Journal Pre-proof

Canonical discriminant analysis of the fatty acid profile of muscle to authenticate beef from grass-fed and other beef production systems: model development and validation

R. Cama-Moncunill, A.P. Moloney, F.T. Röhrle, G. Luciano, F.J. Monahan

PII: S0956-7135(20)30736-2

DOI: https://doi.org/10.1016/j.foodcont.2020.107820

Reference: JFCO 107820

To appear in: *Food Control*

Received Date: 21 August 2020

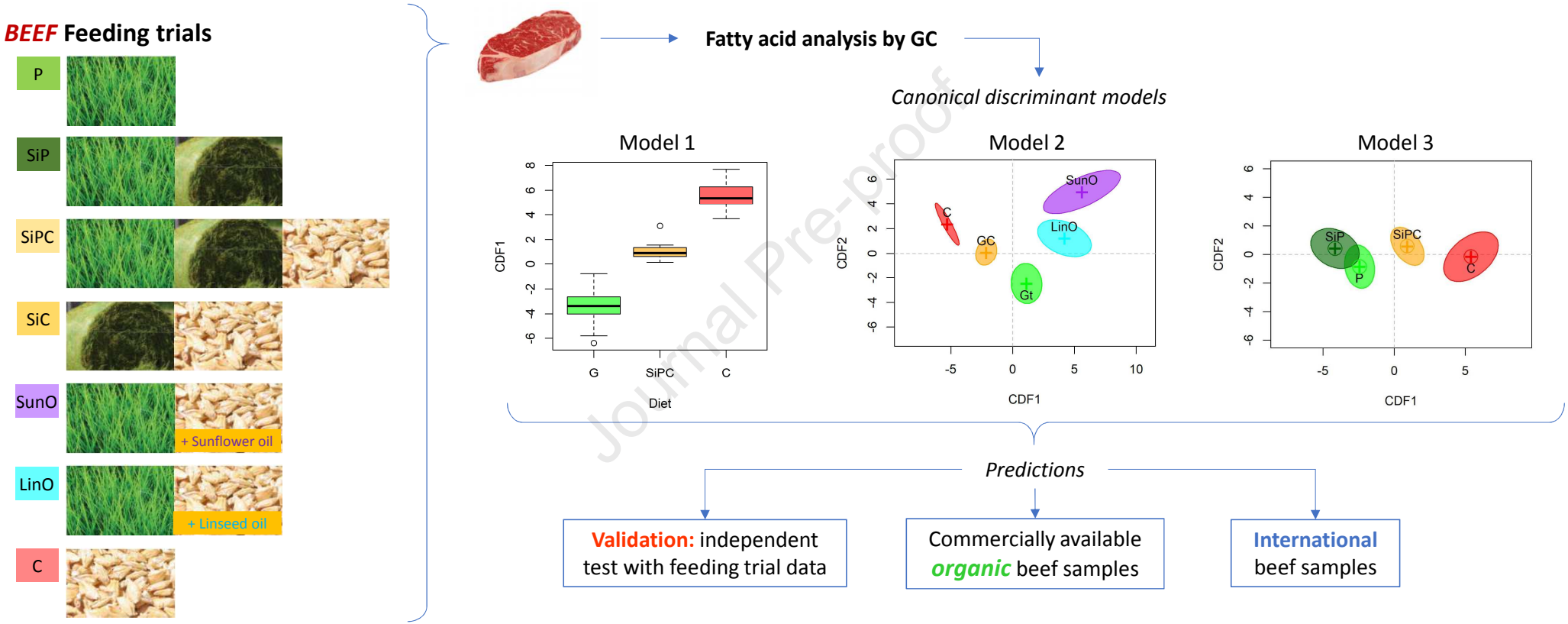
Revised Date: 4 December 2020

Accepted Date: 9 December 2020

Please cite this article as: Cama-Moncunill R., Moloney A.P., Röhrle F.T., Luciano G. & Monahan F.J., Canonical discriminant analysis of the fatty acid profile of muscle to authenticate beef from grass-fed and other beef production systems: model development and validation, *Food Control*, https://doi.org/10.1016/j.foodcont.2020.107820.

Credit author statement

FM and AM conceived and managed the project, reviewed the manuscript and participated in the discussion of the results. RCM carried out the statistical analysis, with the assistance of GL, and took the lead in drafting the manuscript. FR undertook the laboratory analysis and contributed to the preparation of the manuscript.



Canonical discriminant analysis of the fatty acid profile of muscle to authenticate beef from grass-fed and other beef production systems: model development and validation.

R. Cama-Moncunill[1], A.P. Moloney[1,2*], F.T. Röhrle[1], G. Luciano[3] and F.J. Monahan[1]

[1]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland*

[2]*Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland*

[3]*Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy.*

*Correspondence to: aidan.moloney@teagasc.ie, +353469061100

Abstract

The potential of diet-induced differences in the fatty acid profile of muscle to discriminate beef from different feeding systems and its potential use as an authentication tool was investigated. Three canonical discriminant models were built and validated using the fatty acid profile of beef from animals fed solely on pasture or cereal-based concentrates for 11 months or on various pasture/grass silage/concentrate combinations, including concentrates enriched with plant oils. Results indicated that models could successfully discriminate between grass-, partially grass- and concentrate-fed beef (accuracy = 99%) and between grass-fed beef and beef from animals supplemented with plant oils (accuracy = 96%). The approach also showed potential for distinguishing between beef from exclusively pasture-fed cattle and beef from cattle fed on pasture preceded by a period on ensiled grass (accuracy = 89%). Models were also applied to beef samples from 9 different countries. Of 97 international samples, including samples stated to be grass-fed, only 5% were incorrectly classified as Irish-grass-fed beef. These results suggested that the models captured traits in the fatty acid profile that are characteristic of Irish grass-fed beef and that this feature could be used for distinguishing Irish grass-fed beef from beef from other regions.

Keywords

Linear discriminant analysis, pasture, silage, concentrate, vegetable oil, origin.

1. Introduction

Consumer preference for beef produced from specific production systems such as "organic" or "pasture-fed" continues to increase (García-Torres, López-Gajardo, & Mesías, 2016). These systems are perceived as more sustainable, more compatible with animal health and welfare, and as providing wholesome products (Verbeke, Pérez-Cueto, Barcellos, Krystallis, & Grunert, 2010; Daley, Abbott, Doyle, Nader, & Larson, 2010). As the demand for beef from pasture systems grows so does the need for authentication methods capable of distinguishing pasture-fed beef from concentrate-fed beef typically produced in intensive feedlot systems (Monahan, Schmidt, & Moloney, 2018). The geographical origin of beef is also an important consideration for consumers (Monahan et al., 2018). Methods capable of verifying the geographical origin of beef should also be developed, especially as beef produced in a particular region may acquire added value in the marketplace (Cubero-Leon, Peñalver, & Maquet, 2014; Esteki, Shahsavari, & Simal-Gandara, 2019).

The fatty acid profile has been previously used to discriminate between beef from different production systems. Dias et al. (2008) used canonical discriminant analysis (CDA) to differentiate between beef from conventional and organic production systems. CDA was also used by Garcia et al. (2008) to discriminate between grass-fed beef, partially grass-fed beef and concentrate-fed beef, and by Alfaia et al. (2009) to discriminate between beef from cattle fed concentrates for different lengths of time prior to slaughter and beef from pasture-fed animals. More recently, Monteiro, Fontes, Bessa, Prates, & Lemos (2012) used CDA of the fatty acid profile to differentiate between three quality brands of Portuguese beef; Martínez Marín, Peña Blanco, Avilés Ramírez, Pérez Alba, & Polvillo Polo (2013) used CDA to classify beef from bulls fed different ratios of concentrate and maize silage.

The aim of this study was, firstly, to confirm the potential for diet-induced differences in the fatty acid profile of muscle to discriminate between beef from different feeding systems in an Irish context and, secondly, to investigate the potential use of CDA models

based on the fatty acid profile of beef, as tools to authenticate grass-fed beef. The specific objectives of this study were: (i) to develop a reliable CDA model for the discrimination between grass-fed, partially grass-fed and concentrate-fed beef, (ii) to investigate the possible limitations of this model when tested against samples from animals supplemented with sunflower or linseed oils which can alter the fatty acid profile of beef; (iii) to evaluate whether pasture-fed beef could be distinguished from beef from animals receiving a combination of pasture and ensiled grass; and finally (iv) to explore whether CDA models developed for classification of Irish beef production systems captured characteristic traits of Irish grass-fed beef that could be used for discriminating Irish grass-fed beef from beef from other countries.

2. Materials and Methods

2.1. Controlled feeding trials

A description of the Trial A animals and their diets was previously published by Röhrle et al. (2011). In summary, Charolais-Limousin crossbred weanling heifers (n = 98) were weighed and assigned at random to one of four dietary treatments: i) grazed pasture from November to the following October (P, n = 24); ii) grass silage offered *ad libitum* indoors from November to the following April, then grazed pasture from April to October (SiP, n = 24); iii) grass silage offered *ad libitum* indoors from November to the following April, then grazed pasture plus 50% of the dietary dry matter (DM) as a supplementary concentrate from April to October (SiPC, n = 25); iv) concentrate and straw indoors from November to the following October (C, n = 25). The pasture/grass sward consisted of predominately *Lolium perenne L.* The composition of the concentrate was 430 g/kg rolled barley, 430 g/kg pelleted beet pulp, 80 g/kg soybean meal, 35 g/kg molasses, 20 g/kg mineral/vitamin mix and 5 g/kg lime. The daily concentrate ration of all groups was adjusted periodically to the weight gain of animals in the P group. Grass and grass silage were sampled weekly and concentrate and straw were

sampled monthly over the experimental period; all samples were frozen at -20°C until processing for fatty acid analysis. Animals were slaughtered according to European regulations at Meadow Meats Ltd., Rathdowney, Ireland. At 24 h post-mortem, the right *Longissimus thoracis et lumborum* (LTL) muscle was excised from each carcass. *LTL* muscle samples were vacuum packaged and transferred to Teagasc Food Research Centre, Ashtown, Dublin 15 and stored overnight at 4°C after which a 2.5 cm thick subsample was taken between the 10th and 11th rib, vacuum packaged and stored at -20°C until fatty acid analysis. The study was carried out under license from the Irish Government Department of Health and Children and with the approval of Teagasc, the Agricultural and Food Development Authority. All procedures used complied with national and EU regulations concerning experimentation on farm animals

Individual fatty acid data for a second group of animals (Trial B, n = 60) were also used in this study (mean data published by Noci, French, Monahan, & Moloney, 2007; Noci et al., 2005). Briefly, Charolais crossbred heifers were housed and offered grass silage *ad libitum* for two months and then assigned at random to one of the following dietary treatments: v) grazed pasture (SiP2, n = 15); vi) grass silage *ad libitum* plus 3 kg of concentrate offered indoors (SiC, n = 15); vii) grazed pasture plus 1.6 kg of sunflower oil-enriched concentrate (SunO, n = 15); viii) grazed pasture plus 1.6 kg of linseed oil-enriched concentrate (LinO, n = 15). The duration of the dietary treatments was 158 days. The sward consisted of mainly *Lolium perenne L.* The composition of the concentrate fed to the indoor animals (SiC) was 430 g/kg of rolled barley, 430 g/kg of molassed sugar beet pulp, 80 g/kg of soybean meal, 45 g/kg of molasses and 15 g/kg of a mineral/vitamin mix; while the composition of the supplement to the grazing cattle was 670 g/kg of unmolassed sugar beet pulp, 110 g/kg of soybean meal, 50 g/kg of molasses, 20 g/kg of a mineral/vitamin mix and 150 g/kg of sunflower oil or linseed oil. At 24 h post-mortem, *LTL* muscle was excised and stored as described for Trial A.

117

118 **2.2. Irish commercial beef samples**

119 Two sets of Irish commercial beef samples were collected: organic pasture-fed beef striploins
120 (Ir-Org, LTL muscle, n = 18) obtained from a local producer (OmegabeefDirect,
121 Ballymacarbry, Clonmel, Co. Tipperary, Ireland) and samples of unknown dietary
122 background (Ir, n = 8) purchased from a local supermarket (Superquinn, Ballinteer, Dublin
123 16). All samples were stored at -20°C until fatty acid analysis.

124

125 **2.3. International beef samples**

126 Beef samples (97) were collected from 9 countries: Austria (Aus, n = 4), France (Fr, n = 4),
127 Germany (Ger, n = 6), Italy (It, n = 18), Spain (Sp, n = 7), UK (UK, n = 19), Brazil (Br, n =
128 17) and US (n = 22). European samples were obtained frozen from personal contacts of the
129 authors. Brazilian samples were obtained from Dawn Farms Ltd., Naas, Co. Kildare, Ireland.
130 Beef samples from the US were acquired through IdentiGEN Inc. (IdentiGEN North
131 America, Inc. Lawrence, KS), 10 of which were of unknown dietary background (US, n = 10)
132 and 12 reputedly pasture-fed (US-P, n = 12). As far as possible, striploin muscle was
133 obtained but, while samples varied from country to country; all could be classified as beef
134 striploin (LTL muscle), sirloin (*M. gluteus medius*) or round (*M. semimembranosus*). Table 1
135 summarises the various treatments/dietary backgrounds of all sample sets (Trial A, Trial B,
136 commercial and international).

137

138 **2.4. Feed chemical and fatty acid analysis**

139 The chemical composition of feed samples from Trial A, pooled on a monthly basis, was
140 analysed as described by Moloney, Read, & Keane (1996). The fatty acid composition of
141 feedstuffs was determined as described by Sukhija & Palmquist (1988) with the minor
142 modification that toluene was used instead of benzene.

143

144 **2.5. Muscle intramuscular fat and fatty acid analysis**

145 Extraction of intramuscular fat (IMF) and methylation of the fatty acids for Trial A and
146 international samples were conducted as for Trial B (Noci et al., 2005). To determine the
147 IMF in the beef samples, the lipid extract was weighted after drying to a constant weight
148 under a stream of N_2. Results are expressed as g/100 g of muscle. The methylation procedure
149 was carried out directly on the lipid extract, without separation of neutral and polar lipid
150 fractions.

151

152 **2.6. Gas Chromatographic Analysis**

153 Fatty acid methyl esters (FAME) were separated by gas chromatography using a Varian 3800
154 GC (Varian Medical Systems Inc. Palo Alto, CA, USA.) equipped with a CP-Sil 88 capillary
155 column (100 m × 0.25 mm i.d., 0.2 µm film thickness; Chrompack, The Netherlands) and a
156 Varian 8400 autosampler. The injector and the flame ionization detector were kept at
157 constant temperatures of 250 and 260 °C, respectively. The FAME profile of a 2 µl sample
158 injected at a split ratio of 1:50 was determined using the temperature programme described
159 by Shingfield et al. (2003). The total run time was 63 min and H_2 was used as the carrier.

160 Peaks were identified by comparison of retention times with a standard mix of 37
161 FAME (Supelco Inc., Bellefonte, PA, United States) and individual standards (Matreya Inc.,
162 Pleasant Gap, PA, United States) for those FAME not contained in the mix. Fatty acids for
163 which no commercial standards were available were identified by reproducing identical
164 chromatographic conditions as Shingfield et al. (2006) and comparing the retention times to
165 their reference chromatograms. Identified FAME were calculated as g/100 g of total FAME
166 detected using tricosanoic acid (C 23:0) as an internal standard.

167

168 **2.7. Data analysis**

Data analysis was performed in R (R Core Team, 2019) using various packages including A*gricolae*, *MASS, CANDISC, Caret,* and *Klar* as well as in-house functions. Fatty acid data were first examined for non-detected values. If the proportion of non-detected FAME in a treatment or country group was < 50 %, non-detected values were replaced with 0.5 limit of detection (LOD = 0.04 g/100 g of total FAME), if the proportion of non-detected FAME was > 50 %, the FAME was regarded as non-detected for the full treatment group (EPA, 2000). Statistical analysis was performed after correcting for non-detected values and for analyses which require normally distributed data, only FAME having less than 15% non-detected values in each dietary treatment were selected.

One-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test was performed to investigate whether the proportions of individual FAME and families of FAME differed significantly between the feeding groups (P, SiP, SiPC and C) in Trial A. The possibility of classifying beef samples according to the animal's dietary background based on the FAME profile was examined via CDA. Three CDA models were developed using different combinations of Trial A and Trial B data. For Model 1, 3 feeding regimes from Trial A data were considered: G (grass-fed = P + SiP), SiPC and C. For Model 2, five feeding regimes from a combination of Trial A and B datasets were considered: Gt (total grass-fed samples = P + SiP + SiP2), GC (grass and concentrate = SiPC + SiC), C, SunO and LinO. For Model 3, all 4 feeding regimes from Trial A were considered: P, SiP, SiPC and C. A stepwise variable selection procedure was adopted to select the FAME giving the best discrimination between feeding groups based on the results of a leave-one-out cross-validation (CV-LOO) and using a 2% minimum improvement in a model's discriminating ability as a criterion for variable entry. CDA models were then developed based on the selected variables. CDA generates a set of canonical discriminant functions (CDF) that provide the best discrimination between dietary groups (Cui, 2010). The relevance of each CDF was evaluated through the Wilks' lambda test.

The performance of the models was first assessed by CV-LOO, using parameters such as sensitivity, specificity and overall accuracy. In binary classifications, sensitivity refers to the proportion of positive samples that are correctly identified by a model, while specificity refers to the proportion of negative samples that are correctly identified (Han & Kamber, 2011; Tharwat, 2018). For multi-group classification, sensitivity and specificity are calculated for each group (i.e. dietary treatments) by comparing each group to the remaining groups (i.e. a "one versus all" approach) (Kuhn, 2008). Overall accuracy is defined as the ratio between the number of correctly classified samples and the total number of samples (Tharwat, 2018). Models were externally validated by predicting additional samples (i.e., test set) that were not part of the original training set (Jiménez-Carvelo, González-Casado, Bagur-González, & Cuadros-Rodríguez, 2019). Model 1 and 3 were validated using Trial B samples. For Model 2, validation was performed using test sets created by randomly splitting the combined data set (Trial A and Trial B) into training and test sets, 3 times (split ratio = 0.8). Model 2 cross validation and external validation results were expressed as an average of the three repeats. All models were tested against the commercially available Irish samples and the international sample set.

3. Results and discussion

3.1 Chemical composition of feedstuffs

The chemical and fatty acid composition of the dietary components used in Trial A are shown in Table 2. Pasture and grass silage had similar gross compositions, while the concentrate had higher DM digestibility and lower levels of ash, protein and oil B than the forages. Concentrates had higher proportions of C16:0, C18:1*c*9 and C18:2*n*-6, and a lower proportion of C18:3*n*-3 than the pasture and grass silage. Polyunsaturated fatty acids (PUFA) were the main fatty acid family in grass and grass silage (≥ 65 %) and saturated fatty acids

(SFA) predominated in the concentrate (≈ 44%). These results are in general agreement with previous studies (Moloney & Drennan, 2013; Warren et al., 2008).

3.2. Intramuscular fat and fatty acid composition of beef samples

The IMF content and the fatty acid composition of LTL muscle of Trial A animals are presented in Table 3. Muscle from grass-fed animals (P, SiP) had a lower IMF content ($p < 0.01$) than muscle from concentrate-fed animals (C). Muscle from partially grass-fed animals (SiPC) was intermediate, indicating that the higher the concentrate input, the higher the IMF content in muscle. These results are consistent with previous studies (Alfaia et al., 2009; Fruet et al., 2018). To avoid confounding effects of fatness on muscle fatty acid composition, i.e. higher IMF content results in higher levels of individual fatty acids, the fatty acid profile was expressed as proportion of FAME.

The proportions of SFA and monounsaturated fatty acids in muscle were not influenced by diet. Muscle from P and SiP animals had the highest proportion of PUFA, followed by muscle from SiPC animals while muscle from C animals had the lowest proportion. The decrease in the proportion of PUFA in muscle as the amount of dietary concentrates increases agrees with previous studies (Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004). The proportion of *n*-3 PUFA in muscle from P and SiP animals was also higher compared to muscle from SiPC and C animals ($p < 0.01$), indicating that the higher the concentrate input, the lower the proportion of *n*-3 PUFA in muscle reflecting the fatty acid composition of the diet. In contrast, the proportion of *n*-6 PUFA in muscle increased as the amount of concentrate in the diet increased ($p < 0.01$). Muscle from grass-fed beef had the lowest *n*-6:*n*-3 PUFA ratio (≈ 1) followed by SiPC (≈ 2), while muscle from concentrate-fed animals had the highest ratio (6.2). The predominant fatty acid in intramuscular lipid was oleic (C18:1*c*9), followed by palmitic (C16:0) and stearic (C18:0). Linoleic acid (C18:2*n*-6) was the major *n*-6 PUFA while linolenic acid (C18:3*n*-3) was the predominant *n*-3 PUFA.

Muscle from grass-fed animals had lower proportions of C18:2*n*-6 and higher proportions of C18:3*n*-3 compared to muscles from concentrate-fed animals ($p < 0.01$). This outcome was consistent with the composition of the feedstuffs. The C18:2*c*9,*t*11 isomer of conjugated linoleic acid (CLA) and *trans* vaccenic acid (TVA, C18:1*t*11) were higher in grass-fed beef ($p < 0.01$). High levels of CLA and TVA in beef muscle have been previously associated with grass-based diets (Daley et al., 2010; French et al., 2000). Other statistically significant differences between grass and concentrate-fed beef included the proportions of C14:0, C15:0, C16:0, C16:2*c*9,*c*12, C20:3*n*-6, C20:5*n*-3, C22:5*n*-3 and various C18:1 isomers. Overall, differences in the muscle fatty acid composition were largely consistent with previous studies (Alfaia et al., 2009; Daley et al., 2010; French et al., 2000; Garcia et al., 2008; Realini et al., 2004;Warren et al., 2008).

The fatty acid proportions of Trial B samples used for the current study were C18:3*n*-3, C18:2*n*-6, C18:1*t*11, CLA*c*9*t*11, C15:0 and C17:1*c*9. In the same order, the mean proportions of these fatty acids for each treatment group were: 1.37, 2.35, 3.08, 0.73, 0.48 and 0.57 g /100 g total FAME for SiP2; 0.81, 2.60, 1.32, 0.49, 0.42, 0.58 g /100 g total FAME for SiC; 0.87, 3.17, 8.56, 1.78, 0.45, 0.48 g/100 g of total FAME for SunO; 1.34, 2.59, 6.32, 1.26, 0.48, 0.48 g/100 g of total FAME for LinO (Noci et al., 2005, 2007).

The IMF content and the fatty acid proportions of commercially available Irish and international samples are presented in Table 4. Overall, the fatty acid proportions of the Irish samples were intermediate between the proportions for P or SiP and SiPC from Trial A while the fatty acid proportions for the international samples did not clearly align with any of the dietary groups from Trial A. The diversity in fatty acid profile likely reflects variation in production systems across the different countries.

3.3. Discrimination according to dietary background

In this study, three CDA models were developed and evaluated as potential tools for discriminating beef according to its dietary background.

Model 1

Model 1 was developed to discriminate between grass-fed, partially grass-fed and concentrate-fed beef. Data from Trial A were used and P and SiP were combined as G (grass-fed). The stepwise variable selection procedure selected C18:3*n*-3, C18:2*n*-6 and C18:1*t*11 for the discrimination. CDA generated two CDF based on these fatty acids of which only the first function (CDF1), which explained 99.6 % of the between-class variance, was relevant for the discrimination (Wilks' lambda < 0.06). Score values for CDF1 are displayed in Fig. 1a. Beef samples were clearly separated according to animal diet. Muscle from grass-fed animals was associated with low CDF1 score values, muscle from partially grass-fed animals with intermediate values and muscle from concentrate-fed animals with high values. The contribution of each fatty acid to a CDF can be evaluated through the standardized coefficients; while the degree to which each fatty acid is related to the CDF can be better assessed by the structure coefficients (Cui, 2010). Both standardized and structure coefficients for Model 1 are shown in Table S1. The structure coefficients for CDF1 are also displayed in Fig. 1b. C18:3*n*-3 was highly correlated with CDF1 (structure coefficient value of -0.91), followed by C18:1*t*11 (-0.77) and C18:2*n*-6 (0.57). C18:3*n*-3 and C18:1*t*11 influenced the model (CDF1) in a negative direction, indicating that high proportions of C18:3*n*-3 and C18:1*t*11 were associated with grass-based diets; while the positive direction for C18:2*n*-6 indicates that high proportions were related to concentrate-based diets. These relationships agree with the results of ANOVA (Table 3).

Classification results obtained by CV-LOO (Table 5) indicated that Model 1 can successfully classify beef samples according to their dietary background (accuracy = 99%). Group-specific performance corroborated these results. The grass-fed group had a sensitivity

297 of 98% indicating that most of the grass-fed samples were correctly identified and a
298 specificity of 100%, which means that the model did not predict any non-grass-fed beef
299 samples as "grass-fed". These results agree with Garcia et al. (2008) who reported 94, 78 and
300 100% of correctly classified cases (i.e. sensitivity) in cross validation for discrimination
301 between grass-fed beef, partially grass-fed beef and concentrate-fed beef, respectively, and
302 with Alfaia et al. (2009) who reported 100% correct classification of beef from cattle fed
303 concentrates for different times prior to slaughter and beef from pasture-fed animals. Garcia
304 et al. (2008) also reported C18:3*n*-3 and C18:2*n*-6 , among others, as relevant fatty acids for
305 the discrimination between grass and concentrate based diets.
306 The model was further evaluated by predicting the group membership of an
307 independent set of samples of similar dietary backgrounds (SiP2, SiC) and the commercial
308 samples labelled as "organic pasture-fed" (Ir-Org). The predictions are shown in Table 5. All
309 SiP2 and SiC samples were correctly classified as grass-fed and partially grass-fed beef,
310 respectively For the Ir-Org set, 15 samples were classified as "grass-fed" and 3 as "partially
311 grass-fed" (SiPC). This could reflect variations across organic production systems, e.g.
312 inclusion of organic concentrates and differences in the sward type and/or the grazing period
313 (EC, 1999) which would influence the fatty acid composition of beef (Scollan et al., 2006).
314 This highlights the need for discriminant models built using training sets with commercial
315 samples of known dietary background.
316 Model 1 was also tested against SunO and LinO samples. This is important from an
317 authentication perspective since these samples could be erroneously classified as grass-fed
318 due to the effect of dietary vegetable oils on beef fatty acids. Noci et al. (2007) reported that
319 supplementation with sunflower oil decreased the proportion of C18:3*n*-3 and increased the
320 proportions of C18:2*n*-6,CLA*c*9*t*11 and C18:1*t*11 in muscle compared to muscle from
321 unsupplemented grass-fed animals. In contrast, supplementation with linseed oil increased
322 the proportions of CLAc9t11 and C18:1*t*11 but resulted in proportions of C18:3*n*-3 and

C18:2*n*-6 that were similar to those in muscle from unsupplemented grass-fed animals. All SunO and LinO samples were predicted to belong to the grass-fed group (Table 5). Therefore, the model failed to distinguish these samples from true grass-fed beef. In Model 1, a sample is classified as G if it has a low proportion of C18:2*n*-6 and high proportions of C18:3*n*-3 and C18:1*t*11. Thus, the model performed as expected for LinO samples, which had similar proportions of C18:3*n*-3 and C18:2*n*-6 and higher levels of C18:1*t*11 than G samples. Results for SunO samples were somewhat unexpected since the proportions of C18:3*n*-3 and C18:2*n*-6 in SunO samples were more comparable to those observed in partially grass-fed samples (SiPC) than in G samples. However, SunO samples had notably higher proportions of C18:1*t*11 than G samples. These results demonstrated that because of the influence of oil supplementation on the fatty acid profile of beef, new classification models that accounted for this effect were needed.

Model 2

Model 2 was developed to discriminate between grass-fed, partially grass-fed, concentrate-fed, SunO and LinO samples. Five feeding regimes from a combination of Trial A and B datasets were considered: Gt (total grass-fed samples = P + SiP + SiP2), GC (grass and concentrate = SiPC + SiC), C, SunO and LinO. For subsequent external validation, data were randomly split into training (80%) and test (20%) sets 3 times (repeats). For each repeat the stepwise procedure selected the same three fatty acids as for Model 1:C18:1*t*11, C18:2*n*-6 and C18:3*n*-3. CDA then generated three CDF of which only the first two were relevant for the discrimination. On average, CDF1 explained 66.4% of the between-class variation, while CDF2 explained 33.6%. The standard and structure coefficients for one repeat are shown in Table S2. The score plot for CDF1 *vs* CDF2 obtained for one repeat is shown in Fig. 2. Samples were clearly clustered according to animal diet. CDF1 was responsible for the separation of the GC and C groups, while CDF1 in combination with CDF2 separated SunO

and LinO groups from the G group (Fig 2). CDF1 was highly correlated with C18:1*t*11 (~ -0.88) and C18:3*n*-3 (~57); while CDF2 was highly correlated with C18:3*n*-3 (~ -0.72). Thus C18:1*t*11 and C18:3*n*-3 were the main fatty acids for the discrimination which agrees with Noci et al. (2007) who reported significant differences in C18:3*n*-3, and C18:1*t*11 between beef from grass-based diets and beef from diets supplemented with sunflower or linseed oil.

Classification results obtained by CV-LOO are shown in Table 5. The model discriminated between all five feeding regimes with an overall accuracy of 96%. The model correctly classified 48.7 (average of the 3 repeats) out of 50 Gt samples (sensitivity = 97.3%) and misclassified 0.3 samples as GC and 1 sample, as LinO samples. The high specificity for Gt (100%) indicated that the model could successfully distinguish non grass-fed samples from true grass-fed samples. Validation with test samples (20% of the dataset) further demonstrated the model's ability to distinguish between the five feeding regimes. Test samples from GC and LinO groups were 100% correctly classified, while one C sample was predicted as belonging to the GC group and one Gt sample was misclassified as LinO in one of the repeats. The latter, together with CV-LOO results (one Gt sample was classified as LinO in one repeat), suggested that discrimination between Gt and LinO may be more difficult to accomplish than between Gt and SunO. This was expected because Gt and LinO samples had similar proportions of C18:3*n*-3 and C18:2*n*-6. Ir-Org samples were mostly classified as Gt (63% of samples), but also as GC (24% of samples) and LinO (13% of samples). Since the actual diet of cattle in these organic systems is unknown, it is difficult to evaluate whether classifications were correct. Nevertheless, the model did not classify any Ir-Org sample as C, which is the category to which an organic sample would be unlikely to belong.

Model 3

Consumers are increasingly interested in animal welfare and pasture is perceived as a more welfare friendly environment than indoors (Verbeke et al., 2010). Authentication models that could distinguish between beef from grazing animals from animals that were fed a pasture-based ration indoors would be useful in this regard. Model 3 was developed to investigate the possibility of discriminating between two similar grass feeding systems: pasture only for 11 months (P) *vs* grass silage for the first 5 months and pasture for the following 6 months (SiP); in addition to distinguishing each from concentrate-based diets (SiPC and C).

Four fatty acids, i.e. C18:3*n*-3, C18:2*n*-6, C15:0 and C17:1*c*9, were selected during the stepwise variable selection step giving rise to three CDF. CDF1 and CDF2, which explained 97.67% and 2.29% of the between-class variance, respectively, were the only relevant functions for the discrimination (Wilks' lambda CDF1 < 0.06, CDF2 < 0.75). The standardized and structure coefficients of Model 3 are shown in Table S3. The score plot of CDF1 *vs* CDF2 together with the structure coefficients are displayed in Fig. 3. CDF1 was responsible for the discrimination of samples according to their concentrate input and contributed to separation of the P and SiP groups, while CDF2 further separated these groups. C18:3*n*-3 was highly correlated with CDF1 (structure value of -0.93) and was the main fatty for the discrimination between grass-fed (P and SiP), partially grass-fed (SiPC) and concentrate-fed beef (C); while the separation of the P from SiP groups was mostly attributed to C15:0 and C17:1*c*9 and, to a lesser extent, to C18:3*n*-3. High proportions of C15:0 and C17:1*c*9 were associated with a combined silage-pasture diet (SiP) while lower proportions were attributed to an exclusively pasture diet. This is supported by the results of the ANOVA (Table 3). To our knowledge, few studies have compared the effects on the fatty acid profile of beef from cattle fed on pasture, pasture-based ration indoors or combinations of those as in the current study.

Classification results obtained by CV-LOO (Table 5) corroborated results illustrated by the score plot. With an overall accuracy of 89%, Model 3, like Model 1 and 2, could

401 successfully classify SiPC and C samples (sensitivity = 96% for both). However, seven
402 samples (29.1%) from the SiP group were misclassified as P and two samples (8.3%) from
403 the P group were misclassified as SiP. External validation suggested that the model had little
404 ability to differentiate between pasture and silage-pasture diets since 12 out of 15 samples
405 from the SiP2 group were classified as P (Table 5). However, while SiP and SiP2 diets were
406 similar, in SiP, animals were offered grass silage for 5 months before moving to pasture
407 while, in SiP2, animals were offered silage for 2 months. As for Model 1, satisfactory
408 predictions were obtained for SiC samples, with only one sample misclassified as C, and for
409 the Ir-Org samples with no sample classified as C. Model 3 was also used to predict the
410 dietary background of the SunO and LinO samples. With thirteen samples predicted as SiPC
411 and two as C, predictions for the SunO samples were considerably more accurate than those
412 obtained with Model 1. This improvement compared to Model 1 could be attributed to
413 inclusion of C17:1*c*9 as a predictor, which in Model 2 was relevant for the separation of both
414 LinO and SunO samples from grass-fed samples. However, mixed results were obtained for
415 the prediction of the LinO group with six samples classified as P, four as SiP and five as
416 SiPC. This corroborates the need for calibrations, such as in Model 2, that include the
417 characteristic variation of beef from animals fed plant-oil enriched concentrates.

418 Overall, all models could discriminate between grass-fed beef and non-grass-fed beef.
419 Model 1 demonstrated that CDA based on the fatty acid profile of beef can successfully
420 discriminate between grass-, partially grass- and concentrate-based diets and highlighted the
421 need to consider possible variations in the feeding systems such as supplementation with
422 various plant oils. Model 2 by including diets with plant oils had greater applicability; while
423 Model 3 demonstrated that this approach has potential to distinguish between beef from
424 grazing animals and beef from animals offered grazed grass subsequent to ensiled grass.
425 However further validation using pasture/silage combinations are required to improve and
426 evaluate the accuracy of the method.

The proportion of C18:1*t*11 was selected as important predictor for Model 1 and Model 2. However, C18:1*t*11 is often incompletely resolved from C18:1*t*10 during analysis using gas chromatography and there are many more reports in the literature that show CLA *c*9*t*11 alone rather than CLA *c*9*t*11 and C18:1*t*11 e.g. Garcia et al. (2008). Models based on FAME other than C18:1*t*11 may therefore be more applicable to FAME datasets that do not report C18:1*t*11. The stepwise variable selection procedure was repeated excluding C18:1*t*11 as a possible predictor. C18:3*n*-3, C18:2*n*-6 and CLA*c*9*t*11 were selected for the discrimination between G, SiPC and C (Model 1b) and C18:3*n*-3, C18:2*n*-6, CLA*c*9*t*11 and C17.1*c*9 for the discrimination between Gt, GC, C, SunO and LinO (Model 2b). Cross-validation and test results for these models are shown in Supplementary Tables; coefficients in Table S4 (Model 1b) and in Table S5 (Model 2b); score plots in Fig. S1 (Model 1b) and in Fig. S2 (Model 2b). Model 1b had a total accuracy in CV of 98%; while Model 2b had a total accuracy of 96.5%. Thus, if confident quantification of C18:1*t*11 is not possible, accurate models for discrimination between grass-fed, partially grass-fed and concentrate-fed beef could also be used based on the proportions of CLA*c*9*t*11. Similarly, discrimination between Gt, GC, C, SunO and LinO beef samples could be achieved by using the proportions of CLA*c*9*t*11 and C17:1c9. The fact that CLA*c*9*t*11 was selected as a substitute for C18:1*t*11 was expected since both FAME are correlated and increase together in beef in response to an increase in grass or vegetable oil consumption by cattle (Daley et al., 2010; Noci et al., 2005), confirmed by the results of ANOVA in the present study (Table 3)

3.4. Investigation of a characteristic fatty acid profile related to the country of origin

Since the fatty acid profile of beef is highly influenced by the diet of the animal (Scollan et al., 2014), it may be indirectly influenced by the region where animals are raised due to the use of feedstuffs characteristic of that region. In this section, we explored whether the models developed above would capture traits in the fatty acid profile that are characteristic of Irish

grass-fed beef and subsequently, whether the models could be used to authenticate the geographical origin of beef. Since the 3 models were developed based on the variation in the fatty acid profile of Irish beef, we hypothesised that models are rather specific for Irish beef and of the various dietary treatments examined, the grass-fed group may be the more country/region dependent. Hence, our models may be useful to differentiate Irish grass-fed beef from beef from another region. Our exploration, therefore, did not aim to predict the dietary background or origin of the international samples, but to explore whether our models would "misclassify" any of these samples as Irish grass-fed beef.

Models were applied to the commercially available Irish beef samples of unknown dietary background and to the international samples. Predictions obtained using each model are shown in Table 5. Ir samples were mainly classified as grass-fed and partially grass-fed beef (approx. 50% in each category) suggesting that Ir samples came from cattle fed principally grass or in combination with some supplemental concentrate during the finishing period. This is consistent with grass being the main feed constituent in beef production in Ireland (Bord Bia, 2017). Austrian, French and German samples were mainly classified as partially grass-fed (SiPC or GC). However, Model 1 and 3 predicted two German samples as grass-fed. This indicates that if these models were used as an authentication tool to simultaneously verify the origin (Irish) and diet (grass), most of these samples would be classified as partially grass-fed; however, the two German samples would be erroneously labelled as "Irish grass-fed beef". Italian and Spanish beef samples were mainly classified as belonging to the C group. Most of the UK samples were also assigned to the C group; however, 3 to 4 samples, depending on the model, were classified as grass-fed. Similarly, most of the Brazilian samples were identified as partially-grass fed and concentrate-fed, however two samples were identified as grass-fed by Model 3. An aspect to take into account is the type of muscle used in the analysis. For this study however, striploin, sirloin and round

478 muscle were used and according to Pavan & Duckett (2013), little differences exist in the
479 proportions of FAME between these beef cuts.

480 Overall, the low number of samples "misclassified" as Irish grass-fed beef indicated
481 that the models, , captured traits in the fatty acid profile that are characteristic of Irish grass-
482 fed beef and that this feature could be used to distinguish Irish grass-fed beef from beef from
483 other countries. Furthermore, none of the samples from the US, including the pasture-fed
484 samples were classified as Irish grass-fed. This demonstrates that the fatty acid profile could
485 be used to authenticate the country of origin of grass-fed beef but not grass-fed *per se* and
486 supports the hypothesis that the fatty acid profile of grass-fed beef is rather characteristic of
487 the country of origin. These results however are based on a limited number (n = 12) of
488 pasture-fed samples, which may not be representative of US pasture-fed beef. Further
489 validation involving larger sample sizes of beef from various countries/regions and of known
490 dietary background, especially from pasture/grass-based diets, are required to
491 comprehensively evaluate whether CDA models based on the fatty acid profile of Irish beef
492 can successfully discriminate Irish grass-fed beef from grass-fed beef from other countries.
493 Nonetheless, this exploratory analysis indicated that the approach holds potential.
494

495 **4. Conclusion**

496 Beef from different production systems can be discriminated by application of CDA models
497 based on the muscle fatty acid profile of beef. The approach can be successfully applied to
498 distinguish between grass-, partially grass- and concentrate-fed beef as well as distinguishing
499 grass-fed beef from beef fed concentrate supplemented with sunflower and linseed oils. The
500 approach also has potential to discriminate between beef from grazed pasture systems and
501 beef reared in combined pasture and ensiled-grass systems, but further studies are required to
502 comprehensively evaluate this possibility. Models built using fatty acid data from Irish beef
503 raised under various production systems could differentiate Irish grass-fed beef from grass-

fed beef from other regions such as the US. Overall, this study demonstrates that successful classification models based on the proportions of fatty acids in muscle can be developed which, with further development and improvement, could become a reliable authentication tool to support claims of the provenance of beef.

Acknowledgements

The financial support of the Irish Department of Agriculture, Food and the Marine for projects 06/R&D/D/48 (Biomarkers to authenticate Irish grass-fed beef) and 17F252 (Signature of Irish grass-fed Beef) is gratefully acknowledged.

References

Alfaia, C. P. M., Alves, S. P., Martins, S. I. V., Costa, A. S. H., Fontes, C. M. G. A., Lemos, J. P. C., Bessa, R.J.B., & Prates, J. A. M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry*, *114*(3), 939–946. https://doi.org/10.1016/j.foodchem.2008.10.041

Bord Bia. (2017). Sustainable Beef & Lamb Assurance Scheme. Retrieved May 28, 2020, from https://www.origingreen.com/globalassets/origin-green/og-publications/sustainable-beef-and-lamb-assurance-scheme.pdf

Cubero-Leon, E., Peñalver, R., & Maquet, A. (2014). Review on metabolomics for food authentication. *Food Research International*, *60*, 95–107. https://doi.org/10.1016/j.foodres.2013.11.041

Cui, Y. (2010). Discriminant Analyisis. In N. J. Salkind (Ed.), *Encyclopedia of Research Design*. Thousand Oaks: SAGE Publications, Inc. https://doi.org/10.4135/9781412961288

Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal*, *9*(1), 10. https://doi.org/10.1186/1475-2891-9-10

Dias, L. G., Correia, D. M., Sá-Morais, J., Sousa, F., Pires, J. M., & Peres, A. M. (2008).

Raw bovine meat fatty acids profile as an origin discriminator. *Food Chemistry*, *109*(4), 840–847. https://doi.org/10.1016/j.foodchem.2008.01.008

EC. (1999). Council Regulation (EC) No 1804/1999 of 19 July 1999 supplementing Regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs to include livestock production. *Official Journal of the European Communities*, (1804/1999).

Esteki, M., Shahsavari, Z., & Simal-Gandara, J. (2019). Food identification by high performance liquid chromatography fingerprinting and mathematical processing. *Food Research International*, *122*, 303–317. https://doi.org/10.1016/j.foodres.2019.04.025

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., & Moloney, A. P. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science*, *78*(11), 2849–2855. https://doi.org/10.2527/2000.78112849x

Fruet, A. P. B., Trombetta, F., Stefanello, F. S., Speroni, C. S., Donadel, J. Z., De Souza, A. N. M., Rosado Júnior, A., Tonetto, C.J., Wagner, R., De Mello, A. & Nörnberg, J. L. (2018). Effects of feeding legume-grass pasture and different concentrate levels on fatty acid profile, volatile compounds, and off-flavor of the M. longissimus thoracis. *Meat Science*, *140*, 112–118. https://doi.org/10.1016/j.meatsci.2018.03.008

García-Torres, S., López-Gajardo, A., & Mesías, F. J. (2016). Intensive vs. free-range organic beef. A preference study through consumer liking and conjoint analysis. *Meat Science*, *114*, 114–120. https://doi.org/10.1016/j.meatsci.2015.12.019

Garcia, P. T., Pensel, N. A., Sancho, A. M., Latimori, N. J., Kloster, A. M., Amigone, M. A., & Casal, J. J. (2008). Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science*, *79*(3), 500–508. https://doi.org/10.1016/j.meatsci.2007.10.019

Han, J., & Kamber, M. (2011). *Data Mining: Concepts and Techniques* (Vol. 3rd ed). Burlington, MA: Morgan Kaufmann.

Jiménez-Carvelo, A. M., González-Casado, A., Bagur-González, M. G., & Cuadros-Rodríguez, L. (2019). Alternative data mining/machine learning methods for the analytical evaluation of food quality and authenticity – A review. *Food Research International*, *122*, 25–39. https://doi.org/10.1016/j.foodres.2019.03.063

564 Kuhn, M. (2008). Building Predictive Models in R Using the caret Package. *Journal of*
565 *Statistical Software; Vol 1, Issue 5*.

566 Martínez Marín, A. L., Peña Blanco, F., Avilés Ramírez, C., Pérez Alba, L. M., & Polvillo
567 Polo, O. (2013). Selecting the best set of gas chromatography-derived fatty acids to
568 discriminate between two finishing diets using linear discriminant analysis. *Meat*
569 *Science*, *95*(2), 173–176. https://doi.org/10.1016/j.meatsci.2013.04.059

570 Mezgebo, G. B., Monahan, F. J., McGee, M., O'Riordan, E. G., Richardson, I. R., Brunton,
571 N. P., & Moloney, A. P. (2017). Fatty acid, volatile and sensory characteristics of beef
572 as affected by grass silage or pasture in the bovine diet. *Food Chemistry*, *235*, 86–97.
573 https://doi.org/10.1016/j.foodchem.2017.05.025

574 Moloney, A. P., & Drennan, M. J. (2013). Characteristics of fat and muscle from beef heifers
575 offered a grass silage or concentrate-based finishing ration. *Livestock Science*, *152*(2),
576 147–153. https://doi.org/10.1016/j.livsci.2012.12.001

577 Moloney, A. P., Read, M. P., & Keane, M. G. (1996). Effects of ardacin supplementation on
578 rumen fermentation and protein degradability in steers. *Animal Feed Science and*
579 *Technology*, *57*(1), 97–110. https://doi.org/10.1016/0377-8401(95)00842-X

580 Monahan, F. J., Schmidt, O., & Moloney, A. P. (2018). Meat provenance: Authentication of
581 geographical origin and dietary background of meat. *Meat Science*, *144*, 2–14.
582 https://doi.org/10.1016/j.meatsci.2018.05.008

583 Monteiro, A. C. G., Fontes, M. A., Bessa, R. J. B., Prates, J. A. M., & Lemos, J. P. C. (2012).
584 Intramuscular lipids of Mertolenga-PDO beef, Mertolenga-PDO veal and "Vitela
585 Tradicional do Montado"-PGI veal. *Food Chemistry*, *132*(3), 1486–1494.
586 https://doi.org/10.1016/j.foodchem.2011.12.008

587 Moreno, T., Keane, M. G., Noci, F., & Moloney, A. P. (2008). Fatty acid composition of M.
588 Longissimus dorsi from Holstein–Friesian steers of New Zealand and
589 European/American descent and from Belgian Blue×Holstein–Friesian steers,
590 slaughtered at two weights/ages. *Meat Science*, *78*(3), 157–169.
591 https://doi.org/10.1016/j.meatsci.2007.05.028

592 Noci, F., French, P., Monahan, F. J., & Moloney, A. P. (2007). The fatty acid composition of
593 muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant
594 oil-enriched concentrates1. *Journal of Animal Science*, *85*(4), 1062–1073.
595 https://doi.org/10.2527/jas.2006-105

Noci, F., Monahan, F. J., French, P., & Moloney, A. P. (2005). The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: Influence of the duration of grazing. *Journal of Animal Science*, *83*(5), 1167–1178. https://doi.org/10.2527/2005.8351167x

Pavan, E., & Duckett, S. K. (2013). Fatty acid composition and interrelationships among eight retail cuts of grass-feed beef. *Meat Science*, *93*(3), 371–377. https://doi.org/10.1016/j.meatsci.2012.09.021

R Core Team. (2019). R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. https://doi.org/http://www.r-project.org/

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M., & De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science*, *66*(3), 567–577. https://doi.org/10.1016/S0309-1740(03)00160-8

Röhrle, F. T., Moloney, A. P., Black, A., Osorio, M. T., Sweeney, T., Schmidt, O., & Monahan, F. J. (2011). α-Tocopherol stereoisomers in beef as an indicator of vitamin E supplementation in cattle diets. *Food Chemistry*, *124*(3), 935–940. https://doi.org/10.1016/j.foodchem.2010.07.023

Scollan, N. D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S., Hocquette, J.-F., & Moloney, A. P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, *97*(3), 384–394. https://doi.org/10.1016/j.meatsci.2014.02.015

Scollan, N. D., Hocquette, J.-F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science*, *74*(1), 17–33. https://doi.org/10.1016/j.meatsci.2006.05.002

Shingfield, K. J., Ahvenjarvi, S., Toivonen, V., Arola, A., Nurmela, K. V. V, Huhtanen, P., & Griinari, J. M. (2003). Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Animal Science*, *77*(1), 165–179.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., & Beever, D. E. (2006). Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *Journal of Dairy Science*, *89*(2), 714–732. https://doi.org/10.3168/jds.S0022-0302(06)72134-8

628 Sukhija, P. S., & Palmquist, D. L. (1988). Rapid method for determination of total fatty acid
629 content and composition of feedstuffs and feces. *Journal of Agricultural and Food*
630 *Chemistry*, *36*(6), 1202–1206. https://doi.org/10.1021/jf00084a019

631 Tharwat, A. (2018). Classification assessment methods. *Applied Computing and Informatics*.
632 https://doi.org/10.1016/j.aci.2018.08.003

633 U.S. Environmental Protection Agency (EPA). Office of Pesticide Programs. (2000).
634 *Assigning Values to Non-detected /non-quantified Pesticide Residues in Human Health*
635 *Food Exposure Assessments*. Washington, DC: Office of Pesticide Programs, U.S.
636 Environmental Protection Agency.

637 Verbeke, W., Pérez-Cueto, F. J. A., Barcellos, M. D. de, Krystallis, A., & Grunert, K. G.
638 (2010). European citizen and consumer attitudes and preferences regarding beef and
639 pork. *Meat Science*, *84*(2), 284–292. https://doi.org/10.1016/j.meatsci.2009.05.001

640 Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., & Wood, J. D.
641 (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of
642 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat*
643 *Science*, *78*(3), 256–269. https://doi.org/10.1016/j.meatsci.2007.06.008

644

645

646 **Table 1**. Summary table of the data sets and dietary treatments.
647

Dataset	Code	n	Country of Origin	Dietary Background
Trial A (n=98)	P	24	Ireland	Pasture for 11 months.
	SiP	24	Ireland	Grass silage *ad libitum* for 5 months. Pasture for 6 months.
	SiPC	25	Ireland	Grass silage *ad libitum* for 5 months. Pasture plus 50% of dietary DM as concentrates for 6 months.
	C	25	Ireland	Concentrate and straw for 11 months.
Trial B (n=60)	SiP2	15	Ireland	Grass silage *ad libitum* for 2 months. Pasture for 158 days.
	SiC	15	Ireland	Grass silage *ad libitum* for 2 months. Grass silage *ad libitum* plus 3 kg of concentrate for 158 days.
	SunO	15	Ireland	Grass silage *ad libitum* for 2 months. Pasture plus 1.6 kg of sunflower oil-enriched concentrate for 158 days.
	LinO	15	Ireland	Grass silage *ad libitum* for 2 months. Pasture plus 1.6 kg of linseed oil-enriched concentrate for 158 days.
Commercial (n=26)	Ir-Org	18	Ireland	Labelled as organic pasture-fed.
	Ir	8	Ireland	Unknown
International (n=97)	Aus	4	Austria	Unknown
	Fr	4	France	Unknown
	Ger	6	Germany	Unknown
	It	18	Italy	Unknown
	Sp	7	Spain	Unknown
	UK	19	UK	Unknown
	Br	17	Brazil	Unknown
	US	10	US	Unknown
	US-P	12	US	Labelled as pasture-fed.

648

649 **Table 2.** Chemical composition of the feeds used in feeding Trial A (Mean ± SD).

	Grass/Pasture (n=12)	**Grass Silage (n=6)**	**Concentrate (n=12)**
Proximate composition, g/kg DM			
Crude ash	111.2 ± 8.2	109.7 ± 4.2	69.4 ± 14.6
Crude protein	215.4 ± 46.3	167.7 ± 30.9	134.0 ± 22.0
Fat	38.1 ± 6.3	39.9 ± 2.2	19.2 ± 2.9
DM digestibility (g/kg)	770.1	724	866.4
Individual FAME (g/100g FAME)			
C14:0	0.50 ± 0.09	2.89 ± 1.81	0.30 ± 0.45
C16:0	17.63 ± 1.15	18.25 ± 1.14	39.82 ± 1.59
C18:0	2.39 ± 0.83	2.44 ± 0.11	3.38 ± 0.28
C18:1*c*9	2.42 ± 0.67	3.29 ± 0.29	20.88 ± 0.92
C18:2*n*-6	12.67 ± 1.43	15.40 ± 1.20	31.31 ± 1.52
C18:3*n*-3	54.84 ± 4.09	50.43 ± 2.00	2.25 ± 0.81
C20:0	0.48 ± 0.09	0.63 ± 0.05	nd
C22:0	1.06 ± 0.24	1.13 ± 0.11	0.06 ± 0.22
C22:1*n*-9	0.65 ± 0.14	0.34 ± 0.27	nd
C24:0	0.91 ± 0.19	1.00 ± 0.16	0.03 ± 0.1
C24:1	0.49 ± 0.29	0.20 ± 0.16	nd
Families of FAME (g/100g FAME)			
SFA	22.96 ± 1.95	26.34 ± 2.42	43.59 ± 1.43
MUFA	3.56 ± 1.25	3.82 ± 0.48	20.88 ± 1.13
PUFA	67.51 ± 3.42	65.84 ± 2.46	33.56 ± 1.92

650

651 nd = not detected.

652 DM = dry matter.

653 FAME = fatty acid methyl esters.

654 SFA = saturated fatty acids.

655 MUFA = monounsaturated fatty acids.

656 PUFA = polyunsaturated fatty acids.

657

658 **Table 3.** Fatty acid proportion of total intramuscular fat from *LTL* muscle of beef heifers
659 (Trial A) receiving pasture (P), silage followed by pasture (SiP), silage followed by pasture
660 supplemented with concentrate (SiPC) or concentrate (C).

	P (n=24)	**SiP (n=24)**	**SiPC (n=25)**	**C (n=25)**	**SEM**	**p-value**
IMF (g/100g muscle)	3.10^{bc}	2.66^{c}	3.60^{ab}	4.11^{a}	0.199	<0.01
FAME (g/100g FAME)						
C14:0	2.04^{b}	2.00^{b}	2.28^{ab}	2.36^{a}	0.079	< 0.01
C14:1	0.50	0.51	0.64	0.61	0.043	0.04
C15:0	0.36^{b}	0.42^{a}	0.36^{b}	0.27^{c}	0.012	< 0.01
C15:0*iso*	0.14*	0.18	0.12	0.08*	-	-
C15:0*anteiso*	0.36^{ab}	0.38^{a}	0.31^{ab}	0.25^{b}	0.032	0.03
C15:1	nd	0.09*	0.07*	0.12	-	-
C16:0	20.91^{b}	20.66^{b}	22.27^{ab}	24.8^{a}	0.711	< 0.01
C16:0*iso*	1.56^{ab}	1.79^{a}	1.35^{bc}	1.16^{c}	0.105	< 0.01
C16:1*c*9 + C17:0*anteiso*	3.51^{c}	3.53^{bc}	4.24^{ab}	4.65^{a}	0.195	< 0.01
C16:1*t*9 + C17:0*iso*	0.48^{a}	0.53^{a}	0.41^{b}	0.32^{c}	0.016	< 0.01
C16:1*t*11	0.17*	0.21*	0.15*	nd	-	-
C16:1*t*12	0.35	0.38	0.28	0.25	0.035	0.04
C16:1*c*13	nd	0.12*	nd	nd	-	-
C16:2*c*9,*c*12	0.94^{ab}	1.05^{a}	0.73^{bc}	0.57^{c}	0.087	< 0.01
C17:0	0.80^{ab}	0.88^{a}	0.83^{ab}	0.76^{b}	0.029	0.05
C17:1*c*9	0.76^{c}	0.90^{a}	0.88^{ab}	0.79^{bc}	0.028	< 0.01
C18:0	13.22^{a}	12.45^{ab}	11.03^{b}	11.32^{b}	0.484	< 0.01
C18:1*c*9	37.7^{ab}	35.72^{b}	39.34^{a}	40.3^{a}	0.966	< 0.01
C18:1*t*9	0.08*	0.12	0.09*	0.12*	-	-
C18:1*t*10	0.15	0.18	0.16	0.14	0.012	0.13
C18:1*c*11	1.16^{b}	1.14^{b}	1.31^{ab}	1.49^{a}	0.057	< 0.01
C18:1*t*11	2.43^{a}	2.40^{a}	1.79^{b}	0.61^{c}	0.134	< 0.01
C18:1*t*12	0.09*	0.09*	0.08*	0.05*	-	-
C18:1*c*13	0.28^{b}	0.28^{b}	0.35^{ab}	0.36^{a}	0.021	< 0.01
C18:1*t*13	0.33*	0.24*	0.19*	0.12*	-	-
C18:1*c*15 + C18:2.10.14	0.19^{a}	0.19^{a}	0.17^{ab}	0.13^{b}	0.013	< 0.01
C18:1*t*16	0.20	0.22	0.16	0.06*	-	-
C18:2*n*-6	2.20^{b}	2.56^{b}	3.15^{a}	3.49^{a}	0.143	< 0.01
C18:2*c*11,*t*15	0.10*	0.10*	0.10*	nd	-	-
C18:2*t*11,*c*15	0.25	0.30	0.21	nd	-	-
CLA*c*9,*t*11	0.85^{a}	0.86^{a}	0.71^{a}	0.31^{b}	0.042	< 0.01
CLA*t*10,*c*12	nd	0.06*	nd	nd	-	-
C18:2.10.13 + C18:2.11.14	0.22	0.24	0.20	0.05*	-	-
C18:3*n*-3	1.38^{b}	1.70^{a}	0.92^{c}	0.27^{d}	0.054	< 0.01
C20:1*t*9	0.08	0.09	0.13	0.17	-	-
C20:3*n*-6	0.24^{c}	0.27^{bc}	0.32^{ab}	0.38^{a}	0.019	< 0.01
C20:4*n*-6	1.22	1.30	1.16	1.35	0.096	0.50

C20:5*n*-3	0.74[b]	1.02[a]	0.47[c]	0.13[d]	0.049	< 0.01
C22:0	0.27	0.25	0.10*	nd	-	-
C22:2*n*-6	0.18	0.23	0.07*	nd	-	-
C22:5*n*-3	1.03[a]	1.10[a]	0.73[b]	0.37[c]	0.052	< 0.01
C22:6*n*-3	0.08*	0.16	0.07*	nd	-	-
SFA	39.65	39.00	38.64	41.04	1.109	0.43
MUFA	44.42	42.68	45.69	45.30	1.042	0.19
PUFA	9.46[ab]	10.94[a]	8.87[b]	7.04[c]	0.430	< 0.01
PUFA:SFA	0.24[a]	0.29[a]	0.24[ab]	0.18[b]	0.016	< 0.01
n-6	3.87[b]	4.41[ab]	4.72[ab]	5.26[a]	0.253	< 0.01
n-3	3.58[b]	4.38[a]	2.50[c]	0.85[d]	0.145	< 0.01
n-6:n-3	1.08[c]	1.00[c]	1.90[b]	6.19[a]	0.082	< 0.01

SEM = pooled standard error of the means.

[a,b,c,d] different letters within a row indicate a significant difference (P<0.05). Only applicable to FAME that had <15% of non-detected values in all feeding regimes.

* non-detected measurements accounted for 15-50%.

nd: non-detected measurements accounted for >50%.

FAME = fatty acid methyl esters.

CLA = conjugated linoleic acid.

SFA = sum of saturated fatty acids (C14:0 + C15:0 + C15:0*iso* + C15:0*anteiso* + C16:0 + C16:0*iso* + C17:0 + C18:0 + C22:0).

MUFA = sum of monounsaturated fatty acids (C14:1 + C15:1 + C16:1*t*10 + C16:1*t*11 + C16:1*t*12 + C16:1*c*13 + C17:1*c*9 + C18:1*t*4 + C18:1*c*9 + C18.1*t*9 + C18:1*t*10 + C18:1*c*11 + C18:1*t*11 + C18:1*c*12 + C18:1*t*12 + C18:1*c*13 + C18:1*t*13 + C18:1*t*16 + C20.1*t*9).

PUFA = sum of polyunsaturated fatty acids (C16:2*c*9*c*12 + C18:2*n*-6 + C18:2*c*11*t*15 + C18:2*t*11*c*15 + CLA*c*9*t*11 + CLA*t*10*c*12 + C18:2.10.13 + C18:2.11.14 + C18:3*n*-3 + C18:3*c*9*t*11*c*15 + C20:2*n*-6 + C20:3*n*-6 + C20:4*n*-6 + C20:5*n*-3 + C22:2*n*-6 + C22:5*n*-3 + C22:6*n*-3).

n-6: sum of omega-6 fatty acids (C18:2*n*-6 + CLA*t*10*c*12 + C20:2*n*-6 + C20:3*n*-6 + C20:4*n*-6 + C22:2*n*-6).

n-3: sum of omega-3 fatty acids (C18:2*c*11*t*15 + C18:2*t*11*c*15 + C18:3*n*-3 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3)

682 **Table 4.** Fatty acid proportion of total intramuscular fat from commercial and international beef samples with unknown or stated dietary
683 background (mean ± SD).
684

	Ir-Org (n = 18)	**Ir (n = 8)**	**Aus (n = 4)**	**Fr (n = 4)**	**Ger (n = 6)**	**It (n = 18)**	**Sp (n = 7)**	**UK (n = 19)**	**Br (n = 17)**	**US (n =10)**	**US – P (n = 12)**
IMF (g/100g muscle)	3.02 ± 1.21	3.47 ± 0.45	4.33 ± 1.59	5.99 ± 2.10	3.96 ± 1.7	2.83 ± 1.29	2.82 ± 1.1	2.52 ± 1.03	3.60 ± 1.54	6.15 ± 1.03	6.46 ± 1.14
FAME (g/100g FAME)											
C14:0	1.94 ± 0.41	2.19 ± 0.24	1.87 ± 0.15	2.52 ± 0.40	2.33 ± 0.82	1.81 ± 0.52	1.93 ± 0.96	1.47 ± 0.59	2.51 ± 0.55	3.02 ± 0.31	2.57 ± 0.38
C14:1	0.31 ± 0.17	0.76 ± 0.19	0.28 ± 0.01	0.57 ± 0.14	0.73 ± 0.29	0.25 ± 0.16	0.32 ± 0.14	0.40 ± 0.26	0.61 ± 0.22	0.70 ± 0.17	0.46 ± 0.12
C15:0	0.57 ± 0.18	0.40 ± 0.07	0.37 ± 0.01	0.31 ± 0.11	0.45 ± 0.26	0.37 ± 0.12	0.33 ± 0.08	0.38 ± 0.08	0.41 ± 0.14	0.54 ± 0.10	0.52 ± 0.11
C15:0*iso*	0.28 ± 0.05	0.20 ± 0.08	0.15 ± 0.02	0.15 ± 0.05	0.17 ± 0.07	0.13* ± 0.08	0.12 ± 0.04	0.12 ± 0.07	0.26 ± 0.10	0.09 ± 0.03	0.25 ± 0.09
C15:0*anteiso*	0.52 ± 0.13	0.28 ± 0.07	0.21 ± 0.06	0.22 ± 0.11	0.33 ± 0.15	0.61 ± 0.24	0.38 ± 0.22	0.48 ± 0.32	0.43 ± 0.15	0.20 ± 0.09	0.32 ± 0.10
C15:1	0.10* ± 0.10	nd	nd	nd	nd	0.13 ± 0.06	nd	0.05* ± 0.03	nd	nd	nd
C16:0	21.37 ± 1.85	23.15 ± 1.65	23.63 ± 0.88	25.92 ± 1.92	20.98 ± 2.13	21.64 ± 2.85	20.15 ± 5.16	18.85 ± 2.66	21.52 ± 2.29	23.99 ± 1.55	24.79 ± 1.7
C16:0*iso*	1.23 ± 0.64	0.80 ± 0.16	0.72 ± 0.34	0.41 ± 0.26	1.04 ± 0.59	1.93 ± 0.93	1.78 ± 1.54	1.71 ± 0.71	1.13 ± 0.65	0.48 ± 0.24	0.47 ± 0.19
C16:1*c*9 + C17:0*anteiso*	2.94 ± 0.31	4.47 ± 0.80	2.54 ± 0.03	3.65 ± 0.45	4.37 ± 1.65	2.20 ± 0.58	2.97 ± 1.01	2.48 ± 1.18	3.35 ± 0.64	3.42 ± 0.66	2.94 ± 0.51
C16:1*t*9 + C17:0*iso*	0.47 ± 0.24	0.10 ± 0.06	nd	0.09 ± 0.10	0.08* ± 0.06	0.36 ± 0.06	0.09* ± 0.09	0.12 ± 0.08	0.17* ± 0.22	0.20 ± 0.09	0.31 ± 0.22
C16:1*t*10	nd	0.37 ± 0.07	0.27 ± 0.15	0.27* ± 0.17	0.31* ± 0.17	nd	0.18* ± 0.16	nd	0.27* ± 0.24	nd	nd
C16:1*t*11	nd	nd	0.15* ± 0.15	nd	0.06* ± 0.07	0.37 ± 0.22	nd	0.37 ± 0.11	nd	0.10* ± 0.11	0.30 ± 0.24
C16:1*t*12	0.21* ± 0.11	0.20 ± 0.03	0.15* ± 0.08	0.17 ± 0.06	0.21 ± 0.05	0.23 ± 0.05	0.17 ± 0.05	0.14 ± 0.08	0.22 ± 0.10	0.14 ± 0.03	0.14* ± 0.09
C16:1*c*13	0.31 ± 0.16	0.10 ± 0.04	0.17 ± 0.15	0.13 ± 0.13	0.19 ± 0.10	0.31 ± 0.16	0.29 ± 0.27	0.38* ± 0.30	0.21 ± 0.16	0.07 ± 0.05	0.09* ± 0.07
C16:2*c*9*c*12	1.19 ± 0.54	0.62 ± 0.20	0.64 ± 0.43	0.56 ± 0.49	0.95 ± 0.44	1.60 ± 0.82	1.74 ± 1.32	3.05 ± 1.67	0.99 ± 0.51	0.68 ± 0.27	0.62 ± 0.23
C17:0	1.08 ± 0.13	0.81 ± 0.11	1.05 ± 0.09	0.87 ± 0.16	0.82 ± 0.35	0.76 ± 0.19	0.72 ± 0.29	0.79 ± 0.26	1.00 ± 0.22	1.41 ± 0.34	1.10 ± 0.11
C17:1*c*9	0.65 ± 0.13	0.74 ± 0.10	0.54 ± 0.03	0.56 ± 0.06	0.73 ± 0.16	0.40 ± 0.11	0.61 ± 0.21	0.68 ± 0.32	0.77 ± 0.19	1.07 ± 0.32	0.62 ± 0.11
C18:0	17.06 ± 2.02	12.49 ± 1.65	16.61 ± 0.9	15.26 ± 1.91	11.45 ± 3.2	16.53 ± 2.97	13.58 ± 2.18	13.4 ± 2.62	15.74 ± 3.34	12.44 ± 1.31	16.18 ± 2.95
C18:1*t*4	0.19* ± 0.11	0.14 ± 0.06	0.15 ± 0.03	0.16 ± 0.04	0.16 ± 0.04	0.25 ± 0.15	0.19 ± 0.09	0.10 ± 0.06	0.15* ± 0.08	0.38 ± 0.13	0.26 ± 0.11
C18:1*c*9	29.88 ± 2.54	36.82 ± 2.30	36.75 ± 1.81	37.88 ± 1.82	35.94 ± 8.17	26.81 ± 4.21	28.53 ± 7.89	28.77 ± 6.75	34.06 ± 3.32	35.22 ± 3.18	34.06 ± 3.13
C18:1*t*9	0.23 ± 0.05	0.22 ± 0.05	0.22 ± 0.04	0.22 ± 0.03	0.21 ± 0.02	0.27 ± 0.11	0.24 ± 0.12	0.18 ± 0.09	0.20 ± 0.07	0.65 ± 0.20	0.31 ± 0.11
C18:1*t*10	0.43 ± 0.34	0.24 ± 0.08	0.23 ± 0.10	0.31 ± 0.09	0.24 ± 0.07	0.78 ± 0.69	0.63 ± 0.43	0.48 ± 0.39	0.32 ± 0.24	3.30 ± 1.35	0.75 ± 0.55

C18:1*c*11	1.13 ± 0.31	1.27 ± 0.28	0.95 ± 0.08	0.81 ± 0.51	2.20 ± 1.19	1.12 ± 0.24	1.40 ± 0.74	2.03 ± 0.70	1.16 ± 0.31	1.30 ± 0.25	1.03 ± 0.50
C18:1*t*11	2.77 ± 1.31	1.94 ± 1.36	0.91 ± 0.09	0.89 ± 0.38	1.48 ± 0.95	1.13 ± 0.53	0.61 ± 0.45	1.26 ± 2.36	1.51 ± 0.53	0.95 ± 0.50	1.91 ± 0.75
C18:1*c*12	0.07 ± 0.05	0.05* ± 0.02	0.18 ± 0.01	0.20 ± 0.11	0.09 ± 0.02	0.23 ± 0.07	0.13 ± 0.10	0.17 ± 0.12	0.06* ± 0.12	0.18 ± 0.06	0.17 ± 0.11
C18:1*t*12	0.15* ± 0.13	0.15* ± 0.09	0.14 ± 0.11	0.13 ± 0.04	0.15 ± 0.08	0.29 ± 0.12	0.22 ± 0.09	0.10* ± 0.09	0.18 ± 0.13	0.21 ± 0.14	0.18 ± 0.13
C18:1*c*13	0.14 ± 0.05	0.32 ± 0.10	0.16 ± 0.01	0.22 ± 0.07	0.40 ± 0.25	0.14 ± 0.07	0.17 ± 0.05	0.29 ± 0.21	0.26 ± 0.09	0.38 ± 0.12	0.22 ± 0.12
C18:1*t*13	0.22 ± 0.16	0.23 ± 0.14	0.18 ± 0.09	0.25 ± 0.13	0.20* ± 0.15	0.32 ± 0.16	0.12* ± 0.09	0.11 ± 0.07	0.21 ± 0.15	0.28 ± 0.11	0.19 ± 0.09
C18:1*c*15 + C18.2.10.14	0.24 ± 0.09	0.10 ± 0.05	0.09 ± 0.02	0.07 ± 0.03	0.11 ± 0.02	0.15 ± 0.05	0.11 ± 0.08	0.07 ± 0.03	0.15 ± 0.08	0.14 ± 0.05	0.16 ± 0.07
C18:1*t*16	0.24 ± 0.05	0.17 ± 0.04	0.27 ± 0.04	0.18 ± 0.03	0.02 ± 0.06	0.21 ± 0.09	0.10 ± 0.05	0.13 ± 0.06	0.15 ± 0.07	0.08 ± 0.04	0.23 ± 0.03
C18:2*n*-6	3.02 ± 0.98	2.64 ± 0.37	4.16 ± 1.20	2.42 ± 1.39	4.05 ± 2.83	9.16 ± 3.72	8.41 ± 5.93	8.26 ± 4.97	3.96 ± 1.34	4.24 ± 0.70	3.36 ± 0.99
C18:2*c*11*t*15	0.12 ± 0.03	0.12 ± 0.04	0.08 ± 0.03	0.11 ± 0.08	0.15 ± 0.06	0.07* ± 0.04	0.05 ± 0.02	0.09 ± 0.06	0.08 ± 0.04	0.05 ± 0.03	0.10 ± 0.03
C18:2*t*11*c*15	0.39 ± 0.16	0.24 ± 0.12	0.17 ± 0.02	0.13 ± 0.06	0.30 ± 0.19	0.07* ± 0.07	0.06* ± 0.04	0.11 ± 0.13	0.15 ± 0.08	0.11 ± 0.06	0.20 ± 0.11
CLA*c*9*t*11	0.71 ± 0.21	0.74 ± 0.37	0.30 ± 0.03	0.32 ± 0.12	0.82 ± 0.23	0.30 ± 0.11	0.38 ± 0.34	0.40 ± 0.34	0.49 ± 0.20	0.45 ± 0.22	0.70 ± 0.14
CLA*t*10*c*12	0.08 ± 0.05	0.05* ± 0.03	0.03* ± 0.01	nd	0.15 ± 0.15	nd	0.04* ± 0.04	0.03* ± 0.02	nd	nd	0.03* ± 0.01
C18:2.10.13 + C18:2.11.14	0.17 ± 0.04	0.18 ± 0.02	0.20 ± 0.02	0.17 ± 0.06	0.21 ± 0.04	0.11* ± 0.07	0.11 ± 0.04	0.30 ± 0.12	0.10 ± 0.05	0.14 ± 0.05	0.19 ± 0.04
C18:3*n*-3	1.32 ± 0.33	1.19 ± 0.17	1.45 ± 0.26	0.58 ± 0.22	1.50 ± 1.25	0.53 ± 0.27	0.38 ± 0.19	1.03 ± 1.16	0.73 ± 0.31	0.24 ± 0.12	0.57 ± 0.23
C18:3*c*9*t*11*c*15	0.08* ± 0.04	nd	nd	0.05* ± 0.02	0.04* ± 0.03	0.06* ± 0.06	0.13 ± 0.12	0.10 ± 0.04	0.05* ± 0.04	0.07 ± 0.02	0.09 ± 0.03
C20:1*t*9	0.08 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.15 ± 0.05	0.20 ± 0.22	0.09* ± 0.05	0.11 ± 0.03	0.13* ± 0.14	0.13 ± 0.05	0.12 ± 0.04	0.09* ± 0.04
C20:2*n*-6	0.21 ± 0.08	0.12* ± 0.07	0.06* ± 0.05	0.06 ± 0.02	0.09* ± 0.04	0.11 ± 0.05	0.13 ± 0.10	0.20 ± 0.10	0.17* ± 0.11	0.05* ± 0.03	0.09 ± 0.04
C20:3*n*-6	0.11* ± 0.17	0.27 ± 0.05	0.18 ± 0.07	0.22 ± 0.17	0.33 ± 0.14	0.48 ± 0.30	0.66 ± 0.39	0.78 ± 0.34	0.39 ± 0.19	0.24 ± 0.11	0.25 ± 0.07
C20:4*n*-6	1.63 ± 0.78	1.10 ± 0.29	0.83 ± 0.39	0.63 ± 0.53	1.27 ± 0.88	2.74 ± 1.49	3.17 ± 2.11	2.56 ± 1.22	1.43 ± 0.74	0.72 ± 0.38	0.73 ± 0.18
C20:5*n*-3	0.09 ± 0.09	0.67 ± 0.18	0.29* ± 0.21	0.20 ± 0.22	0.55 ± 0.36	0.33 ± 0.31	0.52 ± 0.27	0.27 ± 0.13	0.36 ± 0.25	0.08 ± 0.04	0.10 ± 0.05
C22:0	0.25 ± 0.19	nd	nd	0.03* ± 0.01	0.05* ± 0.02	0.11 ± 0.06	0.12 ± 0.06	0.08* ± 0.07	0.05* ± 0.04	nd	nd
C22:2*n*-6	0.80 ± 0.51	0.20 ± 0.04	0.10 ± 0.04	0.09 ± 0.05	0.19 ± 0.08	nd	0.12* ± 0.11	0.69 ± 0.66	0.24 ± 0.28	0.06* ± 0.07	0.12 ± 0.12
C22:5*n*-3	1.12 ± 0.38	0.91 ± 0.18	0.50 ± 0.21	0.35 ± 0.23	0.77 ± 0.33	0.68 ± 0.39	0.62 ± 0.35	1.11 ± 0.77	0.88 ± 0.43	0.16 ± 0.11	0.30 ± 0.14
C22:6*n*-3	0.20 ± 0.10	0.21 ± 0.12	0.08 ± 0.07	0.08* ± 0.07	0.14 ± 0.07	nd	0.15 ± 0.08	0.17 ± 0.24	0.17 ± 0.09	nd	nd
SFA	44.31 ± 3.09	40.34 ± 2.68	44.64 ± 1.55	45.68 ± 3.41	37.61 ± 5.96	43.89 ± 4.17	39.10 ± 6.72	37.28 ± 3.10	43.05 ± 3.79	42.20 ± 2.59	46.23 ± 4.02
MUFA	37.32 ± 2.52	43.88 ± 1.78	41.80 ± 1.69	43.12 ± 0.63	43.72 ± 8.86	33.36 ± 4.57	34.15 ± 8.83	35.79 ± 7.17	40.61 ± 3.68	45.15 ± 3.39	41.06 ± 3.78
PUFA	11.23 ± 3.00	9.30 ± 1.03	9.09 ± 2.84	5.99 ± 3.44	11.49 ± 5.75	16.33 ± 6.23	16.68 ± 10.22	19.13 ± 8.22	10.21 ± 3.37	7.33 ± 1.34	7.49 ± 1.08
PUFA:SFA	0.26 ± 0.08	0.23 ± 0.04	0.21 ± 0.07	0.14 ± 0.09	0.30 ± 0.13	0.38 ± 0.18	0.48 ± 0.38	0.53 ± 0.25	0.24 ± 0.09	0.17 ± 0.03	0.16 ± 0.03
n-6	5.85 ± 2.24	4.39 ± 0.70	5.35 ± 1.69	3.45 ± 2.07	6.07 ± 3.75	12.53 ± 5.40	12.52 ± 8.36	12.51 ± 6.18	6.22 ± 2.31	5.33 ± 1.02	4.58 ± 1.00

n-3	3.24 ± 0.66	3.33 ± 0.47	2.56 ± 0.74	1.44 ± 0.82	3.41 ± 1.83	1.73 ± 0.89	1.79 ± 0.72	2.76 ± 1.90	2.36 ± 0.91	0.66 ± 0.23	1.29 ± 0.43
n-6:n-3	1.76 ± 0.41	1.33 ± 0.21	2.09 ± 0.12	2.41 ± 0.43	1.81 ± 0.47	8.82 ± 4.77	6.87 ± 2.65	5.51 ± 2.93	2.80 ± 1.17	8.78 ± 2.86	4.19 ± 2.35

Ir-Org: Ireland, organic pasture-fed; Ir: Ireland, unknown; Aus: Austria, unknown; Fr: France, unknown; Ger: Germany, unknown; It: Italy, unknown; Sp: Spain, unknown; UK: unknown, Br: Brazil, unknown; US: unknown. US-P: pasture-fed.

* non-detected measurements accounted for 15-50%.

nd: non-detected measurements accounted for >50%.

FAME = fatty acid methyl esters.

CLA = conjugated linoleic acid.

SFA = sum of saturated fatty acids (C14:0 + C15:0 + C15:0*iso* + C15:0*anteiso* + C16:0 + C16:0*iso* + C17:0 + C18:0 + C22:0).

MUFA = sum of monounsaturated fatty acids (C14:1 + C15:1 + C16:1*t*10 + C16:1*t*11 + C16:1*t*12 + C16:1*c*13 + C17:1*c*9 + C18:1*t*4 + C18:1*c*9 + C18.1*t*9 + C18:1*t*10 + C18:1*c*11 + C18:1*t*11 + C18:1*c*12 + C18:1*t*12 + C18:1*c*13 + C18:1*t*13 + C18:1*t*16 + C20.1*t*9).

PUFA = sum of polyunsaturated fatty acids (C16:2*c*9*c*12 + C18:2*n*-6 + C18:2*c*11*t*15 + C18:2*t*11*c*15 + CLA*c*9*t*11 + CLA*t*10*c*12 + C18:2.10.13 + C18:2.11.14 + C18:3*n*-3 + C18:3*c*9*t*11*c*15 + C20:2*n*-6 + C20:3*n*-6 + C20:4*n*-6 + C20:5*n*-3 + C22:2*n*-6 + C22:5*n*-3 + C22:6*n*-3).

n-6: sum of omega-6 fatty acids (C18:2*n*-6 + CLA*t*10*c*12 + C20:2*n*-6 + C20:3*n*-6 + C20:4*n*-6 + C22:2*n*-6).

n-3: sum of omega-3 fatty acids (C18:2*c*11*t*15 + C18:2*t*11*c*15 + C18:3*n*-3 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3).

700 **Table 5**. Classification results for models 1, 2 and 3 from leave-one-out cross-validation (CV-LOO) and predictions for 3 independent datasets
701 consisting of samples from grass and partially grass-fed animals (validation), samples from animals that received plant oil enriched concentrate
702 ("oil-enriched" samples), and samples from various countries of origin (international samples).

	Model 1				**Model 2***						**Model 3**				
		Predictions				*Predictions*						*Predictions*			
		G	SiPC	C		Gt	GC	C	SunO	LinO		P	SiP	SiPC	C
CV-LOO	G (n = 48)	47	1	0	Gt (n = 50)	48.7	0.3	0.0	0.0	1.0	P (n = 24)	22	2	0	0
	SiPC (n = 25)	0	25	0	GC (n = 32)	0.0	32.0	0.0	0.0	0.0	SiP (n = 24)	7	17	0	0
	C (n = 25)	0	0	25	C (n = 20)	0.0	2.0	18.0	0.0	0.0	SiPC (n = 25)	0	0	24	1
					SunO (n = 12)	0.0	0.0	0.0	10.7	1.3	C (n = 25)	0	0	1	24
					LinO (n = 12)	0.0	0.0	0.0	0.0	12.0					
	Sensitivity (%)	97.9	100	100	Sensitivity (%)	97.3	100	90.0	88.9	100	Sensitivity (%)	91.7	70.8	96.0	96.0
	Specificity (%)	100	98.6	100	Specificity (%)	100	97.5	100	100	98.0	Specificity (%)	90.5	97.3	98.6	98.6
	Accuracy (%)	99.0			Accuracy (%)	96.3					Accuracy (%)	88.9			
Validation	SiP2 (n = 15)	15	0	0	Gt (n = 13)	12.7	0.0	0.0	0.0	0.3	SiP2 (n = 15)	12	3	0	0
	SiC (n = 15)	0	15	0	GC (n = 8)	0.0	8.0	0.0	0.0	0.0	SiC (n = 15)	0	0	14	1
	Ir-Org (n = 18)	15	3	0	C (n = 5)	0.0	0.3	4.7	0.0	0.0	Ir-Org (n = 18)	3	11	4	0
					Ir-Org (n = 18)	11.3	4.3	0.0	0.0	2.3					
"Oil-enriched" samples	SunO (n = 15)	15	0	0	SunO (n = 3)	0.0	0.0	0.0	2.7	0.3	SunO (n = 15)	0	0	13	2
	LinO (n = 15)	15	0	0	LinO (n = 3)	0.0	0.0	0.0	0.0	3.0	LinO (n = 15)	6	4	5	0
International samples	Ir (n = 8)	5	3	0	Ir (n = 8)	4.3	3.6	0.0	0.0	0.0	Ir (n = 8)	4	1	3	0
	Aus (n = 4)	0	4	0	Aus (n = 4)	0.0	4.0	0.0	0.0	0.0	Aus (n = 4)	0	0	4	0
	Fr (n = 4)	0	3	1	Fr (n = 4)	0.0	3.0	1.0	0.0	0.0	Fr (n = 4)	0	0	2	2
	Ger (n = 6)	2	3	1	Ger (n = 6)	1.3	4.3	0.3	0.0	0.0	Ger (n = 6)	0	2	3	1
	It (n = 18)	0	1	17	It (n = 18)	0.0	1.0	17.0	0.0	0.0	It (n = 18)	0	0	0	18
	Sp (n = 7)	0	0	7	Sp (n = 7)	0.0	0.0	7.0	0.0	0.0	Sp (n = 7)	0	0	0	7
	UK (n = 19)	4	1	14	UK (n = 19)	3.0	1.0	14.0	1.0	0.0	UK (n = 19)	1	3	3	12
	Br (n = 17)	0	8	9	Br (n = 17)	0.0	8.0	9.0	0.0	0.0	Br (n = 17)	0	2	7	8
	US (n = 10)	0	0	10	US (n = 10)	0.0	0.0	10.0	0.0	0.0	US (n = 10)	0	0	5	5
	US-P (n = 12)	0	7	5	US-P (n = 12)	0.0	6.7	5.3	0.0	0.0	US-P (n = 12)	0	0	7	5

703

G: grass-fed group (P + SiP); Gt: total grass-fed group (P + SiP + SiP2); GC: grass-concentrate (SiPC + SiC).

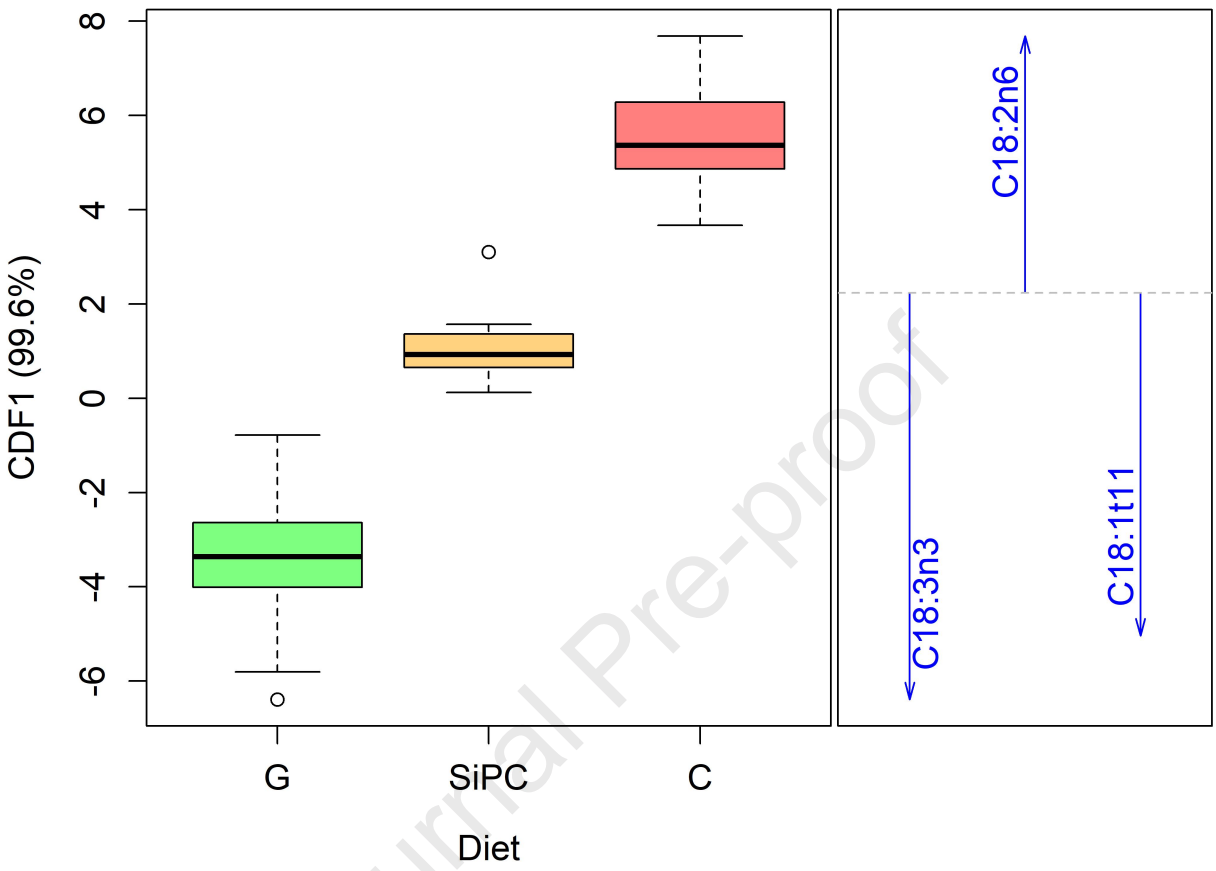
Ir-Org: Ireland, organic pasture-fed; Ir: Ireland, unknown; Aus: Austria, unknown; Fr: France, unknown; Ger: Germany, unknown; It: Italy, unknown; Sp: Spain, unknown; UK: unknown, Br: Brazil, unknown; US: unknown. US-P: pasture-fed.

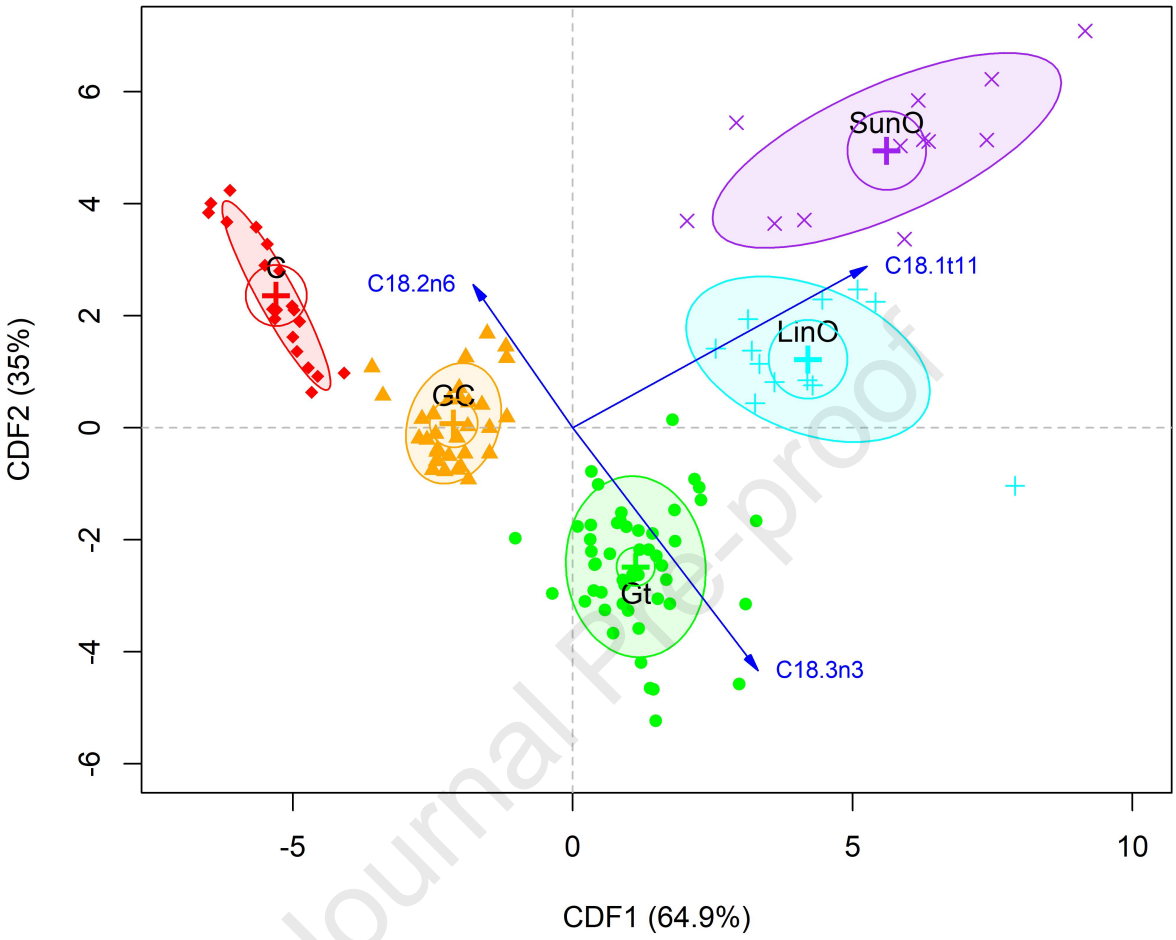
* *model 2* results are the average of 3 repeats resulting from randomly splitting the data into training and test set 3 times (ratio =0.8).

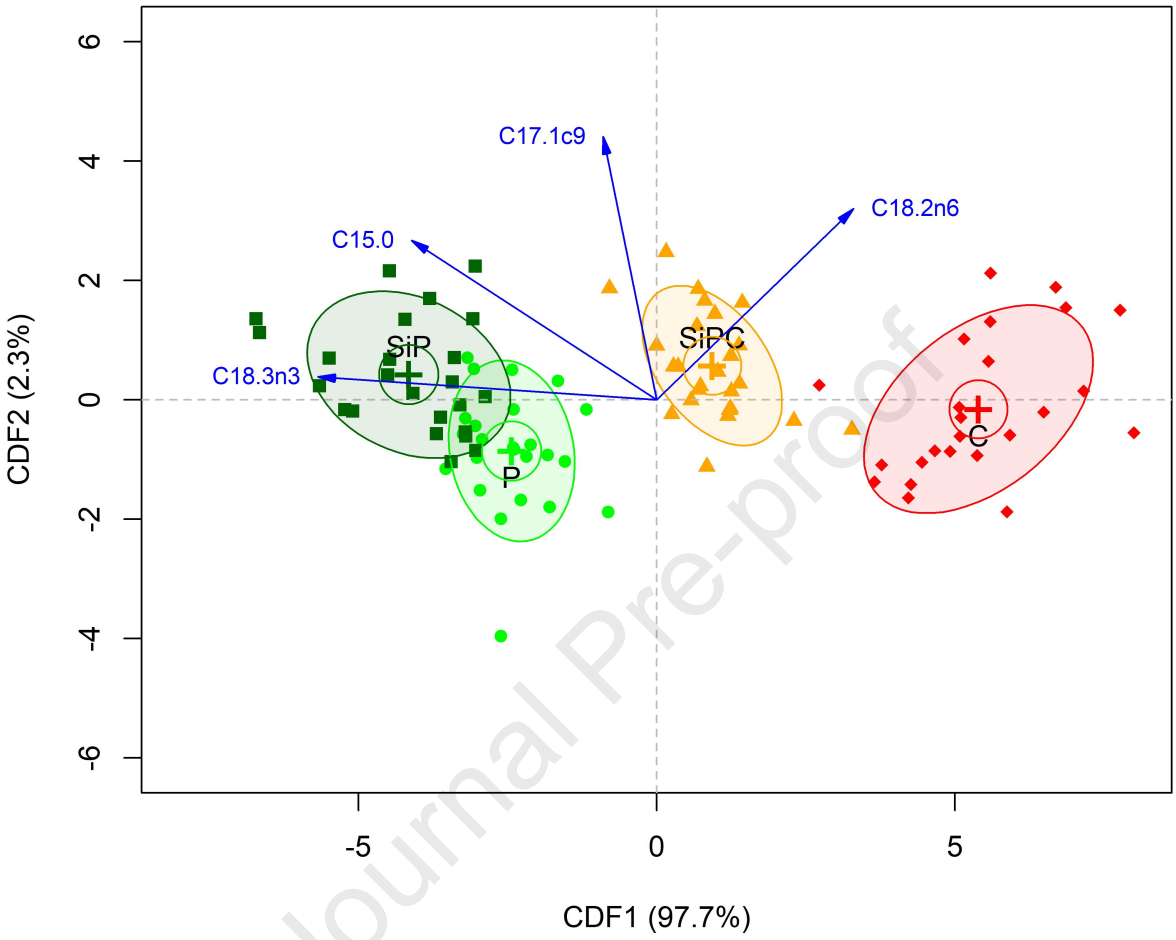
Fig. 1. Canonical score (a) and structure coefficient (b) plots for the 1st canonical discriminant function (CDF1) of model 1.

Fig. 2. Canonical score and structure coefficient plot for the 1st and 2nd canonical discriminant functions (CDF1 and CDF2) of model 2.

Fig. 3. Canonical score and structure coefficent plot for the 1st and 2nd canonical discriminant funtions (CDF1 and CDF2) of model 3.







Highlights

1. Muscle fatty acid profile was used to discriminate beef from various feeding systems
2. Canonical discriminant models were validated with an independent data set
3. Models were applied to an international set of beef samples
4. Beef from cattle fed grass, concentrate or combinations can be discriminated
5. Grass-fed beef can be distinguished from beef supplemented with vegetable oils

Dear Editor,

"Canonical discriminant analysis of the fatty acid profile of muscle to authenticate beef from grass-fed and other beef production systems: model development and validation" by Cama-Moncunill et al.

The authors declare they have no conflict of interest in the work reported in this manuscript.

Yours sincerely,

Aidan Moloney.