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Brief Report

Variation of Fatty Acids in Cool-Season Grasses

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Abstract: Cool-season grass samples were collected and analyzed for omega-6 and omega-3 fatty acid (FA) content. Perennial ryegrass, tall fescue, orchardgrass, and Kentucky bluegrass samples were collected on four harvest dates from various trials conducted in the eastern portion of the Appalachian Mountains. There was a greater change in linolenic acid (C18:3) than linoleic acid (C18:2) concentrations in all forage species and across seasons. Perennial ryegrass had higher levels of linolenic acid compared to the other grasses on most dates other than in August, when it did not provide forage. Linoleic acid concentrations changed less across seasons and were generally lower in tall fescue compared to the other grass species, which tended to contain similar levels. There was a species × date interaction on FA concentrations. Kentucky bluegrass had a peak concentration of linoleic acid in August. Concentration in orchardgrass fluctuated slightly across seasons, while concentrations in tall fescue and perennial ryegrass decreased as the season advanced. Identification of FA concentrations in plant species and managing species diversity in pastures to increase and stabilize the content of omega-3 FA in meat and milk products appears to be a valuable tool for managers to manipulate FA characteristics of products from pasture-based systems.

Keywords: fatty acids; forage; omega-3; omega-6; conjugated linoleic acid; CLA

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1. Introduction

For many years, consumers in industrialized countries have displayed an aversion to dietary fat. For anticipated health reasons, nutritional guidelines advocate a decrease in the consumption of saturated fat [1,2]. However, animal products such as meat and milk are also a source of unsaturated FA, particularly omega-3 FA, vaccenic acid (VA), and conjugated linoleic acids (CLA) [2,3]. Interest in omega-3 FA, VA, and CLA has increased over the last 50 years due to the potential health benefits related to their consumption [4–10]. These FAs are essential in that the human body cannot synthesize them, and they must be obtained directly from diet [11].

Feeding animals feedstuffs rich in omega-3 and omega-6 FA provides the opportunity to manipulate the FA composition in animal products [11–13]. Forages are important low-cost sources of omega-3 and omega-6 FA, precursors of VA and CLA found in animal products [11,14]. A ratio of no more than 4:1, omega-6 to omega-3 FA is considered essential for a healthy human diet [11,15]. The ratio of omega-6 to omega-3 FA in grass-fed beef has been shown to have a favorable relationship of 2:1 [15]. Products high in CLA content may be related to a favorable ratio of these essential FA [2]. The Omega-3 FA, linolenic acid, is the major FA in fresh pasture, constituting 50–75% of its total [2,8,14,16,17] and is most useful for enhancing milk and beef FA quality [13]. This FA enables specific

microflora development that increases the formation and deposition of CLA in tissue [18]. Linolenic acid is also the precursor to two other FA, eicosapentaenoic acid (C20:5) and docosahexaenoic acid (20:6) [9], which are found in fish oil. Linoleic, an omega-6 FA, is the second most prominent FA (18%) in fresh forage and is a precursor to VA and CLA in milk and meat [2,13,14,19]. These FA are formed from incomplete ruminal biohydrogenation of linoleic acid [5,8,9,11,14,19,20]. Animals grazing fresh forage have higher levels of omega-3 FA and CLA in their milk and meat products [5,10,20–24] compared to those consuming conserved forages [5,6,9,24–26] or grain [11,20,27].

The effect of fresh forage consumption on omega-3 FA and CLA in animal products depends mostly on two processes, increasing the supply of precursor FA in the forage crop and reducing the extent of biohydrogenation in the rumen [28]. Biohydrogenation of FA in the rumen is catalyzed by linoleic acid isomerase produced by ruminal bacterium *Butyrivibrio fibrisolvens* [29], whose growth and activity may be affected by dietary intake of linoleic acid and rumen conditions [20]. French et al. [20] suggested that the growth of *B. fibrisolvens* was favored by grass in the diet due to more rapidly fermentable sugar and soluble fiber found in grass, thus promoting a greater production or a decreased utilization of CLA by this ruminal bacterium.

Research suggests that FA composition in plants varies with species [2,3,17,25,30], maturity [16,31,32], season [12,17,33,34], temperature [35,36], conservation methods [6,8,10,26,34,37], fertilization [31,38], and to some extent cultivar [38]. The number and timing of grazing events affect the FA composition of forage [2,9,25,33,34] and management that inhibits the initiation of flowering increases FA levels [19].

Clapham et al. [2], comparing 3 different cool-season forage species grown under green-house conditions that have potential applications in an Appalachian pasture used for finishing beef cattle, observed significant differences in the FA profile of grasses species. Nevertheless, this study reports no field data. Phillip et al. [9] found that cool-season annual forages may be used to extend the grazing season into early spring in cool climates. However, no autumn grazing was investigated in this study. In Canada, Mir et al. [39] study three cool-season species grown in the early part of the season and found a significant species × date interaction from May to June on FA concentration in those grasses. Moreover, in Boufaïed's studies [28,38], cool-season grasses were also tested in the spring and early summer. It was found that harvesting forage at an early stage of growth and choosing species with high FA concentration could be used as a strategy to increase the FA profile in forages. Nonetheless, these studies were run in early spring and early summer, lacking further data for the whole growing season. Dewhurst et al. [25] ran two experiments: one with orchardgrass and tall fescue species as cool-season grasses from July to November, the other with three perennial ryegrass from April to November with three perennial ryegrasses, and observed leaf as an important factor in determining FA concentration. Despite their contribution, only two grass species were tested for the summer and fall harvest. Although their second experiment was run for the whole grazing year, only data for perennial ryegrass was presented. To date, few data are available on the FA profile in perennial cool-season species grown under field conditions during the whole grazing season. This study aimed to measure the content of linoleic and linolenic acid as major FA representatives of omega-3 and omega-6, respectively, and total FA in cool-season perennial grass species present in pastures in the Appalachian Mountains to determine how their content is affected by species and season year long and examine implications for the animal-derived product. These naturalized grasslands are a major source of feed for grazing livestock, with the potential to extend the grazing season into early spring and late fall in this cool mountain climate.

2. Materials and Methods

This study was conducted at the Western Maryland Research and Education Center, Keedysville, Maryland, to measure FA concentration in cultivars of cool-season grass species during the 2002 growing season. Grass stands were seeded in September 1999.

Fertilizer phosphorous and potassium were applied based on soil tests and University of Maryland recommendations. Nitrogen was applied at 336 kg ha^{-1} year^{-1}, as ammonia nitrate, in equal split applications, one in the spring and three after the 1st, 2nd, and 3rd harvest. Fresh forage samples were taken from an experiment containing pure stands of cool-season grasses. The experimental plots were one by three meters in size and replicated three times. The plots were cut four times during the year (9 May, 7 June, 27 August, and 7 November). Grasses were in a vegetative state at each harvest. The species and cultivars collected were: perennial ryegrass (*Lolium perenne* L.): Mara, Belramo, Grand Daddy; tall fescue (Schedonorus arundinaceous (Schreb.) Dumort, previously known as *Festuca arandinacea Schreb*): Barcel, Select, Martin #2; orchardgrass (*Dactylis glomerata* L.): Cambria, Benchmark, Haymate; and Kentucky bluegrass (*Poa pratense* L.): Libertador. Tall fescue and orchardgrass had a three-year yield of 3360 kg DM ha^{-1} (Confidence interval (CI) 376) and 2660 kg DM ha^{-1} (CI 336), respectively. Perennial ryegrass had a two-year yield of 2274 kg DM ha^{-1} (CI 378). Kentucky bluegrass was productive in only one year, yielding 1283 kg DM ha^{-1}. Forage samples were collected with clippers and cut at a 3.8 cm height above the soil. All harvested forage was removed from the plot. Approximately 300 g of forage were collected for FA determination, and 700 g were collected for conventional forage analyses. Immediately after the samples were cut, they were placed in Tyvek bags and placed in a cooler with dry ice. Samples for conventional forage analyses were placed in zipper-top plastic bags and placed on regular ice. Samples for FA determination were preserved in a cooler at −52 °C until they were freeze-dried. Then they were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) to pass a 1 mm screen and stored in plastic bags at −52 °C. These forage samples were analyzed for FA content using near-infrared spectroscopy (NIRS) analysis (Foss System 6500) at the USDA-ARS, Beaver, WV laboratory [40]. Conventional forage composition samples were dried at 60 °C, ground in a Wiley Mill pass to a 1-mm screen, and stored in plastic bags until sent to a commercial forage testing laboratory (Dairy One, Inc. Ithaca, NY, USA) for NIRS analyses. Forage species, cultivars, and harvest date for FA composition were analyzed in a complete randomized design. The GLM procedure in NCSS-2002 statistical software [41] was used to test for species, cultivar, and date effect on forage crude protein (CP) and FA composition. The significance test was at $p < 0.05$, and species and date were fixed effect variables.

3. Results

There were species, date, and interaction effects ($p < 0.05$) on FA concentrations in the sampled forage. When cultivars within species were tested using ANOVA, there were no varietal differences within species ($p = 0.648$). Kentucky bluegrass and orchardgrass had higher levels ($p < 0.001$) of linoleic acid than tall fescue and perennial ryegrass (Table 1). Perennial ryegrass and orchardgrass had higher levels ($p < 0.001$) of linolenic acid than did Kentucky bluegrass and tall fescue. The contribution of linolenic acid to the total FA in the plant material was between 43–62%, whereas linoleic acid content was 17–26%, both within the normal range of 50–75% and 11–22%, respectively, for cool-season forages. Total FA content was greatest ($p < 0.001$) in perennial ryegrass followed in descending order by orchardgrass, Kentucky bluegrass, then tall fescue. Forage CP content was highest ($p < 0.001$) in Kentucky bluegrass, followed by perennial ryegrass, orchardgrass, and tall fescue. Across species and dates, the correlation ($p < 0.001$) between CP and linoleic, CP and linolenic, and CP and total FA were 0.39, 0.32, and 0.45, respectively.

Table 1. Crude protein (CP) and Fatty Acid (FA) content (g/kg dry matter, mean ± SE) in grass species.

Species	N	CP	C18:2 †	C18:3 ††	Total FA
K. bluegrass	12	237 ± 4 ^{a}	8.1 ± 0.2 ^{a}	13.1 ± 0.6 ^{c}	30.1 ± 0.8 ^{b}
T. Fescue	35	183 ± 3 ^{d}	4.9 ± 0.1 ^{d}	12.2 ± 0.3 ^{d}	23.2 ± 0.5 ^{c}
Orchardgrass	34	207 ± 3 ^{c}	7.3 ± 0.1 ^{b}	17.0 ± 0.3 ^{b}	31.8 ± 0.5 ^{b}
P. Ryegrass	25	214 ± 3 ^{b}	5.5 ± 0.2 ^{c}	20.0 ± 0.4 ^{a}	32.1 ± 0.6 ^{a}

† linoleic acid, †† linolenic acid; N = sample size; means with different superscripts within the same column differ ($p < 0.05$).

FA production (kg ha^{-1}) was calculated using the average annual dry matter yield for each species (Table 2). Tall fescue and orchardgrass had higher ($p < 0.001$) production of linoleic acid (C18:2) compared to the other two species. Linolenic acid (C18:3) production was highest ($p < 0.001$) for perennial ryegrass and orchardgrass, followed by tall fescue, and lowest for Kentucky bluegrass. Total FA production was highest ($p < 0.001$) for orchardgrass, followed by tall fescue, perennial ryegrass, and Kentucky bluegrass.

Table 2. Production of fatty acids per area (kg ha^{-1}) in grass species.

Species	N	C18:2 †	C18:3 ††	Total FA
K. bluegrass	12	10.4 ^{d}	16.8 ^{c}	38.6 ^{d}
T. Fescue	35	16.5 ^{b}	41.0 ^{b}	78.0 ^{b}
Orchardgrass	34	19.4 ^{a}	45.2 ^{a}	84.6 ^{a}
P. Ryegrass	25	12.5 ^{c}	45.5 ^{a}	73.0 ^{c}

† linoleic acid, †† linolenic acid; N = sample size; means with different superscripts within the same column differ ($p < 0.05$).

The content of CP and FA in these grasses was affected by date (Table 3). Linoleic acid was higher in May and August and lower in June and November ($p < 0.001$). Linolenic acid content was highest in November, with lower values in August, May, and June ($p < 0.001$). Total FA concentrations were highest in the fall and late summer ($p < 0.001$). The percentage of CP was highest ($p < 0.001$) in the spring and declined in the summer, with a slight increase in late summer and fall.

Table 3. Crude protein (CP) and Fatty Acid (FA) content (g/kg dry matter, mean ± SE) by date in grass species.

Date	N	CP	C18:2 †	C18:3 ††	Total FA
May 8	29	254 ± 3 ^{a}	6.8 ± 0.1 ^{b}	14.3 ± 0.4 ^{c}	28.5 ± 0.5 ^{b}
June 7	30	186 ± 3 ^{d}	6.2 ± 0.1 ^{c}	12.6 ± 0.4 ^{d}	25.3 ± 0.5 ^{c}
August 27	20	193 ± 3 ^{c}	6.9 ± 0.2 ^{a}	16.4 ± 0.5 ^{b}	31.3 ± 0.6 ^{b}
November 7	27	209 ± 3 ^{b}	5.8 ± 0.2 ^{d}	18.9 ± 0.4 ^{a}	32.2 ± 0.5 ^{a}

† linoleic acid, †† linolenic acid; N = sample size; means with different superscripts within the same column differ ($p < 0.05$).

Kentucky bluegrass peaked in linoleic acid concentration in August, while orchardgrass fluctuated across seasons (Table 4). Tall fescue and perennial ryegrass concentration of linolenic acid decreased as the season advanced. In all species, linolenic acid concentrations were highest in the fall ($p < 0.001$).

Table 4. Crude protein (CP) and Fatty Acid (FA) content (g/kg dry matter, mean ± CI) interaction by grass species and date.

Species	Date	N	CP	C18:2 †	C18:3 ††	Total FA
K. bluegrass	8 May	3	282 ± 38	7.2 ± 1.9	12.0 ± 5.0	27.0 ± 6.9
	7 June	3	216 ± 38	7.0 ± 1.9	12.9 ± 5.0	27.5 ± 6.9
	27 August	3	201 ± 38	10.3 ± 1.9	10.6 ± 5.0	32.6 ± 6.9
	7 November	3	251 ± 38	7.8 ± 1.9	16.7 ± 5.0	33.5 ± 6.9
T. fescue	8 May	9	230 ± 12	5.5 ± 0.6	12.1 ± 1.5	23.2 ± 2.1
	7 June	9	190 ± 12	5.0 ± 0.6	11.0 ± 1.5	21.7 ± 2.1
	27 August	9	139 ± 12	4.9 ± 0.6	9.3 ± 1.5	21.5 ± 2.1
	7 November	8	174 ± 19	4.1 ± 0.6	16.3 ± 1.7	26.4 ± 2.3
Orchardgrass	8 May	9	249 ± 12	7.8 ± 0.6	15.3 ± 1.5	31.1 ± 2.1
	7 June	9	174 ± 12	6.6 ± 0.6	12.9 ± 1.5	25.7 ± 2.1
	27 August	8	189 ± 13	7.7 ± 0.6	17.9 ± 1.7	33.7 ± 2.3
	7 November	8	214 ± 14	6.9 ± 0.6	22.0 ± 1.7	36.5 ± 2.3
P. ryegrass	8 May	8	254 ± 13	6.8 ± 0.6	17.8 ± 1.7	32.7 ± 2.3
	7 June	9	163 ± 13	6.4 ± 0.6	13.7 ± 1.5	26.3 ± 2.1
	27 August	0				
	7 November	8	198 ± 13	4.3 ± 0.6	20.5 ± 1.7	32.3 ± 2.3

† linoleic acid, †† linolenic acid; CI = confidence interval (p = 0.05); N = sample size.

4. Discussion

4.1. Total FA

There has been an increasing interest in the FA profile consumed by humans, especially regarding the value of increasing the content of CLA, VA, and omega-3 FA in the diet. This has impacted the interest in developing forage systems that manipulate the FA composition in pasture-raised beef products [2]. The FA profiles within cool-season grasses vary greatly. Dewhurst et al. [25] observed that FA profiles differed among forage species when they received the same management acknowledging a strong genetic basis. In their experiment, they compared three ryegrasses over a growing season involving three or five cutting dates. They observed a higher concentration of FA for all species during the vegetative stage (late April), with a sharp decline during the summer months and an increase during the late season (October-November). They suggested that the decline in concentrations of FA was associated primarily with flowering, which in this case, the ryegrasses tested flowered in late May. They also indicated that there is a potential for grass breeders to produce grasses high in lipids through genetic manipulation of flowering times and flowering propensity, suggesting the importance of management to profit from genetic differences. They also observed that FA content declined when the regrowth interval was expanded (from 20 to 38 days) and suggested the importance of leaf proportion in determining FA concentrations in forage. Harvesting at an early stage of plant development and practicing a frequent harvest regime may result in the grass with a higher FA concentration than later harvest with expanded cut intervals. Bauchart et al. [33] also reported high concentrations of FA in perennial ryegrass during the primary growth in the spring and the third regrowth in the autumn, and a profound decline during the summer, and indicated that the latter parallels the stemmy regrowth. Other research has also reported the concentration of FA to be highest in spring and autumn, with the lowest values during the summer [9,16,17,25,33], which follow the same trend observed in our study. Gilliard et al. [42] expressed that seasonal fluctuations were more compelling than the difference among variety at any given cutting date, pointing to the fact that pasture management and most probably climatic factors are more relevant than variety selection for FA concentration in forages. Dias et al. [43] reported that the increase in FA levels in spring and autumn harvest dates might be due to the influence of lower temperatures on the activation of the desaturase enzymes responsible for inserting double bonds into FA, a process that favors photosynthesis and promotes cold tolerance. Cyril et al. [36] suggested that either the desaturase activity or

the gene expression responsible for synthesizing one or more desaturase enzymes is cold temperature regulated.

Contrary to the increased levels of FA in the spring and the fall harvest, Dewhurst et al. [34] found a progressive increase in total FA concentration from May to November in perennial ryegrass. Their results could be explained by the frequent cutting regime (eight cuts) in their work that would have maintained vegetative growth, and perhaps the flowering process was interrupted. Their findings indicated that the number and grazing cycles affect FA composition in the pasture. Moreover, their plots were fertilized with 52 kg N ha^{-1} per cut date. It has been proposed that N fertilization brings metabolic changes in the plant compared to its structure that could lead to greater production and acquisition of FA in the leaf tissue chloroplast [38]. However, in a recent study, Phillips et al. [9] suggested that soil N levels likely do not influence the FA profile variation in forages.

Boufaïed et al. [38] found that the concentration of FA in orchardgrass was higher during the summer than in the spring, which is similar to our August versus May results for orchardgrass [38]. Mir et al. [39] observed a fat concentration decline in orchardgrass, perennial ryegrass, and tall fescue over the 140 days of sampling during the spring season, followed by a marginal increase in the three forages tested. They commented that as the season progresses, stem material may increase, followed by a further increase in leaf material, thus causing the sharp decline in fat content followed by the increase observed for those forage species.

Crude protein content is frequently reported in forage FA studies and is considered the variable that best predicts total FA content in grasses [13,43]. Glasser et al. [13] expressed that it could be partially explained because CP and FA share the same location in the leaf tissue. Hawke [32] found that short succulent perennial ryegrass composed mainly of leaf tissue, which is characterized by high CP content, contained more lipid than did mature forage, which holds a greater proportion of stems. He indicated that as plants mature, the total FA content decreases, and the proportion of saturated FA increases. Glasser et al. [13] reported that the vegetation stage of forage at harvest is the main factor influencing the FA levels in forages. Thus, NDF increases with advancing maturity, and it is typically lowest in leaf tissue and an important negative correlate of forage FA. Boufaïed et al. [28] observed a decrease in total FA with advancing maturity in timothy. They indicated that the forage lipids are predominantly of leaf origin, and leaf proportion is important in determining FA concentration. Thus, the proportion of leaves decreases in timothy as it matures, with an increase in stems which have half to one-third of the FA concentration of leaves. Phillips et al. [9] point out that as grasses advance in maturity, the leaf-to-stem ratio decreases, which results in a decrease in the membrane lipids of the forage. Phillip et al. [9], after reviewing the literature, suggested that forage species and maturity are the major factors that impact the variation of the total FA contents in forages.

In our study, due to the species-by-date interactions, mixed species pastures may provide a more uniform supply of FA across the growing season in the Appalachian Mountains. This especially applies to perennial ryegrass, which provides the highest concentration of the desired FA when it is available. However, it did not provide harvestable forage in August of the study year. When used in mixtures with other species, perennial ryegrass can provide high FA content when in production. Grass mixture in the pasture can provide forage and desirable FA when perennial ryegrass is not productive during the summer heat.

4.2. Linoleic Acid (C18:2) and Linolenic Acid (C18:3)

Dewhurst and Scollan [16] looked at the changes in FA profile in grass species and varieties during three months of the year (November, July, and August) under a frequent (9-cut) grazing schedule. They found that in a vegetative stage, linolenic acid concentration was greater in Italian and hybrid ryegrasses, intermediate in perennial ryegrass, and lowest in tall fescue. Their findings followed the same trend as our November result, in that perennial ryegrass showed the highest concentration of linolenic acid on that cutting

date. In the following year, they observed less variability in the levels of linolenic acid among harvest dates and commented that this variation might be related to the capability of different species to bloom under the cutting management used. Flowering would have lowered the content of FA in a pasture along with linolenic acid as the main FA component [19,25]. Dewhurst et al. [34] highlighted the importance of avoiding flowering, maintaining high concentrations of linolenic acid in the plant. Glasser et al. [13] found higher amounts of linoleic and linolenic acid in perennial ryegrass, followed by tall fescue, orchardgrass, and timothy. They stated that the vegetative stage was the main variable influencing linolenic acid at harvest in forages. Consistently, Mir et al. [39] reported a higher concentration of linolenic acid in young, vegetative perennial ryegrass as compared with tall fescue. However, they observed higher concentrations of linoleic acid in orchardgrass compared to perennial ryegrass or tall fescue sampled on all cutting dates through the early growing season. Hawke's study [32] found that perennial ryegrass at a young growth stage had a greater amount of linolenic acid and lower amounts of linoleic acid compared to a mature growth stage for each harvest date (September-October, October, November) and suggested that the linolenic acid level was related to the higher lipid content at that growth stage. The fact that linoleic concentration increased with maturity could be explained by the higher levels of linoleic acid in stems rather than leaves [43]. Boufaïed et al. [38] observed that linoleic and linolenic acid concentration in timothy decreases as maturity increases, suggesting that this was because of a decrease in the proportion of leaves in the total plant material. In general, the literature reports a higher proportion of linolenic acid in pastures with the greatest proportion of leaves and CP content [13,43]. When forage grows older, CP content decreases along with total FA and linolenic acid, and the plant experiences an increase in NDF content as well as linoleic acid in total FA [13]. In another experiment, Boufaïed et al. [31] compared linoleic and linolenic acid concentrations among eight cool-season forage species harvested at early heading. They found that timothy, Kentucky bluegrass, meadow brome (Bromopsis biebersteinii (Roem. & Schult.)), and smooth bromegrass had lower concentrations of linolenic acid than tall fescue, orchardgrass, and meadow fescue (*Schedonorus pratensis* or *Festuca pratensis*). In contrast, ryegrass showed the greatest concentrations of this FA in their trial. The linoleic acid concentration was lower for tall fescue compared to the other species, and less variability of this FA was observed among the species tested during their trial.

Boufaïed et al. [31] observed seasonal effects on linolenic acid levels with higher levels in the summer than the spring growth and suggested that it may be due to a higher leaf-to-stem ratio in summer regrowth, as observed for total FA content. The effect of the date on the change of the concentrations of linoleic acid was not as pronounced as linolenic. Diaz [43] reported a greater proportion of linoleic acid in tropical pastures with the highest proportion of leaves regardless of their growth stage. The percentage of linolenic acid in their study varied from 48.7 to 64.7% of the total FA, being at a lower range than the usual percentage in temperate climate plants. They commented that for the survival of plants in cold temperatures, synthesis of unsaturated FA takes place, thus, contributing to the maintenance of membrane fluidity in the chloroplast. Linoleic and linolenic acid is the dominant FA in chloroplast thylakoid membranes and therefore account for the majority of the FA present in pasture plants [43,44]. Research has demonstrated that unsaturated linoleic and linolenic FA are essential for recovering photosystem II complex from a low temperature in the chloroplast thylakoid membranes [45]. The cis double bonds of these unsaturated FA do not pack closely in the thylakoid membrane, and therefore do not crystallize and freeze easily, thereby enhancing membrane fluidity [35,45].

In our research study, there were species, date, and interaction effects on FA concentrations in the sampled forage. Mir et al. [18] reported a species-by-date interaction in a spring trial where the linolenic acid concentration was higher for perennial ryegrass than orchardgrass and tall fescue, declining for all species progressively over the cut-dates. Their results match our May values on those species and dates, where linolenic acid declines towards the summer months. Their observations for linoleic acid levels in orchardgrass

showed an increase across the four cutting dates compared to perennial ryegrass and tall fescue, which were similar on all dates. Dewhurst et al. [25] reported a phylogenetical closeness between ryegrasses and fescues, and this pattern is repeated across the literature, including our study. Clapham et al. [2], in their greenhouse trial, observed that plant material, harvest date, and their interaction had a significant effect on linoleic and linolenic acid concentration as a contribution to total FA. The concentration of this omega-3 FA declined in the plant material between the first and the third harvest in the descending order: perennial ryegrass, orchardgrass, and tall fescue. However, the fractional contribution of each FA to the total FA changed little as the plants grew older. In the same manner, Phillips et al. [9] demonstrated that the concentration of linolenic acid in the total FA content of ryegrass (7%) and wheat (21%) decreased linearly during the grazing interval, May to June (46 days interval). However, the rate of that decline per day was greater for wheat than for rye, which had a more constant value across the interval. Linoleic acid levels were greater in total FA for rye compared to wheat for the majority of the interval. Other studies have found less variability in linoleic acid concentration in herbage across seasons than linolenic acid for perennial ryegrass [9,25,33,34] and orchardgrass. Phillips et al. [9] concluded, based on their review of the literature, that linoleic and linolenic acid levels are primarily determined by forage species and frequency of harvest.

Collectively, our findings and the literature show that linolenic acid (C18:3) concentrations are highest at a vegetative stage in young plants, which can be accomplished by a frequent cut regime. In this manner, higher leaf material and CP in plants are promoted, and flowering would be avoided. Even though the studies were done in different countries under varied climatic and soil conditions, there is a consistency in these observations. Perennial ryegrass shows mostly higher concentrations of this omega-3 FA in all studies, including ours, followed by tall fescue and closely followed by orchardgrass. Kentucky bluegrass showed mostly lower concentrations of this FA in all literature and including our present work. These concentration patterns parallel the production of FA per area calculated in our trial. Across all literature presented, including our findings, seasonal concentrations in all species were highest in the fall followed by spring harvest and were lowest for the summer cuts. These results also parallel the fatty acid production per area calculated for our plots. The temperature may be considered a factor promoting linolenic acid synthesis in cool-season forages. Concerning linoleic acid, its concentrations change less across seasons and are lower in leaf than stem material. Its variation may be defined by the vegetative stage, the number of cuts, and maturity.

5. Conclusions

Our study agrees with research that forage species, date of harvest, and species × date interactions are the major factors determining FA concentrations in cool-season grasses. Selecting cool-season grass species by their total FA or linolenic acid concentration across the growing season under a similar cutting regime, which will increase the forage leaf-stem ratio, could be a tool to increase desirable FA concentrations (omega-3 FA, CLA, and VA) in meat and milk products produced from grass-based livestock systems. As we observed in the present study and across the literature, there is a high correlation between total FA, linolenic acid (C18:3), and CP content in plant tissue, which is related to vegetative growth. Since CP and FA share the same location in the plant tissue, CP determination would be a good indicator of total FA and linolenic acid levels. Shorter regrowth intervals allow for maintaining plants in a vegetative stage, thus, favoring higher levels of linolenic acid and total FA, which are precursors of the healthy-beneficial CLA and VA. From the literature, it can be concluded that perennial ryegrass has a higher content of linolenic acid, followed by orchardgrass and tall fescue. On the other hand, little variability has been observed in linoleic acid (C18:2) levels among species and seasons. We suggest that due to the species-by-date interactions, mixed species pastures may provide a more uniform supply of FA across the growing season. It can also be rescued that linolenic acid concentrations (C18:3) in all species were highest in the fall, followed by spring harvest, and lowest for

the summer cuts. There is value in finishing cattle during the late fall due to the highest availability of linolenic acid and other FA precursors to CLA and TVA that could impact their deposition in the animal products to fulfill consumer demands. Further studies are warranted to determine how species and grazing management interaction impact omega-3-FA, CLA, and TVA content in ruminant animal products from cool grasses fed systems in the Appalachian region.

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