Pasture feeding is strongly associated with a beneficial fatty acid (FA) profile of ruminant animal products, which has spurred an increased consumer demand for grass-fed meat and dairy products (Benbrook et al., 2018). Fresh grazing provides a large supply of the n-3 polyunsaturated FA α-linolenic acid (ALA), which is the chief FA in vegetative forages and an important source of the desired FA content and profile of ruminant milk and meat products (Elgersma, 2015). Traditional perennial pasture in temperate regions of the world is often composed of C3 photosynthetic (i.e., cool-season) forage species, which can have limited grazing availability during the hot dry months of the “summer slump.” Because of this, there is a growing interest in C4 photosynthetic (i.e., warm-season) annual forage species, which can exceed the productivity of cool-season species. However, little research into FA has been performed (O’Kelly and Reich, 1976; Vargas M. et al., 2013; Bainbridge et al., 2017; Dias et al., 2017). The content of ALA and total fatty acids (ΣFA) decreases in cool-season forage crops with advancing maturity (Dewhurst et al., 2001; Elgersma et al., 2003, 2005; Dias et al., 2017). Maturity-associated decreases in ALA and ΣFA content, as well as lower proportions of ALA within ΣFA, may be due, at least in part, to growth of pseudostem fractions, which contain less ALA and ΣFA than do laminae fractions (Dewhurst et al., 2001; Elgersma et al., 2003; Clapham et al., 2005; Glasser et al., 2013). Maturity-associated decreases in ALA and ΣFA content, as well as lower proportions of ALA within ΣFA, may be due, at least in part, to growth of pseudostem fractions, which contain less ALA and ΣFA than do laminae fractions (Dewhurst et al., 2001; Elgersma et al., 2003, 2005; Dias et al., 2017). Because many warm-season annual grass species elongate and mature with large pseudostem fractions relative to the elongation of cool-season grass species (Atkinson et al., 2016), we hypothesize that pearl millet [Cenchrus americanus (L.) Morrone], an important summer annual forage crop, will exhibit significant decreases in ALA and ΣFA content with advancing maturity, primarily because of the increasing dry matter (DM) proportion of the pseudostem fraction. The objective of this study was to determine the extent to which

Fatty Acids Decrease in Pearl Millet Forage from Relative Increases of Pseudostem

C. P. Goossen, J. Kraft, and S. C. Bosworth*
the content and proportion of ALA, as well as the content of \( \Sigma FA \), change within plant fractions as they mature.

**Materials and Methods**

Five replicate pearl millet (‘Wonderleaf’) samples were collected from a commercial farm field in Highgate Center, VT, USA (44°58' N, 73°01' W) using handheld electric clippers (Gardena Accu Grass Shears ComfortCut, Husqvarna Professional Products Inc.), six times over 19 d (18 July–5 Aug. 2016; Table 1). Pearl millet samples ranged from early vegetative stage on Day 1 to late boot stage on Day 19. Replicate samples from each sampling date were separated between lamina (separated immediately above the ligule) and pseudostem fractions, respectively. Lamina and pseudostem samples (<150 g fresh weight) were placed in separate paper bags, microwaved onsite at maximum power (800 W) for 1 min before transport to a drying room, and dried as described in Goossen et al. (2018).

**Forage Analysis**

Nutritive quality (crude protein [CP] and neutral detergent fiber [aNDF]) and FA analyses were performed as described by Goossen et al. (2018). Whole plant measures were calculated for each replicate as weighted averages by dry weight of the constituent plant fractions, that is, whole plant FA g kg\(^{-1}\) DM = (lamina FA g kg\(^{-1}\) DM × lamina proportion) + (pseudostem FA g kg\(^{-1}\) DM × pseudostem proportion). Variance of whole plant means estimates were calculated as the sum of the variances of the lamina and pseudostem fractions, and two times their covariance \( s^2_{\text{whole plant}} = s^2_{\text{lamina}} + s^2_{\text{pseudostem}} + 2 \cdot \text{cov}_{\text{lamina, pseudostem}} \). The small amount of pseudostem material present at the first sampling date necessitated compositing dried samples for FA analysis, and as such, variance estimates could not exist for that fraction and could not be calculated for the whole plant measure at that time point.

**Statistical Analysis**

The GLM procedure in SAS version 9.4 (SAS Institute, Cary, NC) was used to perform a one-way analysis of covariance on plant fractions, using height as covariate. Differences were considered significant with a Tukey’s honest significant difference test adjusted \( P < 0.05 \). The CORR procedure in SAS was used to generate partial correlation coefficients.

**Results**

There was a substantial difference of ALA and \( \Sigma FA \) content and ALA proportion of \( \Sigma FA \) between lamina and pseudostem fractions (Fig. 1). With increasing height, neither lamina nor pseudostem fractions had a rate of change of ALA content or proportion different than zero. However, the rate of \( \Sigma FA \) content reduction in the pseudostem fraction was more than twice that of the lamina fraction (slopes = –0.11, –0.04, respectively).

**Table 1.** Sampling date, days after seeding, growth stage, height, and lamina mass ratio (LMR) of pearl millet, Highgate Center, VT, 2016.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>18 July</th>
<th>21 July</th>
<th>26 July</th>
<th>29 July</th>
<th>1 Aug.</th>
<th>5 Aug.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after seeding</td>
<td>47</td>
<td>50</td>
<td>55</td>
<td>58</td>
<td>61</td>
<td>65</td>
</tr>
<tr>
<td>Growth stage†</td>
<td>Early veg.</td>
<td>Veg.</td>
<td>Elong.</td>
<td>Elong.</td>
<td>Flag leaf</td>
<td>Boot stage</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>54</td>
<td>74</td>
<td>107</td>
<td>117</td>
<td>133</td>
<td>139</td>
</tr>
<tr>
<td>LMR‡</td>
<td>0.96</td>
<td>0.87</td>
<td>0.75</td>
<td>0.69</td>
<td>0.62</td>
<td>0.43</td>
</tr>
</tbody>
</table>

† veg. = vegetative; elong. = elongating.
‡ LMR = (lamina dry matter/lamina + pseudostem dry matter).

**Fig. 1.** (A) Alpha-linolenic acid (ALA) content, (B) proportion, and (C) total fatty acid (\( \Sigma FA \)) content of pearl millet over 19 d, by plant fraction (least squares means and standard error of means, \( n = 5 \) replicate samples, except composited pseudostem samples at the first collection). Whole plant measure is a weighted mean of plant fractions.
Lamina mass ratio (LMR; lamina DM/lamina + pseudostem DM), CP, and aNDF were all highly correlated with the three FA measures on a whole plant basis (Table 2). Within plant fractions, lamina CP content was a stronger correlate of lamina ALA and ΣFA content than either LMR or lamina aNDF. However, lamina CP content was not associated with ALA proportion, with which LMR showed a slight negative correlation. In pseudostems, LMR was the strongest correlate with ALA and ΣFA content, although not with ALA proportion, which was more strongly correlated with pseudostem CP content.

### Discussion

In agreement with our hypothesis, ALA and ΣFA content and ALA proportion declined rapidly with advancing maturity on a whole plant basis (Fig. 1). The ALA and ΣFA content declines were markedly less in lamina fractions, and ALA proportion was unchanged in lamina and pseudostem fractions.

Alpha-linolenic acid and ΣFA content declines were minor within lamina fractions, possibly a result of cell wall thickening as the laminae aged. Alpha-linolenic acid is vital for chloroplast function, which may explain why the ALA proportion of ΣFA did not decrease within the lamina fraction. The decline of ALA and ΣFA in the pseudostem fraction is likely due (i) to the greater lignification of living cells within this structurally important plant component and (ii) to a greater proportion of non- or minimally metabolically active tissue (e.g., pith, xylem), with consequently fewer lipid-rich membranes and very few ALA-rich chloroplast membranes.

This study provides evidence that declines in ALA and ΣFA content associated with advancing maturity in pearl millet are largely a function of a greater DM proportion of pseudostem fractions, which inherently contain less ALA and ΣFA. These findings corroborate the work of Dias et al. (2017) investigating *Pennisetum purpureum* Schumach. This has profound management implications for ruminant milk and meat producers concerned with the FA profile of their products, who utilize these warm-season annual forage species. Alpha-linolenic acid and ΣFA content decrease minimally within laminae fractions, providing an opportunity for management practices, such as reduced stocking pressure in grazing systems, and/or high mowing/chopping height, to capture a greater proportion of laminae material in older stands.

### Acknowledgments

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### References


### Table 2. Pearson correlation coefficients (n = 30) of lamina mass ratio, crude protein, and neutral detergent fiber content with alpha-linolenic acid (ALA) content, proportion, and total fatty acid (Σ FA) content of a weighted mean of constituent fractions (calculated whole plant basis) as well as in lamina and pseudostem fractions of pearl millet.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>LMR†</th>
<th>CP‡</th>
<th>aNDF§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>P value</td>
<td>r value</td>
</tr>
<tr>
<td>Whole plant</td>
<td>ALA (g kg⁻¹ DM)</td>
<td>0.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>ΣFA (g kg⁻¹ ΣFA)</td>
<td>0.86</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>LMR†</td>
<td>0.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lamina</td>
<td>ALA (g kg⁻¹ DM)</td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>ΣFA (g kg⁻¹ ΣFA)</td>
<td>−0.46</td>
<td>0.01</td>
</tr>
<tr>
<td>Pseudostem</td>
<td>ALA (g kg⁻¹ DM)</td>
<td>0.43</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>ΣFA (g kg⁻¹ ΣFA)</td>
<td>0.02</td>
<td>0.93</td>
</tr>
</tbody>
</table>

† LMR = lamina mass ratio (lamina dry matter/lamina + pseudostem dry matter).
‡ CP = crude protein content of each plant fraction, and weighted mean on a whole plant basis.
§ aNDF = neutral detergent fiber content of each plant fraction, and weighted mean on a whole plant basis.


