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The fatty acid profile and stable isotope ratios of C and N of muscle from cattle that grazed grass or grass/clover pastures before slaughter and their discriminatory potential

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Abstract

Consumption of grazed pasture compared to concentrates results in higher concentrations, in beef muscle, of fatty acids considered to be beneficial to human health. Little information is available on the influence of the type of grazed forage. Our objectives were to determine 1) the effect of inclusion of white clover in a grazing sward on the fatty acid profile of beef muscle and 2) the potential of the fatty acid profile and stable isotope ratios of C and N to discriminate between beef from cattle that grazed grass-only or grass/clover swards before slaughter. A total of 28 spring-born Charolais steers grazed from March until slaughter in October, either on a perennial ryegrass (*Lolium perenne *L.) sward that received approximately 220 kg N/ha or a perennial ryegrass–white clover (*Trifolium repens *L.) sward that received 50 kg N/ha. The longissimus muscle from cattle finished on grass/clover had a higher ($P < 0.05$) proportion of C18:2 and C18:3 but a lower ($P < 0.05$) proportion of conjugated linoleic acid and δ15N value than animals finished on the grass-only sward. Discriminant analysis using the fatty acid data showed that, after cross-validation, 80.7% of grass/clover and 86.1% of grass-only muscle samples were correctly classified. Discriminant analysis using the stable isotope data showed that, after cross-validation, 95.7% of grass/clover and 86.5% of grass-only muscle samples were correctly classified. Inclusion of white clover in pasture is likely to have little effect on healthiness of meat for consumers. However, changes in fatty acids and stable isotopes can be used to distinguish between grass/clover-fed and grass-only-fed beef.

Keywords

beef • discrimination • fatty acids • grass/clover • stable isotopes

Introduction

The majority of systems of beef production practised in temperate climates seek to make the best use of locally available forage and inexpensive concentrates. Grazed pasture is an important component of many production systems in Ireland (O'Riordan and O'Kiely, 1996). To maintain sustainable, economically competitive and welfare-friendly systems of beef production, it is necessary to further increase the production and utilisation of grazed pasture. For beef production, swards containing a significant proportion of white clover (*Trifolium repens* L.) may afford savings in relation to expensive chemical N inputs to grassland and may result in lower N_2O emissions (Ledgard *et al*., 2009). Moreover, since the seasonal dry matter (DM) distribution of clover is such that more growth occurs in the second half of the year at a time when grass growth naturally declines (Gilliland *et al*., 2009), it complements the demand of the grazing animal. Consequently, there is currently increasing interest in including clover in perennial ryegrass (*Lolium perenne* L.)-based swards for grazing cattle, continuing a long-standing but somewhat cyclical interest in this topic internationally and in Ireland (Blaser *et al*., 1956; Ryan *et al*., 1984).

Consumer interest in the relationship between diet and health has focussed attention on the composition of food and in particular on its fatty acid composition. While beef is considered to be rich in saturated fatty acids (SFAs), it is also a source of polyunsaturated fatty acids (PUFAs), particularly omega (n)-3 PUFA and conjugated linoleic acid (CLA) which are considered to be beneficial to human health (Simopoulos, 2002; Yaqoob *et al*., 2006). Plants have the unique ability to synthesise *de novo* α-linolenic acid (18:3n-3), and forages such as grass and clover contain a high proportion (50–75%) of total total fatty acids as 18:3n-3 (Dewhurst *et al*., 2006). Exploiting herbage as a source of n-3 PUFA is an important nutritional strategy for enhancing the content of n-3 PUFA in beef since a proportion of dietary PUFA escapes biohydrogenation in the rumen and is deposited in tissue (Scollan *et al*., 2014). Thus, when compared to concentrates, feeding fresh grass results in higher concentrations of n-3 PUFA in muscle lipids, beneficially contributing to not only an increase in the PUFA:SFA ratio and a decrease in the n-6:n-3 PUFA ratio but also an increase in the deposition of CLA (*cis*-9, *trans*-11) (Scollan *et al*., 2014). While there are many comparisons of grazed grass with concentrate feeding, little information is

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available on the influence of the type of grazed forage on the fatty acid profile. Our first objective therefore was to determine the effect of inclusion of clover in a grazing sward on the fatty acid profile of beef muscle.

Increasingly, consumers are interested in the provenance of their food, and pasture-based systems have come to be regarded by some consumers as more environmentally and animal-welfare-friendly alternatives to intensive/feedlot systems of production (Broom *et al.,* 2013; Li *et al*., 2016). Animal products from such systems, for example, those labelled as 'grass-based', can command a price premium but require methods to validate the system of production and make it less likely that counterfeit products are fraudulently sold under such labels. Several approaches have been examined in this regard (Monahan *et al*., 2013; Moloney *et al*., 2014). Beef from cattle finished on pasture or concentrates could be distinguished based on their fatty acid composition (Alfaia *et al.,* 2009) or stable isotope ratios (Osorio *et al.,* 2011). However, no studies have been reported on discriminating between beef from cattle finished on different pastures, even though grass-only versus grass/clover pasturing will likely receive different sustainability and environmental ratings from consumers in the future (Broom *et al*., 2013). Accordingly, our second objective was to determine the potential of both the fatty acid profile and the C and N stable isotope composition to discriminate between beef from cattle that grazed grass-only or grass/clover swards before slaughter.

Materials and methods

Experimental design and animal management

This study was carried out under license from the Irish government's Department of Health (HRB, 2011), and all procedures used complied with national regulations concerning experimentation on farm animals. A total of 28 Charolais-sired male bull calves (mean birth date: 16 March) grazing a common pasture with their dams (Limousin/Friesian) were selected for this study. The animals were castrated in September using a burdizzo plus local anaesthetic, weaned on 30 October and housed on 25 November. For the winter, the calves were accommodated in a slatted floor shed and offered a predominantly perennial ryegrass silage *ad libitum* +1.5 kg proprietary concentrate daily. At the end of the winter, the animals were weighed (consecutive days), assigned at random to one of the two experimental swards and turned out to pasture on 15 March. The experimental swards were a conventionally managed perennial ryegrass and a perennial ryegrass–white clover (grass/clover) sward. The grass/clover sward was established by conventionally re-seeding a representative portion of an established perennial ryegrass sward approximately 24 months before the experiment commenced. The total N input to the grass/clover sward was 50 kg/ha which was applied in the form of urea in the spring. The total N input to the grass swards was 220 kg/ha which was applied as urea (100 kg) in spring and as calcium ammonium nitrate in summer. The animals rotationally grazed 21 paddocks of each sward (target pre-grazing herbage mass was 1,200 kg DM/ha) to a residual sward plate metre (Jenquip Agri Business Centre, Fielding, New Zealand) height of 50 mm. At turnout, 11 paddocks were closed. After harvesting grass for conservation as silage, these plots were introduced to the grazing management. The animals spent, on average, 2 days in each paddock. The animals were weighed on 9 October and 10 October, when they were removed for slaughter.

Pre-grazing herbage was sampled in duplicate from alternative paddocks as described by French *et al*. (2001). One sample was manually separated into grass, clover and weeds, and each component dried at 40°C for at least 48 h and weighed to calculate the white clover content (DM basis). The other sample was frozen until pooled on a monthly basis for the analysis of chemical composition.

Post-slaughter carcass measurements and sampling

On the day of slaughter, after weighing, animals were transported 150 km to a commercial slaughter facility and slaughtered at random within 1 h of arrival. After slaughter, cold carcass weight (hot carcass weight ´ 0.98) was recorded, and the carcasses were classified for fat score (1 = leanest and 5 = fattest) using the EU Beef Carcass Classification Scheme. At 48-h post-mortem, a Minolta Chromameter Cr3000 (Minolta Camera Co., Osaka, Japan) was used to measure lightness (L), redness (a) and yellowness (b) of subcutaneous fat on the loin and rump of each carcass as described by Dunne *et al*. (2004). The sides were cold boned at 48-h post-mortem, and a steak (2.5 cm thick) was collected from the *Musculus longissimus dorsi* (LD) and frozen for compositional analysis.

Chemical analysis

Intramuscular fat and moisture contents of thawed minced LD samples were determined using an automated, integrated microwave moisture and methylene chloride fat extraction method (Bostian *et al*., 1985) on a CEM moisture/solid analyser (Model AVC 80; CEM Corp., Matthews, NC, USA). Intramuscular fat was extracted from blended LD, and the fatty acids were quantified as their fatty acid methyl esters using gas chromatography as previously described (French *et al.,* 2000). Individual fatty acids were identified with reference to fatty acid standards, and the *cis*-9 and *trans*-11 CLA isomer was identified with reference to the CLA standard mix generously provided by M. Pariza (The Food Research Institute, University of Wisconsin, Madison, WI, USA).

Thin slices of LD (5 g) were freeze-dried (Edwards Pirani 501 freeze dryer; Edwards Ltd, Crawley, UK) for 48 h and pulverised using a ball mill (TypeMM2; Glen Creston Ltd, Stanmore, UK). The milled muscle samples were then stored in plastic bags under vacuum desiccation. Total lipid was extracted from the milled samples using hexane/isopropanol (3:2, v/v) according to the method by Radin (1981). The defatted muscle was separated from the solvent mixture by vacuum filtration and air-dried overnight in a container covered with aluminium foil to protect samples from light. Lipid-free muscle weighing 0.9–1.1 mg was loaded into tin capsules for dual C and N isotope analysis. Natural abundance stable isotope ratios of C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) in lipid-free muscle and feed samples (below) were measured by continuous flow isotope ratio mass spectrometry as described by Osorio *et al*. (2011). Stable isotope ratios were expressed using conventional δ notation in units of per mil (‰) relative to a suitable standard and defined as follows:

$$\delta(‰) = [(R_{sample} - R_{reference}) - 1] \times 1000$$

where R_{sample} is the isotope ratio in the sample and $R_{reference}$ is the isotope ratio in the reference material. Results are referred to Vienna Pee Dee Belemnite (V-PDB) for carbon and atmospheric N_2 for nitrogen.

Monthly composite herbage samples were oven dried at 40°C for 48 h and milled (1-mm sieve). General feed analyses were carried out as previously described by Moloney and O'Kiely (1995). For fatty acid analysis, lipid was extracted from the forages and methylated as described by Sukhija and Palmquist (1988) and methyl esters were quantified as described by French *et al*. (2000). For isotope analysis, 3.5–4.5 mg of the dried milled feed samples were packed into ultra-clean tin capsules.

Statistical analysis

All statistical analyses were carried out using GenStat (14th edition; VSN International Ltd., Hemel Hempstead, UK). Data were subjected to analysis of variance using a model that had sward type as the main effect and initial live weight as a covariate, where indicated. Discriminant analysis was performed to evaluate the possibility of differentiating LD muscle according to the diet of the animals. The stable isotope ratio data were subjected to discriminant analysis directly. The ability of the determined discrimination function to separate the data into the dietary classes was tested using a Chi-square analysis. Fatty acid data were subjected to forward stepwise discriminant analysis. As an initial analysis indicated a strong correlation between individual fatty acids and fatty acid classes (SFA, monounsaturated fatty acid [MUFA], PUFA) or ratios (PUFA:SFA, n-3 PUFA:n-6 PUFA), only individual fatty acids were considered in the final analysis. Variable selection was based on bootstrapping, and the determined discriminant function was tested as described earlier. For each discriminant function, its robustness was tested using a bootstrapping approach and the success of the discrimination was measured as the proportion of cases correctly assigned in the cross-validation.

Results

The proportion of clover in the grass/clover pasture increased from animal turnout until slaughter in October (Figure 1). There was little clover evident in the grass-only sward (Figure 1). The chemical composition of the pastures (Table 1) was largely similar. The oil content of the pastures was similar,

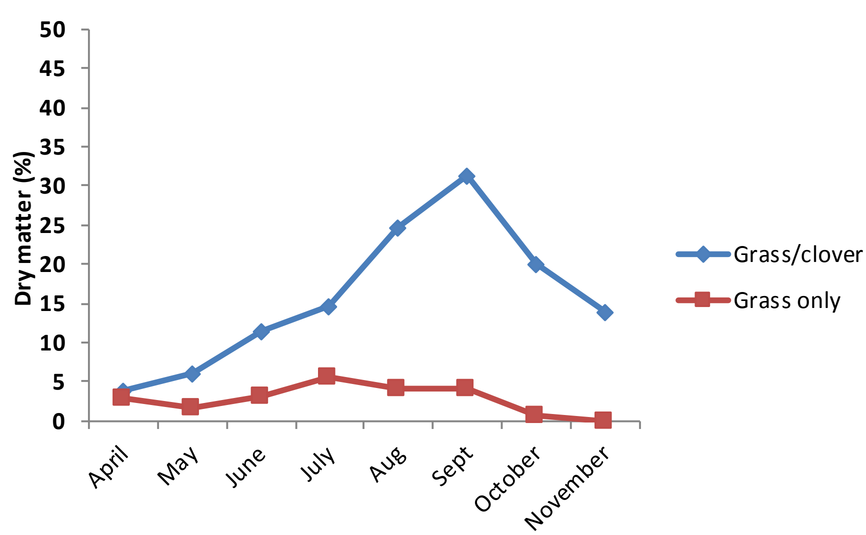

Figure 1. Proportion of clover in the grass/clover and grass-only pastures during the growing season.

Table 1. The mean (s.d.) botanical and chemical composition of the forages

	Grass/clover	Grass only
Grass (g/kg DM)	793 (91.7)	910 (42.2)
Clover (g/kg DM)	145 (80.2)	38 (24.6)
Weeds (g/kg DM)	62 (33.4)	52 (25.5)
DM (g/kg)	186 (21.7)	176 (7.9)
Crude protein (g/kg DM)	195 (32.2)	192 (26.6)
DM digestibility (g/kg)	812 (23.5)	811 (28.7)
Ash (g/kg DM)	112 (12.3)	106 (12.2)
Oil (g/kg DM)	25.6 (4.95)	24.1 (3.06)
Fatty acids (g/kg DM)	31.4 (5.31)	32.0 (6.24)
Fatty acids (g/kg fatty acid methyl esters)		
$C_{14:0}$	5.3 (2.96)	4.9 (3.58)
$C_{16:0}$	198.6 (14.64)	218.3 (25.88)
$C_{16:1}$	12.7 (12.51)	10.1 (10.05)
$C_{18:0}$	25.4 (11.21)	26.3 (13.83)
$C_{18:1}$	21.0 (5.66)	24.5 (4.57)
$C_{18:2}$	132.0 (11.38)	127.3 (15.54)
$C_{18:3}$	511.5 (42.91)	469.4 (52.98)
SFA	286.0 (25.96)	314.6 (41.99)
MUFA	55.9 (26.06)	57.2 (15.16)
PUFA	658.2 (44.55)	628.3 (50.99)
$\delta^{15}N$(‰)	4.88 (0.97)	5.88 (1.14)
$\delta^{13}C$(‰)	–30.24 (0.64)	–30.37 (0.56)

DM, dry matter; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

and no fatty acids with <12 carbons were detected. The total and dominant SFA (C16:0) proportion tended to be lower in the grass/clover pasture. The total and dominant PUFA (C18:3n-3) proportion tended to be higher in the grass/clover sward. The grass/clover pasture tended to have a lower $\delta^{15}N$ value than the grass-only pasture, while the $\delta^{13}C$ value was similar for both pastures.

Production and carcass-related data are summarised in Table 2. There was no difference in mean slaughter weight or growth rate between steers reared on grass-only or grass/clover swards. Mean carcass weight was similar for steers reared on grass-only or grass/clover swards, and carcasses had similar fat and conformation scores. There was little difference in subcutaneous fat colour other than "C" or saturation, which, when averaged over the carcass, was higher ($P < 0.05$) for carcasses from steers reared on the grass-only sward.

Data for intramuscular fat concentration and its fatty acid composition are summarised in Table 3. There was no difference in intramuscular fat concentration but muscle from cattle reared on grass/clover had a higher proportion ($P < 0.05$) of C12:0, C18:2, C18:3, total PUFA ($P < 0.1$) and n-6 PUFA ($P < 0.1$) but a lower proportion ($P < 0.05$) of C17:1, CLA and unidentified fatty acids than cattle reared on the grass-only sward.

Muscle from cattle reared on the grass/clover sward had a lower ($P < 0.05$) $\delta^{15}N$ value, but there was no difference in $\delta^{13}C$ value of muscle from cattle reared on the two swards (Table 3).

From the stepwise discriminant analysis of the individual fatty acid data, C16:1, C18:3 and C20:5 were selected as giving the best discrimination. The Mahalanobis squared distance between the grass/clover and grass-only groups using this discriminant function was 2.876, and the F-test of the distance had $P = 0.003$. In the original classification, 12 samples in the grass/clover group were correctly classified and 13 samples in the grass-only group were correctly classified (Figure 2). In the cross-validation, 80.7% of grass/clover samples and 86.1% of grass-only samples were correctly classified (overall classification error of 16.6%).

Based on the discriminant analysis using the $\delta^{15}N$ and $\delta^{13}C$ values, the Mahalanobis squared distance between the grass/clover and grass-only groups was 7.149 and the F-test of the distance had $P < 0.001$. In the original classification, all samples (i.e. 14) in the grass/clover group were correctly classified and 12 samples in the grass-only group were correctly classified (Figure 3). In the cross-validation, 95.7% of grass/clover samples and 86.5% of grass-only samples were correctly classified (overall classification error of 8.9%).

Table 2. Initial and final weight and carcass characteristics of beef cattle that grazed grass/clover clover or grass only swards prior to slaughter

	Grass/clover	Grass only	s.e.d.	Significance
Initial weight (kg)	439.4	425.9	15.27	
Final weight (kg)	632.2	609.4	18.88	
Final weight (kg)[a]	626.6	615.1	14.48	
Growth (g/day)	923	878	68.1	
Carcass weight (kg)	342.2	330.0	10.25	
Carcass weight (kg)[a]	339.2	333.1	7.87	
Fat score[b]	3.54	3.46	0.227	
Conformation[c]	3.14	3.14	0.173	
Fat colour[d]: rib				
L	70.9	69.1	1.47	
a	4.3	3.4	0.68	
b	12.8	12.5	1.56	
C	71.6	75.4	2.20	
H	13.5	13.0	1.63	
Fat colour: round				
L	68.3	69.7	1.21	
a	5.1	4.7	0.64	
b	15.3	17.3	1.08	
C	71.5	74.9	2.05	
H	16.2	18.0	1.11	
Fat colour: mean				
L	69.6	69.4	1.02	
a	4.7	4.1	0.48	
b	14.0	14.9	1.16	
C	71.3	74.9	1.76	*
H	14.8	15.5	1.18	

[a]Adjusted using initial weight as a covariate. [b]Fat score 5^+ (highest) to 1^- (lowest), (5^+ is 15). [c]Conformation score as EUROP where E^+ (highest) to P^- (lowest) (E^+ is 15). [d]Colour coordinates. a, redness; b, yellowness; C, chroma; H, hue angle; L, lightness.

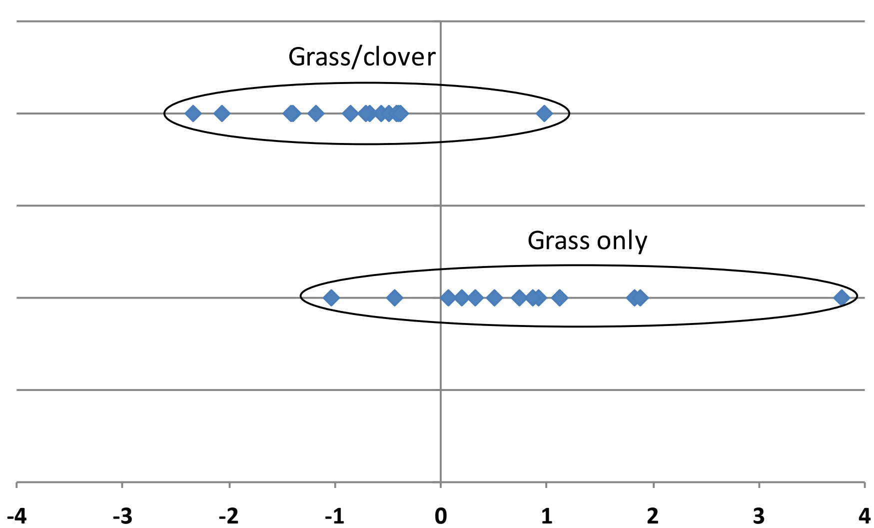

Figure 2. Plot of individual observation discriminant scores against the discriminant function based on fatty acid composition of muscle lipids.

Table 3. Composition, fatty acid profile and stable isotope ratios of *M. longissimus* muscle of beef cattle that grazed grass/clover or grass only swards prior to slaughter

	Grass/clover	Grass only	s.e.d.	Significance
Composition (g/kg)				
Fat	27.4	29.2	4.53	
Moisture	734.5	735.9	4.17	
Fatty acids (g/100 g fatty acid methyl esters)				
$C_{10:0}$	0.05	0.06	0.004	
$C_{12:0}$	0.08	0.07	0.004	*
$C_{14:0}$	2.60	2.51	0.147	
$C_{14:1}$	0.55	0.51	0.051	
$C_{15:0}$	0.49	0.50	0.029	
$C_{16:0}$	26.49	25.85	0.465	
$C_{16:1}$	3.40	3.45	0.149	
$C_{17:0}$	1.10	1.14	0.041	
$C_{17:1}$	0.94	1.01	0.032	*
$C_{18:0}$	15.64	15.66	0.596	
$C_{18:1}$	36.49	37.67	0.875	
$C_{18:2}$	3.08	2.51	0.255	*
$C_{18:3}$	1.61	1.23	0.138	*
Cis-9, *trans*-11 $C_{18:2}$	0.47	0.58	0.045	*
$C_{20:0}$	0.08	0.07	0.006	
$C_{20:1}$	0.14	0.12	0.169	
$C_{20:2}$	0.03	0.03	0.008	
$C_{20:3}$	0.17	0.15	0.026	
$C_{20:4}$	0.68	0.62	0.082	
$C_{22:1}$	0.19	0.16	0.021	
$C_{20:5}$	0.48	0.48	0.072	
$C_{22:5}$	0.69	0.61	0.083	
$C_{22:6}$	0.05	0.06	0.006	
Unidentified	4.51	4.96	0.211	*
SFA	46.53	45.87	0.728	
MUFA	41.69	42.91	0.949	
PUFA	7.26	6.25	0.606	0.10
n-6 PUFA	3.76	3.15	0.332	0.065
n-3 PUFA	3.00	2.52	0.287	
PUFA:SFA	0.16	0.14	0.014	
n-6:n-3 PUFA	1.27	1.25	0.027	
$C_{18:2}$: $C_{18:3}$	1.93	2.03	0.068	
Desaturase index[a]	0.47	0.49	0.009	
δ^{15} N(‰)	8.20	9.12	0.132	***
δ^{13}C(‰)	–27.20	–27.50	0.167	0.08

[a]Desaturase index = (C14:1 + C16:1 + C18:1)/(C14:1 + C16:1 + C18:1 + C14:0 + C16:0 + C18:0).
M. longissimus, *Musculus longissimus*; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Discussion

Herbage

The proximate composition, fatty acid profile and stable isotope ratios of C and N of the grass-only sward were within the ranges previously observed at this location (Noci *et al*., 2007; Osorio *et al*., 2011). The pattern of clover growth within the grass/clover sward is typical of the seasonal distribution curve for white clover in Ireland (Gilliland *et al*., 2009). With respect to the fatty acid proportions, the higher PUFA proportion in the grass/clover sward reflects the higher PUFA proportions sometimes reported for white clover compared to perennial ryegrass (Boufaïed *et al*., 2003; Wyss and Collomb, 2010) and the increasing proportion of white clover as the experiment progressed. This is illustrated in Figure 5 for the proportion of C18:3 in sward lipid which increased in the grass/clover sward as the proportion of clover increased.

The significantly higher $\delta^{15}N$ values of the grass-only sward likely reflect a system-wide enrichment of ^{15}N in conventional grassland farms, where the use of mineral fertilizers results in highly positive N input–output balances (associated with larger N losses preferentially of light ^{14}N) and/or the fact that legumes are isotopically lighter when actively fixing atmospheric N_2 that has a defined $\delta^{15}N$ value of zero (Schmidt *et al*., 2005; Kriszan *et al*., 2014).

Cattle growth

While Yarrow and Penning (2001) reported an increase in cattle growth as the proportion of clover in the sward increased, in general in the literature, there is little difference in growth rates between cattle that grazed a fertilised grass sward and a grass/clover sward (Bax and Schils, 1993; O'Riordan, 1995; Tyson *et al*., 1997) as is the case in the current study.

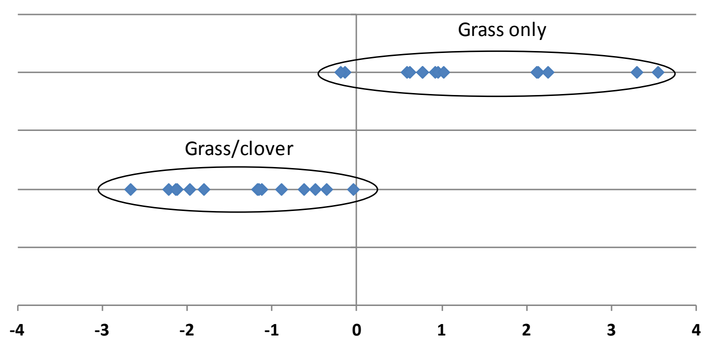

Figure 3. Plot of individual observation discriminant scores against the discriminant function based on stable isotope composition of muscle.

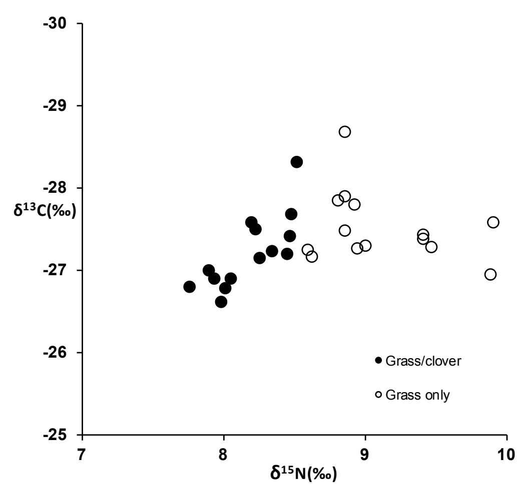

Figure 4. $\delta^{13}C$ versus $\delta^{15}N$ values of muscle from heifers that grazed a grass-only or a clover/grass sward before slaughter.

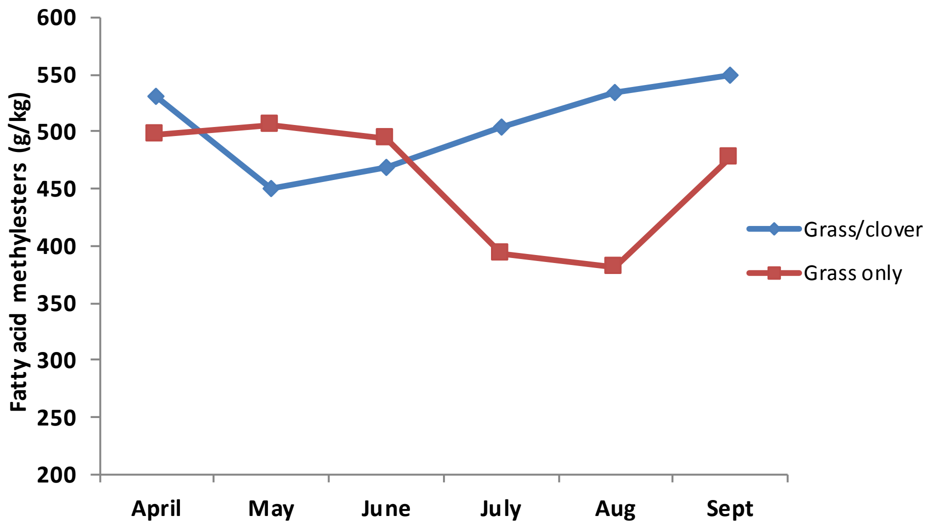

Figure 5. The proportion of linolenic acid (C18:3) in the lipids of the grass/clover and grass-only pastures during the growing season.

With regard to carcass weight, in a 3-year study using similar pastures to those used in the current study, O'Riordan (1995, 1996) reported a higher mean carcass weight for cattle that grazed the grass/clover sward prior to slaughter in years 1 and 2 (19 and 22 kg, respectively, $P < 0.05$), but the response in the third year (7 kg; O'Riordan and Travers, 1997) was not significant but similar to the non-significant 6 kg seen in the current study. The differences between studies and year within a study most likely reflect the proportion of clover in the pasture. Nevertheless, that carcass yield was not lower for cattle that grazed the grass/clover sward compared to the fertilised grass sward highlights the potential cost-reduction due to the substitution of inorganic N fertiliser with clover.

Fatty acid composition

As more lipids are deposited in muscle, the MUFA proportion of total lipid increases and the PUFA proportion decreases (Moreno *et al*., 2008). Thus, changes in fatness due to differences in energy intake *per se* can confound the effects of diet on fatty acid composition of intramuscular fat. In the current study, there was no effect of pasture type on intramuscular fat concentrations in LD. Consequently, the confounding effect of differences in intramuscular fat concentrations on fatty acid composition was avoided.

There have been many comparisons of grazed grass with concentrate feeding with respect to the fatty acid composition of muscle lipid (Scollan *et al*., 2014). The general findings are that, compared to concentrates, feeding fresh grass results in higher concentrations of n-3 PUFA in muscle lipids, contributing to beneficial changes in the PUFA:SFA and n-6:n-3 PUFA ratios and an increased deposition of CLA (*cis*-9, *trans*-11) (Scollan *et al*., 2014). In contrast, little comparable information is available on the influence of the type of grazed forage and in particular consumption of mixed ryegrass and clover pasture.

The fatty acid profile of LD from cattle that grazed the grass-only sward was largely similar to that previously reported for this location (Noci *et al*., 2007). Consistent with the current study, Scollan *et al*. (2002a, 2002b) reported that, when compared to a grass-only pasture, cattle that grazed a grass/white clover pasture had a higher proportion of C18:3 n-3 and C18:2 n-6 in total muscle lipids which contributed to an increase in the PUFA:SFA ratio, but the CLA proportion was unaffected. The higher proportion of PUFA in muscle lipids from cattle that grazed the grass/clover sward compared to the grass-only sward may reflect not only higher consumption (not measured) but also a decrease in rumen biohydrogenation due to the increased passage rate frequently reported for white clover (Dewhurst *et al*., 2009). The lower proportion of CLA in muscle lipids from the grass/clover group in the current study is consistent with the latter hypothesis.

The increased proportion of C18:3 in muscle lipids of cattle that grazed a grass/clover sward rather than a grass-only sward may be interpreted as beneficial to the health of the consumer. However, the published reference intake values for human beings are 2 g and 250 mg/day for C18:3n-3 and eicosapentaenoic acid (EPA) +docosahexaenoic acid (DHA), respectively (EFSA, 2009). A 100 g serving of beef from the cattle that grazed the grass/clover sward would supply 44 mg of C18:3 and 15 mg of EPA + DHA compared to 36 and 16 mg, respectively, for beef from cattle that grazed the grass-only sward. These compare with C18:3 values of 25 and 36 mg/100 g muscle for beef labelled as conventional or organic, respectively, in the UK supermarkets, but both assumed by the authors to be from 'forage-based production systems' (Kamihiro *et al*., 2015). In the current study, neither beef could be labelled as a 'source' of omega-3 fatty acids according to the EFSA (2009) definition.

The stable isotope composition of muscle reflected that of the swards offered. In a comparison of conventional and organic-labelled beef purchased from Irish supermarkets, there was little difference in $\delta^{13}C$ between July and October when Irish conventional beef cattle are likely to be at pasture (Bahar *et al*., 2008). In the same period, the $\delta^{15}N$ was higher for conventional beef than organic-labelled beef (Bahar *et al*., 2008). The higher $\delta^{15}N$ in beef from cattle that grazed the grass-only sward in the current study is consistent with these findings, suggesting a dilution of cattle muscle N with isotopically lighter, clover-derived N which, in turn, derives from atmospheric N_2. Devincenzi *et al*. (2014) recently reported a linear decrease in the $\delta^{15}N$ in the muscle of lamb that grazed cocksfoot pasture as the proportion of alfalfa (a nitrogen-fixing legume) in the pasture grazed increased.

Authentication of pre-slaughter diet

Consumers are increasingly interested in the provenance of beef, and the methods that validate the system of production are thus required. Beef from cattle finished on pasture or concentrates could be distinguished (94–100% correct classification after cross-validation) based on their relatively large (compared to the current study) differences in fatty acid composition (Garcia *et al*., 2008; Alfaia *et al*., 2009). When the intramuscular fatty acid profile was more similar, due to the supplementation of grazing cattle with concentrates at 0.7% or 1.0% of live weight, correct classification without cross-validation was 78% (Garcia *et al*., 2008). Similarly, Monteiro *et al*. (2012) reported a correct classification, after cross-validation, of 82–87% for veal calves reared according to the local protected geographical indication and protected designation of origin rules which had similar intramuscular fatty acid profiles, while Dias *et al*. (2008) reported a correct classification, without cross-validation, of 100% for beef produced according to a 'traditional' or organic production system which had similar fatty acid profiles. The current study demonstrates that the relatively small differences in the fatty acid profile can also be used to distinguish beef produced from different sward types, with correct classification after cross-validation within the range described earlier.

A potential limitation to the use of fatty acid profile as an indicator of production system is that non-grass sources of fatty acids could give C18:3, C18:2 or CLA contents in meat similar to those derived from grass (Scollan *et al*., 2014). As the potential of stable isotope ratio measurements of C and N to differentiate between beef produced from pasture and concentrates and between organic and conventional beef has been demonstrated (Schmidt *et al*., 2005; Osorio *et al*., 2011), this approach was also examined in the current study. Osorio *et al*. (2011) also compared the stable isotope composition of muscle from cattle that either grazed grass or were offered ensiled grass during the winter and subsequently grazed a grass-only pasture for 6 months before slaughter. The $\delta^{13}C$ values were similar (-27.7 versus -27.6), the δN^{15} values differed by 0.3 (‰) and little discrimination was obtained in a plot of muscle δN^{15} values against muscle $\delta^{13}C$ values. In contrast, the plot of muscle $\delta^{15}N$ values against muscle $\delta^{13}C$ values from the current study (Figure 4) shows clear discrimination, indicating the power of this approach even when the difference between mean isotope values is small. In the study by Devincenzi *et al*. (2014), the discriminant analysis between muscle samples from lambs that grazed 100% cocksfoot or cocksfoot with 25% alfalfa included (the most relevant comparison to the current study) had an overall correct classification of 76.5%. This compares to the 91% correct classification in the current study.

Conclusions

Inclusion of white clover in a perennial ryegrass pasture can maintain cattle performance, thereby reducing the need to purchase artificial fertiliser N. The impact of white clover inclusion on the fatty acid composition of muscle from cattle that grazed the pasture is likely to have little effect on the health of the consumer but can be used to distinguish grass-/clover-fed beef from grass-only-fed beef. Stable isotope ratio measurements of N have additional potential in this regard, because they reflect differences between soil-derived N in grasses and isotopically lighter, atmospheric N_2 fixed by symbiotic legumes.

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