation	<i>Probabilities</i>								
Treatment comparisons									
1 vs. 2, fresh grass vs. wilted grass	NS	**	*	**	*	**	**	**	**
1 vs. 3, fresh grass vs. grass hay	**	*	**	*	†	**	**	**	**
1 vs. 4, fresh grass vs. haylage	NS	NS	NS	NS	NS	**	NS	NS	NS
1 vs. 9, fresh grass vs. silage	NS	NS	*	NS	NS	**	**	**	*
2 vs. 4, wilted grass vs. haylage	NS	**	**	NS	*	NS	**	**	**
3 vs. 4, grass hay vs. haylage	**	**	**	NS	NS	NS	**	**	**
3 vs. 9, grass hay vs. silage	**	*	**	**	NS	NS	**	**	**
4 vs. 9, haylage vs. silage	NS	NS	**	*	NS	NS	**	**	**
(5, 6, 10, 11) vs. (4, 9), with vs. without LAB inoculant	NS	NS	NS	NS	NS	NS	†	**	*
(7, 8, 12, 13) vs. (4, 9), with vs. without formic acid	NS	NS	*	NS	NS	NS	**	*	*
(5, 10) vs. (6, 11) 10 ⁵ vs. 10 ⁶ CFU ^x of LAB g ⁻¹ FM	NS	NS	NS	NS	NS	NS	†	NS	NS
(7, 12) vs. (8, 13), 2 vs. 6 L formic acid Mg ⁻¹ FM	NS	NS	NS	NS	NS	NS	NS	NS	NS

^zTFA = total fatty acids.

^yLAB = Lactic acid bacteria (*Lactobacillus plantarum*).

^xCFU = colony forming units.

^wStandard error of the mean.

† Non-significant at 0.10 > P ≥ 0.05, and *, ** significant at P < 0.05, P < 0.01, respectively; NS, non-significant at P ≥ 0.10.

tial DM concentrations of 230, 210, 250, and 310 g DM kg⁻¹ of fresh matter (FM) at the four growth stages, respectively.

Data on timothy FA concentrations were analyzed by ANOVA using the GLM procedure of the SAS Institute, Inc. (1985) according to a split-split plot design with four replicates per treatment. Growth stages of timothy were assigned to the main plots, P fertilization to the sub-plot, and N fertilization to the sub-sub plot. Quantitative contrasts were defined for each factor. For growth stages, linear and quadratic components were calculated based on sampling dates, which were equally spaced in time. For this experiment and the following ones, statistical testing was done at 5% significance unless noted otherwise.

Experiment 2: Timothy in Conserved Forages

In this experiment, we studied the influence of conservation methods on the FA concentration of timothy (cv. Champ). Twelve conservation methods, plus fresh grass as a control, were applied to four replicates of harvested timothy (Table 2). A timothy field (148 m²) at Saint-Joseph-de-la-Pointe-de-Lévy (Lat. 46°48'N, Long. 71°05'W) was fertilized with 80 kg N ha⁻¹ on 28 May; it was harvested with hand clippers at the early heading stage on 14 June 1999, leaving a 5-cm stubble. The forage yield for the harvested surface was approximately 2.95 t of DM ha⁻¹. The fresh forage material (230 g DM kg⁻¹) was separated into two parts.

One part was spread out uniformly on wire netting; it was field-wilted for either a few hours to reach 400 g DM kg⁻¹ FM (wilted grass), or for 3 d to obtain 850 g DM kg⁻¹ FM (grass hay). The second part of the fresh material and the wilted grass were used to make silage. These forages were chopped to a theoretical length of 13 mm with a home-made electric chopper. The chopped material was distributed in plastic trays in portions of 2 kg of FM. Formic acid was diluted with water at two concentrations: 2/18 and 6/14 (vol/vol) solutions. A lactic acid bacteria (*Lactobacillus plantarum*) inoculant (Biomax 5, CHR Hansen, Biosystems, Milwaukee, WI) was diluted with sterile water at two concentrations: 0.05 and 0.50 g L⁻¹. The four solutions were sprayed at 20 mL kg⁻¹ of FM on corresponding forage trays to make silages containing either 2 or 6 L formic acid Mg⁻¹ of FM, or 10⁵ or 10⁶ colony forming units (CFU) g⁻¹ of FM. Similarly, water was sprayed at the same rate on wilted and fresh material to make control haylage and silage. Then, the content of each tray was hand mixed with the specified additives. Approximately 1.1 to 1.4 kg FM of chopped material, treated with water or additives, was placed in a polyvinyl chloride laboratory silo (40-cm length, 7.5-cm inside diameter) sealed with a plastic cap at one end. To simulate compaction in silos, a pressure of 1200 kPa was applied to the ensiled forage with a hydraulic cylinder. After filling and compacting the material, the silos were sealed with plastic

Table 3. Fatty acid concentrations of forage species in spring growth and summer regrowth (exp. 3)

Species	Growth	Fatty acid (mg g ⁻¹ of DM)								TFA ^z
		C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	
Orchardgrass	Spring	0.06	0.09	2.83	0.28	0.29	0.73	2.60	7.76	14.68
	Summer	0.10	0.13	3.49	0.41	0.34	0.74	3.47	11.81	20.52
Timothy	Spring	0.04	0.10	2.83	0.27	0.28	1.12	3.15	6.76	14.59
	Summer	0.07	0.13	3.62	0.43	0.41	1.13	4.63	11.16	21.63
Alfalfa	Spring	0.08	0.13	4.08	0.37	0.75	1.12	3.53	6.97	17.06
	Summer	0.12	0.17	4.35	0.48	0.84	1.09	3.67	6.73	17.48
Red clover	Spring	0.06	0.12	3.73	0.46	0.74	1.56	3.82	8.61	19.13
	Summer	0.12	0.17	4.63	0.65	0.88	1.54	4.52	9.39	21.94
SEM ^y		0.008	0.011	0.165	0.019	0.019	0.093	0.183	0.387	0.783
Source of variation		Probabilities								
Species (contrasts)										
Grasses vs. Legumes		**	**	**	**	**	**	**	**	†
Timothy vs. Orchardgrass		*	NS	NS	NS	NS	**	**	†	NS
Alfalfa vs. Red clover		NS	NS	NS	**	NS	**	*	**	**
Growth		**	**	**	**	**	NS	**	**	**
Growth × Species (contrasts)										
Growth × (Grasses vs. Legumes)		*	NS	NS	NS	NS	NS	**	**	**
Growth × (Timothy vs. Orchardgrass)		NS	NS	NS	NS	*	NS	†	NS	NS
Growth × (Alfalfa vs. Red clover)		NS	NS	†	NS	NS	NS	NS	NS	NS

^zTFA = total fatty acids.^yStandard error of the mean.

† Non-significant at 0.10 > P ≥ 0.05, and *, ** significant at P < 0.05, P < 0.01, respectively; NS, non-significant at P ≥ 0.10.

caps equipped with a pressure release valve. Laboratory silos were kept at 38°C for 2 wk, at 30°C for the next 2 wk, at 25°C for 2 more weeks, and then at room temperature (20 to 23°C) to complete 60 d of fermentation. Then, silos were opened and emptied. Where necessary, the mouldy portions of silage at both ends of the silo were discarded. The remaining silage was frozen (-20°C) and lyophilized (model 50 SRC; Virtis Co., Gardiner, NY). Samples of fresh grass, wilted grass, and grass hay were also frozen and lyophilized.

Data were analyzed as a completely randomized design with four replicates per treatment using the GLM procedure of the SAS Institute, Inc. (1985). A set of contrasts was defined a priori for testing differences among treatments (Table 2).

Experiment 3: Growth Period and Species

Fatty acid concentrations of alfalfa (*Medicago sativa* L. 'AC Caribou'), red clover (*Trifolium pratense* L. 'AC Charlie'), orchardgrass (*Dactylis glomerata* L. 'Okay'), and timothy (cv. Champ) harvested in both spring growth and summer regrowth were examined (Table 3). A trial with four replicates was established at the Jean-Charles-Chapais Farm of Agriculture and Agri-Food Canada at Saint-David-de-l'Auberivière (Lat. 46°46'N, Long. 71°11'W) in 1997. For spring growth, all forage plots (1.5 m × 1.5 m) were harvested at a 5-cm height on 14 June 1999 when alfalfa and red clover were at the early flowering stage (10% bloom), timothy at the 50% heading stage, and orchardgrass at the flowering stage. For summer regrowth, all forage plots were harvested 36 d later, on 20 July 1999. Plots were fertilized with 16 kg P ha⁻¹ as triple phosphate and 62 kg K ha⁻¹ as muriate of potash after the first harvest. Grass plots also

received 50 kg N ha⁻¹ as calcic ammonium nitrate.

Data were analyzed by ANOVA using the GLM procedure of the SAS Institute, Inc. (1985) as a randomized complete block design with the REPEATED option where the growth period was considered a repeated measurement on each plot. Orthogonal contrasts were defined to compare: grasses to legumes, the two grasses (timothy vs. orchardgrass), and the two legumes (alfalfa vs. red clover).

Experiment 4: Species and Cultivars

The FA concentration was determined in different forage species and cultivars grown at the Normandin Research Farm of Agriculture and Agri-Food Canada (Lat. 48°51'N, Long. 72°32'W) in 1999. Twelve forage species, including eight grasses and four legumes with a total of 34 cultivars (Table 4), were harvested from 7.5 m² plots (1.5 m × 5 m). The annual ryegrass was seeded on 14 May and harvested on 15 July 1999 at a vegetative stage and when it reached a 25-cm height. All perennial grasses were seeded in 1998, but Kentucky bluegrass (*Poa pratensis* L.) was seeded in 1996 and tall fescue (*Festuca arundinacea* Schreb.) in 1997. Perennial grasses were harvested when they reached the early heading stage: meadow fescue, meadow bromegrass (*Bromus riparius* Rehmann), and Kentucky bluegrass on 4 June; orchardgrass and smooth bromegrass (*B. inermis* Leyss.) on 7 June; the timothy cultivars Tiller and Toro on 9 June; tall fescue on 10 June; and the timothy cultivars Champ and Climax on 14 June 1999. The legumes were harvested at 10% bloom: white clover (*Trifolium repens* L.) on 4 June; birdsfoot trefoil (*Lotus corniculatus* L.) on 10 June; alfalfa between 21 and 23 June; and red clover on 25 June.

Table 4. Fatty acid concentrations of forage species and cultivars (exp. 4)

Family	Species	Cultivar	Fatty acid (mg g ⁻¹ of DM)								TFA ^z	
			C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3		
Grasses	Annual rygrass	Maris Ledger	0.05	0.11	4.79	0.90	0.49	0.89	3.97	20.56	31.77	
		Aubade	0.06	0.13	4.63	0.70	0.41	1.34	4.01	15.22	26.50	
	Meadow fescue	Mimer	0.07	0.12	4.27	0.69	0.32	1.69	3.43	12.73	23.32	
		Epic	0.06	0.13	4.20	0.67	0.31	1.40	3.48	11.89	22.13	
		Bartura	0.06	0.10	4.08	0.59	0.27	1.71	3.39	11.26	21.46	
	Orchardgrass	Benchmark	0.06	0.11	4.09	0.58	0.40	0.78	3.72	12.28	22.02	
		Juno	0.05	0.12	3.99	0.54	0.37	0.78	3.64	11.47	20.97	
		Kay	0.05	0.09	3.76	0.56	0.35	0.73	3.86	11.40	20.80	
		Okay	0.06	0.10	3.90	0.54	0.38	0.82	3.69	11.01	20.50	
	Tall fescue	Kokanee	0.05	0.08	4.03	0.62	0.35	1.04	2.77	11.82	20.77	
		Montebello	0.06	0.09	3.98	0.62	0.35	1.04	2.69	11.60	20.43	
		Courtenay	0.05	0.08	3.68	0.58	0.33	0.91	2.61	10.83	19.07	
	Smooth bromgrass	Bravo	0.05	0.08	3.55	0.52	0.28	0.64	3.32	10.46	18.90	
		Saratoga	0.05	0.08	3.31	0.45	0.23	0.56	3.18	9.41	17.27	
	Timothy	Toro	0.05	0.14	3.68	0.56	0.39	1.14	4.12	9.63	19.71	
		Tiller	0.05	0.15	3.62	0.52	0.37	1.17	3.89	9.00	18.78	
		Champ	0.05	0.14	3.45	0.43	0.36	1.06	3.72	7.87	17.07	
		Climax	0.05	0.14	3.33	0.42	0.35	1.08	3.60	7.30	16.26	
	Kentucky bluegrass	Troy	0.05	0.10	3.61	0.57	0.37	1.05	3.31	8.23	17.30	
Rosy		0.05	0.09	3.77	0.67	0.31	0.99	2.89	8.14	16.92		
Meadow bromgrass	Paddock	0.05	0.07	3.32	0.46	0.22	0.78	2.98	8.18	16.07		
	Fleet	0.05	0.07	3.40	0.47	0.20	0.81	2.82	7.92	15.75		
Legumes	White clover	California	0.07	0.13	5.22	0.88	0.84	1.46	4.62	16.52	29.76	
		Merit	0.07	0.11	4.74	0.77	0.82	1.44	4.65	15.55	28.13	
	Trefoil	Leo	0.70	0.20	5.09	0.57	0.65	1.68	4.71	11.64	25.25	
		Upstart	0.69	0.19	4.79	0.57	0.60	1.37	4.29	11.63	24.13	
	Red clover	AC Charlie	0.05	0.09	4.16	0.58	0.80	1.66	5.11	9.54	22.00	
		Marino	0.06	0.11	4.11	0.53	0.80	1.67	5.30	9.29	21.88	
		Walter	0.06	0.11	4.05	0.57	0.83	1.73	4.94	9.27	21.56	
		Atlas	0.06	0.10	3.92	0.53	0.79	1.85	4.61	8.86	20.72	
	Alfalfa	120	0.08	0.14	4.25	0.43	0.78	0.87	3.75	6.91	17.20	
		Arrow	0.08	0.14	4.10	0.40	0.76	1.16	4.05	6.90	17.59	
		Oneida VR	0.08	0.14	4.00	0.38	0.73	1.00	3.59	6.33	16.24	
		5262	0.07	0.13	4.02	0.38	0.76	1.02	3.61	6.04	16.03	
	LSD _{0.05} ^y		0.04	0.02	0.40	0.08	0.05	0.29	0.31	1.20	2.00	
	SEM ^x		0.014	0.007	0.143	0.030	0.019	0.102	0.110	0.425	0.707	
	Source of variation			<i>Probabilities</i>								
	Species (contrasts)											
	Grasses vs. legumes			**	**	**	†	**	**	**	**	**
	Ryegrass vs. other grasses			NS	†	**	**	**	NS	**	**	**
	Timothy vs. orchardgrass			NS	**	*	*	NS	**	NS	**	**
Alfalfa vs. red clover			*	**	NS	**	*	NS	**	**	**	
Cultivars within species			NS	*	NS	**	*	NS	**	**	**	

^zTFA = total fatty acids.^yLSD_{0.05} = least significant difference ($P = 0.05$) when comparing means within the same species.^xSEM = standard error of the mean when comparing means within the same species.† Non-significant at $0.10 > P \geq 0.05$, and *, ** significant at $P < 0.05$, $P < 0.01$, respectively; NS, non-significant at $P \geq 0.10$.

The annual ryegrass received 22 kg N ha⁻¹, 39 kg P ha⁻¹, and 75 kg K ha⁻¹ as a 5-20-20 mixed fertilizer at seeding and 30 kg N ha⁻¹ as calcic ammonium nitrate at the two- to four-leaf stage of growth. Meadow bromegrass and meadow

fescue received 30 kg N ha⁻¹, tall fescue 40 kg N ha⁻¹, Kentucky bluegrass 41 kg N ha⁻¹, timothy 51 kg N ha⁻¹, and smooth bromegrass and orchardgrass 57 kg N ha⁻¹. For perennial grasses, N fertilization was applied prior to the

start of growth in spring 1999, but a maintenance P and K fertilization was applied in fall 1998.

Data were analyzed with Genstat 5 (Genstat 5 Committee 1993) according to a split-plot experimental design with species as main plots and cultivars as subplots. Replicate plots were nested within species for practical reasons; the calculated error variance for species would be less than that for completely randomized or randomized complete-block designs. Simple orthogonal contrasts were used to test differences between grasses and legumes, annual ryegrass and all perennial grasses, timothy and orchardgrass, and alfalfa and red clover. Cultivar means, the SEM, and LSD (5%) were calculated. Because all species were grown at different sections in the field, a preliminary statistical analysis was done to check for any systematic trends across the field. Only five grass species (timothy, orchardgrass, smooth bromegrass, meadow fescue, and meadow bromegrass) were used for this analysis because they were seeded the same year and they were located across the length of the field. The five grass species were numbered in relation to their position in the field. The positions in the field were considered as the main plots and the cultivars as subplots. The degrees of freedom for the positions were partitioned into three components: linear, quadratic, and deviations. The deviation term was used to test the linear and quadratic components. The linear and quadratic components were not significant, which indicates no systematic trends across the field; the calculated error variance for species would not be seriously underestimated.

Chemical Analyses

Fresh forage samples (500 g of FM) from expts. 1, 3, and 4 were dried at 55°C in a forced-air oven for approximately 2 d. Dried samples and all lyophilized samples from exp. 2 were ground through a 1-mm screen (Standard Model 3, Arthur H. Thomas. Co, Philadelphia, PA).

Forage samples from the first experiment were mineralized using a mixture of sulfuric acid and selenious acids, as described by Isaac and Johnson (1976), and total N concentration was measured on a QuickChem 8000 Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI) using a method based on salicylate, hypochlorite, and sodium nitroprusside. The NDF of forage samples were determined using the ANKOM Fiber Analyzer (Model No: Ankom 200, Ankom Technology, Fairport, NY), using sodium sulfite and α -amylase in the NDF procedure.

Fatty acids of ground forage samples were extracted and methylated by a one-step procedure using toluene as solvent (Sukhija and Palmquist 1988). Methyl nonadecanoate was used as an internal standard. Fatty acid methyl esters were quantified by gas chromatography using a HP 5890 chromatograph (Hewlett Packard Co., Palo Alto, CA), under the following conditions: 60-m \times 0.32-mm DB-23 capillary column, 0.25 μ m film thickness, H₂ as carrier gas, 2.8 cm³ min⁻¹ volumetric flow rate, injector split 1/100 at 240°C, septum purge vent at 2 mL min⁻¹, flame ionization detector at 250°C, and 15 kPa of heat pressure. The initial temperature was 150°C, which was increased 5°C min⁻¹ up to 200°C. Fatty acid methyl esters in toluene were directly

injected through the split injection port. Peak area of each FA was measured using a Turbochrom 3 analytical system (version 3.3; PE Nelson, Cupertino, CA). Each peak was identified and quantified using pure methyl ester standards (Alltech, Deerfield, IL).

RESULTS AND DISCUSSION

Growth Stage (Experiment 1)

Concentrations of C16:0, C16:1, C18:2, C18:3, and TFA decreased with advancing maturity, whereas concentrations of C12:0, C14:0, C18:0, and C18:1 were not significantly affected by maturity in timothy (Table 1). The TFA concentration in timothy declined linearly from the first to the fourth growth stage. This result agrees with Gervais and St-Pierre (1979) who found a reduction in ether extract concentration with advancing maturity. They reported concentrations of timothy ether extract of 46, 33, 23, 20, 18, and 13 mg g⁻¹ DM at the vegetative, stem elongation, boot, heading, blooming, and seed ripening stages. Another Québec study reported a similar decline in ether extract concentration with increasing maturity in orchardgrass and smooth bromegrass (Gervais 1991). Brouwer (1944) also observed that the concentration of ether extract in grass fell from 53 mg g⁻¹ DM at the 5-cm height growth stage to 32 mg g⁻¹ DM at the seed ripening stage; TFA concentration decreased from 37 to 16 mg g⁻¹ DM.

The proportion of leaves in the forage, expressed by the leaf to weight ratio (LWR), decreases with maturity in timothy (Bélanger and McQueen 1996) and stems have half to one-third of the FA concentration of leaves (Jarrige et al. 1995). Therefore, the decrease in the proportion of leaves with advancing maturity may explain, in part, the decline in concentrations of C16:0, C16:1, C18:2, C18:3, and TFA from stem elongation to early flowering.

N and P Fertilization (Experiment 1)

Nitrogen fertilization increased C14:0, C16:0, C16:1, C18:1, C18:2, C18:3, and TFA concentrations (Table 1). Concentrations of C12:0 and C18:0 were not affected by N fertilization. The effects of N fertilization depended on growth stage for C12:0, C16:1, and C18:3; the decrease in C16:1 and C18:3 concentrations with maturity occurred at an earlier growth stage when timothy was not fertilized with N. An increase of TFA with N fertilization was also reported in orchardgrass; Lefebvre et al. (1967) reported that high N applications (240 kg N ha⁻¹) in orchardgrass increased total lipids and TFA concentrations in leaves compared with lower levels of N fertilization (24, 50, and 120 kg N ha⁻¹).

In the present study, P fertilization had no significant effect on concentrations of total and individual FA, except for that of C18:1 (Table 1), which was 6.1% lower ($P = 0.03$) in P-fertilized plots; P was not deficient because P fertilization had no significant effect on DM yield (data not shown).

The majority of lipids and FA in forage plants are localized in the chloroplasts of leaf tissues, which also contain all the pigments, chlorophyll, and carotenoids (Jarrige et al. 1995). Bélanger and McQueen (1998) reported that LWR decreased in timothy with increased levels of N fertilization.

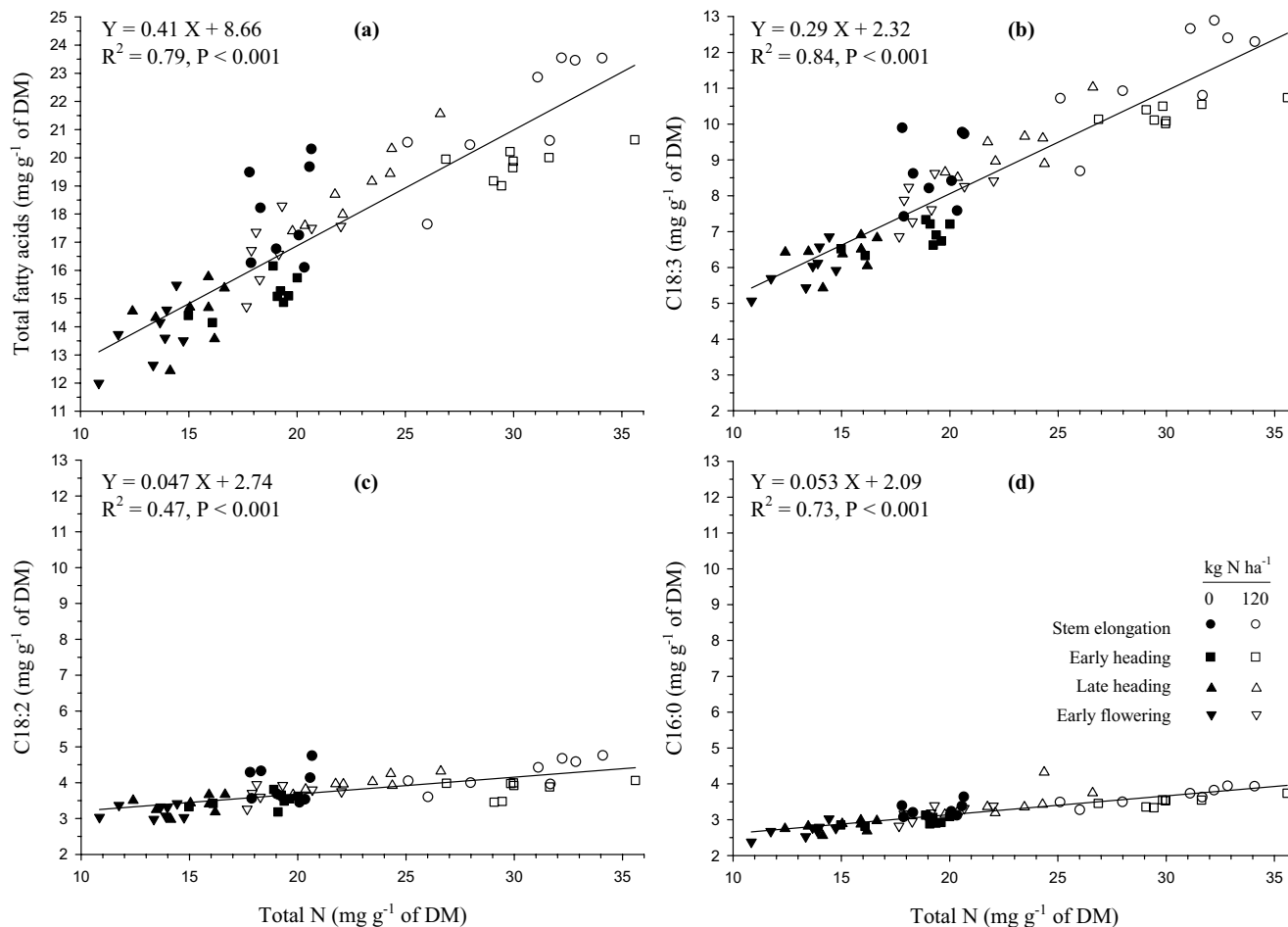


Fig. 1. Relationship between concentrations of total N and total fatty acids (a), C18:3 (b), C18:2 (c), and C16:0 (d) in timothy harvested at four growth stages and fertilized with two levels of N and two levels of P. Data points (64) from each N application (2), P application (2), growth stage (4), and replicates (4) are presented. The effect of P fertilization (0 vs. 45 kg P ha⁻¹) on total fatty acid concentration was not significant, and is not shown in this figure.

Therefore, the increase in TFA concentration of timothy after N application cannot be explained by an increase of the LWR. Under the conditions for the present experiment, NDF concentration, or structural component of the forage biomass, was less with 120 kg N ha⁻¹ (603 mg g⁻¹ DM) than when no N was applied (627 mg g⁻¹ DM). Conversely, the metabolic component, which corresponds to the forage biomass without the structural components, consists essentially of cellular contents (Bélanger et al. 2001) and was greater when N was applied. In the present experiment, total N concentration in timothy varied from 10.8 to 35.6 mg g⁻¹ DM and it was higher in plots fertilized with 120 kg N ha⁻¹ (25.6 mg g⁻¹ DM) than in control plots (16.5 mg g⁻¹ DM). Furthermore, a linear relationship was found between timothy total N concentration and TFA, C18:3, C18:2, and C16:0 concentrations (Fig. 1). These results are consistent with those of Kemp et al. (1965), which show a linear relationship between crude protein and FA concentrations in samples of permanent pastures, orchardgrass, perennial ryegrass, and hay. The increase in the metabolic component,

including chloroplasts, with N fertilization could cause a greater synthesis and accumulation of lipids and FA in the plant. Nitrogen fertilization could also have a direct effect on the FA metabolism in the plant. However, further research is required to confirm these hypotheses.

Our results indicate that C18:2, C18:3, and TFA concentrations in timothy are greatest at early stages of development with N fertilization.

Conservation Methods (Experiment 2)

The conservation treatments applied to timothy after harvest had a significant effect on most FA concentrations (Table 2). Fresh grass had higher concentrations of C14:0, C16:0, C16:1, C18:1, C18:2, C18:3, and TFA than wilted grass and grass hay. Fresh grass contained higher concentrations of C18:1 than haylage and silage. Haylage contained higher concentrations of C14:0, C16:0, C18:2, C18:3, and TFA compared with wilted grass and grass hay, but lower concentrations of C16:0, C16:1, C18:2, C18:3, and TFA than silage.

The decrease in the majority of FA in wilted grass and grass hay compared with fresh grass (contrasts 1 vs. 2 and 1 vs. 3, Table 2), the decreases in C14:0, C16:0, C18:2, C18:3, and TFA concentrations in hay compared with haylage and silage (contrasts 3 vs. 4 and 3 vs. 9), plus the decrease in concentrations of C16:0, C16:1, C18:2, C18:3, and TFA in haylage as compared to silage (contrast 4 vs. 9) were related to the effects of wilting. Dewhurst and King (1998) reported similar results in perennial ryegrass, which was wilted 2 or 68 h on a laboratory bench before ensiling. Extended wilting of ryegrass leads to a significant reduction in total and individual FA, with a marked reduction in the proportion of C18:3. In orchardgrass, Yang and Fujita (1997) reported a slight decrease in the amount of total and individual FA, especially C18:2 and C18:3, in hay as compared with fresh grass. In the present experiment, the lower concentrations of C16:1, C18:1, C18:2, C18:3, and TFA in wilted than in fresh grass would be due, in part, to oxidative degradation and, therefore, the loss of unsaturated FA during wilting (Dewhurst and King 1998). Brouwer (1944) reports that a moderate destruction of unsaturated FA was observed during artificial drying and conservation of dried grass. Thus, the wilting process of forage prior to ensiling is a major factor in substantial losses of FA.

Silage had higher concentrations of C16:0, C18:2, C18:3, and TFA than fresh grass. This increase was significant but probably of minor biological importance. This effect was likely due to either a loss of some components (such as volatile FA and CO₂) during fermentation or a loss of soluble components in silage effluent, with a resultant increase in concentrations of other components. Mayne and Gordon (1986) reported that DM losses during ensiling were 13.4% in unwilted and 6% in wilted perennial ryegrass silages stored in 100-t-capacity bunker silos. With very wet forage, effluent DM losses can exceed 10%, whereas very little effluent is produced with forage ensiled at DM contents of 250 to 350 g kg⁻¹ (McDonald et al. 1991). The DM content of typical silage effluent varies between 20 and 100 g kg⁻¹ DM (Graves and Vanderstappen 1993). In the current experiment, there was no production of effluent in haylage made from wilted grass ensiled at 400 g kg⁻¹ DM. Therefore, we can assume that a small loss of DM occurred in haylage, which could explain, in part, the lack of difference in TFA concentrations between haylage and fresh grass.

Lough and Anderson (1973) report that *trans* C18:2, *trans* C18:1, and C16:0 concentrations were higher in esterified FA of ensiled mixed grasses than in fresh grasses. The presence of *trans* isomers of C18:2 and C18:1 and the lower proportion of C18:3 in silage may be related to an isomerisation and biohydrogenation of unsaturated FA occurring in silage (Lough and Anderson 1973). In the present study, the concentration of C18:3 was higher in silage than in fresh grass. No *trans* isomers of C18:2 or C18:1 were detected in silage, even though the method used allowed the detection of these fatty acids (Chouinard et al. 1997), which indicates a low rate of unsaturated FA biohydrogenation during silage fermentation.

Silage Additives (Experiment 2)

Haylage and silage inoculated with LAB (10⁵ and 10⁶ CFU g⁻¹ FM) had lower concentrations of C18:3 and TFA than haylage and silage without LAB inoculant (Table 2). Decreased C16:0, C18:2, C18:3, and TFA concentrations occurred with added formic acid to haylage or silage. Dewhurst and King (1998) reported a restricted fermentation when formic acid or formalin was added to wilted and unwilted perennial ryegrass silages, which led to lesser quantities of acetic, lactic, and butyric acids. They observed small but significant differences in FA concentration with lower proportions of C18:2, C18:3, and TFA when formalin was added to ryegrass silage as compared with control silage. The lower fermentation observed with silage additives may reduce the loss of fermentable components. Although not determined in the present experiment, the reduced fermentation and DM loss may explain the lower concentration of TFA in silage inoculated with LAB or treated with formic acid as compared with silage without additives. The rate of LAB inoculant (10⁵ vs. 10⁶ CFU g⁻¹ FM) had no significant effect on individual FA concentrations in haylage and silage except for C18:2, which tended to be lower with the higher dose of inoculant. Furthermore, there was no significant difference between the two additions of formic acid (2 vs. 6 L Mg⁻¹ FM) for the concentration of individual FA in haylage and silage ($P > 0.10$).

Growth Period and Species (Experiment 3)

Individual FA and TFA concentrations of orchardgrass, timothy, and red clover were significantly ($P < 0.01$) higher in summer regrowth than in spring growth except for C18:1 (Table 3). The growth period × (grasses vs. legumes) interaction was significant for C18:2, C18:3, and TFA, due principally to a larger increase in the concentration of these FA for grasses than legumes. Total FA concentration was higher in summer regrowth than in spring growth by 48.0% in timothy and 39.7% in orchardgrass, but by only 14.7% in red clover and 2.5% in alfalfa. Greater TFA concentration in grass species in the summer regrowth contradicts the results of a study conducted on perennial ryegrass; Bauchart et al. (1984) in France found that the TFA concentration was at a maximum when ryegrass was harvested at the primary growth (32 mg g⁻¹ DM), declined slowly during the first regrowth (22 mg g⁻¹ DM), decreased more quickly during the stemmy second regrowth (12 mg g⁻¹ DM), but increased sharply during the leaf regrowth (31 mg g⁻¹ DM) period.

Grasses and legumes differed significantly ($P < 0.01$) in concentrations of individual FA. The two grasses, orchardgrass and timothy, contained more C18:3 than the two legumes, alfalfa and red clover, primarily in summer regrowth, but concentrations of C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, and C18:2 were higher in legumes than in grasses. Consequently, the TFA concentration did not differ between grasses and legumes. Timothy had higher concentrations of C18:1 and C18:2, but a lower concentration of C12:0 than orchardgrass. For the two legumes, red clover had greater concentrations of C16:1, C18:1, C18:2, C18:3, and TFA than alfalfa.

The forage lipids are predominantly of leaf origin (Harfoot 1981) and leaf proportion is important in determining FA concentration. Dewhurst et al. (2001) analyzed the FA composition of three ryegrass species cultivated in Aberystwyth, UK, over a growing season, with three or five cuts. All species had high concentrations of FA and a high proportion of C18:3 during vegetative growth (late April) and FA levels declined markedly after this date, recovering by autumn. Concentrations of FA were highest in early and late season but lowest during summer months; they suggest that this pattern of change can be explained by the leaf proportion. Bélanger and McQueen (1998) reported that the proportion of leaves in timothy was higher in summer regrowth (LWR = 0.5) than in spring growth (LWR = 0.3). In alfalfa, Tremblay et al. (2002) reported that the LWR of 27 cultivars seeded in three replicates during 2 yr and harvested during the 2 subsequent production years was lower in spring growth (0.39) than in summer regrowth (0.45). Onstad and Fick (1983) also reported a lower LWR in spring than in regrowth of alfalfa at the same physiological age. Consequently, the greater difference of the LWR between spring growth and summer regrowth in timothy than in alfalfa in the cited studies could explain, in part, the greater difference in TFA concentration between the growth periods for timothy.

Species and Cultivar (Experiment 4)

Species varied significantly for individual and total FA concentrations (Table 4). The C18:3, C18:2, and C16:0 were the three most abundant FA; for all cultivars, they accounted for 88% of TFA (Table 4). These results are consistent with those reported previously indicating that the major FA of forage plants are represented quantitatively by C18:3, C18:2, and C16:0, but C18:1 and C16:1 are present in lower concentrations (Harwood 1980). Harfoot and Hazlewood (1988) reported that the FA composition of forage fat was dominated by the polyunsaturated FA C18:3 and C18:2. The concentration of C18:1 is generally minor in leaves as compared with C18:2 and C18:3 (McDonald et al. 1988).

The concentration of C18:3 varied from 6.04 mg g⁻¹ in 5262, an alfalfa cultivar, to 20.56 mg g⁻¹ of DM in Maris Ledger, an annual ryegrass cultivar. The concentration of C18:2 ranged from 2.61 mg g⁻¹ of DM in Courtenay, a tall fescue cultivar, to 5.30 mg g⁻¹ of DM in Marino, a red clover cultivar. The C16:0 concentration varied from 3.31 mg g⁻¹ of DM in Saratoga, a smooth bromegrass cultivar, to 5.22 mg g⁻¹ of DM in California, a white clover cultivar. The C18:1, C18:0, C16:1, C14:0, and C12:0 FA were present in lower concentrations; their concentrations varied from 0.55 to 1.85 mg g⁻¹ of DM for C18:1, from 0.20 to 0.84 mg g⁻¹ of DM for C18:0, from 0.38 to 0.90 mg g⁻¹ of DM for C16:1, from 0.07 to 0.20 mg g⁻¹ of DM for C14:0, and from 0.05 to 0.70 mg g⁻¹ of DM for C12:0. The TFA concentrations ranged from 15.75 mg g⁻¹ of DM in Fleet, a meadow bromegrass cultivar, to 31.77 mg g⁻¹ of DM in Maris Ledger.

Legumes had higher concentrations of C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, and TFA and lower concentrations of C18:3 than grasses (Table 4). However, a large vari-

ation was observed among species within each family (grasses and legumes). The annual ryegrass had higher concentrations of C16:0, C16:1, C18:0, C18:2, C18:3, and TFA compared with perennial grasses evaluated in this study. Fertilization practices were different for annual than perennial grasses, and annual ryegrass was harvested on 15 July at a vegetative stage when it reached a 25-cm height, but the first harvest of all other grasses was taken between 4 and 14 June at the early-heading stage. The difference in management practices may explain, in part, the higher concentration of individual and total FA in annual ryegrass as compared with other grasses.

In a study with eight temperate grass species, Dewhurst et al. (2001) reported that orchardgrass had the lowest C18:1 concentration and timothy had the highest C18:2 concentration. In a second study comparing three ryegrass (*Lolium*) species over a growing season, they noticed that all species had high concentrations of FA and a high proportion of C18:3 during vegetative growth in late April and that FA levels declined markedly after this date, recovering by autumn. This report was similar to the results of our fourth experiment in which annual ryegrass had higher TFA, C16:0, and C18:3 concentrations than all perennial grasses, and orchardgrass had lower C18:1 concentration than timothy but similar amounts of C18:2. In our third experiment, however, orchardgrass had lower C18:1 and C18:2 concentrations than did timothy.

The cultivar within species source of variation was significant for C14:0, C16:1, C18:0, C18:2, C18:3, and TFA concentrations. Based on the LSD test, the difference among cultivars was significant in annual ryegrass and timothy for TFA concentration, in annual ryegrass, meadow fescue, orchardgrass, and timothy for C18:3 concentration, and in timothy, Kentucky bluegrass, trefoil, red clover, and alfalfa for C18:2 concentration. Timothy was the only species in which the difference among cultivars was simultaneously significant for all C18:2, C18:3, and TFA concentrations. The early-maturing timothy cultivars Toro and Tiller had high concentrations, the medium-maturing cultivar Champ had intermediate ones, and the late-maturing cultivar Climax had low C18:2, C18:3, and TFA concentrations. Bartura, a meadow fescue cultivar, contained less C18:3 than Mimer. Among the orchardgrass cultivars, Benchmark had a higher C18:3 concentration than Okay. Rosy, a Kentucky bluegrass cultivar, had a lower concentration of C18:2 than Troy. Leo, a trefoil cultivar, contained more C18:2 than Upstart. The red clover cultivars Walter, Marino, and AC Charlie had higher concentrations of C18:2 than Atlas. In alfalfa cultivars, Arrow had a higher concentration of C18:2 than Oneida VR and 5262.

The annual ryegrass cultivar Maris Ledger had higher concentrations of TFA and C18:3 than Aubade. Maris Ledger and Aubade are two different types of ryegrass grown as summer annuals in the Atlantic provinces of eastern Canada. Maris Ledger is an Italian ryegrass cultivar, which is used for pasture and is suitable for close grazing, whereas Aubade is a Westerwolds ryegrass cultivar used for silage and pasture and is suitable for cutting and grazing. These two annual ryegrasses differed considerably in their

growth. When grown as summer annual grass, Italian ryegrass (Maris Ledger) remains at a vegetative stage and is leafy with few stems; leaves make up 60 to 80% of the whole crop. Westerwolds ryegrass (Aubade) is stemmy at maturity and has a leaf content of 40 to 60% (Kunelius 1991). Since FA in forages are predominantly of leaf origin (Harfoot 1981), the higher proportion of leaves in Maris Ledger than Aubade could explain its higher TFA concentration.

The above results showing a difference in FA composition and TFA concentration among cultivars within a species suggest that selection for those parameters might be possible. Further research is required to determine the impact of increased C18:3 concentration in forages on the milk C18:3 content.

CONCLUSIONS

The C18:3, C18:2, and C16:0 were the most abundant FA in all forage species and they averaged, respectively, 51, 18, and 19% of TFA. Concentrations of C16:0, C18:2, C18:3, and TFA in timothy decreased, respectively, by 15, 16, 31, and 23% between stem elongation and early flowering stage. In timothy, fertilization of 120 kg N ha⁻¹, compared with none, increased concentrations of C16:0, C18:2, C18:3, and TFA by 18, 12, 40, and 26%, respectively, whereas P fertilization had no significant effect on FA concentration. The wilting process of timothy prior to ensiling is a significant factor in substantial losses of FA. Significant variation among species was observed for all FA concentrations and this variation was as large within grasses as within legumes. In grasses, an annual ryegrass cultivar had the highest C18:3 concentration, whereas a timothy cultivar had the lowest. In legumes, a white clover cultivar and an alfalfa cultivar had, respectively, the highest and the lowest C18:3 concentrations. Concentrations of C18:2, C18:3, and TFA were higher in summer regrowth than in spring growth and the increase was more important in grasses than in legumes. Increasing polyunsaturated FA in forages can be achieved by harvesting timothy at an early stage of development and as fresh grass, by increasing N fertilization of timothy, or by choosing species with higher FA concentration such as white clover or annual ryegrass.

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