FATTY ACID VARIATION BETWEEN FORAGE SPECIES AND WITHIN POPULATIONS AND FATTY ACID CONTENT OF BEEF FINISHED ON PASTURE WITH DIFFERENT FORAGE SPECIES

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DEDICATION

TO:
My family
My parents Michael and Victoria Dierking
and
My wife Emily

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ABSTRACT

We describe two experiments; the first experiment was conducted in a greenhouse to minimize environmental effects. Four forage species – orchardgrass (*Dactylis glomerata* L.), tall fescue [*Lollium arundinaceum* (Schreb.) S.J. Darbysh = *Schedonorus arundinaceus* (Schreb.) Dumort.], perennial ryegrass (*Lolium perenne* L. ssp. *perenne*), and alfalfa (*Medicago sativa* L. ssp. *sativa* and *falcata* (L.) Arcang) were used to determine among and within variation. Perennial ryegrass contained the largest amount of α-linolenic and total FA while alfalfa possessed the greatest amount of linoleic acid. Additionally, populations within each species showed significant variation for nearly all FAs examined. Additionally, α-linolenic and total FA had the greatest correlation to the phenotypic characteristic total chlorophyll. The second experiment was a field-scale grazing trial with steers finished on three pasture treatments: tall fescue, tall fescue with red clover (*Trifolium pretense* L.), and tall fescue with alfalfa. The pasture treatments were different for crude protein, neutral detergent fiber (NDF) digestible neutral detergent fiber (dNDF, on a proportion basis), *in vitro* true digestibility (IVTD) and FAs myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), steric (C18:0), oleic, (C18:1) linoleic (C18:2), and total FAs. Steers (*Bos taurus* L.) grazing pastures with either red clover or alfalfa had greater average daily gains than cattle grazing tall fescue alone. However, differences found in pasture FAs did not translate into differences for any FA examined in beef. Therefore, it seems that factors other than forage FA concentration, like cattle genetics, are more important than the type of forage being fed to produce beef with high amounts of omega-3 fatty acids.

LITERATURE REVIEW

INTRODUCTION

Western diets are high in saturated fatty acids (SFAs) commonly found in fried foods. High amounts of SFA and the lack of omega-3 (*n*-3) fatty acids are linked to health problems such as obesity, heart disease, and diabetes (Sumeca and Miller, 2000; Weiss et al., 2004). Fatty acids (FAs), particularly *n*-3 and omega-6 (*n*-6), are of great importance to human health with the *n*-6:*n*-3 ratio a common benchmark of dietary acceptability. The typical western diet contains an extremely high *n*-6:*n*-3 ratio, (on the order of 15:1), that can lead to essential FA deficiencies when compared to eastern diets, where the ratio is near 4:1 (Trautwein, 2001).

Currently, dietitians, physicians, and health professionals prescribe a diet higher in polyunsaturated fatty acids (PUFA), lower in SFA and with a lower *n*-6:*n*-3 ratio. The majority of PUFAs in the human diet originate from plants and fish. Forage plants found in pastures are high in PUFA, specifically linoleic acid (C18:2, *n-6*) and α-linolenic acid (C18:3, *n-3*). While cattle can be finished on pasture, nearly all of the beef cattle finished in the United States are fed high concentrate diets before slaughter rather than forage. Several studies have compared concentrate to forage-based diets to determine how fat in finished beef is altered. In studies where concentrate diets are compared to forage-based diets, the FA profiles in beef are altered with increasing amounts of PUFA, decreasing amounts of SFA and a lower *n*-6:*n*-3 ratio when animals are fed a forage-based diet (Dannenerger et al., 2005; Nuernberg et al., 2005; Poulson et al., 2004; French et al., 2000).

Factors that Alter Fatty Acids in Forages

Fatty acid variation within forage species is currently unknown. Previous research demonstrates that fresh forage contains high amounts of PUFA in the form of α-linolenic acid (50 -75% of total FA concentration) along with lower amounts of linoleic acid and a sparse amount of SFA (Hawke, 1973). While perennial ryegrass (*Lolium prenne* L.) has been most widely studied, Clapham et al. (2005) analyzed the FA content of several forage species and found that α-linolenic followed by linoleic and palmitic acid were the predominate FAs. Dewhurst et al. (2001) compared three ryegrass species and found linoleic and α-linolenic differing by as much as 0.34 g kg^{-1} and 4.02 g kg^{-1}, respectively. Similarly, Boufaied et al. (2003) found legumes contained 1.3 times more linoleic acid than grass species while grasses contained 1.1 times more α-linolenic acid than legumes on average. Additionally, FA concentration decreases as plants mature, making forage management a central determinant in FA consumption by livestock (Clapham et al., 2005).

Factors other than species that affect FA concentration in forage include cultivar, cutting date/interval, storage method, and nitrogen fertilization. When comparing cultivars within a species, nearly all FAs examined have been found to be different (Boufaied et al., 2003; Gilliland et al. 2002; Clapham et al. 2006). The most important factors influencing the FA content of fresh forage appear to be cutting date and interval, which reflect maturity differences. A cutting interval of 20 compared to 38 days increased the concentration of all FAs examined in perennial ryegrass and Italian ryegrass (*Lolium multiflorum* L.) (Dewhurst et al., 2001). Also, seasonal fluctuations were assessed in perennial ryegrass between April and November. It was found that during the

months of June and July, forages contained the least amount of total FAs. Elgersama et al. (2003) showed values decreasing to about 2.7 and 17.4 g kg^{-1} for linoleic and α-linolenic, respectively, while Dewhurst et al. (2001) reported values as low as 1.4 and 4.4 g kg^{-1}. However, forages collected in April and November contained the highest amounts, reaching 2.8 g kg^{-1} for linoleic and 12.9 and 10.4 g kg^{-1} for α-linolenic, respectively (Dewhurst et al., 2001).

Storage method (i.e. silage, haylage, or hay) impacts FA content with dried or wilted forages containing less and ensiled forages generally containing more FA than fresh forage. Fresh pasture, when compared to hay (850 g kg^{-1}) or grass cut and allowed to wilt before pasturing (400 g kg^{-1}), had significantly higher amounts of myristic, palmitic, palmitoleic, oleic, linoleic, α-linoleic, and total FA. The FA concentrations using those forage preservation methods ranged from 3.77 to 3.87 mg g^{-1} and 8.11 to 8.25 mg g^{-1} for linoleic and α-linolenic, respectively, compared to 4.54 and 9.26 for fresh forage. However, silage (230 g kg^{-1}) had significantly higher amounts of palmitic, linoleic, α-linolenic, and total FA. Haylage (400 g kg^{-1}) was nearly identical to fresh forage with no differences for any FA examined except oleic acid (Boufaied et al., 2003). This work is supported by Dewhurst and King (1998), where silage wilted for only 2 h contained significantly higher α-linolenic and total FA than silage wilted for 68 h.

Lastly, nitrogen fertilization influences FA concentrations in forage, although the mechanism is currently not understood. Elgersma, et al. (2005) and Boufaied et al. (2003) found that linoleic and α-linolenic levels increased with nitrogen application rates in perennial ryegrass and timothy **(***Phleum pratense* L.), regardless of maturity. They reported values ranging from 3.19 to 3.97 mg g^{-1} and 5.96 to 8.71 mg g^{-1} at 0 kg ha^{-1} N for linoleic

and α-linolenic, respectively. When the N was increased to 120 kg ha^{-1}, linoleic had a larger range from 3.71 to 4.26 mg g^{-1} and α-linolenic also increased from 7.9 to 11.43 mg g^{-1} (Boufaied et al. 2003). Similar values were reported by Elgersma et al. (2005) when nitrogen rates between 0 and 100 kg ha^{-1} were applied and forage was harvested at various maturities.

Much research has focused on environmental conditions and utilization methods which modify FA content, while little research has examined genetic variation within species. Genetic variation is crucial for providing the basis for trait improvement; however it is also important to separate the influence of environment from genetics for FA production. Phenotypic traits that appear to correlate with FA include date of maturity, the stay-green trait, leaf color, and traits that minimize lipid oxidation. These traits modify FA content by altering the growth stage of the plant, the chloroplast content, or the degradation of membranes (Dewhurst et al., 2001). In addition to these traits, winter hardiness also impacts the FAs found in forages. Samala et al. (1998) found increased PUFA concentrations in the lipid membranes of winter hardy varieties of bermudagrass after the plants were subjected to a cold treatment. However, the FA differences are greater between different species compared to the differences between cultivars of the same species. Further, it remains to be demonstrated if intraspecific differences in the FA content of forages can influence the FAs in beef. In a study utilizing different perennial ryegrass cultivars as sources of pasture, dairy cattle increased milk CLA content while grazing the cultivar containing the greatest PUFA concentrations compared to cattle consuming the cultivar lowest in PUFA (Elgersma, 2003).

Fatty acid transformations in the rumen

One of the most difficult problems that food and animal scientists face is the fate of FAs in the rumen. When PUFAs, like linoleic and α-linolenic acid, enter the rumen, the process of biohydrogenation begins. Rumen bacteria immediately begin the process of saturating these FAs; however, this conversion has no clear purpose. It has been postulated to occur because the microbes contained in the rumen utilize the PUFA to make a range of other FAs that can be incorporated into their membranes (Harfoot and Hazelwood, 1997). Alternatively a more probable cause is the toxic role PUFA have on the microfauna of the rumen, and thus the microbes seek to detoxify their environment (Kemp and Lander, 1984). Through the process of detoxifying the rumen, between 60 to 95% and 80-100% of linoleic and α-linolenic are hydrogenated, respectively (Doreau et al. 1997). To prevent biohydrogenation, many researchers have attempted to treat PUFAs with rumen "protected" substances like formaldehyde-treated proteins, calcium associated salts, and fatty acyl amides (Doreau et al., 1997). Only the formaldehyde treatment has shown much promise as a solution for reducing rumen biohydrogenation.

Currently it is known that two groups of rumen bacteria must be present to convert both linoleic and α-linolenic FA to either oleic or steric FA. These bacterial groups are categorized by the molecules they hydrogenate. Bacterial group A consists of *Butyrivibrio fibrisolven* and other yet unidentified bacteria that hydrogenate all of the linoleic and α-linolenic acids entering the rumen to transvaccenic acid (C18:1 *trans*11). Bacterial group B consists of two species of *Fuscocillus* and a gram-negative rod species. Others may belong to this group but are undefined. This group hydrogenates oleic acid

(C18:1 *cis*9), transvaccenic acid, and linoleic to steric acid (Harfoot and Hazelwood, 1997).

One mechanism cattle employ to partially overcome rumen biohydrogenation of PUFA is the use of the Δ^9-desaturase enzyme. This enzyme converts transvaccenic acid to CLA in muscle tissues (i.e. endogenous formation). However, the *cis*9, *trans*11 isomer of CLA is the only PUFA molecule synthesized from transvaccenic acid in muscle tissues. The other isomers of CLA along with C18:3 FA are not produced endogenously. Therefore, if the activity of Δ^9-desaturase is increased, more steric and transvaccenic acid can be converted to CLA. Another option is to increase the amount of PUFA in the feed ration to further inhibit biohydrogenation by overwhelming the bacteria in the rumen before PUFAs enter the intestine. Once there, these acids are absorbed, transferred to, and are unaltered in tissues. However, if plant lipids in the ratio exceed 2 to 4% of dietary intake, fiber digestion is likely to be depressed in cattle (Jenkins, 1994). Therefore, by understanding the total variation and the genetic control of PUFAs in forages, greater amounts can enter the system and begin to decrease the hydrogenation of these acids by the rumen bacteria.

Factors Known to Influence Fatty Acids in Beef and Consumer Quality

Many factors can affect fat content and composition in beef. The principal ones include breed, age, genetic disposition, and diet; of these, diet has the greatest influence on changing the composition of fats present in muscle tissue. Albrecht et al. (2006) found that the body structure of breeds (i.e. light or heavily muscled), affect the amount of fat present. The intramuscular fat of meat can have an extensive range from 0.63% to 5.45% at 24 months of age depending on the breed of cattle (Albrecht et al., 2006).

As animals mature, the amount of fat present in both subcutaneous adipose and intramuscular tissues increases. The intramuscular fat, averaged over four breeds of cattle, began at 0.12% at two months of age and increased to 3.78% at 24 months (Albrecht et al., 2006). With this increase in fat, specific FAs increase as an animal's age increases as well, with the proportion of unsaturated to saturated fat increasing regardless of the feed source (i.e. concentrate vs. pasture). This was demonstrated by Huerta-Leidenz et al. (1996) where cattle initially grazing native pasture and then fed a concentrate diet had the level of monounsaturated FA MUFA:SFA and unsaturated FA UFA:SFA increase from approximately 0.93 and 0.98 to 1.48 and 1.56, respectively. This increase was due to the increase in C18:1 and C18:2 but not C18:3. Noci et al., (2005) showed a similar trend, with cattle being fed longer on pasture having slightly higher UFA:SFA ratio. Additionally, as cattle remained on pasture for long durations, the *n*-6:*n*-3 ratio decreased from 2.21 to 1.46 (Noci et al. 2005).

Pasture diets not only take longer to fatten cattle versus typical feedlot diets, but also alter meat composition. This was shown in Australia where cattle for Asian markets were backgrounded on pasture only (P1), pasture with protein pellets (P2), or pasture with a "forage crop" (P3). After backgrounding, the steers were finished either in a feedlot or on pasture. Steers backgrounded in the P1 group had lighter finishing weights, less back fat, and less intramuscular fat than either of the other groups. Additionally, pasture finished animals were inferior in all respects to grain finished animals including finishing gain, weight, meat yield, and fat content (Robinson et al., 2001). This study clearly demonstrates that cattle either backgrounded or finished on pasture deposit less fat and have lighter finishing weights, resulting in a yield reduction. Consequently,

compared to cattle finished in feedlot, cattle finished on pasture must be fed for a longer period if animals are to be harvested at the same weight.

Another factor involved is that different muscle tissues in beef are known to differ in fat composition. When comparing the muscle tissues *Triceps brachii*, *Longissimus dorsi* and *Semimembranosus,* the *Triceps brachii* contained 1.2 mg g^{-1} more fat and had greater *cis*-9, *trans*-11concentrations but contained equal amounts of total CLAs in the raw state compared to the other muscles (Lorenzen et al., 2007). However after cooking, the *Triceps brachii* had the greatest *cis*-9, *trans*-11 and total CLAs while *Semimembranosus* tissue contained the greatest PUFAs, PUFA:SFA and *n*-6:*n*-3 ratios (Lorenzen et al., 2007).

Lastly, the palatability of beef has been evaluated after animals were finished on pasture versus concentrate diets. Varying results have been reported, but generally few differences have been described for off flavors (Lorenzen et al., 2007; Nuernberg et al., 2005; Poulson et al., 2004). In addition to the variable tested above, Lorenzen et al. (2007) found differences in juiciness and overall consumer liking for concentrate finished animals compared to the pasture finished steers, but that the values obtained for the pasture finished cattle were above the acceptable point of 5.0 on a 9.0 scale. From these trials, it is noteworthy that beef produced on pasture compared to grain not only contained a higher PUFA content in the forms of CLA and α-linolenic FA, but that the quality of beef is not jeopardized in human taste and texture experiments.

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FATTY ACID VARIATION BETWEEN FORAGE SPECIES AND WITHIN POPULATIONS

ABSTRACT

There is a growing market for animals raised under organic practices with the meat from these animals having a positive influence on human health. Since these animals are often finished on pasture, there is a greater amount of omega-3 fatty acid (FA) as well as conjugated linoleic acids (CLA) present in the meat. The objective was to determine the variation among and within forage species commonly grown in the lower Midwest for FA composition providing the best sources of unsaturated fat and omega-3 FAs for cattle finished on pasture. A secondary objective was to determine if there are any phenotypic traits in forage that are associated with specific fatty acids. The forages analyzed included multiple cultivars of orchardgrass (*Dactylis glomerata* L.), tall fescue [*Lollium arundinaceum* (Schreb.) S.J. Darbysh = *Schedonorus arundinaceus* (Schreb.) Dumort.], perennial ryegrass (*Lolium perenne* L.), and alfalfa (*Medicago sativa* L. ssp. *sativa* and *falcate*). As expected, variation existed between species, but significant differences ($P<0.05$) were found within forage species also. Grasses maintained higher amounts of α-linolenic (C18:3) acid compared to alfalfa while alfalfa, had larger amounts of linoleic acid (C18:2). Correlations between phenotypic traits and specific FAs were found; plant chlorophyll had the greatest correlation. Overall, there does not seem to be a large amount of within species variation that breeders could use in a plant breeding program to make large increases in FA content; however, by determining chlorophyll content it may be possible to make an assessment of the levels of FA present.

INTRODUCTION

The composition of fats from food animals has become a major human health concern. Several studies show that feed sources or additives impact the quality of meat or milk from ruminant animals by affecting fatty acid (FA) composition (Dhiman et al., 1999; French et al., 2000). It is then inferred that different pasture forages, like different concentrate diets, may have an impact on the FA characteristics due to their different FA profiles and concentrations between and within species. One feed source that has been widely promoted in the scientific and popular press is the use of pasture, instead of high-energy concentrates, to produce meat or dairy products with increased concentrations of "healthy" fats (Mandell et al., 1998; Dannerberger et al., 2005; Lorenzen et al, 2006).

Several types of FAs can be found within different forages; the most prominent is α-linolenic (C18:3), followed by linoleic acid (C18:2) and palmitic acid (C16:0). These FA levels range from 45-70, 10-25, and 15-25% of the total FA, respectively, in many of the forage species examined (Dewhurst et al., 2001; Boufaied et al., 2003; Clapham et al. 2006). Linoleic and α-linolenic acid are of primary importance in producing beef and milk with high levels of omega-3 FAs and conjugated linoleic acids (CLA) (Scollan et al., 2001); these "good fats" are found primarily in ruminant products, produced on forage diets (Chin et al., 1992). Omega-3 FAs and CLAs have been shown to reduce cardiovascular disease, diabetes, cancers, and obesity (Pariza, 2004). The primary CLAs found in ruminant products include the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers, with the former elevated to a greater degree when animals are fed forage-based diets (Jiang et al., 1996; Lorenzen et al., 2006). In feeding trials with beef ,CLAs and omega-3 FAs

increased while the omega-6 to omega-3 ratio decreased when animals were fed dietary supplements with high amounts of omega-3 FAs or a forage diet, (Scollan et al., 2001).

Studies examining forages for lipid content have shown that environmental factors play a large role in the proportions and amounts of FAs found in these forage crops. Such factors include light (Dewhurst and King, 1998), cutting interval, and season of year (Dewhurst et al., 2001). Additionally, plant fertilization alters the amount of FA present. As nitrogen fertilization increases, so do the lipid levels, particularly α-linolenic acid (Boufaied et al., 2003; Elgersma et al., 2005). Plant age also has an effect, with mature forages generally containing lower FA concentrations compared to immature forages, mostly due to the decrease in the leaf-to-stem ratio (Boufaied et al. 2003). Lastly, forage harvested for hay, haylage, or wilted prior to grazing, shows a decrease in most FAs, particularly polyunsaturated fatty acids (PUFA) concentrations, compared to fresh forage (Dewhurst and King, 1998; Boufaied et al. 2003; Elgersma et al., 2003).

Plant genetics also impacts this trait, as FA profiles can distinguish grass species at a single harvest but not across harvests due to the environmental conditions causing fluctuations in FAs concentrations over time (Dewhurst et al., 2001). These FA reside in the membranes of cells and organelles rather than the oil bodies found in oil seeds. Therefore, an increase in lipids would require either more membranes or the formation of oil bodies in forage tissues. The creation of oil bodies has been accomplished using the transgene acyl CoA:diacylglycerol acyltransferase (DGAT) from *Arabidopsis thaliana* under the control of a constitutive promoter in tobacco (Bouvier-Nave et al., 2000). This is the first committed step in the production of triacylglycerides. Genes controlling the saturation level, known as FAD2, 3, 6, 7, and 8, are responsible for the formation of

monounsaturated fatty acids MUFA and PUFA in the endoplasmic reticulum or chloroplasts (Roberts et al., 2002). However, this is the limit that is currently known about the genetic components controlling this trait in forages. Therefore, more information regarding the variation within a species and the heritability of these traits is needed.

The objective of this study was to determine the variation among and within four different forage species over several harvests at the same maturity. A secondary objective was to identify potential phenotypic characteristics that may be associated with individual FAs. This study focused on forage species common to pastures and hayfields in the lower Midwest: orchardgrass, tall fescue, perennial ryegrass, and alfalfa. This information is crucial for the development of plant varieties to contain higher amounts of polyunsaturated FA as well as identifying correlated traits.

METHODS AND MATERIALS

Plant Materials, Planting, Harvesting, and Storage

Populations of four different forage species (Table 2.1) were obtained for testing from both private and public resources. The four species were orchardgrass, tall fescue, perennial ryegrass, and alfalfa. Within each species, 20 populations of orchardgrass and tall fescue, 19 populations of perennial ryegrass, and 21 populations of alfalfa were tested. Populations within each species were selected to give a wide range in adaptation, genetic background, and agronomic performance. Individual populations were selected based on criteria that have been considered to influence or correlate with specific FAs. These included winter hardiness, fall dormancy, color (i.e. chlorophyll content), maturity, and ploidy level.

Plants were grown from seed under greenhouse conditions with a 14 h day length from late September 2006 through early January 2007. Seeds were sown into Custom™ Containers 600C that measured 23 cm x 21.6 cm x 18 cm and contained a soil comprised of 40% sand, 45% silt, and 15% clay. Soil was amended with N, P, and K as recommended by the University of Missouri soil testing lab. After germinating, seedlings were thinned to stand densities common in field settings (Roberts and Gerrish, 2001). Orchardgrass was sown at 100 seeds per pot and then thinned to 60 plants, tall fescue and perennial ryegrass were planted at 50 seeds per pot and thinned to 35 plants, and alfalfa was seeded at 40 seeds per pot and thinned to 27 plants. Greenhouse temperatures were recorded with a StowAway® TidbiT® temperature logger. Temperature readings were recorded every five minutes. Temperatures in the greenhouse over all harvests averaged 21.1 +/- 9.3 ^{o}C.

After seeding, plants were allowed to establish for four to five weeks, and then the initial growth was cut to a 5-cm stubble and discarded. After the initial cutting, all plant material 5 cm above the soil was harvested three times over a 72 d period (24 d harvest schedule). At each of the three harvests, all forage from each plant was placed in 50 mL conical tubes, frozen with liquid N and then kept on dry ice until placed into a lyophilizer. After lyophilization, samples were stored at -20 ^{o}C. Because of their small size, samples were ground using coffee grinders for 30 sec. and then returned to the freezer until they were scanned using near infrared (NIR) spectroscopy.

NIR measurements

All 720 samples (80 entries x 3 replications x 3 harvests) were analyzed by NIR spectroscopy with a FOSS NIRSystems (Silver Spring, MD) model 6500 near-infrared

spectrophotometer with WinISI Winscan software, version 1.50 (Infrasoft Int., Port Matilda, PA). Samples were scanned twice from 400 to 2498 nm, Log 1/reflectance was recorded, and scans were averaged. Next, 100 samples were selected for calibration development; these included 40 alfalfa and 60 grass samples. Calibrations were developed by regressing spectral data against chemical data for the 100 calibration samples. Spectral pretreatments included standard normal variance (SNV), detrending (DT), and conversion to second derivatives as described by Duckworth (2004). Chemometric procedures included modified partial least squares regression as described by Westerhaus et al. (2004). Optimum calibrations were identified by low values for standard error of calibration and cross-validation as well as high values for coefficient of determination and 1-variance ratio. When calibration statistics indicated unsuccessful quantification of a particular FA, that calibration was not used; this occurred when attempting calibrations for FAs C14:0 and C16:1. Successful calibrations were obtained for the FAs C12:0, C14:1, C16:0, C18:0, C18:1, C18:2, and C18:3. Total FA is represented by the sum of each FA examined in this experiment. The corresponding statistics can be seen in Table 2.1, which are similar to those reported by Foster et al. (2006).

Fatty Acid Determination for NIR Calibration

The calibration set (n=100) was extracted for FA analysis using the one-step method described by Sukhija and Plamquist (1988) and amended by Clapham et al. (2006). Ground samples equilibrated to room temperature when 250 mg of tissue was weighed and mixed with 1 mL of internal standard (IS) C17:0, 1 mL methanolic HCl (prepared daily), and 500 uL of hexane. Samples were vortexed, placed in a water bath at

70 ^{o}C for 2 h, then removed and allowed to equilibrate to room temperature. Once the samples reached room temperature, 2.5 mL of 60 mg g^{-1} potassium carbonate and 1 mL of hexane were added. Samples were vortexed and then centrifuged at 1750 rpm for 10 minutes. The top hexane layer was then removed and filtered using Supelclean ENVI-carb (Supelco, Bellefonte, PA) SPE tube with 250 mg of anhydrous sodium sulfate to remove any water. The samples were then stored at -20 ^{o}C until they could be analyzed.

The samples were analyzed with an Agilent 6890 GC and a model 7683 automatic liquid sampler with a flame ionization detector (Agilent Technologies, Santa Clara, CA). A sample of 2 μL was injected and split 50:1 into a WCOT fused silica, chemically bonded capillary column (Chrompack CP-SIL 88 fused silica column100 m long, 0.25 mm inside diameter, 0.2 μm film thickness; Varian, Walnut Creek, CA). Helium gas at 20 mL min^{-1} was used as the carrier gas. The temperature ramp for the GC was as follows: 70 ^{o}C for 1 min; increase to 180 ^{o}C at 30 ^{o}C min^{-1}, hold for 1 min; increase to 220 ^{o}C at 2 ^{o}C min^{-1}; increase to 240 ^{o}C at 10 ^{o}C min^{-1}, hold for 0.5 min; for a total run time of 29.7 min. Injector temperature was set at 280 ^{o}C; detector temperature was set at 300 ^{o}C. Reference standard GLC-63b (Nu-Check Prep, Elysian, MN) was used to quantify and identify samples by their retention times. Samples were analyzed for C12:0, C14:0, C14:1, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3. Heptadecanoic acid (C17:0, 0.4 mg mL^{-1} in hexane; Matreya, Pleasant Gap, PA) was used as the internal standard.

Chlorophyll Content Determination for NIRS Calibration

The same 100 NIRS calibration samples were analyzed for chlorophyll concentrations. Chlorophyll was determined by weighing 5 mg of dried, ground material

into a 15 mL tube then adding 5 mL of methanol. The samples were then vortexed every 5 min. for 30 min. A 1 mL aliquot was taken and placed in a 2 mL micro-centrifuge tube and centrifuged at 10,000 rpm for 1 min. Absorbance was recorded at wavelengths of 700, 665, and 652 nm using a Hewlett Packard 5453 spectrophotometer and analyzed using HP UV-Visible ChemStation software. Chlorophyll *a, b,* and total chlorophyll content were determined using equations described by Porra, et al. (1989).

Phenotypic Data

Phenotypic data of winter hardiness, fall dormancy, color (i.e. chlorophyll content), maturity, and ploidy level was either determined in the lab or was obtained from the seed supplier. These measurements were then standardized based on comparative check samples used at the suppliers location. These values were then compared to the levels of each FA.

Statistical Analysis

Pots were arranged in the greenhouse in a completely randomized design (CRD) as described by Steel and Torrie (1980). Each species was replicated three times and each plant was harvested three times. Fatty acid data were analyzed by analysis of variance using PROC GLM in SAS statistical software (SAS Inst. Inc., Cary, NC). Trait correlations to FA concentrations for each species were performed using PROC CORR in SAS statistical software (SAS Inst. Inc.). To determine if a difference exist between species for chlorophyll and harvests for alfalfa traits of fall dormancy and winter hardiness the correlation coefficients were tested for each trait correlation.

RESULTS AND DISCUSSION

Species Differences

Between the forage species tested (orchardgrass, tall fescue, perennial ryegrass, and alfalfa), significant differences ($P < 0.01$) existed for all FAs examined. Alpha-linolenic was the predominate FA, comprising 60-76% of the total FA concentration between the four species (Tables 2.3-2.6). Across all three harvests, perennial ryegrass, tall fescue, orchardgrass, and alfalfa had an average of 31.9, 28.4, 26.7, and 24.8 mg g^{-1} α-linolenic acid, respectively (Figure 2.1). Linoleic and palmitic acid were similar to each other in concentration, comprising 7-19% and 11-16% of the total FA, respectively, when averaged across the species. The grasses contained similar amounts of linoleic acid, averaging 3.81 mg g^{-1}, while alfalfa had significantly greater ($P < 0.01$) levels with a mean value of 6.22 mg g^{-1}. Grass species contained 4.63 mg g^{-1} of palmitic acid, which was less than the 5.66 mg g^{-1} in alfalfa.

The remaining FAs make up on average 6.4% of the total FAs analyzed with lauric acid having the smallest fraction at approximately 0.03 mg g^{-1} or 0.07% of the total. Perennial ryegrass contained more lauric acid than any of the other forages tested. Myristoleic acid exhibited the greatest variation ranging from 0.44 to 3.19 mg g^{-1} in alfalfa and perennial ryegrass, respectively. The myristoleic acid levels increased with each successive harvest with tall fescue and perennial ryegrass having significantly ($P < 0.01$) larger amounts than orchardgrass or alfalfa. Steric and oleic FAs were similar in their amounts averaging 0.6 mg g^{-1} and equaling 1.5% of the total FA concentration. Orchardgrass had the least amount of steric and oleic acid while tall fescue and perennial

ryegrass had similar amounts and alfalfa contained the greatest of these FAs averaged over all harvests.

These values are similar to those reported by Clapham et al. (2006) but are two- to three-fold greater than reports from field data (Dewhurst et al., 2001; Boufaied et al., 2003). However, the amount of individual FAs on a percentage basis of the total FA concentration is similar to samples collected under field conditions. (Dewhurst et al., 2001; Boufaied et al., 2003; Clapham et al. 2006). This trend suggests that forage species must maintain certain FA proportions for the continued work of the lipid bilayers in organelles such as chloroplasts and other cellular systems. Therefore, it may be easier to increase total amount of FA present by management practices or genetic advancement rather than increase a specific FA.

The forages were harvested at vegetative stages to mitigate the effects of maturity on FA concentrations, and harvest schedules were based on approximate maturity levels found in rotational grazing schemes. However, the FA concentration in all species was influenced ($P < 0.01$) by harvest, which shows the capricious nature of fatty acids (Figure 2.1). Lauric, palmitic, oleic, α-linolenic, and total FA decreased in concentration at each consecutive harvest. Ultimately, these FA concentrations were 5 to 25% lower at the third harvest compared to the first harvest. In contrast, myristoleic and linoleic acid increased at each successive harvest. By the third harvest, myristoleic acid concentrations were 1.6 times greater than at the initial harvest while linoleic acid increased 5% over the same period. The response of steric acid to harvest depended on species; concentrations in orchardgrass, tall fescue, and perennial ryegrass trended up over successive harvests while those in alfalfa trended downward (Figure 2.1).

When evaluating the chlorophyll content it responded in an opposite fashion to steric acid. Among the grasses, chlorophyll *a*, *b*, and total concentration decreased by 14.8, 7.9, and 12.8% from the first harvest to the last harvest. On the other hand, chlorophyll *a*, *b*, and total concentrations in alfalfa increased by 3.9, 7.6, and 1.8%, respectively, from the initial harvest to the last (Figure 2.2). There must be some mechanism(s) that is controlling the chlorophyll content differently between the grasses and legume tested. It may be a possibility that the membrane structure is somewhat different between the legume species than the grasses species generating this difference in chlorophyll. However, it might also be that since the alfalfa is able to fix N, unlike the grass species, the N content was slowly decreasing in the potted grasses while the alfalfa plants were able to accumulate more N as the study progressed giving these differing responses.

Others have shown that as plant maturity increases, FA concentrations decrease as a result of an increase in stem-to-leaf ratio or proportion of lamina in plant material (Gilliland et al., 2002; Elgersma et al., 2003) This suggests that if these forages were rotationally grazed on shorter cycles, FA concentrations would remain relatively stable compared to longer intervals between grazing events. However, the values reported here and by Clapham et al. (2006) might over estimate the FA levels that would accumulate in a field setting. Both our results and those of Clapham et al. (2006) show that by growing these plants in a greenhouse, that the FA values are nearly double those reported from field grown materials (Dewhurst et al., 2001; Boufaied et al., 2003). It does suggest that biotic and abiotic stresses in the field play a greater role than plant genetics in determining the concentration of FAs present in pastures.

Population Differences

Among populations tested, the effect of harvest was found to be significant; however, few interactions between populations and harvests existed. Thus, the data for each population was pooled across all three harvests for analysis (n=9 for each population) and is shown in Tables 2.3, 2.4, 2.5, and 2.6. Within each species, significant ($P < 0.05$) differences were found for each FA with two exceptions. Myristoleic and steric acid concentrations did not differ in the tall fescue populations (Table 2.4). However, the discovery that myristoleic and steric acid were not different within the tall fescue population might be of little consequence because the two only comprise 6% of the total FA content. The perennial ryegrass populations had the lowest variation for FA levels (Table 2.5), while alfalfa had the greatest variation in palmitic, linoleic, and α-linolenic, and total FA content (Table 2.6).

For total FA, orchardgrass populations ranged from 38.0 to 43.0 mg g^{-1}, tall fescue ranged from 40.3 to 46.0 mg g^{-1} and perennial ryegrass ranged from 45.2 to 49.0 mg g^{-1}. Total fatty acid concentrations in alfalfa were nearly identical to those in orchardgrass but had a slightly wider range (37.5 to 45.0 mg g^{-1}). For α-linolenic acid, perennial ryegrass populations contained the highest amounts with 'Caravelle' containing the greatest concentration at 34.2 mg g^{-1}. The alfalfa populations had the largest range of α-linolenic acid (21.6 to 27.1 mg g^{-1}), but the lowest concentration of all the populations. Orchardgrass and tall fescue were both intermediate with α-linolenic ranging from 24.1 to 29.6 and 27.0 to 31.7 mg g^{-1}, respectively. A noteworthy trend occurring within the populations of all species is that there is not just one single FA that is high, but that all FAs seem to follow the trend of being either high or low throughout a single population.

This means that if α-linolenic acid is high, linoleic, palmitic, and the remaining FAs are high as well. This trait gives plants the ability to maintain rather constant lipid proportions.

In contrast to the grasses, alfalfa contained the highest levels of linoleic and palmitic acids. Linoleic acid averaged 15% of the total FA ranging from 5.5 to 7.1 mg g^{-1} which is about 2 to 3 mg g^{-1} greater ($P < 0.05$) than any of the grasses (Table 2.6). Palmitic acid was similar to that of linoleic acid comprising 14% of the total FAs in alfalfa and having values ranging from 5.2 to 6.3 mg g^{-1}. This is compared to 4.1 to 4.8 mg g^{-1} for orchardgrass and tall fescue and 4.9 to 5.4 mg g^{-1} for perennial ryegrass (Tables 2.3 through 2.5). Additionally, steric and oleic acid levels were the highest among the alfalfa populations ranging from 0.71 to 0.79 mg g^{-1} and 0.65 to 0.89 mg g^{-1}, respectively. For the same FA orchardgrass had the lowest range from 0.34 to 0.6 mg g^{-1} and 0.38 to 0.49 mg g^{-1}, respectively.

The population differences reported here are larger than the ranges for tall fescue, orchardgrass, and alfalfa reported by Boufaied et al. (2003), or the ranges reported by Gilliland et al. (2002) for perennial ryegrass. However, when comparing the results of Boufaied et al. (2003) to Gilliland et al. (2002), the differences between species are nearly the same. These differences may be due to the amount of material screened, genetic background of the populations, or environment (i.e. field vs. greenhouse). Of those three factors, environment seems to be the most influential factor as Clapham et al. (2006) reported similar variation to our results with only three varieties of chicory (*Cichorium intybus* L.). Therefore, it seems that the removal of environmental factors accentuates the genetic component of each population.

Fatty Acid Correlation to Phenotypic Traits

Phenotypic traits that are easily quantifiable are important tools that may be used by breeders to enhance other traits. In the case of FAs, several traits may be related to the polyunsaturated fats or other FA present in the plant. Dewhurst et al. (2002) suggested that the stay–green trait allows for continued chloroplast integrity, which prevents reductions in polyunsaturated FAs due to chlorophyll breakdown. Additional traits that can influence FA concentration include maturity and ploidy, which were suggested by Dewhurst et al. (2001) and investigated by Gilliland et al. (2002). Lastly, the phenotypic trait of winter hardiness is used as an indicator of winter survival. The ability to survive cold temperatures is thought to be responsible in part by the ability to alter FA concentrations (Levit, 1980). Therefore, it is suspected that the plants may have inherent high or low levels of particular FAs, most notably polyunsaturated FAs that can be identified by one or more phenotypic characters.

Ploidy level and maturity did not correlate to any of the FAs examined in our study. This result is in contrast to that reported by Gilliland et al. (2002) where it was found that perennial ryegrass diploids had significantly ($P < 0.05$) greater linoleic acid but tetraploids had significantly ($P < 0.05$-0.001) more α-linolenic acid. In addition, Gilliland et al. (2002) found that early flowering cultivars contained greater linoleic acid concentrations than later maturing cultivars, but the opposite was true for α-linolenic acid. However, this result was not consistent throughout their experiment which may lead to these traits not being as important as other traits measured in our study.

Winter hardiness was correlated ($P > 0.01$) to linoleic acid in orchardgrass (r = 0.28) and ($P > 0.05$) in tall fescue to α-linolenic acid (r = 0.18) (data not shown). Samala et al., (1998) reported that the winter hardy bermudagrass variety 'Midiron' had a higher percentage of α-linolenic acid after 21 days of cold treatment than the non-tolerant 'U3' variety. In contrast, winter hardiness in alfalfa had a much stronger correlation to individual FAs when compared to that of the grass species. Both palmitic and linoleic acid were correlated ($P > 0.01$) to winter hardiness (r = 0.61) (Figure 2.4). In addition, α-linolenic acid correlated at r = 0.41, significant at $P < 0.01$ (data not shown). For this study, winter hardiness, maturity, and ploidy ratings were received from the plant material source and combined through the use of similar checks for each species. A higher correlation might have been detected if all plants were screened for these traits under the same experimental conditions.

Fall dormancy is a relative ranking that describes the ability of alfalfa to terminate growth as temperatures begin to cool in autumn. Alfalfa populations ranged from a fall dormancy rating of 1 through 9. Like the winter hardiness ratings, alfalfa's fall dormancy correlated well with palmitic and linoleic acid. Palmitic, oleic, linoleic, α-linolenic, and total FA were significantly correlated to fall dormancy, however, palmitic and linoleic acid had highest correlation coefficients at 0.54 and 0.59, respectively (Figure 2.4). Oleic had a correlation coefficient of 0.45 while α-linolenic and total FA had respective values of 0.32 and 0.42 (data not shown). Therefore, it seems that an increase in the winter survivability, measured either by winter hardiness or fall dormancy has some basic impact on the FA levels even under non-freezing conditions. This result is supported by Alarcón Zúñiga (2003), where roots of several alfalfa cultivars differing in winter

hardiness levels were compared for FA concentrations and winter injury; cultivars with higher concentrations of linoleic, palmitic, and α-linolenic acid in autumn experienced less injury over winter. Correlation coefficients for linoleic acid ranged from -0.41 to -0.71 whereas α-linolenic acid varied from -0.38 to -0.51 and palmitic acid was 0.36 (Alarcón Zúñiga 2003).

Out of the phenotypic traits tested, total chlorophyll most closely correlated to FAs, particularly to α-linolenic and total FAs. Correlation coefficients for these two parameters ranged from 0.75 to 0.95 and 0.80 to 0.93, respectively (Figure 2.3). Both chlorophyll *a* and *b* correlated well with α-linolenic and total FAs, along with other FAs, but the greatest correlation coefficients were produced when chlorophyll *a* and *b* were combined into total chlorophyll. This result may have great implications as a rapid, species-dependent, quantifiable trait that relates to the α-linolenic and total FA concentration without the need for lengthy laboratory analysis.

This relationship was initially discussed by Mayland et al. (1976) when comparing total chlorophyll and total FAs with increasing N fertilization. Using perennial ryegrass, wheatgrass (Agropyron desertorum (Fisch.) Schult.), and wheat (*Triticum aestivum* L.), they found correlation coefficients of 0.89-0.93, 1.00, and 0.96, respectively, for each species. These results are similar to the correlation coefficients found for the grasses in our study and support the fact that nearly 70% of the lipids within a plant cell reside in chloroplast membranes (Taiz and Zeiger, 2006). However, this correlation is indicative of the amount of membrane structures contained within the chloroplasts, and not the amount of total chloroplasts. When working with chlorophyll deficient mutants in grass and broadleaf species it was observed that the amount of

chloroplasts was nearly identical but the amount of thylakoids per granum, total grana, and the stroma lamellae structures were reduced by nearly half when compared to wildtype plants (Goodchild et al., 1966; Schmidt et al., 1966; Keck and Dilley, 1970; Bolton et al., 1978; and Kirchhoff et al., 1989). Additionally, Bolton et al. (1978) using a barley chlorophyll *b* mutant found that steric and oleic levels were elevated compared to the wild-type, but that levels of α-linolenic and total FA were reduced in the mutant. Further, it has been reported that α-linolenic increases while linoleic decreases after etiolated plants are exposed to light (Tremolieres and Lepage 1971; Roughan and Boardman, 1972). Therefore, by increasing either the number of membranes contained in chloroplasts or number of chloroplasts, an increase in the levels of FAs present is plausible.

Conclusions

Overall, there were differences in fatty acids, both between species and within populations, of perennial ryegrass, tall fescue, orchardgrass and alfalfa. Perennial ryegrass contained the greatest concentrations of α-linolenic and total FAs while tall fescue and orchardgrass were intermediate and alfalfa possessing the lowest concentration. However, alfalfa contained the greatest concentrations of palmitic and linoleic acids while concentrations in the grass species was considerably less. Other FAs were also found to be different between species and among populations but have little biological significance due to their small percentage (4.1 to 10.0%) of total fatty acid. Perennial ryegrass had the least variation in fatty acid concentrations for the populations tested, although tall fescue populations did not have differences for myristoleic and steric acids. Alfalfa had the greatest variation for all FAs examined and there may be an

opportunity to select for and increase particular FAs within the *Medicago* genus. The phenotypic traits of maturity and ploidy level had no influence on FA concentrations, even though they have in other reports. Winter hardiness and fall dormancy, especially in alfalfa, may have a greater influence for both polyunsaturated and saturated FA levels than the other phenotypic traits other than chlorophyll. Total chlorophyll levels had the greatest correlation to α-linolenic and total FAs.

Indeed there seems to be an overall relatedness in that the majority of the plants FA reside in the chloroplast. These FA correlate to the amount of chlorophyll present, therefore to accommodate the increased levels of chlorophyll, the chloroplast must create more internal membrane structures for the chlorophyll to reside. Additionally, for a plant to remain functional under cold temperatures (i.e. winterhardy) the fluidity of the membrane needs to be maintained in the chloroplast. This is partially accomplished by increasing the amount of polyunsaturated FAs in those membranes. From these data it is apparent that there are underlying genetic differences in addition to phenotypic traits we tested that associate with particular fatty acids. However, in FAs, grass species there may not be great opportunity to increase particular FA levels due to the limited amount of variation found.

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Table 2.1. Near-infrared reflectance spectroscopy (NIR) calibration for the quantification of eight fatty acids and chlorophyll *a*, *b*, and total chlorophyll in orchardgrass, tall fescue, perennial ryegrass, and alfalfa.

Fatty Acid	Math Treatment[a]	Scatter Correction[b]	N	Mean	SEC[c]	SECV[d]	R^{2e}	1-VR[f]
				----------(%)----------				
Lauric Acid (C12:0)	2, 12, 8, 1	None	96	0.026	0.0017	0.0022	0.83	0.73
Myristoleic Acid (C14:1)	2, 4, 4, 1	SNV and DT	79	1.49	0.24	0.35	0.97	0.93
Palmitic Acid (C16:0)	2, 4, 4, 1	SNV and DT	96	5.04	0.18	0.21	0.94	0.91
Steric Acid (C18:0)	2, 4, 4, 1	SNV and DT	89	0.66	0.04	0.05	0.95	0.90
Oleic Acid C18:1)	2, 12, 8, 1	None	92	0.61	0.09	0.09	0.72	0.68
Linoleic Acid (C18:2)	2, 4, 4, 1	None	96	4.71	0.39	0.48	0.92	0.88
α-Linolenic Acid (C18:3)	2, 4, 4, 1	None	93	26.83	0.85	1.31	0.96	0.91
Chlorophyll *a*	2, 2, 2, 1	None	94	9.93	0.47	0.73	0.91	0.79
Chlorophyll *b*	2, 4, 4, 1	None	93	2.81	0.12	0.19	0.92	0.80
Total Chlorophyll	2, 4, 4, 1	None	94	12.76	0.57	0.86	0.92	0.81

Data are expressed on a dry matter basis. [a] Math treatment designations: derivative order, gap, first smoothing, and second smoothing, respectively. [b] Scatter corrections were none or standard normal variance (SNV) and detrend (DT) transformations. [c] SEC, standard error of the calibration. [d] SECV, standard error of the cross validation. [e] R^2, coefficient of determination for calibration. [f] 1-VR, one minus the variance ratio (the ratio of unexplained variance to total variance), is the coefficient of determination for cross validation during modified partial least squares regression.

Table 2.2. Forage species and populations tested for fatty acid concentrations.

Species	Population	Maturity	Ploidy Level	Winter hardiness	Fall Dormancy	Source
Orchardgrass	Ambassador	Early	Tetraploid	Excellent	NA	DLF
	Baridana	Mid	Tetraploid	Excellent	NA	Barenbrug Seed
	Barlemas	Late	Tetraploid	Poor	NA	Barenbrug Seed
	Benchmark	Early	NA	Moderate	NA	FFR Coop
	Boone (PI 578555)	Early	NA	Excellent	NA	GRIN
	Chinook (PI 311033)	Early	NA	Excellent	NA	GRIN
	Columbian ecotype (PI 308542)	Early	NA	NA	NA	GRIN
	Dolcea (PI 596697)	Late	Diploid	Moderate	NA	GRIN
	Early Arctic	Late	Tetraploid	Excellent	NA	Northstar Seed
	Haymaster	Late	Diploid	NA	NA	FFR Coop
	Intensive	Late	Tetraploid	Excellent	NA	Barenbrug Seed
	Iranian ecotype (PI 380819)	NA	NA	NA	NA	GRIN
	Juno (PI 381116)	Mid	NA	Excellent	NA	GRIN
	Latar (PI 578561)	Late	NA	NA	NA	GRIN
	Ludovic	Mid	Diploid	Excellent	NA	DLF Trifolium
	Mammoth	Early	Tetraploid	Excellent	NA	DLF
	Okay	Late	Diploid	Excellent	NA	PICKSEED
	Palestine (PI 578563)	NA	Tetraploid	Poor	NA	GRIN
	Pennlate (PI 578554)	Late	NA	Moderate	NA	DLF
	Potomac (PI 578553)	Early	Tetraploid	Moderate	NA	DLF

Table 2.2. cont.

Species	Population	Maturity	Ploidy Level	Winter hardiness	Fall Dormancy	Source
Tall Fescue	Atlas	Early	Hexaploid	Excellent	NA	ProSeeds
	AuTrimph	Early	Hexaploid	Poor	NA	DLF
	Barcarella	Mid	Hexaploid	Moderate	NA	Barenbrug Seed
	Bariane	Late	Hexaploid	Excellent	NA	Barenbrug Seed
	Barollex	Late	Hexaploid	Excellent	NA	Barenbrug Seed
	Barvetia (PI 601384)	NA	Hexaploid	NA	NA	GRIN
	Chilean ecotype (PI 427127)	NA	Hexaploid	NA	NA	GRIN
	Courtenay (PI 578728)	Late	Hexaploid	Moderate	NA	Northstar Seed
	Drover	Early	Hexaploid	Moderate	NA	Barenbrug Seed
	Festorina	Mid	Hexaploid	Excellent	NA	SRO Forage
	Forager (PI 600739)	Early	Hexaploid	NA	NA	GRIN
	Fuego	Mid	Diploid	Excellent	NA	DLF Trifolium
	Jesup	Mid	Hexaploid	Excellent	NA	Pennington Seed
	Jesup MaxQ	Mid	Hexaploid	Excellent	NA	Pennington
	Kentucky 31	Mid	Hexaploid	Moderate	NA	
	Russian ecotype (PI 314684)	NA	Hexaploid	NA	NA	GRIN
	Select	Early	Hexaploid	Excellent	NA	ProSeeds
	Stockman	Mid	Hexaploid	NA	NA	SRO Forage
	TF1 (PI 636645)	NA	Hexaploid	Excellent	NA	GRIN
	Tuscany II	NA	Hexaploid	NA	NA	SRO Forage

Table 2.2. cont.

Species	Population	Maturity	Ploidy Level	Winter hardiness	Fall Dormancy	Source
Perennial Ryegrass	Anaconda	Early	Tetraploid	Excellent	NA	DLF Trifolium
	BAR1M	Early	Diploid	Excellent	NA	Barenbrug Seed
	Barnhem	Late	Diploid	Moderate	NA	Barenbrug Seed
	Barvestra (PI 403849)	Early	Diploid	NA	NA	GRIN
	Bastion	Early	Tetraploid	Moderate	NA	PICKSEED
	Bolivian ecotype (PI 306292)	Early	NA	Poor	NA	GRIN
	Caravelle (PI 600775)	Mid	Diploid	Poor	NA	GRIN
	Compliment	Late	Diploid	Moderate	NA	DLF Trifolium
	Fanal	Late	Tetraploid	Poor	NA	DLF Trifolium
	Loretta (PI 600768)	Late	Diploid	NA	NA	GRIN
	New Zealand ecotype (PI 462335)	Early	NA	NA	NA	GRIN
	Norlea (PI 278773)	NA	NA	Excellent	NA	GRIN
	Odenwalder (PI 276666)	NA	Diploid	NA	NA	GRIN
	PR2 (PI 639822)	NA	NA	Excellent	NA	GRIN
	Quartet	Late	Tetraploid	Moderate	NA	AMPAC Seed
	Raunui (PI 462341)	Early	NA	NA	NA	GRIN
	Remington	Mid	Tetraploid	Excellent	NA	Barenbrug Seed
	Tonga	Mid	Tetraploid	Excellent	NA	AMPAC Seed
	Turkish ecotype (PI 340105)	Late	NA	NA	NA	GRIN

Table 2.2. cont.

Species	Population	Maturity	Ploidy Level	Winter hardiness	Fall Dormancy	Source
	6200HT	NA	NA	Excellent	2	Garst
	6420	NA	NA	Excellent	4	Garst
	Afghan ecotype (PI 212106)	Early	NA	Moderate	NA	GRIN
	Cody (NSL 4101)	NA	NA	NA	2	GRIN
	Demnate town (PI 516892)	Early	NA	Poor	NA	GRIN
	Evergreen	NA	NA	Excellent	4	NK
	FC24280 (PI 405064)	Mid	Diploid	Excellent	NA	GRIN
	Forcast 1001	Early	Tetraploid	Excellent	4	Dairyland Seed
	Forcast 3001	Late	Tetraploid	Excellent	3	Dairyland Seed
	Genoa	NA	NA	Excellent	4	NK
Alfalfa	Moapa (W6 22308)	NA	NA	Poor	8	GRIN
	PGR12483 (PI 467974)	Early	NA	Excellent	NA	GRIN
	PHI variety P	NA	NA	Excellent	4	Pioneer
	PHI variety Q	NA	NA	Poor	8	Pioneer
	PHI variety R	NA	NA	Poor	9	Pioneer
	Rambler (PI 255962)	Mid	NA	Excellent	1	GRIN
	San Francisco (PI 478553)	Early	NA	Poor	NA	GRIN
	Saudi Arabian ecotype (PI 183261)	Early	NA	Poor	NA	GRIN
	Severyanka (PI 502449)	Late	Diploid	Excellent	1	GRIN
	Turkish ecotype (PI 464767)	Late	Tetraploid	Moderate	NA	GRIN
	WL342	NA	NA	NA	4	W-L Seed

NA not available; PHI Pioneer Hi-Bred International Inc. varieties; PI and NLS lines available through Germplasm Resources Information Network (GRIN).

Table 2.3. Differences in fatty acid content between populations of orchardgrass. Values represent averages of three replications over three harvests (n=9 per population).

Population	lauric	myristoleic	palmitic	steric	oleic	linoleic	α-linolenic	Total
	----------mg g^{-1} dry matter----------							
Ambassador	0.027	1.34	4.40	0.45	0.45	4.07	27.16	40.89
Baridana	0.027	1.50	4.41	0.50	0.45	3.81	27.04	40.75
Barlemas	0.023	2.24	4.11	0.55	0.38	3.77	24.10	38.18
Benchmark (PI 538331)	0.026	1.25	4.38	0.44	0.45	4.07	26.89	40.50
Boone (PI 578555)	0.029	1.05	4.82	0.44	0.47	4.49	29.62	43.92
Chinook (PI 311033)	0.029	1.14	4.61	0.43	0.49	4.21	28.94	42.85
Columbian ecotype (PI 308542)	0.027	1.39	4.38	0.47	0.44	3.96	26.96	40.62
Dolcea (PI 596697)	0.026	2.17	4.43	0.60	0.49	4.11	27.05	41.88
Early Arctic	0.025	1.91	4.31	0.50	0.38	4.14	25.24	39.50
Haymaster	0.027	1.71	4.42	0.51	0.45	4.12	26.91	41.14
Intensiv	0.028	1.26	4.57	0.45	0.46	4.23	28.08	42.09
Iranian ecotype (PI 380819)	0.025	0.77	4.35	0.34	0.49	3.75	27.18	39.91
Juno (PI 381116)	0.026	1.46	4.22	0.50	0.43	3.67	25.84	39.15
Latar (PI 578561)	0.024	1.61	4.22	0.47	0.43	3.91	25.09	38.74
Ludovic	0.027	1.46	4.39	0.52	0.45	3.90	26.77	40.52
Mammoth	0.027	1.03	4.42	0.38	0.44	4.00	27.40	40.71
Okay	0.024	1.69	4.26	0.50	0.40	3.87	25.44	39.18
Palestine (PI 578563)	0.024	1.59	4.17	0.49	0.44	3.49	25.27	38.48
Pennlate (PI 578554)	0.024	1.86	4.21	0.56	0.45	3.92	25.83	39.85
Potomac (PI 578553)	0.027	1.50	4.49	0.48	0.44	4.20	27.19	41.33
SEM	0.0000074	0.67	0.07	0.01	0.004	0.07	6.64	5.64
†LSD (0.05)	0.0025	0.76	0.24	0.10	0.06	0.25	2.40	2.21

† Fisher's Protected LSD (0.05)

Table 2.4. Differences in fatty acid content between populations of tall fescue. Values represent averages of three replications over three harvests (n=9 per population).

Population	lauric	myristoleic	palmitic	steric	oleic	linoleic	α-linolenic	Total
	----------mg g^{-1} dry matter----------							
Atlas	0.026	1.48	4.46	0.51	0.60	3.56	28.14	41.78
AuTrimph	0.024	1.43	4.30	0.49	0.56	3.56	27.14	40.51
Barcarella	0.024	1.85	4.37	0.54	0.59	3.62	27.41	41.39
Bariane	0.025	1.97	4.51	0.57	0.62	3.59	28.39	42.67
Barolex	0.025	2.13	4.50	0.60	0.60	3.64	27.46	41.95
Barvetia (PI 601384)	0.025	1.36	4.47	0.49	0.61	3.66	28.45	42.07
Chilean ecotype (PI 427127)	0.026	1.29	4.71	0.47	0.61	3.98	29.48	43.57
Courtenay (PI 578728)	0.025	2.12	4.48	0.59	0.62	3.53	28.20	42.56
Drover	0.024	1.39	4.20	0.50	0.54	3.43	27.25	40.34
Festorina	0.025	1.69	4.28	0.53	0.56	3.58	27.01	40.66
Forager (PI 600739)	0.026	1.68	4.40	0.56	0.58	3.67	28.92	42.85
Fuego	0.027	1.45	4.42	0.51	0.62	3.47	28.83	42.34
Jesup	0.026	1.83	4.51	0.55	0.60	3.58	28.70	42.80
Jesup MaxQ	0.026	1.62	4.46	0.51	0.60	3.42	28.26	41.89
Kentucky 31	0.026	1.81	4.52	0.53	0.63	3.48	28.54	42.54
Russian ecotype (PI 314684)	0.024	1.65	4.46	0.52	0.62	3.49	28.09	41.85
Select	0.027	1.69	4.54	0.51	0.65	3.45	29.17	43.03
Stockman	0.026	1.86	4.44	0.54	0.62	3.41	28.47	42.36
TF1 (PI 636645)	0.029	1.63	4.78	0.52	0.67	3.63	31.74	46.00
Tuscany II	0.025	2.06	4.48	0.55	0.61	3.38	28.29	42.40
SEM	0.0000042	0.39	0.05	0.007	0.003	0.09	3.84	3.77
†LSD	0.0019	NS	0.21	NS	0.05	0.28	1.82	1.81

† Fisher's Protected LSD (0.05)

Table 2.5. Differences in fatty acid content between populations of perennial ryegrass. Values represent averages of three replications over three harvests (n=9 per population).

Population	lauric	myristoleic	palmitic	steric	oleic	linoleic	α-linolenic	Total
	----------mg g^{-1} dry matter----------							
Anaconda	0.029	2.74	4.91	0.68	0.60	3.88	30.80	46.65
BAR 1M	0.028	2.55	4.94	0.67	0.58	3.88	30.29	45.95
Barnhem	0.027	2.83	4.93	0.72	0.64	3.68	29.40	45.22
Barvestra (PI 403849)	0.029	2.53	4.94	0.66	0.60	3.78	31.06	46.60
Bastion	0.031	2.28	5.09	0.65	0.62	3.90	32.12	47.69
Bolivian ecotype (PI 306292)	0.031	1.82	5.17	0.60	0.65	3.97	32.95	48.20
Caravelle (PI 600775)	0.031	1.99	5.18	0.64	0.71	3.77	34.19	49.51
Compliment	0.028	2.93	4.91	0.74	0.61	3.81	29.81	45.83
Fanal	0.028	3.19	4.94	0.77	0.62	3.85	29.88	46.27
Loretta (PI 600768)	0.032	1.59	5.25	0.57	0.68	4.07	33.83	49.02
New Zealand ecotype (PI 462335)	0.032	0.77	5.37	0.47	0.72	3.88	34.60	48.84
Norlea (PI 278773)	0.029	2.64	5.03	0.69	0.63	3.92	31.38	47.31
Odenwalder (PI 276666)	0.030	1.89	5.05	0.60	0.65	3.72	32.92	47.87
PR2 (PI 639822)	0.031	2.12	5.10	0.61	0.62	3.93	32.77	48.17
Quartet	0.031	2.21	5.26	0.65	0.61	4.21	32.42	48.38
Raunui (PI 462341)	0.031	1.79	5.32	0.59	0.68	4.10	33.26	48.78
Remington	0.028	2.58	4.89	0.69	0.60	3.69	30.73	46.21
Tonga	0.030	2.26	5.12	0.67	0.63	3.98	32.04	47.73
Turkish ecotype (PI 340105)	0.029	2.19	5.01	0.63	0.61	3.85	31.04	46.36
SEM	0.0000076	0.80	0.07	0.013	0.003	0.09	7.24	5.64
†LSD	0.002	0.83	0.26	0.11	0.05	0.28	2.51	2.22

† Fisher's Protected LSD (0.05)

Table 2.6. Differences in fatty acid content between forage populations of alfalfa. Values represent averages of three replications over three harvests (n=9 per population).

Population	lauric	myristoleic	palmitic	steric	oleic	linoleic	α-linolenic	Total
	----------mg g^{-1} dry matter----------							
6200HT	0.023	0.77	5.19	0.76	0.65	5.54	21.59	37.53
6420	0.027	0.50	6.03	0.75	0.74	6.77	27.14	44.96
Afgan Ecotype (PI 212106)	0.024	0.62	5.77	0.73	0.72	6.30	24.73	41.89
Cody NSL 4101	0.025	0.65	5.61	0.74	0.71	6.24	24.74	41.72
Demnate town (PI 516892)	0.024	0.68	5.38	0.76	0.66	5.87	23.70	40.07
Evergreen	0.027	0.68	5.86	0.75	0.72	6.49	26.17	43.70
FC24280 (PI 405064)	0.025	0.63	6.25	0.74	0.89	7.13	26.10	44.75
Forcast 1001	0.025	0.55	5.68	0.74	0.70	6.20	24.89	41.79
Forcast 3001	0.026	0.59	5.74	0.75	0.70	6.38	24.87	42.05
Genoa	0.027	0.67	5.93	0.74	0.68	6.68	26.15	43.88
Moapa (W6 22308)	0.025	0.55	5.55	0.73	0.68	6.02	25.35	41.90
PGR12483 (PI 467974)	0.024	0.54	5.64	0.73	0.73	6.11	25.55	42.31
PHI variety P	0.023	0.65	5.44	0.76	0.66	5.94	23.60	40.07
PHI variety Q	0.026	0.61	5.64	0.74	0.71	6.11	25.57	42.40
PHI variety R	0.023	0.68	5.21	0.73	0.65	5.55	22.25	38.09
Rambler (PI 255962)	0.023	0.70	5.64	0.75	0.71	6.26	24.07	41.16
San Francisco (PI 478553)	0.024	0.44	5.22	0.71	0.67	5.57	23.62	39.25
Saudi Arabian ecotype (PI 183261)	0.026	0.82	5.35	0.79	0.66	5.48	24.11	40.23
Severyanka (PI 502449)	0.025	0.78	5.97	0.74	0.77	6.83	25.67	43.78
Turkish ecotype (PI 464767)	0.023	0.63	5.76	0.73	0.74	6.59	24.30	41.77
WL342	0.027	0.65	5.98	0.76	0.74	6.61	26.43	44.20
SEM	0.0000022	0.02	0.05	0.0009	0.002	0.10	2.81	4.22
†LSD	0.0014	0.14	0.22	0.02	0.04	0.29	1.56	1.91

† Fisher's Protected LSD (0.05); PHI varieties received from Pioneer Hi-Bred International, Inc.

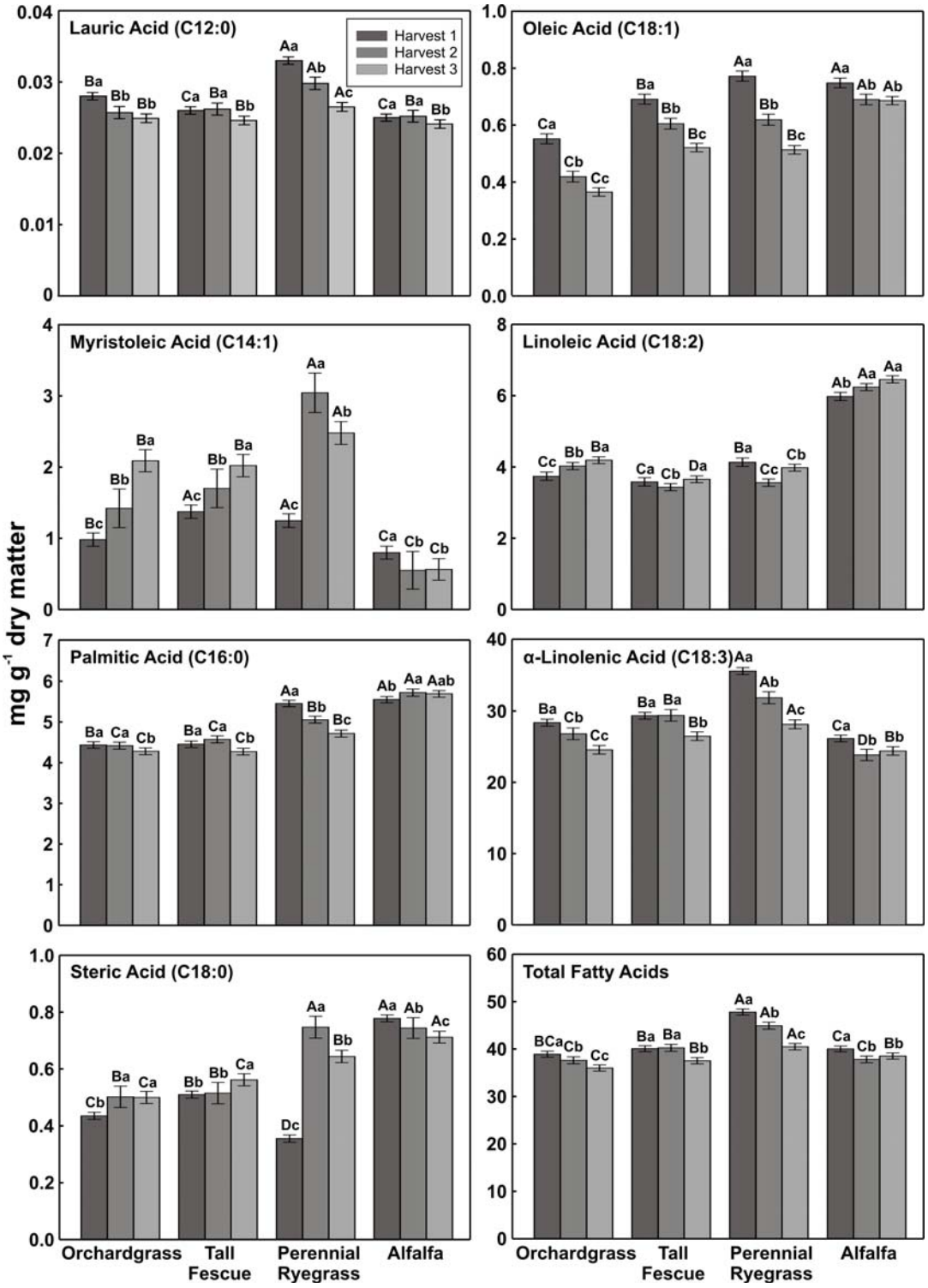

Figure 2.1. Differences between species for lauric (C12:0), myristoleic (C14:1), palmitic (C16:0), steric (C18:0), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3), and total fatty acids. Values are from the individual harvests 1, 2 and 3 across all species. Different capital letters indicate significant ($P < 0.05$) differences for an individual harvest across species. Different lower case letters indicate significant ($P < 0.05$) differences between individual harvests within each species. Error bars represent 2SEM.

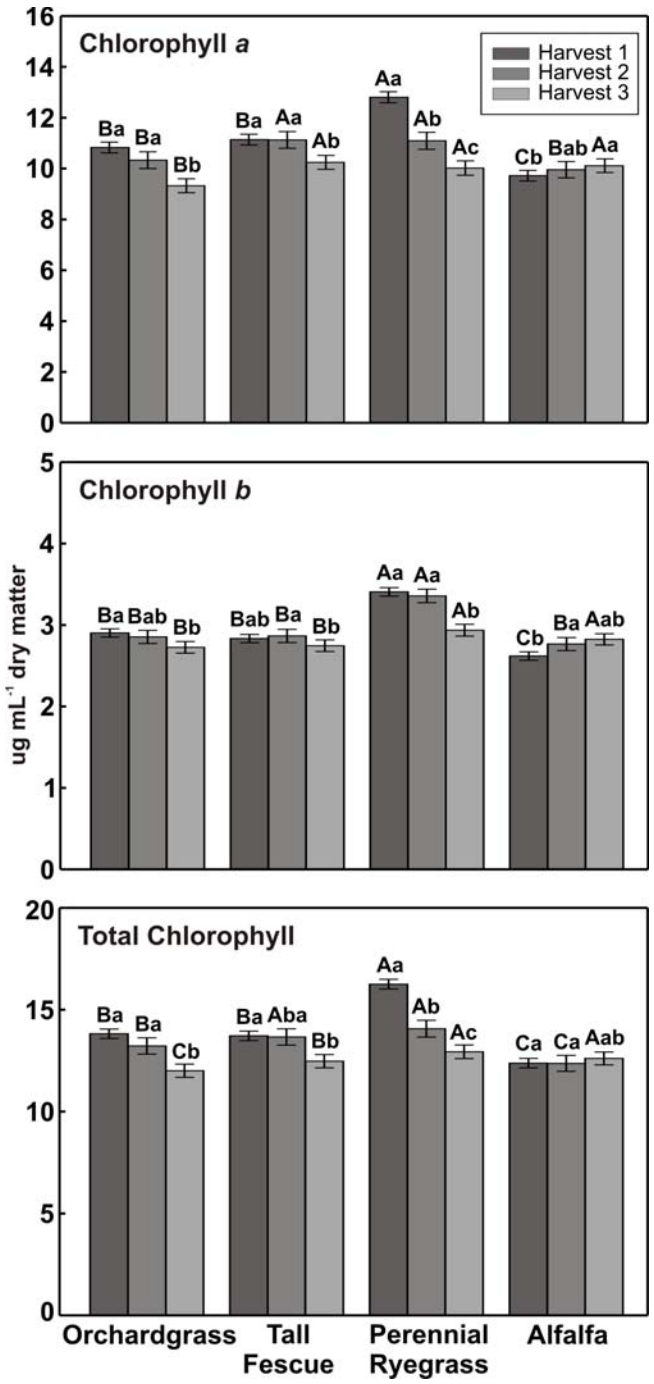

Figure 2.2. Differences between species for chlorophyll *a*, chlorophyll *b*, and total chlorophyll determined by NIR. Values are from the individual harvests 1, 2 and 3 across all species. The first letter indicates significant differences for an individual harvest across all species. The second letter indicates significant differences between individual harvests for each species.

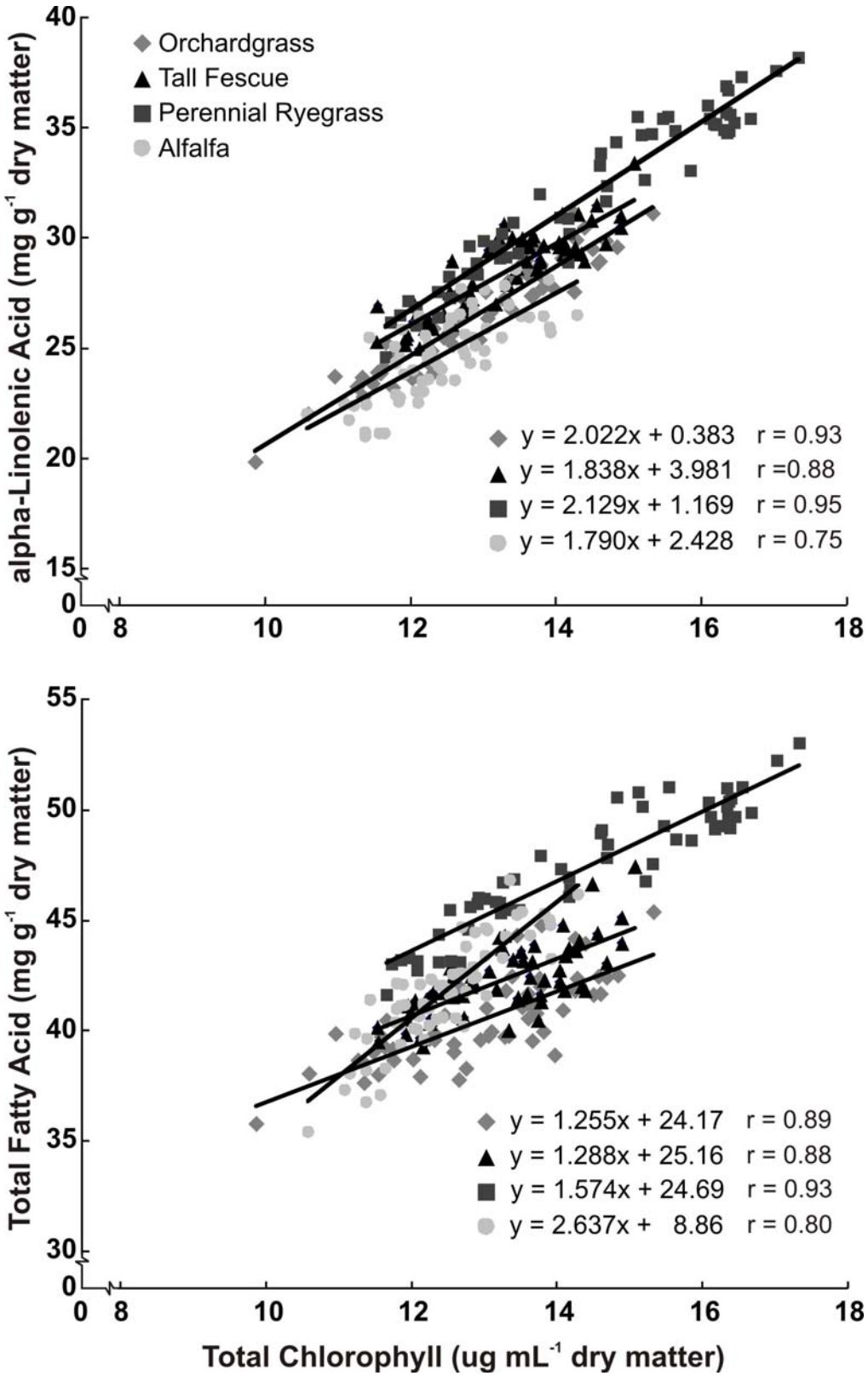

Figure 2.3. α-linolenic acid and total fatty acids correlation with total chlorophyll.

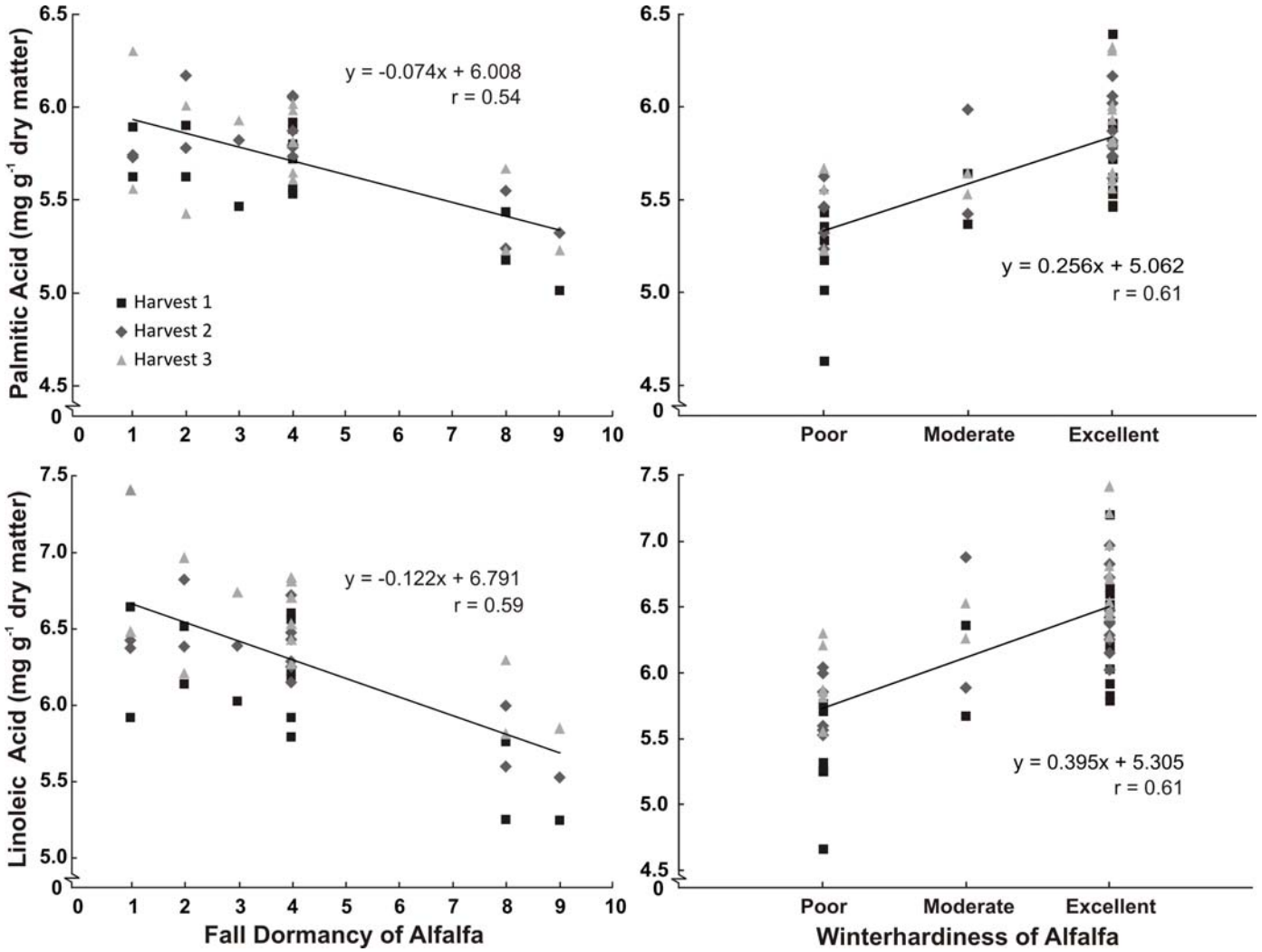

Figure 2.4. Fatty acids correlated to fall dormancy rating and winter hardiness in alfalfa over three harvests.

FATTY ACID CONTENT OF BEEF FINISHED ON PASTURE WITH DIFFERENT FORAGE SPECIES

ABSTRACT

Consumers are ever more concerned with the types and quantity of fat present in the food products they consume. This is leading to a shift in the way food is produced, in particular the animal industry increasing the number organic and naturally finished meats. The objective of this study was to determine if pastures composed of grass only or in grass legume mixtures would impact the FA composition of the meat of beef steers (*Bos taurus* L.). There were three pasture treatments that included only tall fescue [*Lollium arundinaceum* (Schreb.) S.J. Darbysh = *Schedonorus arundinaceus* (Schreb.) Dumort.], or tall fescue combined with either red clover (*Trifolium pretense* L.) or alfalfa (*Medicago sativa* L. ssp. *sativa* L.). Beef steers (n=9-10) rotationally grazed each pasture treatment composed of 12 individual paddocks. Forage from treatments were different for crude protein (CP), in vitro true digestibility (IVTD) and the fatty acids (FA) myristric (C14:0), palmitic (C16:0), palmitoleic (C16:1), steric (C18:0), oleic (C18:1), linoleic (C18:2), and total fatty acid. Steers grazing mixtures with either red clover or alfalfa had greater average daily gains (ADG) than cattle grazing tall fescue alone. Additionally, steers from the RC treatment had larger ribeye areas (REA) and greater finishing weights than those in the ALF treatment. However, the differences found between pasture treatments in FA concentration did not translate into any differences in the FA concentration of meat harvested from steers. Thus, it is concluded that although the pastures contained different FA concentrations, with the levels of legumes present, does not influence the FA content of beef.

INTRODUCTION

Recently, there has been a movement by both dietitians and the health conscious public to promote the consumption of healthier forms of livestock products. The current model of finishing animals, in particular beef, is to have these animals consume a high concentrate diet such as corn (*Zea mays* L.) or corn byproducts prior to slaughter. This system has led to meat products that contain high amounts of saturated fat and an imbalance in the ratio between omega-6 and omega-3 (*n*-6:*n*-3) polyunsaturated fatty acids (PUFA). In western diets, the *n*-6:*n*-3 ratio approaches 15:1; however, a more appropriate level would be 2:1 or less (Simopoulos, 1998). Meat from pasture finished animals has greater amounts of *n*-3 fatty acids (FA) and also has the added benefit of greater levels of conjugated linoleic acid (CLA). Both *n*-3 and CLA have been shown to lower the risk of heart disease, diabetes, and obesity as well as contain anticarcinogenic properties (Leaf and Kang, 1998; Sumeca and Miller, 2000; Weiss et al., 2004).

This information has led to many studies in dairy and beef that compare the current feeding practices to feeding animals a forage diet (either pasture or conserved forages), or supplemental oils high in *n*-3 FA. Dairy cattle provided a high-quality forage diet produce milk that has elevated levels of both *n*-3 and CLA (Dhiman et al., 1999). Similarly, beef cattle being finished on pasture diets, rather than traditional concentrate diets, also have meat products with improved nutritional quality (Dannenerger et al., 2005; French et al., 2000). When comparing high concentrate diets to those based on forages, the *n*-3 fraction of PUFA increased with a concomitant reduction of the SFA component found in the intramuscular fat (Dannenerger et al., 2005; Nuernberg et al.,

2005; Poulson et al., 2004; French et al., 2000). It was thought that adding a lipid source high in *n*-3 to the ration, such as linseed oil or fish oil, would allow the cattle to attain both high rates of gain and possess a higher proportion of PUFA (Scollan et al., 2001). Supplementing beef cattle with fish oil increased the amounts of long-chain PUFA and decreased the *n*-6:*n*-3 while linseed oil increased the amounts of short-chain PUFA (Scollan et al., 2001). Additionally, CLA concentrations increase with increasing amounts of forage in the diet (French et al., 2000). However, supplementing livestock with CLA did not increase CLA in meat as much as grazing cattle on high-quality pasture (Poulson et al., 2004).

Although it has been established that forage-based diets improve the FA quality of meat and milk products, few studies have evaluated the effects of forage species on FA content in livestock products. Dairy cattle fed red clover silage produced milk with greater amounts of linoleic and alpha-linolenic acid and lower amounts of saturated FA when compared to grass silage (Vanhatalo et al., 2007). Additionally, dairy cows fed red clover-grass silage produced milk with more PUFA and a lower *n*-6:*n*-3 ratio than those fed white clover (*Trifolium repens* L.)-grass silage (Steinshemn et al., 2007). Similarly, when lambs grazed pastures with high amounts of white clover and alfalfa, the intramuscular fat of the *longissimus thoracis* contained more PUFA than that of lambs that grazed perennial ryegrass or a "biologically diverse" pasture (Lourenco et al., 2007). Our objective was to determine if the FA concentrations in pasture-finished beef are altered by adding legumes to tall fescue pastures.

MATERIALS AND METHODS

Animal Pretreatment and Measures

Crossbred Angus steers were obtained from a local livestock auction in January and February, 2007 and were overwintered on a diet of corn, distillers dried grain with solubles (DDGS), chopped hay, and limestone in a 65%, 25%, 9%, and 1% proportion, respectively. The diet was mixed through a Knight 3020 reel mixer on a feed truck and fed in fenceline bunks at 08:00 daily. Steers were moved to pasture treatments (described below) in late March or early April 2007, and grazing continued through July 30, 2007. Pasture treatments included tall fescue only, tall fescue + red clover, or tall fescue + alfalfa, hereafter referred to as TF, RC, ALF, respectively. The pasture treatments were located at New Franklin, MO (39^{o} 1’ 2” N, 90^{o} 44’ 14” W) on a Menfro silt loam soil (Fine-silty, mixed, superactive, mesic Typic Hapludalfs). The paddocks in each treatment were rotationally grazed throughout the duration of the experiment based on forage availability. In March, alfalfa stands began to grow quickly due to above normal temperatures, and steers were able to begin grazing this treatment on 28 March. However, March was followed by a cold April where temperatures were below freezing. This killed much of the growth that occurred in March (Figure 3.2). Due to the weather conditions and associated forage availability, steer placement on the RC and TF pastures was delayed by 7 and 13 days, respectively to the ALF treatment. Cattle weights were recorded before placement on pasture treatments in mid-March (ALF 419 kg SEM 7.74, RC 434 kg SEM 6.39, and TF 450 kg SEM 8.81) and when removed from the treatments in late July. Additionally, a 5 g subcutaneous fat sample was taken from the tailhead of each animal in early March and analyzed for initial FA concentrations (analysis described

below). This initial data was used to stratify cattle within treatments as well as provide a covariate for analysis. The RC treatment had trees dispersed throughout the pastures that provided shade for the duration of the experiment. Livestock in the TF and ALF treatments had access to artificial shade structures from late June to the end of July. At the completion of the experiment the cattle where harvested by captive bolt stunning followed by exsanguination at a commercial packing plant in Omaha, NE.

Ultrasound

Beef steers prior to harvest had an ultrasound image taken of the ribeye to determine if size was impacted by forage treatments. Ultrasonic images were captured by AUSkey System Software (Animal Ultrasound Services, Ithaca, NY) using a 500V Aloka (Corometrics Medical Systems, Inc., Wallingford, CT) ultrasound machine with a 3.5-MHz transducer fitted to a custom beef animal standoff. The ultrasound technician was trained by Animal Ultrasound Services for live beef evaluations, transducer head placement, image collection, and interpretation. Ribeye area (REA) was captured between the 12th and 13th rib on the left side of each steer. Copious amounts of commercial vegetable oil were applied to the area being measured to decrease sound wave attenuation.

Forage Measurements, Sampling, and Management

Forage yield was estimated weekly using a FARMWORKS rising plate meter (Feilding, NZ). Briefly, 50 readings were recorded from each of 12 paddocks per treatment weekly and the rising plate meter was calibrated every 21 d during the grazing season. To calibrate the rising plate meter, ten pre or twelve post-grazing 0.8 x 4.6 m strips were cut to a 2 cm height with a tractor-mounted, flail-type harvester from the most

recently grazed and next-to-be-grazed paddock in each treatment. Apparent animal intake and pasture DM disappearance (PDMD) was determined from pre- and post-grazing measurements (Casler et al., 1998). A composite hand sample of approximately 300 g was collected from the harvested strips for an individual paddock. The forage sample was used to determine the DM content of the paddock by drying the sample using a forced-air oven for 24h at 90^{o}C. The sample was subsequently ground using a cyclone mill (Udy Corp., Ft. Collins, CO) to pass a 1 mm screen.

The ground hand sample, collected from the harvested material, was used to determine ash concentration due to the additional soil collected by the flail harvester. The ash was determined by placing a 1 g DM sample into an Isotemp Muffle Furnace (Fisher Scientific, Pittsburg, PA) at 550 ^{o}C for 2 h. Forage samples collected by hand separately from the harvested material at the time of harvest from the same paddock were used as a control ash sample in determining the additional ash percentage from the flail harvested samples. This value was used to correct the DM to estimate the PDMD for each paddock.

Prior to the beef steers entering a new paddock in the rotational grazing scheme, additional pasture samples were collected to determine crude protein (CP), neutral detergent fiber (NDF), *in vitro* true digestibility (IVTD), and FA composition. These samples were collected by cutting forage to 2 to 3 cm at 25 random locations within the paddock the animals were being placed. The samples were immediately frozen using liquid N and placed on dry ice until they could be stored in a freezer at -20 ^{o}C. All samples were then lyophilized and ground as described above.

Nitrogen concentration of forage samples was determined by thermoconductivity from a 100 mg sample analyzed by a Leco TruSpec FP-428 (LECO Corp., St. Joseph,

MI). The N concentration was then multiplied by 6.25 to obtain CP. Neutral detergent fiber was determined with an ANKOM 200 Fiber Analyzer (ANKOM Technology, Fairport, NY). *In vitro* true digestibility determinations were made by placing 250 mg (+/- 5 mg) of sample ANKOM F57 filter bags. Bags were pretreated with acetone for 3-5 minutes and then dried to remove any surfactant that would inhibit microbial digestion. Samples were then placed into a Daisy^{II} Incubator for 48 hours at a 39℃ +/- 0.5℃ followed by a washed with neutral detergent fiber solution as described by Spanghero et al. (2003). Rumen fluid was taken from a cannulated dry Holstein cow maintained on a forage diet. From these analyses, digestible NDF (dNDF) as a proportion of NDF was calculated (NRC, 2001).

Each pasture treatment consisted of three main pastures which were broken into four paddocks for a total of 12 paddocks per treatment (Figure 3.1). To maintain pasture in a vegetative state cattle were moved to a new paddock on average every 3.2 days. When growth in some paddocks became excessive and mature the forage was removed by making silage. The forage removal was determined by both the plate meter values along with visually determining the maturity level of the forage. Silage harvests were made once for the TF and ALF treatments in late May, while the RC treatment was harvested in late June.

Fatty Acid Determination

Fatty acids from pasture samples were extracted using the one-step method described by Sukhija and Plamquist (1988) and amended by Clapham et al. (2006). Ground samples were equilibrated to room temperature where 250 mg of leaf tissue was weighed and mixed with 1 mL of internal standard (IS) C17:0, 1 mL methanolic HCl

(prepared daily), and 500 uL of hexane. Samples were vortexed, placed in a water bath at 70^{o}C for 2 hours, then removed and allowed to equilibrate to room temperature. Once the samples reached room temperature, 2.5 mL of 60 mg g^{-1} potassium carbonate and 1 mL of hexane were added. Samples were vortexed and then centrifuged at 1750 rpm for 10 min. The top hexane layer was then removed and filtered using Supelclean ENVI-carb (Supelco, Bellefonte, PA) SPE tube with 250 mg of anhydrous sodium sulfate to remove any water. The samples were then stored at -20 ^{o}C until they could be analyzed.

The samples were analyzed with an Agilent 6890 gas chromatograph and a model 7683 automatic liquid sampler with a flame ionization detector (Agilent Technologies, Santa Clara, CA). A sample of 2 μL was injected and split 50:1 into a WCOT fused silica, chemically bonded capillary column (Chrompack CP-SIL 88 fused silica column100 m long, 0.25 mm inside diameter, 0.2 μm film thickness; Varian, Walnut Creek, CA). Helium at 20 mL min^{-1} was used as the carrier gas. The temperature ramp for the GC was as follows: 70 ^{o}C for 1 min; increase to 180 ^{o}C at 30 ^{o}C min^{-1}, hold for 1 min; increase to 220 ^{o}C at 2 ^{o}C min^{-1}; increase to 240 ^{o}C at 10 ^{o}C min^{-1}, hold for 0.5 min; for a total run time of 29.7 min. Injector temperature was set at 280 ^{o}C; detector temperature was set at 300 ^{o}C. Reference standard GLC-63b (Nu-Check Prep, Elysian, MN) was used to quantify and identify samples by their retention times. Samples were analyzed for C12:0, C14:0, C14:1, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2 and C18:3. Heptadecanoic acid (C17:0, 0.4 mg mL^{-1} in hexane; Matreya, Pleasant Gap, PA) was used as the internal standard. Total FA is represented by the sum of each FA examined in this experiment.

At the time of animal harvest, *Longissimus dorsi* samples were collected, vacuumed sealed, allowed to age 14 days, and then stored at -20 ^{o}C. These samples along with the tissue samples collected from the tail head of steers before being placed on their respective pasture treatments, were ground and lyophilized. Total crude fat and individual FAs were determined by the University of Missouri Experiment Station Chemical Laboratories describe by the AOAC official methods 954.02 and 965.49 and AOCS official methods Ce1h-05 and Ce2-66. The samples were analyzed with an Agilent Technologies gas chromatograph, model 7890A, equipped with a 7683 Series autosampler (Agilent Technologies, Santa Clara, CA). A sample was injected into a SP-2330 column (Supelco, St. Louis, MO), 30 meter x 0.25 mm, 0.20 micron-bonded phase thickness. FA reference standard, FAMQ-005, was purchased from AccuStandard (New Haven, CT) and used to identify and quantify samples by their retention times. Samples were analyzed for C14:0, C14:1, C15:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1t9, C18:1n9, C18:1n7, C18:2n6, C18:3n3 C18:4n3, C20:0, C20:1n9, C20:3n3, C20:4n6 C20:4n3, C20:5n3, C22:0, C22:1n9 C22:5n3, C22:63n3, C24:0, C24:1n9, and total CLA.

Statistical Analysis

Pastures systems were set in a completely randomized design (CRD) design as described by Steel and Torrie (1980). All pasture samples were analyzed using PROC GLM in SAS (SAS Inst. Inc., Cary, NC). Pasture samples collected from each paddock prior to cattle entering represents a replication for that pasture treatment. Mean separation was performed using orthogonal contrasts between TF and pastures with legumes and between RC and ALF treatments. Cattle represent the experimental unit where 9-10 animals (i.e. replications) were used per pasture treatment. The FA data from meat was

analyzed using PROC GLM in SAS (SAS Inst. Inc.) and subcutaneous fat samples taken prior to pasture placement were used as covariates for intramuscular fat analysis.

RESULTS AND DISCUSSION

Pasture Nutritive Value

The ALF treatment supplied cattle with significantly greater ($P < 0.01$) CP than either RC or TF. Forage from ALF treatment averaged 216 g kg^{-1} CP while that of TF and RC pasture treatments had mean values of 164 and 172 g kg^{-1}, respectively (table 3.1). The reason RC pastures were not different from TF pastures is most likely a direct result of the botanical composition of RC compared to ALF pastures. The RC treatment only averaged 16.5% red clover while the ALF treatment was comprised of 38.4 % alfalfa. However, both pasture treatments with legumes had higher IVTD than the TF treatment. The RC and ALF treatments were identical, averaging 864 g kg^{-1} IVTD, while TF averaged 849 g kg^{-1} (table 3.1). The high level of protein, especially in the ALF treatment, and the high digestibility of all treatments, reflected by the IVTD values, is likely due to the rotational grazing methodology that kept forage relatively high in nutritive value.

The concentrations of NDF were the greatest for the TF treatment averaging 509 g kg^{-1}. The RC treatment had an intermediate NDF concentration at 473 g kg^{-1} while the ALF treatment contained the least at 389 g kg^{-1} (table 3.1). The dNDF (as a proportion of NDF) averaged 658, 718, and 708 g kg^{-1} for ALF, RC, and TF treatments respectively (table 3.1). The dNDF concentration in the RC treatment was different from the ALF treatment however, TF was not different than either of the legumes. These data show that the paddocks of RC and TF have greater amounts of digestible fiber than that contained

in the ALF treatment. Although the ALF treatment contained significantly less NDF than the other pasture treatments, the digestibility of that fiber was much less than the RC or TF treatments.

The CP values for TF are nearly identical to those reported by Asay et al. (2002) while the IVTD level they reported was slightly higher. They evaluated 10 cultivars of tall fescue over two years and 5 irrigation regimes and had a CP mean value of 169 g kg^{-1} and an IVTD average of 860 g kg^{-1}. The RC treatment was also found to have similar CP levels as those described by Mourino et al., (2003) averaging 196 g kg^{-1} over three years; however, their IVTD levels were much lower with a mean of 761 g kg^{-1} even though the red clover percentage was slightly higher at 21%. The ALF treatment had considerable more CP than that the 174 g kg^{-1} reported by Marten and Jordan (1979) even though the alfalfa component in both studies was nearly identical at 40%. However, the grass component of the pasture in their study was comprised of orchardgrass (*Dactylis glomerata* L.) and smooth bromegrass (*Bromus inermis* L.) where ours was tall fescue. Additionally, the CP levels from our study are similar to a monoculture alfalfa pasture described by Hoveland et al. (1988).

Fatty Acid Content of Forage

Pastures were found to have significant differences in FA between the TF treatment and the pastures with legumes. These included FA myristic, palmitic, palmitoleic, steric, oleic, linoleic and total FA being either greater or less than in TF compared to either RC or ALF (Table 3.1). Additionally, palmitic, palmitoleic, and steric acid (Table 3.1) differed between RC and ALF treatments. The FA trend for each pasture treatment over the growing season can be observed in Figure 3.3. Linoleic acid was

greatly impacted by the addition of legumes. For instance, ALF and RC treatments contained greater ($P < 0.01$) concentrations of linoleic acid than TF treatment with treatment means of 4.67, 4.32, and 3.91 mg g^{-1}, respectively. In addition, palmitic acid differed for all three treatments. The ALF treatment contained the greatest concentration averaging 4.69 mg g^{-1}, followed by the RC at 4.01 mg g^{-1} and the TF treatment possessing the smallest concentration at 3.85 mg g^{-1}. The ALF and RC treatments contained 7.7, 9.8, and 9.0 % greater concentrations of palmitoleic, steric, and oleic acid, respectively, when compared to TF. Additionally, the palmitoleic and steric acid were different between the ALF and RC treatments with the ALF treatment containing 0.05 and 0.06 mg g^{-1}, more, respectively. Even though palmitoleic, steric, and oleic acid were different between pasture treatments they only comprise approximately 6.5% of the total FA. Total FA concentrations were only different between the TF and legume treatments with ALF containing 28.39 mg g^{-1}, RC 28.182 mg g^{-1}, and TF 27.21 mg g^{-1} of total FA (table 3.2).

These FA results are similar to other reports (Dewhurst et al., 2001; Boufaied et al., 2003) for tall fescue. Compared to our study, however, the red clover and alfalfa reported by Boufaied et al. (2003) contained approximately 37 and 56% less α-linolenic acid, respectively. The lower α-linolenic acid is partially due to the fact that Boufaied et al. (2003) used pure stands of red clover and alfalfa whereas our pastures were mixed grass/legume stands. Oddly enough, the TF treatment had the smallest α-linolenic acid content. Grasses normally comprise greater amounts of α-linolenic than legume species (Boufaied et al., 2003). However, the higher α-linolenic acid in the ALF and RC pastures may be due to the stage at which they were utilized by the cattle. The red clover and alfalfa tested by Boufaied et al. (2003) were harvested at about 10 % bloom whereas

samples collected for this study rarely had blooms when grazed. It has been shown that immature plants, or plants harvested frequently, contain greater levels of FAs (Dewhurst et al., 2001; Boufaied et al., 2003; Elgersma et al. 2003b; Elgersma et al. 2003c; Elgersma et al., 2005).

Beef Quality

When comparing the FA concentration of beef on either a percent of total fat or dry matter (mg g^{-1} DM) basis, no differences were observed between treatments tested (Table 3.3 and 3.4). This result was somewhat unexpected, first because of the differences found in FA content between the pasture treatments, and secondly because of reports that dairy cattle, beef cattle, and sheep grazing pastures containing red clover had significantly higher amounts on *n*-3 FA. Research regarding different forage diets is limited, but that which has been conducted is primarily with dairy cattle. Elgersma et al., (2003a) reported milk differences for CLA (C18:2 *c*9,*t*11), along with lower SFA and elevated PUFA from cows fed a perennial ryegrass cultivar high in linoleic and α-linolenic acid. However, a majority of these studies compare a diet composed of red clover to grass only diets. Milk fat contained significantly higher PUFA and *n*-3 FA when diets containing between 40 and 100% red clover were compared to diets with grass only or white clover pasture/silages (Dewhurst et al., 2003a; Vanhatalo et al., 2007; Steinshemn et al., 2007). Additionally, differences in meat, abomasum, or duodenal flow of FA were found in lamb and beef on grazing pasture types containing approximately 35% or greater red or white clover (Lee et al., 2003; Lourenco et al., 2007, Scollan et al., 2006a). In contrast, Fraser et al., (2007) found that when 25% of the diet of beef steers was red clover fed for part of the study, only three FA in meat- C18:1n-7, C18:3n-3, and

the ratio of C18:2:C18:3 – were different compared to animals given perennial ryegrass during the same period. Therefore, our RC treatment may have not contained enough red clover to affect the intramuscular fat content as observed in other studies.

Other possibilities might include are cattle preferentially grazing tall fescue and not red clover, or the grazing duration was not sufficient to alter the FA content of the steers. However, we observed that when the cattle on the ALF treatment entered a new paddock, the alfalfa portion was typically grazed before the tall fescue. Similar results were reported by Rutter et al., (2004) where dairy cattle consuming pasture of mix proportions of white clover and perennial ryegrass had 63 % of their diet of white clover. Therefore it seems that our cattle on the RC treatment would preferentially consume the red clover prior to grazing the tall fescue. Also, the grazing period for our study was between 110 to 123 d; perhaps this duration was not sufficient for the accumulation of PUFA differences to be perceived; although, Noci et al. (2005) was able to detect difference in heifers at 158 days of grazing.

There are natural and synthetic compounds known to aid in rumen bypass of PUFA to the lower gut. Red clover possesses polyphenol oxidase (PPO) (Winters et al., 2003; Lee et al., 2006). Even though biohydrogenation of α-linolenic acid is 86 to 94% in the rumen, when cattle consume red clover or grasses, nearly 2.4 times more α-linolenic is able to escape the rumen when animals are fed red clover (Dewhurst et al., 2003b). Although the level of PPO was not assessed in this experiment, it appears that the level of red clover present in our pastures was not sufficient to appreciably disrupt the biohydrogenation process in the rumen. Relating our red clover levels and the levels of Fraser et al. (2007), a minimal level between 16.5 and 25% of red clover in the diet must

be attained before the PPO levels reach a critical level to allow the PUFA contained in the forage to bypass the rumen increasing PUFA content in the meat. It seems preferable that a much higher percentage, greater than 40% be used, for the PPO activity in red clover to dramatically increase the PUFA found in both milk and meat products.

While pasture contains only short-chain PUFA (C 18:2 and 18:3), there is a marked increase in all PUFA (short- and long-chain) along with CLAs when cattle consume high forage diets. There has been a great effort to identify diets that enhance the deposition of these FA into the intramuscular fat. When comparing grain diets to forage only diets, the CLA content increases approximately 1.6- to 2.9-fold in forage-fed animals (French et al., 2000; Engle and Spears, 2004; Lorenzen et al., 2006). Even when CLA is added to the diet, beef grazing pastures had superior CLA content (Poulson et al., 2004). However, the CLA percentages reported by French et al., (2000), Noci et al (2005), and Engle and Spears (2004) are 2 to 3 fold higher than the CLA content (0.31%) from our study. Also, the *n*-6:*n*-3 ratio for our study averaged 4.9 which is about 3 times greater than reported levels (French et al., 2000; Engle and Spears, 2004; Noci et al 2005). The increase in this ratio is contributed to the large amount of linoleic acid (7.2%) comprising the *n*-6 FA component. This is nearly 2 to 3 times higher than animals that have finished on grain- or forage-based diets. Duckett et al. (1993) however reported that steers at 16 mo of age had a linoleic acid content of 6.5%, similar to our results.

Although diet greatly influences the final composition of meat, genetic disposition of animals also plays a role in determining the amounts of intramuscular FA present. Almost certainly this was a factor in our study where Angus was the common cross. Pure bred Angus cattle have been shown to possess a higher amounts (mg g^{-1}) of SFA, PUFA,

and a higher *n*-6:*n*-3 ratio in meat; however, on a percent basis the PUFA content ratio is dramatically lower when compared to Brahman and Romosinuano breeds (Dinh, 2003). When these breeds were crossed, the Angus crosses contained lower SFA compared to their pure bred counterparts (Dinh, 2003). Laborde et al., (2001) found a similar trend with Red Angus containing a greater amount (mg g^{-1}) of SFA and PUFA compared to Simmental, with significantly higher amounts of *n*-3 and *n*-6:*n*-3 ratio. These breed differences have also been observed between Galloway, German Holstein, and White-Belgian Blue cattle (Nürnberg et al., 1999). Crossbred analysis has shown some genetic control for FA characteristics with trait heritabilities ranging from 14 to 36% along with having moderate correlations to specific FAs (Pitchford et al, 2002). Additionally, sires have been shown to influence the FA content of their progeny. Progeny from specific Japanese Black Wagyu sires have significantly increased SFA and monounsaturated fatty acid MUFA content (Oka et al., 2002). These breed differences are seemingly due to enzymatic activity related to gene expression and/or enzyme function, such as stearoyl CoA desaturase (delta-9-desaturase), which is related to FA production (Scollan et al., 2006b). Therefore, choosing livestock that perform well on pasture and are predisposed to produce high amount of PUFA relative to other FA is essential to producing optimal FA profiles in meat from this feeding scheme.

Beef Performance

Steer ADG was different ($P > 0.05$) between the TF treatment and legume treated pastures. steers in TF treatment had the lowest gain at 0.24 kg d^{-1} while steers grazing in the RC treatment gained the most at 0.40 kg d^{-1} (Table 3.2). This difference in weight gain is partially indicative of the significantly higher IVTD of the pasture with legumes.

However, between pastures with legumes, steers in the ALF treatment had lower gains than steers in the RC treatment. The ADG of the cattle on the TF and ALF treatments were lower than the results of Lorenzen et al. (2006) while the cattle on the RC treatment was nearly the same. According to the NRC (2000), the CP requirement for steers weighing between 435 and 475 kg ranges from 6.5 to 13.0% and the TDN requirement ranges from 50 to 90% depending on expected ADG. In all treatments, CP from pasture exceeded these requirements. Although the IVTD values cannot be directly compared to the TDN values provided by NRC, the digestibility was high (>84 %). Additionally, heat stress and shade may have played a factor in the ADG for the RC treatment. Finishing cattle provided with shade in either feedlots or pasture systems was shown to positively impact ADG (McDaniel and Roark 1956; Mitlohner et al., 2002). Shade types have also shown to impact ADG with natural shaded animals having greater ADG than those shaded by artificial structures (McDaniel and Roark 1956).

The ribeye area (REA) estimated by ultrasound before steers were harvested showed no significant differences in size between any of the pasture treatments. Steer REA taken from the ultra sound averaged 73.61 cm^2 over all pasture treatments (Table 3.2). Finish weight between the ALF and RC treatments was different ($P < 0.05$); however, this finish weight may be skewed due to the RC and TF treatments being held on the pretreatment diet longer than in the ALF treatment. The delayed start of the RC and TF treatments were due to slow pasture growth resulting from a late spring freeze in early April. However, if steers were placed on pastures at the same time and weight the TF treatment might have been different from the legume treated pastures since the ADG was significantly different for the TF and RC treatments. Although the cattle had

different ADG it does not appear to be a result of the PDMD. Across all treatments the PDMD was similar with steers in the ALF treatment consuming 17.4 kg animal^{-1} d^{-1}, those in the RC treatment at 17.6 kg animal^{-1} d^{-1}, and steers in TF treatment having the greatest intake at 18.1 kg animal^{-1} d^{-1}.

Initially, experiments evaluated how the FA composition of forage fed beef differed from those finished on various concentrate diets. However, from these studies it was apparent that the cattle consuming forage-based diets required a longer time to finish due to the reduced concentration of energy in forage-based diets. This lower ADG is apparent from our work where the cattle ranged from 0.24 to 0.40 kg d^{-1}. With slower rates of gain, the time required for the animals to finish is extended from approximately 80-180 days in the feedlot to nearly 18 months on pasture. However, this extended period on pasture influences the FA characteristics enhancing the PUFA and lowering overall SFA content as well as increasing CLA and *n*-3 FA (Noci et al., 2005).

Conclusions

In conclusion, improved cool-season grass and legume pastures contain different levels of FA and can provide nearly double the CP required for finishing steers as well as forage that has exceptional digestibility. However, the FA composition of the distinctive pasture types did not influence the intramuscular fat composition in beef steers. This may be due to the low level of PPO contained in the RC treatment. Perhaps the percentage of red clover in this study was not adequate to increase the PUFA of the intramuscular fat. The duration of the experiment also may have not been long enough to allow the accumulation of PUFA that would typically be seen in an animal consuming only a forage diet throughout. Additionally, using cattle breeds with a genetic disposition to

produce many of the healthy FA, in particular PUFA, and convert forages to animal gain more efficiently is of considerable importance.

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Table 3.1. Percent of legume in pasture, crude protein (CP), neutral detergent fiber (NDF), digestible neutral detergent fiber (dNDF) as a proportion of NDF ,and *in vitro* true digestibility (IVTD) from the pasture treatments through the duration of the experiment. Grain pretreatment was not included in the analysis.

Pasture Treatment	N	Legume	CP	NDF	dNDF	IVTD
		---%---	----------g kg $^{-1}$ DM----------			
Grain (pretreatment)		0	149	142	590	942
Tall Fescue	27	0	164	509	708	849
Tall Fescue + Alfalfa	47	38.36	216	389	658	864
Tall Fescue + Red Clover	33	16.45	172	473	719	864
SEM			381	1415	3273	889
Tall fescue vs. Legumes			<0.001	<.001	NS	<.001
Red Clover vs. Alfalfa			<0.001	<.001	.008	NS

NS non-significant

Table 3.2. Fatty acids levels determined from the pasture treatments through the duration of the experiment. Grain pretreatment was not included in the analysis.

Pasture Treatment	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total
	----------mg g^{-1} DM----------								
Grain (pretreatment)	0.11	0.67	8.00	0.11	1.41	12.73	29.39	1.15	53.57
Tall Fescue	0.11	3.52	3.85	0.54	0.46	0.71	3.91	14.10	27.21
Tall Fescue + Alfalfa	0.09	2.54	4.69	0.61	0.54	0.80	4.66	14.48	28.39
Tall Fescue + Red Clover	0.09	3.30	4.01	0.56	0.48	0.76	4.33	14.66	28.18
SEM	0.004	2.22	0.11	0.01	0.006	0.02	0.27	4.05	7.11
Tall fescue vs. Legumes	0.086	NS	0.0001	0.0004	0.0001	0.059	0.0005	NS	0.01
Red Clover vs. Alfalfa	NS	NS	0.0001	0.0025	0.0001	NS	NS	NS	NS

NS non-significant

Table 3.3. Pasture treatment effects on ADG, REA, and finishing weight of beef steers.

Treatment	ADG (kg d^{-1})	REA (cm^2)	Finish Weight (kg)
Tall Fescue	0.2	73	476
Tall Fescue + Alfalfa	0.3	71	456
Tall Fescue + Red Clover	0.4	77	480
SEM	0.12	6.7	25.2
Tall Fescue vs. Legumes	0.03	NS	NS
Red Clover vs. Alfalfa	0.10	0.08	0.05

NS non-significant

Table 3.4. Effect of pasture treatment on fatty acid proportion of total lipids in fat taken from the *Longissimus dorsi* of beef steers.

Fatty Acids, proportion of total fatty acids x 100	TF	ALF	RC	SEM	P value[g]
Myristic (C14:0)	2.41	2.32	2.22	0.12	NS
Myristoleic (C14:1)	0.42	0.38	0.41	0.016	NS
Pentadecanoic (C15:0)	0.43	0.44	0.43	0.003	NS
Palmitic (C16:0)	21.83	21.92	21.11	3.51	NS
Palmitoleic (C16:1)	2.94	2.73	2.80	0.19	NS
Margaric (C17:0)	1.05	1.01	1.03	0.013	NS
Heptadecanoic (C17:1)	1.07	0.88	1.06	0.009	NS
Stearic (C18:0)	15.04	15.98	15.52	1.10	NS
Elaidic (C18:1*t9*)	2.94	3.26	2.84	0.174	NS
Oleic (C18:1*n9*)	32.47	31.20	31.30	12.08	NS
Trans vaccenic (C18:1*n7*)	0.30	0.56	0.63	1.27	NS
Linoleic (C18:2*n6*)	7.03	7.03	7.53	3.02	NS
α-Linolenic (C18:3*n3)*	0.63	0.80	0.73	0.02	NS
Stearidonic (C18:4*n3)*	0.15	0.19	0.13	0.01	NS
Arachidic (C20:0)	0.04	0.07	0.03	0.005	NS
Eicosenoic (C20:1*n9*)	0.03	0.05	0.03	0.003	NS
Eicosatrienoic (C20:3*n3)*	0.00	0.00	0.00	0.00	NS
Arachidonic (C20:4*n6*)	2.35	2.24	2.64	1.20	NS
Arachidonic (C20:4*n3)*	0.00	0.00	0.00	0.00	NS
Eicosapentaenoic (C20:5*n3*; EPA*)*	0.44	0.52	0.44	0.05	NS
Docosanoic (C22:0)	0.00	0.00	0.00	0.00	NS
Erucic (C22:1*n9*)	0.00	0.00	0.00	0.00	NS
Docosapentaenoic(C22:5*n3*; DPA)	0.67	0.78	0.80	0.08	NS
Docosahexaenoic (C22:6*n3*; DHA)	0.00	0.00	0.00	0.00	NS
Lignoceric (C24:0)	0.03	0.03	0.03	0.006	NS
Nervonic (C24:1*n9*)	0.02	0.00	0.00	0.001	NS
Total CLA[a]	0.29	0.33	0.32	0.006	NS
Total SFA[b]	40.84	41.77	40.36	5.48	NS
Total MUFA[c]	40.16	39.02	39.03	10.48	NS
Total PUFA[d]	11.54	11.88	12.59	8.92	NS
UFA:SFA ratio	1.26	1.22	1.27	0.009	NS
MUFA:SFA ratio	0.98	0.93	0.96	0.004	NS
PUFA:SFA ratio	0.27	0.25	0.28	0.007	NS
Total *n*-6[e]	9.66	9.60	10.49	6.50	NS
Total *n*-3[f]	1.88	2.29	2.10	0.35	NS
n-6:*n*-3	5.40	4.21	5.05	0.32	NS

[a]Sum of c9,t11 and t10,c12.
[b]Sum of all even chain fatty acids from C14:0 toC24:0 + C15:0 and C17:0.
[c]Sum of C14:1, C16:1, C17:1, all C18:1, C20:1, C22:1, and C24:1.
[d]Sum of total n-6, total n-3, CLAs c9,t11, and CLA t10,c12.
[e]Sum of C18:2, C18:3n-6, C20:2, C20:3n-6, C20:4, and C22:2.
[f]Sum of C18:3n-3, C20:3n-3, C20:5, C22:5, and C22:6.
[g]P-value > 0.05 are listed as non-significant (NS)

Table 3.5. Effect of pasture treatment on fatty acid concentration of total lipids in fat taken from the *Longissimus dorsi* for beef steers.

Fatty Acids, concentration of total fatty acids (mg g^{-1})	TF	ALF	RC	SEM	P value[g]
Myristic (C14:0)	2.25	2.56	2.05	0.12	NS
Myristoleic (C14:1)	0.40	0.44	0.39	0.05	NS
Pentadecanoic (C15:0)	0.39	0.46	0.36	0.02	NS
Palmitic (C16:0)	20.19	23.76	18.79	61.63	NS
Palmitoleic (C16:1)	2.72	2.98	2.51	1.39	NS
Margaric (C17:0)	0.96	1.08	0.89	0.12	NS
Heptadecanoic (C17:1)	0.96	0.91	0.88	0.06	NS
Stearic (C18:0)	13.60	16.81	13.06	23.22	NS
Elaidic (C18:1*t9*)	2.71	3.52	2.51	1.23	NS
Oleic (C18:1*n9*)	29.83	34.01	27.41	120.33	NS
Trans vaccenic (C18:1*n7*)	0.17	0.35	0.34	0.45	NS
Linoleic (C18:2 *n6*)	6.15	6.75	5.91	0.86	NS
α-Linolenic (C18:3*n3)*	0.56	0.80	0.60	0.03	NS
Stearidonic (C18:4*n3)*	0.14	0.22	0.13	0.01	NS
Arachidic (C20:0)	0.04	0.10	0.03	0.007	NS
Eicosenoic (C20:1*n9*)	0.02	0.07	0.03	0.005	NS
Eicosatrienoic (C20:3n3)	0.00	0.00	0.00	0.00	NS
Arachidonic (C20:4*n6*)	2.01	2.00	1.96	0.14	NS
Arachidonic (C20:4*n3)*	0.00	0.00	0.00	0.00	NS
Eicosapentaenoic (C20:5*n3;* EPA*)*	0.38	0.48	0.36	0.02	NS
Docosanoic (C22:0)	0.00	0.00	0.00	0.00	NS
Erucic (C22:1*n9*)	0.00	0.00	0.00	0.00	NS
Docosapentaenoic(C22:5*n3;* DPA)	0.58	0.71	0.63	0.01	NS
Docosahexaenoic (C22:6*n3;* DHA)	0.00	0.00	0.00	0.00	NS
Lignoceric (C24:0)	0.02	0.03	0.01	0.003	NS
Nervonic (C24:1*n9*)	0.01	0.00	0.00	0.0003	NS
Total CLA[a]	0.28	0.37	0.30	0.02	NS
Total SFA[b]	37.43	44.81	35.20	217.51	NS
Total MUFA[c]	36.82	42.28	34.07	191.76	NS
Total PUFA[d]	10.10	11.32	9.89	1.66	NS
Total *n*-6[e]	8.44	9.11	8.17	1.10	NS
Total *n*-3[f]	1.67	2.21	1.72	0.13	NS

[a]Sum of c9,t11 and t10,c12.
[b]Sum of all even chain fatty acids from C14:0 toC24:0 + C15:0 and C17:0.
[c]Sum of C14:1, C16:1, C17:1, all C18:1, C20:1, C22:1, and C24:1.
[d]Sum of total n-6, total n-3, CLA c9,t11, and CLA t10,c12.
[e]Sum of C18:2, C18:3n-6, C20:2, C20:3n-6, C20:4, and C22:2.
[f]Sum of C18:3n-3, C20:3n-3, C20:5, C22:5, and C22:6.
[g]P-value > 0.05 are listed as non-significant (NS)

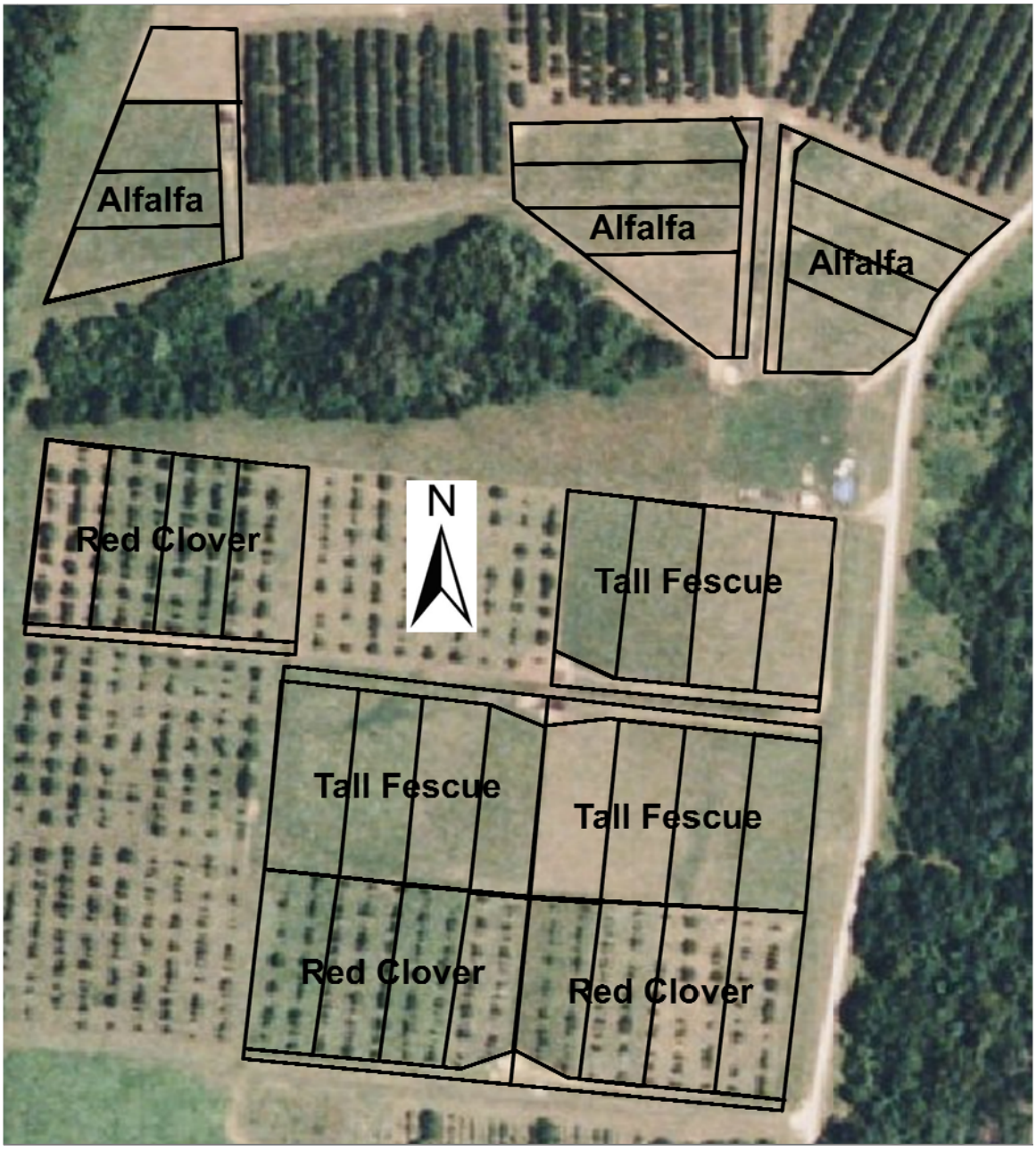

Figure 3.1. Map of experimental grazing pastures with treatments tall fescue, tall fescue with red clover, and tall fescue with alfalfa located at the University of Missouri Horticulture and Agroforestry Research Center (HARC) for spring 2007.

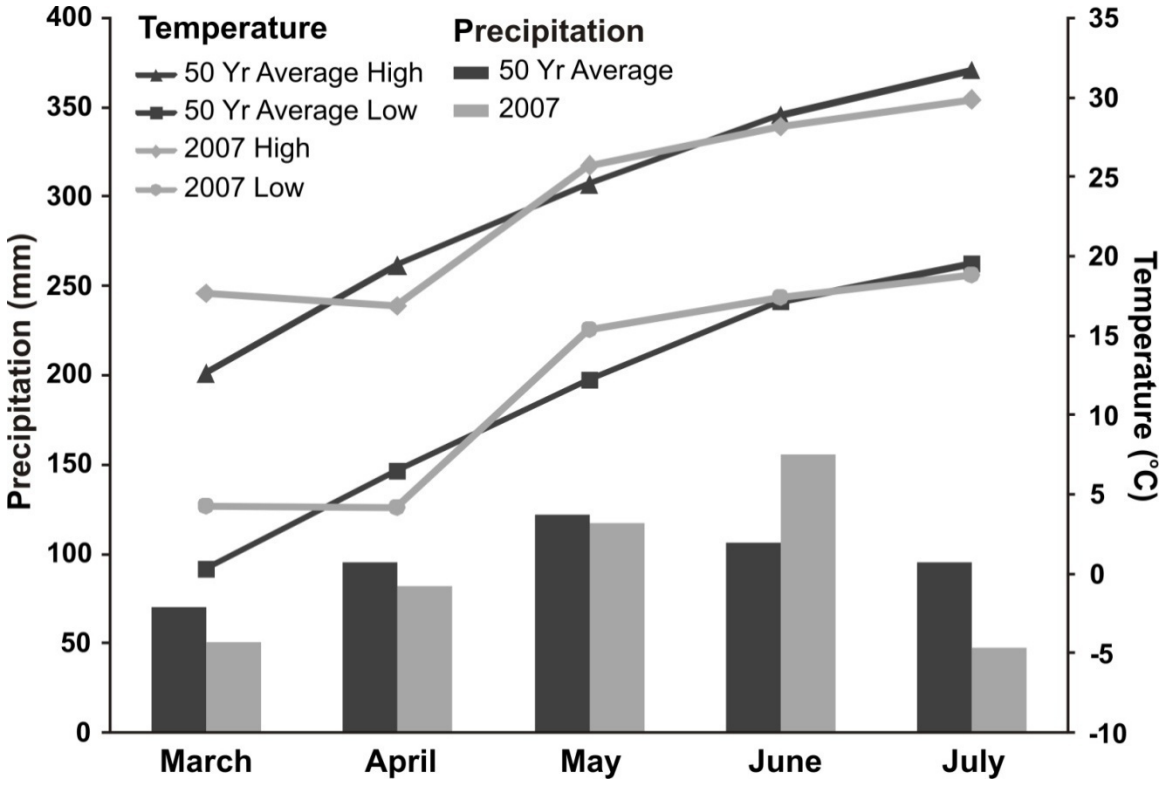

Figure 3.2. Monthly total precipitation with average monthly high and low air temperature at New Franklin, MO, during the spring and summer of 2007. Historic averages represent 50 years.

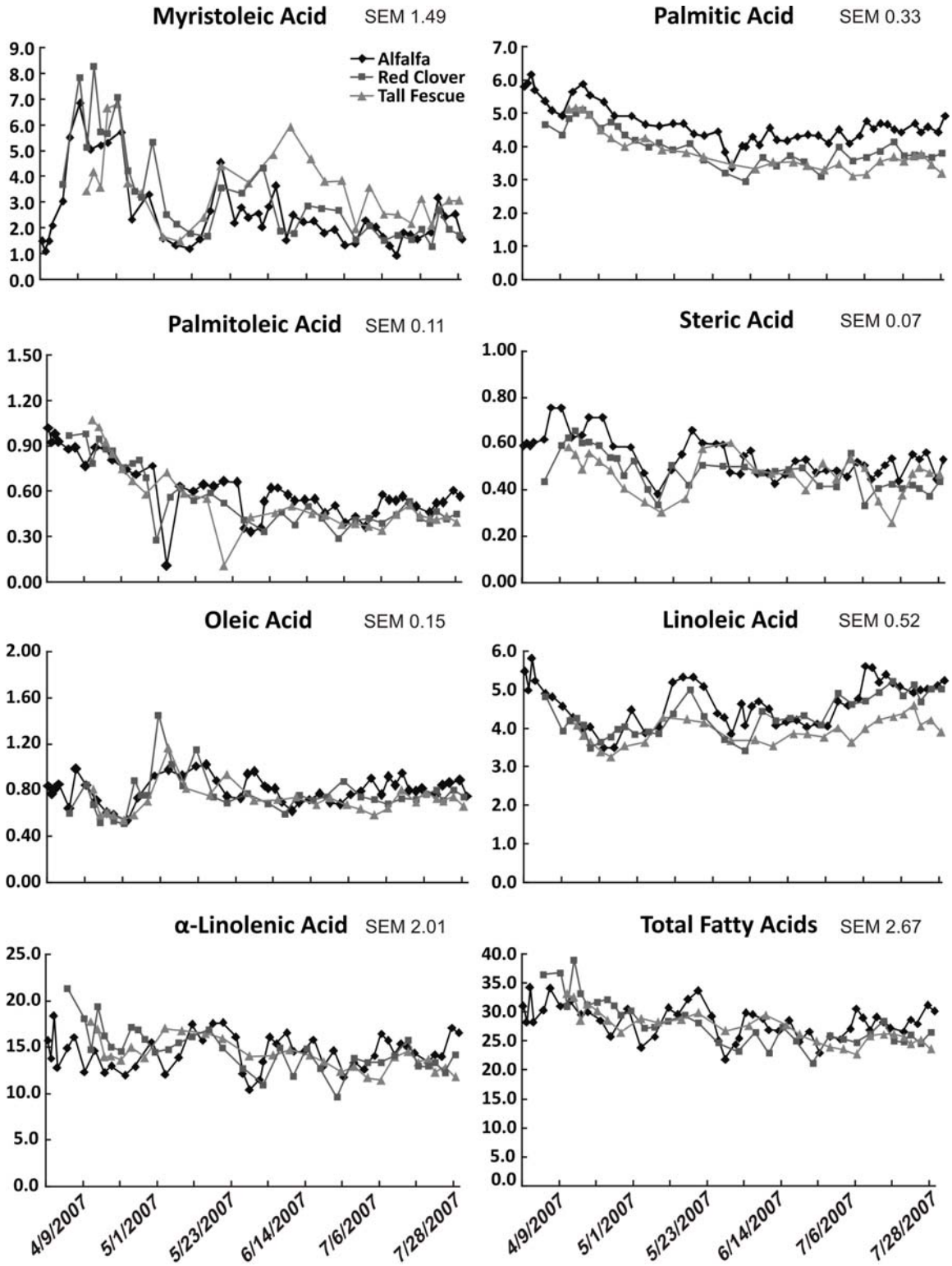

Figure 3.3. Fatty acid trends of three pasture treatments over the course of the trial from 3/29/2007 to 7/29/2007.