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Article

Phenotypic Variation and Relationships between Fatty Acid Concentrations and Feed Value of Perennial Ryegrass Genotypes from a Breeding Population

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Abstract: Fatty acid (FA) concentration and composition of forage has recently gained interest due to potential opportunities for improving FA profile of ruminant products (meat and milk). Twenty perennial ryegrass genotypes from an experimental breeding population and four genotypes from an experimental mapping population were used to assess (1) genotypic variation, and (2) associations between FAs and other important chemical constituents (i.e., protein, carbohydrate and fibre). Mean total FA (TFA) concentration was 23.8 g kg⁻¹ DM, ranging from 14.5 to 33.8 g kg⁻¹ DM; 89% to 95% of which was comprised of six individual FAs, namely, palmitic acid (C16:0), *trans*-3-hexadecenoic acid (C16:1 Δ^3), stearic acid (C18:0), oleic acid (C18:1 Δ^9), linoleic acid (C18:2 $\Delta^{9,12}$) and α -linolenic acid (C18:3 $\Delta^{9,12,15}$). Mean crude protein (CP), water-soluble carbohydrate (WSC), neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were: 133, 188, 447 and 240 g kg⁻¹ DM, respectively. Genotypes from the mapping population differed for: WSC ($p = 0.015$), C16:0 ($p = 0.034$), C18:0 ($p < 0.001$), C18:3 $\Delta^{9,12,15}$ ($p = 0.012$) and TFA ($p = 0.025$). Genotypes from the breeding population differed ($p < 0.001$) for all measured components except CP ($p = 0.078$). Higher FA concentration was generally associated with higher CP concentration and lower WSC, NDF and ADF. Selectively breeding for higher FA concentrations may alter the overall feed value of perennial ryegrass, however further investigation is needed to fully understand the relationship between FA concentration and feed value and the possible implications for ruminant nutrition.

Keywords: *Lolium perenne*; lipids; crude protein; water-soluble carbohydrates; feed value

1. Introduction

Perennial ryegrass (*Lolium perenne* L.; PRG) is one of the most commonly grown grass species in Western Europe due to its high productivity and digestibility [1,2]. As such, this species has received much attention within forage breeding programmes to improve beneficial traits and the range of grass breeding objectives has continuously expanded since the early 20th century. Breeding initially focused on improving agronomic characteristics such as yield, persistency and disease resistance to increase output [3,4]. Nutritional qualities of forage such as dry matter digestibility and dry matter intake were then targeted, followed more recently by water-soluble carbohydrate (WSC) in grasses and crude protein (CP) in legumes (through improved nitrogen fixation) [5]. This has led to distinctive forage varieties with quality traits such as high-sugar grasses being created [6].

Improvements in forage quality facilitate improvements in ruminant production and efficiency. Forages also play a key role in other livestock related issues such as economics, sustainability, environmental impacts, animal welfare and consumer demands for enhanced quality and functionality of products; further encouraging the development of new and novel forage varieties [5,7]. One such novel forage trait attracting increasing attention is fatty acid (FA) concentration and composition. Interest in this particular trait has arisen mainly through increasing consumer demand for grass-fed ruminant products (meat and milk), owing to the perceived health benefits associated with such products [8,9]. Breeding forage for higher FA concentrations offers an opportunity to further improve the FA profiles of meat and milk [10–12]. Other benefits of selectively breeding for increased FAs may also include increased energy density of forages [13] which may provide an opportunity to further improve ruminant efficiency [14–16], and the potential use of high lipid forage as a non-seed biomass oil crop [17,18].

Although seasonal and environmental factors play a substantial role in FA concentration and composition of forages [19], several studies have highlighted a significant genetic contribution, signifying the potential to selectively breed for this trait [20,21]. Moreover, Hegarty et al. [22] have successfully identified regions of the PRG genome which are associated with FA concentration using traditional marker-assisted selection (MAS) carried out on a mapping population. It was expected that the rapid developments in molecular genetics and genomics technologies during the past decade or so would vastly accelerate the plant breeding process by reducing the requirement for costly and time-consuming field-scale phenotyping [23].

Yet integration of genetic approaches such as MAS into plant breeding programmes has been slow [24], with few reports of successful employment of MAS for forage crops in particular [25]. As such, many new and novel forage crop cultivars are still being sourced from breeding populations that are predominantly based on phenotypic selection. To produce a novel cultivar in this way takes between 15 and 20 years for a species such as PRG [26]. Furthermore, it is critical to consider and understand the consequences selectively breeding for a specific trait may have on other traits of importance, and how this may affect overall cultivar acceptance and performance. The aim of the study was therefore to (1) characterise the phenotypic variation in FA concentrations present in a breeding population of PRG, and (2) explore the resulting changes in chemical composition and how this may impact overall feed value.

2. Materials and Methods

2.1. Experimental Design

The study was conducted at the Institute of Biological, Environmental and Rural Sciences (IBERS), Gogerddan, Aberystwyth University (52°25' N, 04°05' W). Four 'benchmark' genotypes from an Aurora x AberMagic F1 PRG mapping population, which were previously been phenotyped for FA concentration [22], and twenty 'experimental' genotypes from an intermediate heading 13th generation PRG breeding population were used in the study. Each genotype mother plant was vegetatively propagated in April 2012, whereby mature single PRG tillers were transplanted into 6" pots containing John Innes No.1 potting compost to give four clones of each genotype. Pots were arranged in a randomized block design with four replicates equating to a total of 96 plants which were maintained under poly-tunnel conditions (average temperature was 12.4 ± 3.86 °C between planting and harvesting). Actively reproductive flowering heads were cut back every two weeks to encourage tillering. All plants were harvested within a two-hour window using hand shears in July 2012. All plant material 5 cm above the soil was collected from each plant, placed into a zip-lock bag and temporarily stored in a polystyrene box containing ice. Harvested samples were stored at -20 °C then freeze dried and ground using a Tecator Cyclotec 1093 (FOSS UK Ltd., Warrington, UK) fitted with a 1 mm screen. Ground samples were stored at -20 °C until laboratory analysis.

2.2. Forage Chemical Composition

2.2.1. Crude Protein, Water-Soluble Carbohydrate and Fibre

Near-infrared reflectance spectroscopy (NIRS) was used to predict CP, WSC, NDF and ADF concentrations. Ground plant samples were scanned at 2 nm intervals over the wavelength range 400 to 2500 nm in reflectance mode, using a scanning monochromator (FOSS NIRSystems 6500, FOSS UK Ltd., Warrington, UK). Data were collected using WinISI II software (Version 1.02a, FOSS, Infrasoft International, Port Matilda, PA, USA) and spectra stored as $\log 1/R$ where R is the diffuse reflectance. Data over wavelength range 1100 to 2498 nm were used for prediction of CP, WSC, NDF and ADF concentrations using WinISI 4 software (Version 4.6.8, FOSS Analytical, FOSS UK Ltd., Warrington, UK). Reliability of NIRS predictions were checked using wet chemistry using a subset of samples.

2.2.2. Fatty Acids

The one-step extraction and methylation procedure by Sukhija and Palmquist [27] was used to extract FAs and convert to fatty acid methyl esters (FAME). Tricosanoic acid (C23:0) methyl ester was used as an internal standard. Fatty acid methyl esters were separated and quantified by GC with flame ionisation detector (GC-FID; CP-3800 with PAL Autosampler, Varian Inc., Palo Alto, CA, USA) equipped with a CP-Sil 88 chemically bonded for FAME column (Agilent Technologies UK Ltd., Berkshire, UK). Sample injection volume was 1 μL with a split ratio of 1:25 and He as the carrier gas at a constant flow rate of 1.5 mL/min. Injection temperature was 250 °C and detector temperature 255 °C. The temperature profile of the oven was 70 °C, held for 0 min and then increased to 170 °C at 20 °C min^{-1} ; this was held for 25 min, then increased to 190 °C at 1 °C min^{-1} and held for 0 min, followed by an increase to 230 °C at 2.7 °C min^{-1} and held for 3 min. This gave a total run time of 68 min. Peaks were identified using a 37 component FAME standard (S37, Supelco, Poole, Dorset, UK) and quantified using the internal standard (C23:0). Varian Star v.6.41 software (Varian Inc., Palo Alto, CA, USA) was used to capture and handle data.

2.3. Statistical Analysis

Chemical composition data were analysed using Genstat 19th edition (VSN International Ltd., Hemel Hempstead, UK). Dry matter percentage (DM%) and concentrations of CP, WSC, NDF, ADF and FA (total and individual) were tested by ANOVA using a nested treatment design (genotype within population) in a randomized block with four replicates, p -values of ≤ 0.05 were deemed significant. Principal components analysis (PCA) was applied to the data to further explore the differences and relationships in chemical composition amongst genotype means.

3. Results

3.1. Fatty Acid and Chemical Composition

Results for DM% and other chemical constituents (CP, WSC, NDF and ADF) are shown in Table 1. Populations differed for DM% ($p = 0.041$), WSC ($p < 0.001$), NDF ($p < 0.001$) and ADF ($p < 0.001$). Although significant, the numerical difference in DM% between the two populations was minor. Average WSC concentration for the 'experimental' genotypes from the breeding population was 34.4% higher than the average for the mapping population genotypes. Average NDF and ADF concentrations for the breeding population genotypes were 7.04% and 7.69% lower compared to the mapping population average, respectively. The breeding population also had a tendency ($p = 0.057$) to be lower in CP, with a 10% difference between the average of the two populations. The 'benchmark' genotypes selected from the mapping population only differed for WSC ($p = 0.015$), where a 51.0% difference was observed between genotype 86 which had the lowest WSC concentration (114 g kg^{-1} DM) and genotype 182 which had the highest (193 g kg^{-1} DM). The 'experimental' genotypes selected from the breeding population differed for DM% ($p < 0.001$), WSC ($p < 0.001$), NDF ($p < 0.001$) and ADF

($p < 0.001$). The 'experimental' genotypes ranged from 20.8% to 29.0% DM, with a 32.9% difference between the lowest and highest values. Like the 'benchmark' genotypes, a 51.2% difference between lowest and highest WSC concentration was observed for the 'experimental' genotypes, which ranged from 153 to 257 g kg⁻¹ DM. Neutral detergent fibre differed by 30.8% between the genotypes with the lowest and highest values, while the difference for ADF was 43.9%. Crude protein did not significantly differ between the 'experimental' genotypes from the breeding population ($p = 0.078$), although CP concentrations ranged from 99.1 to 154 g kg⁻¹ DM (equivalent to a 43.3% difference).

Table 1. Dry matter percentage and feed value (g kg⁻¹ DM) of perennial ryegrass genotypes from two distinct populations.

Variable	DM%	CP	WSC	NDF	ADF
Population					
Mapping	23.7	144	146	475	256
Breeding	24.8	131	196	441	237
s.e.d.	0.53	7.2	9.8	7.6	5.0
<i>p</i>	0.041	0.057	<0.001	<0.001	<0.001
Mapping Population Genotypes					
81	22.8	150	126	494	271
86	22.2	158	114	487	261
103	25.2	141	152	459	243
182	24.6	128	193	458	251
s.e.d.	1.00	13.4	18.4	14.3	9.4
<i>p</i>	0.104	0.386	0.015	0.143	0.168
Breeding Population Genotypes					
27	28.3	119	257	367	183
39	26.3	119	166	482	255
115	23.8	144	173	451	232
132	24.8	135	179	456	243
134	24.5	149	177	454	243
148	24.2	149	171	446	238
204	24.4	154	175	426	216
213	24.0	145	229	373	180
216	25.7	135	187	436	227
223	25.5	130	153	499	271
231	25.3	120	179	501	282
235	24.4	111	222	444	249
238	24.8	127	187	454	241
298	20.8	154	181	404	210
301	24.0	146	188	424	229
307	24.9	122	224	429	244
314	29.0	100	248	441	245
320	25.0	99.1	239	456	259
329	23.4	128	222	399	209
332	23.0	126	173	480	278
s.e.d.	1.09	14.6	20.0	15.6	10.2
<i>p</i>	<0.001	0.078	<0.001	<0.001	<0.001

DM%, dry matter percentage; CP, crude protein; WSC, water-soluble carbohydrate; NDF, neutral detergent fibre; ADF, acid detergent fibre; s.e.d., standard error of difference; *p*, probability.

Fatty acid results are presented in Table 2. Mean TFA concentration was 23.8 g kg⁻¹ DM, ranging from 16.8 to 29.0 g kg⁻¹ DM; between 89% and 95% of which was comprised of six individual FAs, namely palmitic acid (C16:0), *trans*-3-hexadecenoic acid (C16:1 Δ^{13}), stearic acid (C18:0), oleic acid (C18:1 Δ^9), linoleic acid (C18:2 $\Delta^{9,12}$) and α -linolenic acid (C18:3 $\Delta^{9,12,15}$). Overall mean concentration for these individual FAs was: 4.00, 0.397, 0.380, 0.591, 3.57 and 13.3 g kg⁻¹ DM, respectively. Population averages differed for concentrations of C16:1 Δ^{13} ($p < 0.001$) and C18:3 $\Delta^{9,12,15}$ ($p = 0.023$), with the average for the 'experimental' genotypes from the breeding population being 22.5% and 12.2% lower in

these FAs, respectively, compared to the mapping population average. Differences for C16:0, C18:2 $\Delta^{c9,12}$ and TFA were approaching significance ($p < 0.10$) and no difference was observed for C18:0 ($p = 0.497$) and C18:1 Δ^{c9} ($p = 0.227$). Within the mapping population, the ‘benchmark’ genotypes differed for C16:0 ($p = 0.034$), C18:0 ($p < 0.001$), C18:3 $\Delta^{c9,12,15}$ ($p = 0.012$) and TFA ($p = 0.025$) concentration with respective differences of: 20.9%, 38.4%, 42.7% and 31.3% observed between the lowest and highest concentration values. The ‘experimental’ genotypes from the breeding population differed for all individual FA and TFA concentrations ($p < 0.001$), with observed differences between lowest and highest values ranging from 36.5% for C16:0 up to 91.0% for C16:1 Δ^{t3} .

Table 2. Individual and total fatty acid concentration (g kg⁻¹ DM) of perennial ryegrass genotypes from two distinct populations.

Variable	C16:0	C16:1 Δ^{t3}	C18:0	C18:1 Δ^{c9}	C18:2 $\Delta^{c9,12}$	C18:3 $\Delta^{c9,12,15}$	TFA
Population							
Mapping	4.11	0.441	0.376	0.602	3.46	14.2	24.7
Breeding	3.90	0.352	0.383	0.579	3.68	12.5	23.0
s.e.d.	0.113	0.0235	0.0101	0.0192	0.124	0.70	0.96
<i>p</i>	0.061	<0.001	0.497	0.227	0.078	0.023	0.087
Mapping Population Genotypes							
81	4.18	0.451	0.345	0.559	3.60	14.3	24.9
86	4.56	0.467	0.438	0.619	3.53	17.4	28.6
103	4.01	0.465	0.423	0.588	3.49	13.7	24.2
182	3.70	0.381	0.297	0.644	3.20	11.3	20.9
s.e.d.	0.211	0.0439	0.0189	0.0360	0.232	1.30	1.79
<i>p</i>	0.034	0.441	<0.001	0.354	0.620	0.012	0.025
Breeding Population Genotypes							
27	3.85	0.298	0.407	0.529	3.62	12.0	22.5
39	3.53	0.289	0.315	0.564	3.51	9.95	19.6
115	4.38	0.384	0.425	0.651	4.09	13.8	25.2
132	4.14	0.391	0.448	0.633	4.04	14.0	25.1
134	4.17	0.351	0.378	0.557	3.80	13.7	24.4
148	4.50	0.500	0.533	0.562	3.67	15.4	26.6
204	4.24	0.388	0.342	0.674	4.11	14.1	25.4
213	4.32	0.387	0.416	0.713	4.21	14.2	25.9
216	3.88	0.416	0.390	0.639	3.54	12.4	23.1
223	3.86	0.336	0.362	0.496	3.50	11.9	22.2
231	3.39	0.239	0.298	0.536	3.03	9.19	18.1
235	3.76	0.293	0.347	0.527	3.38	11.6	21.5
238	3.96	0.324	0.409	0.567	3.80	11.8	22.6
298	4.29	0.523	0.467	0.593	4.56	16.9	29.0
301	4.04	0.341	0.401	0.533	3.71	14.1	24.8
307	3.55	0.341	0.336	0.553	3.53	11.1	20.9
314	3.12	0.196	0.339	0.664	3.18	7.76	16.8
320	3.33	0.275	0.353	0.465	3.07	9.98	19.0
329	4.03	0.448	0.393	0.518	3.84	15.2	26.2
332	3.60	0.311	0.292	0.608	3.38	11.7	21.4
s.e.d.	0.231	0.0479	0.0207	0.0392	0.253	1.42	1.96
<i>p</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

C16:0, palmitic acid; C16:1 Δ^{t3} , *trans*-3-hexadecenoic acid; C18:0, stearic acid; C18:1 Δ^{c9} , oleic acid; C18:2 $\Delta^{c9,12}$, linoleic acid; C18:3 $\Delta^{c9,12,15}$, α -linolenic acid; TFA, total fatty acids; s.e.d., standard error of difference; *p*, probability.

3.2. Relationships between Fatty Acid Concentrations and Herbage Chemical Composition

The relationships between TFA concentration and concentrations of CP, WSC, NDF and ADF across all genotypes are shown in Figure 1. Higher TFA concentration was strongly associated with higher CP concentration ($R^2 = 0.76$, $p < 0.001$). Negative relationships were observed between TFA concentration and WSC and ADF concentration, both with an R^2 of 0.19 ($p = 0.034$). No significant relationship was observed between TFA and NDF ($p = 0.159$). Similar trends were also observed for individual FA concentrations (Table 3). Crude protein was positively correlated ($p < 0.05$) with five of the main individual FAs (C16:0, C16:1 Δ^{t3} , C18:0, C18:2 $\Delta^{c9,12}$, C18:3 $\Delta^{c9,12,15}$) with R^2 values ranging from 0.28 to 0.81. Water-soluble carbohydrate was negatively correlated with C16:0, C16:1 Δ^{t3} and C18:3 $\Delta^{c9,12,15}$ ($p < 0.05$; $R^2 = 0.22$ to 0.26). Negative correlations were also observed between NDF and C18:2 $\Delta^{c9,12}$ ($p < 0.05$; $R^2 = 0.26$), and between ADF and C18:0 ($p < 0.05$; $R^2 = 0.21$) and C18:2 $\Delta^{c9,12}$ ($p < 0.001$; $R^2 = 0.42$).

Table 3. Pearson's correlation coefficients for the relationships between fatty acid concentrations and feed value ($n = 24$).

	C16:0	C16:1 Δ^{t3}	C18:0	C18:1 Δ^{c9}	C18:2 $\Delta^{c9,12}$	C18:3 $\Delta^{c9,12,15}$
CP	0.90 ^c	0.79 ^c	0.53 ^b	0.35	0.66 ^c	0.87 ^c
WSC	-0.50 ^a	-0.51 ^a	-0.14	-0.09	-0.11	-0.47 ^a
NDF	-0.20	-0.15	-0.36	-0.20	-0.51 ^a	-0.24
ADF	-0.38	-0.27	-0.46 ^a	-0.31	-0.65 ^c	-0.36

CP, crude protein; WSC, water-soluble carbohydrate, NDF, neutral detergent fibre; ADF, acid detergent fibre; C16:0, palmitic acid; C16:1 Δ^{t3} , *trans*-3-hexadecenoic acid; C18:0, stearic acid; C18:1 Δ^{c9} , oleic acid; C18:2 $\Delta^{c9,12}$, linoleic acid; C18:3 $\Delta^{c9,12,15}$, α -linolenic acid; ^a significant at $p < 0.05$; ^b significant at $p < 0.01$; ^c significant at $p < 0.001$.

The PCA biplot is shown in Figure 2 where the first two principal components (PC) combined explained 81.2% of the variation. More than half of the variability within the data was explained by PC1 (57.2%) which was defined by individual FAs, TFA and CP, with approximately equal positive loadings for all except C18:1 Δ^{c9} . Water-soluble carbohydrate, NDF and ADF characterised PC2 which explained a further 24% of the variation, where NDF and ADF had positive loadings and WSC negative loadings. The 'experimental' genotypes from the breeding population were evenly dispersed across both PCs whereas the 'benchmark' genotypes from the mapping population tended to cluster in the top right corner. Four particularly interesting genotypes stand out in the PCA biplot, two from the mapping population (81 and 86) and two from the breeding population (27 and 213). The two mapping population genotypes expressed similarly low levels of WSC but had differing FA concentration, with genotype 86 being higher in FA concentration. Equally, genotype 27 and 213 from the breeding population had comparably high WSC concentration but again differing levels of FA concentrations.

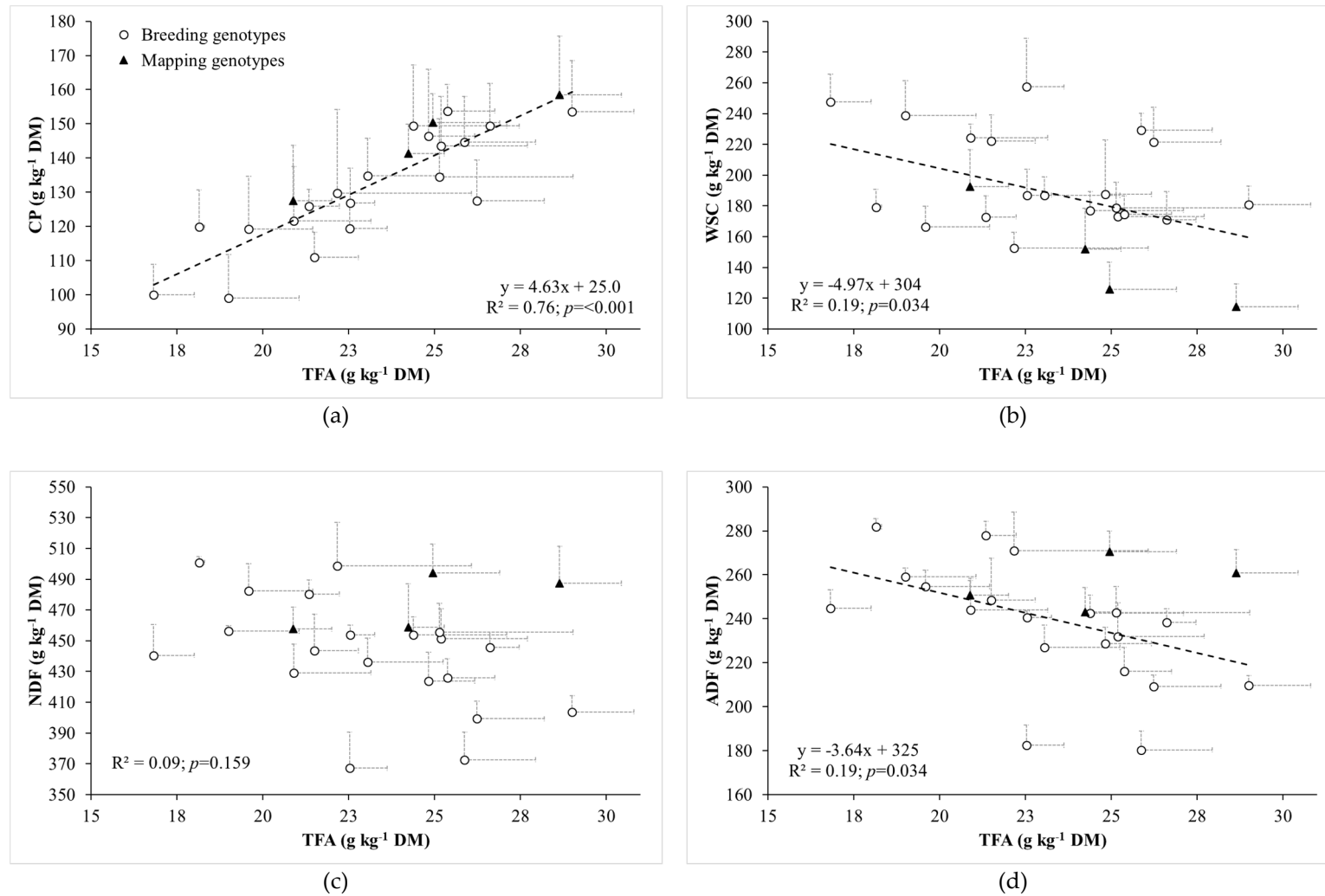


Figure 1. Relationships between (a) crude protein (CP), (b) water-soluble carbohydrates (WSC), (c) neutral detergent fibre (NDF) and (d) acid detergent fibre (ADF) with total fatty acid (TFA) concentrations ($n = 24$). Grey dashed lines indicate standard error of the mean for each genotype.

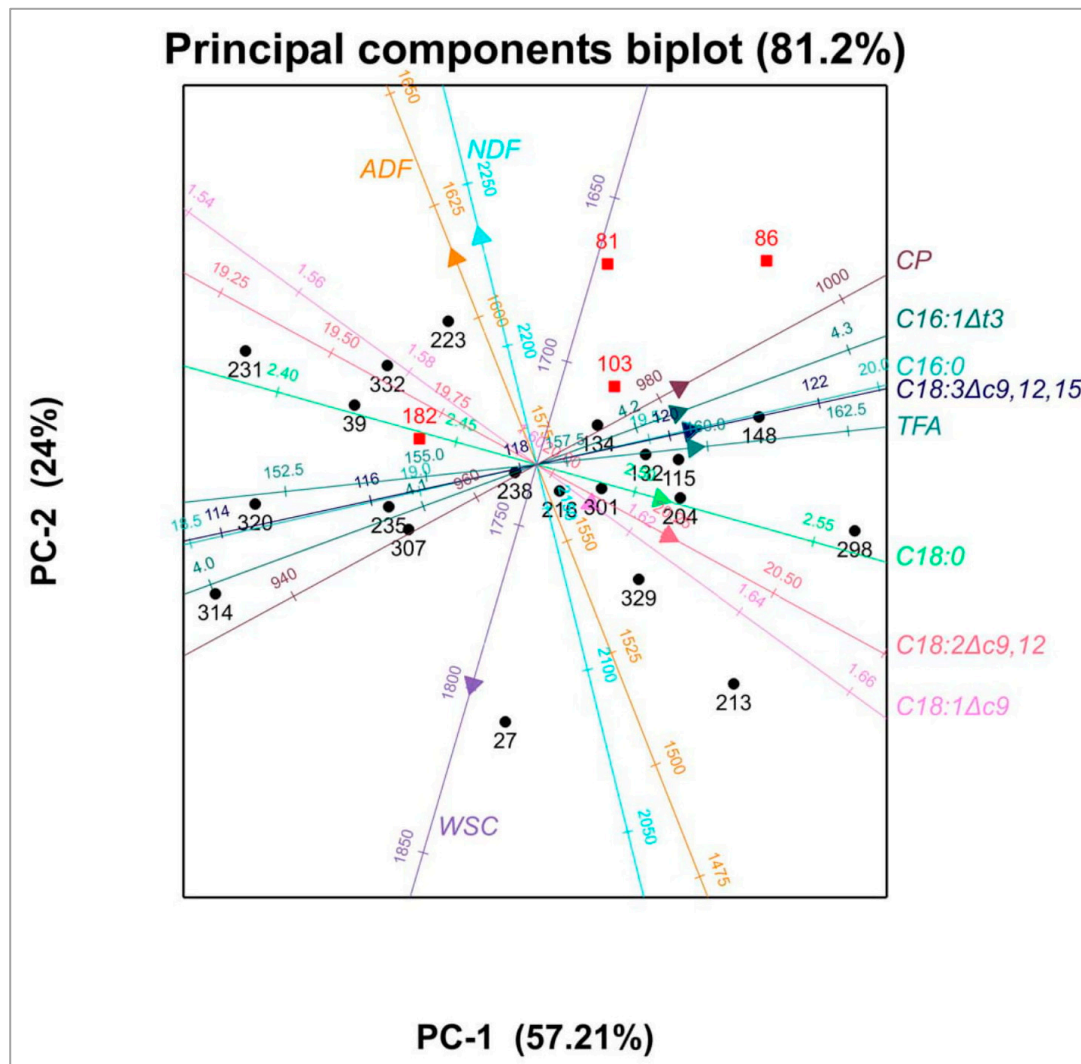


Figure 2. Bi-plot of the first and second principal components. Genotypes from the breeding population are indicated by a black circle [●] and genotypes from the mapping population are indicated by a red square [■]. Vector labels: CP, crude protein; WSC, water-soluble carbohydrate; ADF, acid-detergent fibre; NDF, neutral detergent fibre; C16:0, palmitic acid; C16:1 Δ t³, *trans*-3-hexadecenoic acid; C18:0, stearic acid; C18:1 Δ c⁹, oleic acid; C18:2 Δ c^{9,12}, linoleic acid; C18:3 Δ c^{9,12,15}, α -linolenic acid; TFA, total fatty acids.

4. Discussion

Crude protein results of the present study are comparable to the typical ranges of CP concentrations reported for fresh grass [28–31]. Water-soluble carbohydrate results were lower than the ranges reported by Gilliland et al. [32] and Palladino et al. [21] for harvests of PRG under similar management. This disparity in WSC concentrations between the present study and previously reported values is likely due to differences in the varieties used in the studies, the environmental conditions (poly-tunnel vs. field) and management of the plants (single harvest vs. multiple harvests). Fibre concentrations (NDF and ADF) were also within the typical ranges reported for grasses [33].

Total FA concentrations found in the present study agree with other published data for July harvests of PRG. Dewhurst et al. [34], Elgersma et al. [35] and Van Ranst et al. [36] reported TFA values ranging from 2.2 to 2.6% DM, though Dewhurst et al. [20] observed marginally lower TFA concentration. Differences in FA concentrations have previously been shown at species level [20,37,38], cultivar level [21,35,39] and genotype level [22], signifying that, despite the fact environmental factors can have

substantial effects on FA concentrations, there is still a significant underlying genetic contribution to this trait.

The relationships found in the present study between FA concentrations and CP, WSC, NDF and ADF agree with previous studies which have investigated such relationships. Elgersma et al. [40] investigated the effects of N fertilisation and regrowth interval on the FA concentrations of a PRG sward and found a strong positive relationship between CP and $C18:3\Delta^{c9,12,15}$ ($R^2 = 0.90$, $p < 0.001$), with similar results for CP and TFA. Boufaied et al. [37] also reported high R^2 values (ranging from 0.47 to 0.84) for the relationships between N concentrations and TFA, $C18:3\Delta^{c9,12,15}$, $C18:2\Delta^{c9,12}$ and $C16:0$ for timothy (*Phleum pratense*) grass harvested at varying growth stages with different levels of N and P fertilisation. This strong positive association found between protein and FAs is likely explained by the fact that both constituents are present in significant quantities within chloroplasts, which consist of approximately 35%–50% protein and 20%–30% lipid on a DM basis [41]; hence, higher concentrations of protein and lipid are typically found in leaf versus stem tissue. In contrast, WSC concentrations are typically found to be higher in stem tissue, particularly pseudostem [42,43]. Consequently, WSC and CP are widely acknowledged to be negatively correlated [44], owing to these constituents being predominantly present in different parts of the plant (stem vs. leaf, respectively). This also explains the negative correlation observed in the present study between WSC and FA concentrations. Similarly, Palladino et al. [21] reported respective negative correlations of -0.20 , -0.11 and -0.12 for TFA, $C18:3\Delta^{c9,12,15}$ and $C18:2\Delta^{c9,12}$ versus WSC. While there is no published data on the relationship between FAs and fibre in fresh herbage, a negative association between these two constituents has been reported for silage [19,45]. The underlying mechanism driving this negative correlation between FA and fibre concentrations may again be explained by the ratio of leaf to stem. Although probable explanations for the relationships between FA concentrations and feed value observed in the present study have been given, further investigation is required to confirm these hypotheses.

Within forage breeding programmes, it is vitally important to recognise and understand how targeted selection of one or a few traits of interest will alter the overall characteristics and feed value of the progeny. The relationships presented here and elsewhere suggest that selectively breeding for higher FA concentrations in PRG would in theory also increase CP and decrease WSC and fibre concentrations in subsequent generations. This would lead to lower WSC:CP ratios, which in turn may have an undesirable consequence on nitrogen use efficiency (NUE) at animal level (i.e., the proportion of nitrogen consumed versus that excreted in the milk) [46]. To achieve improved NUE, the advised target WSC:CP ratio is ≥ 1.5 [44]. The WSC:CP ratios in the present study ranged from 0.72 to 2.48, where generally only genotypes with lower TFA concentrations exceeded the advised target ratio (e.g., genotypes 314, 231 and 320). However, little is currently known about how FA concentration and composition may influence NUE. Hypothetically, the higher energy density of FAs relative to WSC may offset the reduction in WSC and thus negate any undesirable reduction in NUE. In any case, investigation into the effect of FA concentration and composition on NUE, as well as other agronomically important characteristics, is needed.

Within a PCA biplot, vectors which are close to any principal component axis (i.e., have high absolute cosine values) are important in explaining that particular component [47]. As such, the present study found that most of the variation between genotypes (57.2% by PC1) was due to differences in FA and CP concentration, with a further 24% of the variation (PC2) explained by WSC and fibre. Mapping population genotypes were mostly grouped towards the top right quadrant of the bi-plot, reflecting their lower WSC and higher fibre, CP and FA concentrations. The breeding population genotypes on the other hand were evenly dispersed along PC1 with some vertical scattering along PC2, illustrating that much of the variation between these genotypes was due to a combination of differences in CP and FA concentrations. As aforementioned, four genotypes particularly stood out on the PCA biplot which expressed distinctive combinations of the chemical constituents measured in the present study. Two mapping population genotypes (81 and 86) expressed similarly low levels of WSC concurrent with differing FA concentrations whilst two genotypes from the breeding population (27 and 213) expressed

similarly high levels of WSC in parallel with differing FA concentrations. These would be excellent candidate genotypes for further investigation into the synergies between WSC, FAs and CP particularly in the context of NUE, although they could provide beneficial information for other agronomically relevant traits.

Although Hegarty et al. [22] successfully identified regions of the PRG genome associated with FAs using MAS, the transfer and applicability of this information to current grass breeding programmes is at present limited. It is envisaged that these genetic markers may be transferable to breeding populations in the future, provided there is sufficient genetic similarity between the mapping and breeding populations. Hence, the present work primarily focussed on investigating genotypes from the most current breeding population, as this is the genetic pool from which new grass varieties arise. The results presented here provide further evidence in support of the strong genetic basis of FA concentration and provide an initial insight into the quantities and variation in FA concentrations present within a breeding population. This is an important first step on the route to developing novel grass varieties with enhanced FA profiles for the benefit of ruminant production, meat and milk quality and alternative energy crop production. Considerably more time and effort will be required to achieve this ambition as it takes approximately 15 to 20 years to produce new commercial cultivars for PRG [26]. Nevertheless, the results presented in this paper are very encouraging in terms of the prospect of being able to selectively breed for this novel trait.

5. Conclusions

Breeding population genotypes were generally higher in WSC and lower in CP, fibre and FA concentrations compared to those from the mapping population. Sizeable variation was observed between individual genotypes which was predominantly due to differences in CP and FA concentrations (as indicated by the multivariate analysis), which further demonstrates the genetic component of forage FA concentration and composition and the prospect of selectively breeding for this novel trait. Fatty acid concentrations were positively correlated with CP and negatively correlated with WSC and fibre. Selection for higher FA concentrations may affect other agronomically important characteristics such as NUE. However, data in relation to this is currently lacking. Furthermore, the multivariate analysis did highlight some genotypes that do not seem to follow these general trends. These particular genotypes warrant further investigation to help identify and understand the biological mechanisms that result in certain genotypes bucking the trend and to explore the multifaceted benefits such genotypes may provide.

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