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Communication

Variation in Fatty Acids Concentration in Grasses, Legumes, and Forbs in the Allegheny Plateau

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Abstract: This study was conducted to determine the fatty acid (FA) content in pasture grasses, legumes, and non-leguminous forbs in northeast West Virginia. Grass, legume, and forb plant material were collected from rotationally stocked pastures and analyzed for crude protein (CP), linoleic acid (C18:2), α-linolenic acid (C18:3), and total FA content. Species within botanical classes varied in FA content. Forbs had the highest linoleic acid (C18:2) content followed by legume and grass species. Grasses and forbs had the highest α-linolenic acid (C18:3) content. Forbs had the highest total FA content. These field data were combined with FA data from the research literature to evaluate the correlation of CP concentration with fatty acid concentration. Likewise, after accounting for CP, the summer months caused a decrease while forbs caused an increase in α-linolenic acid (C18:3) content. Vegetative growth and leafiness are the major determinants of FA content in pasture forage. Grazing management to benefit vegetative growth and the presence of desirable forbs in tune with seasonal changes are valuable tools to increase desirable FA profiles in milk and meat products that may be of benefit to human health.

Keywords: fatty acids; content; pasture; α-linolenic acid; linoleic acid; CLA; conjugated linoleic acid; vaccenic acid

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1. Introduction

Animal products such as meat and milk are a source of essential unsaturated FA, particularly omega-3-FA and conjugated linoleic acids (CLA), which have health benefits related to their consumption [1–3]. The value of increasing the concentration of desirable FA in ruminant products has received greater attention in the past four decades due to the potential of these FA to reduce the risk of occurrence or progression of cancer, cardiovascular, and metabolic diseases [4–6]. The use of concentrate base-diets to accelerate animal growth rates diminishes the level of omega-3 FA in red meat [7,8] and milk [9,10]. Forage lipids are the most economic source of these FA [3,11] and an important strategy would be to increase the delivery of FA from plants into animal products [3,5].

Lipids in forage are part of a complex matrix and their release in the rumen is different than that of oils and fats added to rations that can adversely affect rumen function when fed in high concentrations [11]. Lipolysis, the hydrolysis of the ester bonds by microbial lipase, and biohydrogenation of polyunsaturated FA take place in the rumen and converts unsaturated FA to saturated FA [12,13]. Ruminal microbial lipase acting upon dietary lipids mainly releases polyunsaturated FA, which is toxic to rumen microbes [14]. Biohydrogenation to less toxic saturated FA takes place, especially to C18:0, reducing this negative impact on rumen microbes [10,14]. Long exposure to the biohydrogenation process reduces the deposition of unsaturated FA in the meat and milk of ruminants [15]. It has been observed that forage high in secondary metabolites such as polyphenols, alkaloids, and carotenoids has an antibiotic effect on biohydrogenation. This may protect omega-3 FA against ruminal microbial fermentation, allowing the unsaturated FA to reach the intestinal absorption by the host animal [7].

The most abundant omega-3-FA found in grass is α-linolenic (C18:3) acid (50–75% of total FA) [1,2,9,16]. Linoleic (C18:2) acid (18% of total FA) and α-linolenic (C18:3) acid in fresh pasture are precursors to the beneficial omega-3-FAs: eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) which are formed in the animal tissue [3,11,17]. Several studies report that grazing animals have higher levels of those beneficial FA in their meat and milk products [3,5,10,18–22]. The forage-to-concentrate ratio in an animal's diet influences the biohydrogenation pathway used for linoleic (C18:2) and α-linolenic (C18:3) acid [23]. Biohydrogenation of linoleic and α-linolenic acid are both characterized by an initial isomerization of the cis double bond, resulting in the production of rumenic acid (cis9, trans11–18:2) and rumelenic acid (cis9, trans11, cis15–18:3), respectively [23].

In ruminants, CLA is produced from intermediates of the biohydrogenation of polyunsaturated FA in the rumen such as vaccenic acid (VA, t11-C18:1), the common intermediate of linoleic (C18:2), and linolenic acids (C18:3) [14]. The ruminal bacterium, Butyrivibrio fibrisolvens is recognized as participating in the biohydrogenation of FA, with CLA being an intermediate in the process [14]. Phillips et al. [24] stated that cattle with a ration high in α-linolenic (C18:3) acid seem to develop specific microflora that increases the production and deposition of CLA in tissues.

Vaccenic acid (t11–18:1) is also a precursor of CLA that results from Δ9 desaturation in animal tissue [10]. Lahlou's [10] study showed that endogenous rather than ruminal synthesis was more likely responsible for higher CLA content in milk when cows were fed pasture compared to conserved feeds. These results concur with Yang's [20] study that reported higher activity of Δ9-desaturase in adipose tissue of grazing beef compared to feedlot cattle. Clapham et al. [25] suggested that the amount of CLA in pasture-raised beef can be influenced by the plant species in the pasture. Wu et al. [26] measured the CLA concentration in milk when feeding grasses alone (kentucky bluegrass, quackgrass, and smooth bromegrass) vs. mixed grasses and red clover; they found 50 percent more CLA (14.0 vs. 9.2 mg g^{-1}) in milk produced by animals fed clover and grasses than those fed grasses alone.

Boufaïed et al. [27] postulated that FA concentrations in forages depend on many factors including species, leaf age, and growth stage, which is supported by other literature [15,16,21,25]. The highest amounts of unsaturated FA and crude protein occur in the vegetative growth stage, followed by their decreasing as plants enter the reproductive stage [17,27]. Different agronomic practices such as wilting, shading, silage additives, number and timing of cuts, and fertilization also affect the concentration of omega-3 FAs in forage, having the potential to impact FA content in ruminant products [17,20,28–31]. The application of N fertilizer influences the FA concentration in forages by increasing the leaf/stem ratio since FA content is higher in the leaf compared to the stem component of plants [16,30]. An understanding of the factors that influence the FA content and composition of forages could help producers make effective harvest management decisions, thus improving the quality of their forages [20], and the associated FA profile in animal products [5,16,19,32].

Naturalized grasslands are a major source of feed for livestock in the Allegheny Plateau, which is a large plateau in the northern Appalachian Mountain region of the eastern United States. These grasslands provide pasture and conserved forage for beef and dairy cattle, sheep, and goats, and contain grasses, legumes, and forbs with species abundance differing from farm to farm depending on management and climate (determined by elevation, location relative to the Allegheny Front, and latitude). These grasses and legumes are predominantly of European origin and include the same cool-season forage species that are planted under more intensive farming systems. However, in this region, many of the pastures and meadows are older stands and are not commonly renovated beyond frost seedings of improved legumes such as red or white clover. Quantitative FA data for forbs in the region's pastures is lacking, and little substantial information on FA profile in forbs from university experiments in the Allegheny Plateau is available. Clapham et al. [25] tested 13 different forages, including forbs, legumes and grasses in a greenhouse trial, for FA determination. Although they used a wide range of species, which have potential applications in the Appalachian region for grass-fed animals, no field data was presented. Elgersma et al. [11] compared a variety of legumes and forbs to a grass–clover mixture during the whole growing seasons in two consecutive years; however, their experimental forbs species do not frequently occur in the Allegheny Plateau pastures. When Boufaied et al. [33] studied the effects of twelve grass and legume species in eastern Canada, their experiment was focused only on part of the grazing season, in spring and summer. Cabiddu et al. [16] analyzed a database from various pastures containing pure and mixed swards, including annual ryegrass, legumes, and forbs. However, their data were compared for only two phenological stages in the Mediterranean region. The goals of this study were to obtain novel data on linoleic (C18:2) and linolenic (C18:3) acid content in grasses, legumes, and forbs commonly found in pastures on the Allegheny plateau, to follow the change in these FA across the year, and to compare the concentration of FAs in these species to the FA content in these and other forage species reported in the research literature.

2. Material and Methods

This study was conducted to determine linoleic (C18:2), linolenic (C18:3), and total FA concentrations in grasses, legumes, and forbs during the 2002 growing season. Forage samples were collected from pastures and hay meadows managed as part of a commercial beef cattle operation in Preston County, West Virginia. Pastures were rotationally stocked. Hay meadows are paddocks within a grazing system where hay is harvested one time in the spring and then the paddocks are rotationally stocked for the remainder of the year. Spring grazing began when pasture swards reached a 20 to 25 cm height. Hay meadows were harvested when grasses in the sward reached the early heading stage. Regrowth intervals before grazing events were three weeks in the spring and five to six weeks during the summer and early fall. The plant species sampled represented the grasses, legumes, and forbs in the pastures that were being consumed by the livestock and could potentially impact the FA profiles in animal products harvested from these pastures. The sample harvests were taken in May, June, July, September, and November. The species sampled were: tall fescue (*Schedonorus arundinaceus* (*Schreb*) *Dumort*); orchardgrass (*Dactylis glomerata F.H.Wigg*); buckhorn plantain (*Plantago lanceolata* L.); common plantain (*Plantago rugelii Decne*); dandelion (*Taraxacum officinale* L.); curly dock (*Rumex crispus* L.); milk weed (*Asclepias* L.); quackgrass (*Elymus repens* (L.) *Gould*); smooth bromegrass (*Bromus inermis* Leyss); red clover (*Trifolium pratense* L.); and white clover (*Trifolium repens* L.). Samples of each species were taken at random within paddocks prior to grazing. Botanical samples were collected with clippers at a 3.8 cm height above the soil. Approximately 300 g per species were collected for FA determination and 700 g for forage nutrient analyses. The same pastures were sampled each month, and samples were composited by species. Immediately after the samples were cut they were put in Tyvek bags and placed in coolers with dry ice for the determination of FA. Samples for forage nutrient analysis were placed in zipper-lock bags with regular ice. Samples for FA determination were preserved in a freezer at −80° C until they were freeze-dried, after which they were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) through a 1-mm screen, and stored in plastic bags at −80 °C. These forage samples were later sent to the Ruminant Fermentation Profiling laboratory at West Virginia University (Morgantown, WV, USA) where they were analyzed for FA using wet chemistry analysis [34]. Forage samples for nutrient analysis were dried at 60° C and ground in a Wiley Mill through a 1-mm screen and stored in plastic bags until sent to a commercial forage testing laboratory (Dairy One, Inc., Ithaca, NY, USA) for analyses. Forage species and harvest date effects ($p < 0.05$) on crude protein (CP), neutral detergent fiber (NDF), and FA composition were analyzed using a one-way analysis of variance procedures of NCSS [35]. A randomized block experimental design, with months being the repeated measures, was used to evaluate species' effect on the response variables.

For the second phase of this study a database was built using the data from the study, data from a previous experiment with cool-season grasses [35], and data from the research literature that studied factors affecting the FA composition in forages [12,18,27,36,37]. Publications were included when they reported crude protein, linoleic acid (C18:2), α-linolenic acid (C18:3), and total FA concentrations in the forage. Multiple regression was used to compare FA to CP content in forages, with months as a measure for seasonal effect, and with months and species pooled into groups differing from the regression average ($p < 0.05$). Including all individual species in the regressions increased regression R^2 and reduced the SDreg, with only a few species having FA contents significantly different than the CP based regression mean. Retaining a large number of species in the regression whose FA content is not significantly different from the regression mean provides an R^2 larger than is justified. Forbs and grasses having FA contents different than the regression mean ($p < 0.05$) were pooled and the regressions were run again. Statistical procedures were conducted using NCSS software [35].

3. Results

3.1. Grass, Legume, and Forb Field Data

The mean concentrations of major FA (C18:2, and C18:3), crude protein, and neutral detergent fiber in the grasses, legumes, and forbs are presented in Table 1. Due to the species' life cycles, some plants were not present during all months, so they were not always available for analysis. Plant species did not differ significantly in CP content ($p = 0.134$), but did differ in NDF ($p < 0.001$), C18:2 ($p = 0.012$), C18:3 ($p = 0.045$), and total FA ($p = 0.011$). As botanical groups, forbs tended ($p < 0.052$) to have greater concentrations of linoleic acid (C18:2) than grasses, with legumes in between and not significantly different from the other two groups. Botanical groups were not different in α-linolenic acid (C18:3) concentration ($p = 0.393$) or total FA concentrations ($p < 0.555$).

Table 1. Crude protein (CP), neutral detergent fiber (NDF), linoleic acid (C18:2), α-linolenic acid (C18:3), and total fatty acid (Total FA) content in forbs, grasses, and legumes consumed by livestock in West Virginia pastures (mean g kg^{-1} dry matter ± SD).

Species		N †	CP	NDF	C18:2	C18:3	Total FA
					g kg^{-1}		
Forbes	Aster	1	212	335	5.3	12.5	24.6
	Buckhorn Plantain	5	188 ± 25	340 ± 39	5.0 ± 0.7	16.6 ± 0.9	30.1 ± 1.6
	Common Plantain	5	211 ± 31	332 ± 25	4.8 ± 1.3	17.2 ± 4.0	31.5 ± 6.4
	Curly Dock	4	260 ± 40	407 ± 100	8.3 ± 2.5	20.8 ± 2.6	39.8 ± 4.8
	Dandelion	2	227 ± 1	351 ± 58	8.6 ± 1.5	21.4 ± 0.8	42.6 ± 3.0
	Milkweed	1	247	224	6.1	17.7	34.8
Grasses	Orchardgrass	4	199 ± 38	566 ± 54	5.2 ± 1.4	23.6 ± 2.5	38.3 ± 2.5
	Quackgrass	3	234 ± 36	540 ± 82	4.8 ± 1.0	19.8 ± 6.9	33.5 ± 8.5
	Smooth Bromegrass	5	209 ± 60	590 ± 44	4.5 ± 2.3	15.9 ± 3.3	28.4 ± 6.9
	Tall Fescue	3	200 ± 35	518 ± 50	4.0 ± 0.4	17.1 ± 3.7	28.6 ± 2.9
Legumes	Red Clover	5	222 ± 25	385 ± 41	5.6 ± 1.1	16.8 ± 3.1	31.7 ± 4.5
	White Clover	5	252 ± 7	314 ± 30	5.6 ± 1.1	16.8 ± 3.1	31.7 ± 4.5

Number of months sampled †.

3.2. Combined Field and Literature Data

The field data combined with data available in the research literature produced a data set of 182 forages containing a wide range of CP, linoleic acid (C18:2), α-linolenic acid (C18:3), and total FA content (Table 2). The content of CP and FAs were highly correlated (Table 3). Total FA content was more highly correlated to α-linolenic acid (C18:3) than linoleic acid (C18:2) content. Linoleic acid (C18:2), and α-linolenic acid (C18:3) content were less highly correlated.

Table 2. Crude protein (CP), linoleic acid (C18:2), α-linolenic acid (C18:3), and total fatty acids (Total FA) content (g kg^{-1}) in forage grasses, legumes, and forbs in the combined dataset (12, 18, 26, 28, 37, 38).

Measure	*n*	Mean	SD	Min	Max
CP	182	206	50	77	338
C18:2	182	6.0	1.8	0.5	10.9
C18:3	182	17.3	6.4	7.0	42.5
Total FA	182	31.1	8.3	14.4	62.8

Table 3. Correlation between crude protein (CP), total fatty acids (FA), linoleic acid (C18:2), and α-linolenic acid (C18:3) in grasses, legumes, and forbs (n = 182) (12, 18, 26, 28, 37, 38).

	CP%	C18:2	C18:3	Total FA
CP	1.00	0.40	0.43	0.54
C18:2		1.00	0.27	0.54
C18:3			1.00	0.94
Total FA				1.00

Regressions describing the effect of CP, Month, and pooled species group on the FA content in the grass, legume, and forb forage accounted for 39%, 61%, and 62% of the variation in C18:2, C18:3, and total FA, respectively (Table 4). The unexplained regression errors, expressed as the SD about the regressions (square root of the mean square error), were 1.4, 4.0, and 5.1 g kg^{-1} DM; or 13%, 11%, and 11% of the observed ranges in C18:2, C18:3, and total FA, respectively. The forage CP content accounted for much of the monthly and species effect on FA content. August had above-average C18:2 content. May, June, and July had the greatest negative impact on C18:3. August and September had a lesser negative impact on C18:3. May, June, and July had a negative impact on total FA content. Forbs and grasses (FnG) having above-average C18:2 content were Kentucky bluegrass, chicory, and dandelion. Forbs and grasses having above-average C18:3 content were buckhorn plantain, chicory, dandelion, galeg, perennial ryegrass, and triticale. Forbs and grasses having above-average total FA content were chicory, curly dock, dandelion, galeg, and perennial ryegrass.

Table 4. Regression analysis of total fatty acid (TFA), linoleic acid (C18:2), and α-linolenic acid (C18:3) content (g kg^{-1}) in forage grasses, legumes, and forbs based on their crude protein (CP) content (g kg^{-1}), second-order seasonal effect (Months 2 to 9), and pooled species groups that differed from the regression average (n = 182) (12, 18, 26, 28, 37, 38).

Regression	R^2	SD_{reg} †	AAPE ††
C18:2 = 2.3 + 0.0159 CP + 2.1 FnG + Month Month August = 1.6 ± 0.4	0.39	1.4	27
FnG—Forbs and grasses containing more C18:2 FA than the regression average (p < 0.05) were bluegrass, chicory, and dandelion.			
C18:3 = 6.5 + 0.0642 CP + 6.6 FnG + Month Month May, June, and July = −5.8 ± 0.7 August and September = −3.9 ± 0.9	0.61	4.0	19
FnG—Forbs and grasses containing more C18:3 FA than the regression average (p < 0.05) were buckhorn plantain, chicory, dandelion, galega, perennial ryegrass, and triticale.			
TFA = 13.8 + 0.0900 CP + 10.9 FnG + Month Month May, June, and July = −5.3 ± 0.8	0.62	5.1	13
FnG = Forbs, and grasses containing more TFA than the regression average (p < 0.05) were chicory, curly dock, dandelion, galega, and perennial ryegrass.			

† SDreg—standard deviation about the regression or square root of the mean square error. †† AAPE—average absolute percent error.

4. Discussion

4.1. Total FA

Clapham et al. [25] found that herbage from grasses, legumes, and forbs varied greatly in their FA profiles and followed the pattern of the dominant FA. They reported that chicory contained the most total FA, followed by orchardgrass, tall fescue, and perennial ryegrass with the lowest content being in white clover. Clapham's study demonstrated that total FA concentration was greatly influenced by plant material, harvest, and their interaction, and was highest for all plant materials at the first harvest but declined with time. Elgersma et al. [11] found that total and individual FA concentrations differed between forbs, legumes, and a perennial ryegrass–white clover mixture when plots were harvested four times per year to a residual stubble height of 7 cm. They observed that total FA concentrations were lowest in lucerne, ribwort plantain, chicory, and yellow sweet clover, and highest in caraway and birdsfoot trefoil. They reported higher total FA concentrations in forbs compared to legumes and grasses, where the grasses often had the lowest content, although not always. Other researchers [16,17,27,38] have reported a progressive increase in total FA concentration from May to November. Dewhurst et al. [17] indicated that leaf proportion is important in determining FA concentrations due to forage lipids being predominantly of leaf origin. Frequent cutting inhibits flowering and increases FA levels [3] due to promoting vegetative growth. Most of the literature indicates that total FA concentration in grass and legumes is highest in the spring, decreases during the summer, and increases again in the fall [11,25,37,39,40]. Elgersma [11] found that FA concentrations were generally lowest in the second cut (July) and highest in the first and fourth cuts in middle May and late October, respectively. Boufaïed et al. [30] found that the concentration of FA was higher during the summer than in the spring for orchardgrass and timothy, but to a lesser degree in red clover and alfalfa. Cabiddu et al. [16] observed that the phenological stage had no significant effect on the FA profile of Mediterranean forbs. Goosen et al. [29] observed that as pearl millet matured, total FA content decreased by 55 to 56% within the pseudostems and on a whole-plant basis, but had only a 10% decrease in leaf tissue. The decline in total FA content as maturity advanced was a function of a greater DM proportion of pseudostem which contained less total FA. They also observed a slight decline in total FA within the laminae, suggesting a possible thickening of the cell wall as it ages. They also reported that lamina CP content had a strong correlation with total FA. Their finding agrees with Clapham et al. [25] and Glasser et al. [28] who reported that as the growth stage progressed and forage grew older, CP decreased along with total FA content. These observations agree with our results. Forage CP is highly correlated to total FA content across grasses, legumes, and forbs (Table 3) and species is a good indicator of forage maturity. After accounting for CP content, the lowest total FA content occurred in May, June, and July, when plants were flowering or being impacted by summer weather. Forages that had above-average total FA content were forbes and perennial ryegrass (Table 4).

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

Boufaïed et al. [30] compared the effect of 12 forage species on α-linolenic (C18:3) acid content. They observed that annual ryegrass had the highest content of linolenic acid (C18:3) whereas meadow bromegrass had the lowest. In legumes, they found that white clover had a higher concentration of linolenic acid (C18:3) than did red clover and alfalfa. Timothy had a higher concentration of linoleic (C18:2) acid than orchardgrass, white clover had a higher concentration of linoleic (C18:2) acid than alfalfa, and annual ryegrass had higher linoleic (C18:2) concentrations than did meadow bromegrass and tall fescue. Between legumes, red clover had higher linoleic (C18:2) acid concentrations, and alfalfa had the lowest [29]. In a second study, they found that orchardgrass and timothy contained more α-linolenic (C18:3) acid than did alfalfa or red clover. No difference in α-linolenic (C18:3) content between the two types of grass was observed, whereas there was a difference between the two legumes, with red clover having a higher concentration of linolenic (C18:3) acid than alfalfa. For linoleic (C18:2) acid they found that linolenic (C18:3) acid was higher in legumes than in grasses. Bauchart et al. [41] found that in ryegrass α-linolenic (C18:3) acid represented 55 to 66% of total fatty acids but in alfalfa, it was only 40% of total fatty acids (1.6 g.100 g^{-1} DM). Makmur et al. [15] observed that in ten tropical forages the content of α-linolenic (C18:3) acid was higher in legumes than in grasses, whereas linoleic (C18:2) acid levels were higher in the grass than in legumes. Clapham et al. [25] reported that α-linolenic (C18:3) acid was the dominant FA in 13 types of grass, legumes, and forbs grown under greenhouse conditions. In their study grasses had a uniform composition of α-linolenic (C18:3) acid across species and the fractional contribution of α-linolenic acid to total FA was lower and more variable in forbs than in grasses. Their data demonstrated that herbage from grasses, legumes, and forbs varies greatly in α-linolenic (C18:3) acid. A greater content of α-linolenic (C18:3) was found in forbs, where chicory had the highest content, follow by galega, then the grasses (orchardgrass, perennial ryegrass, and tall fescue), and lower for white clover. Elgersma et al. [12] in a study of forbs, legumes, and grasses found that birdsfoot trefoil had the highest proportion of α-linolenic acids, whereas caraway and lucerne had the lowest content. Linoleic (C18:2) acid proportions were found to be greater in caraway and lowest in birdsfoot trefoil, yellow sweet clover, and a grass–clover mixture. When Cabiddu et al. [16] studied Mediterranean pastures, they found that the level of α-linolenic (C18:3) acid in subclover was higher than in a daisy forb (*Chrysanthemum coronarium*). Their results showed that the linoleic (C18:2) acid content (expressed as a percentage of total FA) was statistically different in the forage species, and the daisy forb (*C. coronarium*) was higher in linoleic acid (C18:2) than sulla (*Hedysarum coronarium*).

Boufaïed et al. [27] found that linoleic and α-linolenic acids were in greater concentrations during the summer than in the spring in grasses and legumes. They suggested that their result was probably because of the higher leaf-to-weight ratio (express as the dry weight of leaves to the whole plant dry weight, it is expressed in mg g^{-1}) in summer regrowth. Alpha-linolenic (C18:3) acid concentrations in orchardgrass and red clover were higher in summer regrowth than in spring growth, whereas in alfalfa α-linolenic (C18:3) acid concentrations were similar during the two growth periods. They also observed that the quantity of linolenic (C18:3) acid tended to become lower as the plants passed from the vegetative to the reproductive stage. Linoleic (C18:2) acid concentrations in orchardgrass and red clover were higher in summer regrowth than in spring growth, whereas alfalfa was similar during the two growth periods. As in the Elgersma [11] study, they observed that the levels of linoleic (C18:2) acid tended to become lower as the plants passed from the vegetative to the reproductive stage. Moreover, Addis et al. [41] reported that when four of the same pasture species: daisy forb (*C.coronarium* L.), annual ryegrass (*Lolium rigidum Gaudin*), burr medic (*Medicago polymorpha*), and sulla (*H. coronarium*) were mowed twice daily cutting the sward at a stubble height of 4 to 6 cm and kept in a range 300 to 400 mm during the experimental period, burr medic and sulla forages were rich in linoleic and α-linolenic (C18:3) acid during winter growth and spring reproductive stage, respectively. Goosen et al. [29] studied α-linolenic acid in pearl millet (*Cenchru americanus* (L.) Morrone), from the early vegetative stage to the late boot stage. They found that as pearl millet matured, α-linolenic (C18:3) acid and total FA content decreased 55 to 56% within the pseudostems and the whole plant, but only by 10% in the laminae. They observed that the α-linolenic (C18:3) acid proportion of total FA decreased by 14% on a whole plant basis. They commented that since α-linolenic (C18:3) acid is essential for chloroplast function this may explain why the α-linolenic (C18:3) acid proportion of total FA did not decrease within the lamina fraction. Goose et al. [29] reported that lamina CP content had a strong correlation with lamina α-linolenic (C18:3) acid as found in other literature [24,25,27]. They also stated that as the growth stage progressed and the forage grew older, CP decreased along with α-linolenic (C18:3) acid content. This pattern is similar to Dewhurst et al. [17] who suggested that these variations are due to the decrease in the proportion of leaves that are richer than stems in membrane lipids and thus in α-linolenic (C18:3) acid. Clapham and coworkers [25] found that the concentration of FA declined as plants developed, but the fractional contribution of α-linolenic acids to total FA remained relatively stable over time. They indicated that plant material, harvest time, and their interaction had a significant effect on linoleic (C18:2) and α-linolenic (C18:3) acid concentration. In their study, the concentration of α-linolenic (C18:3) acid declined in all plant materials between the first and the third harvest, and for linoleic (C18;2) the decline ranged from 28% in ryegrass and white clover to 53% in rape, turnips, and borage.

Based on the data in the presented literature, it can be concluded that α-linolenic (C18:3) acid content declines as the growing season advances, correlated to the plant leaf tissue and its decline in CP content as forages grow older. Grasses tend to have a greater content of α-linolenic (C18:3) acid than legumes with some forb species exceeding the content in grasses. Forbs contain a higher content of linoleic (C18:2) acid, followed closely by legumes with a lower content in grasses, and a trend to fluctuate across seasons, being higher during the summer. Our regression summary of the data is in line with this. After accounting for forage CP as a measure of leafiness and maturity, linoleic (C18:2) FA content was above the regression average in August, and the plant species kentucky bluegrass, chicory, and dandelion across all studies. Similarly, α-linolenic C18:3 content was a function of forage CP with C18:3 content being lowest in May, June, and July, and highest in the forbs buckhorn plantain, chicory, dandelion, galega, perennial ryegrass and triticale. It appears that CP is probably the best single indicator of forage FA content across species with deviations from this relation due to major differences in species and environments.

4.3. Effect of CP on FA Content in Forages

Crude protein has been reported as the variable most closely correlated with forage FA content [13,25,27,28,42]. Glasser et al. [28] suggested that the close relationship between CP and FA content in forages is partially explained by the location of CP and FA in the photosynthetic organs in leaves. They found that for each 10 g/kg DM increase in CP total FA increased by 0.8 g/kg, while the content of linolenic acid (C18:3) increased by 0.5 g/kg, and linoleic acid (C18:2) decreased. They explained that this linkage between CP and FA content is closely related to the proportion and age of photosynthetically active leaves in the forage. Consistently, Loor et al. [13] reported a greater concentration of CP and FA in immature forage which had a higher ratio of leaf tissue to stem tissue as supported by Glasser and coworkers [28] results who observed that young forages with high nutritional value are also those that have higher FA content and composition yielding a high nutritional quality in animal-derived products. Our results concur with these findings on the high correlation of CP to total FA and linolenic acid (C18:3) contents.

5. Conclusions

Collectively the data show that forage quality expressed as CP content, along with distinctive forbs and grasses, and to a lesser extent, a month is a valuable tool for managing the desirable FA content in pasture forage. These desirable omega-3 FA, linoleic (C18:2), and α-linolenic (C18:3), are precursors to VA and CLA in meat and milk produced by livestock pastured on the Allegheny Plateau. Based on this study, forbs are a forage resource that provides increased omega-3 FA content in the pasture and a potential increase in VA and CLA in derived animal products. A characteristic of forbs is their flexibility under different climatic conditions and environments, which makes them complementary to the grasses and legumes in pastures. Among future research studies, it would be interesting to investigate the variation in FA profile in individual forbs during the whole grazing season and also at different phenological stages. Grasses tend to provide more total FA and α-linolenic (C18:3) acid than legumes, but less linoleic (C18:2) acid than legumes. The season is an important factor in FA concentration in the pasture but much of the seasonal effect was accounted for by the CP content in the forage. The highest levels of FAs tend to be in the beginning and at the end of the grazing season, and the lowest across the summer months. We propose that pasture management to keep forage in a vegetative stage with a high CP content is the key factor to attain high levels of omega-3 FA precursors for beneficial FA profiles on the forage-finished animal products. If not already present, introducing forbs such as plantain and chicory can assist in this effort. Future studies should determine how to manage forage species, individually and in pastures containing grasses, legumes, and forbs during the whole grazing season to capture a greater proportion of FA in animal products according to consumer demands.

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