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Fatty acid concentration of plasma, muscle, adipose and liver from beef heifers fed an encapsulated n-3 polyunsaturated fatty acid supplement

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ABSTRACT

Increasing the content of polyunsaturated fat in the human diet is a priority for reducing cardiovascular disease and cancer risks. Beef has the potential to contribute to the polyunsaturated fat content in the human diet; however, ruminants cannot synthesise many long-chain fatty acids *de novo*; they require dietary supplementation. The objectives of the current study were to evaluate (i) the effect of a partially rumen protected n-3 long-chain polyunsaturated fatty acid **(LC-PUFA)** dietary supplement on the fatty acid composition of muscle (*Longissimus dorsi*), adipose and liver tissues of beef heifers and (ii) the usefulness of blood plasma as a predictor of tissue concentrations of specific fatty acids. Charolais crossbred heifers ($n = 20$) were assigned to one of two isolipid dietary treatments namely palmitic acid (control) or an n-3 LC-PUFA supplement for a 91-day period. Blood plasma and adipose tissue samples were taken to determine the temporal effect of these diets on fatty acid composition (days 0, 10, 35 and 91), while liver and muscle samples were taken following slaughter. Dietary lipid source did not influence animal growth rate or body condition score. At day 91, the percentage differences between control and n-3 LC-PUFA heifers in concentrations of eicosapentaenoic acid were +61, +176 and +133 % in liver, muscle and adipose, respectively. For docosahexaenoic acid, at the same time point, the percentage differences were +57, +73 and +138 % for liver, muscle and adipose, respectively. Medium-to-strong positive correlation coefficients were evident for liver and plasma fatty acids, in particular, there were positive relationships with concentrations of total saturated fatty acid (**SFA**), total n-6 PUFA and total n-3 PUFA. This trend also extended to both the ratio of PUFA to SFA (slope (β_1) = 0.56 ± 0.167, intercept (β_0) = 0.56, $R^2 = 0.61$, $P < 0.05$) and the ratio of n-6 to n-3 PUFA ($\beta_1 = 0.15 \pm 0.054$, $\beta_0 = 0.24$, $R^2 = 0.52$, $P < 0.05$). A strong correlation was also detected in the ratio of n-6 to n-3 in plasma and muscle tissue of heifers fed the n-3 LC-PUFA diet ($\beta_1 = 0.53 \pm 0.089$, $\beta_0 = -0.31$, $R^2 = 0.83$, $P < 0.001$). The results of this study show that the n-3 LC-PUFA can be readily increased through targeted supplementation and that plasma concentrations of n-3 LC-PUFA are useful predictors of their concentrations in a number of economically important tissues.

Implication

The findings of this study highlight that producers can include polyunsaturated fatty acid supplementation in cattle finishing regimes, as a method to enhance the proportion of healthy long-chain fatty acids in beef. The decrease in n-6:n-3 ratio, following polyunsaturated fatty acid supplementation, could be used as a marketing tool to highlight positive subsidiary effects for the perception of beef within the human diet. This study also highlighted the potential of utilising blood plasma to predict the fatty acid content of some important edible tissues; these proxies could be used to assist beef processors in their decision on the most suitable market for carcasses.

Introduction

Evidence suggests that consumption of long-chain n-3 polyunsaturated fatty acid (**n-3 LC-PUFA**) such as eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) by humans has the potential to positively influence health and reduce the prevalence of a number of diseases (Calder, 2014). It has been reported that a large proportion of n-3 LC-PUFA intake is derived from beef and lamb products, reflecting the increased importance of red meat in the n-3 LC-PUFA intake of Western societies (Stark et al., 2016). Unfortunately, red meat has been associated with the increased pathogenesis of a number of

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conditions such as cancer, diabetes and cardiovascular disease (Wolk, 2017). The high concentration of saturated fatty acid (**SFA**) and *trans* fatty acids found in red meat has, in particular, been associated with the increased prevalence of these diseases (Vahmani et al., 2015). However, a potential solution may lie in the enrichment of beef with n-3 LC-PUFA.

In order to exert a substantial change in the fatty acid composition of beef it is imperative that protected dietary lipid sources are employed (Wood and Enser, 2017). The benefits of ruminal protection of n-3 LC-PUFA are two fold, firstly helping to stabilise the fatty acids during storage and passage through the rumen (Chikunya et al., 2004), and secondly reducing the production of *trans* fatty acids traditionally associated with microbial biohydrogenation activity of n-3 LC-PUFA in the ruminant fore stomach (Chikunya et al., 2004). Previous work by our group (Kenny et al., 2007), using an unencapsulated supplement, reported low incorporation in muscle; therefore, using such a supplement would not provide a beef product with the possibility of enhanced market value. As such the first objective of the current study was to assess the effect of an encapsulated n-3 LC-PUFA dietary supplement on the fatty acid composition of muscle, adipose and liver tissues of beef heifers.

Determining the fatty acid composition of beef tissues following the supplementation of the bovine diet with n-3 LC-PUFA generally requires invasive and time-consuming tissue biopsies. Previous studies have shown blood plasma to be a good predictor of concentrations of n-3 LC-PUFA in a number of reproductive organs, and in determining indices of stearoyl-CoA desaturase activity in beef cattle, being both relatively non-invasive and rapid (Archibeque et al., 2005; Childs et al., 2008b). As a result, the second objective of this study was to evaluate the usefulness of blood plasma as a predictor of concentrations of specific fatty acids in a number of edible tissues.

Material and methods

All procedures conducted in this experiment were performed in accordance with the European Communities (Amendment of Cruelty to Heifers Act 1876) Regulations 2005 (S.I. No. 613/2005) administered by the Department of Health and Children of the Irish Government and complied with all relevant Irish national and EU legislation.

Heifers, diets and feeding regime

Eighteen month old Charolais crossbreed heifers ($n = 20$) with a mean ± SE live weight of 473.14 ± 5.41 kg and a body condition score (**BCS**; Lowman et al., 1976) of 3.64 ± 0.06 units were blocked on live weight and BCS and randomly assigned, within block, to one of two dietary treatments ($n = 10$ per diet). All heifers were individually fed a barley straw (1.50 kg DM) and concentrate (6 kg DM) based ration, supplemented with either palmitic acid (Palmit 80; SFA 151 g) as a control or 334 g of a partially rumen protected, n-3 LC-PUFA supplement. Rumen protection was achieved via encapsulation in a pH sensitive matrix which remained intact at rumen pH but was degraded at the lower pH of the abomasum. Fish oil (**FO**) derived from anchovy, sardine and salmon and distilled to concentrate the n-3 PUFA content was used. Both supplements were provided by Trouw Nutrition, Belfast, Northern Ireland. The n-3 PUFA supplement was estimated to provide approximately 140 g/head/day, of n-3 LC-PUFA, based on the fatty acid composition of the diet as determined in an earlier study (Childs et al., 2008b). The ingredient composition and chemical analysis of the concentrates and straw and fatty acid compositions of the diets are presented in Supplementary Table S1 and Supplementary Table S2, respectively. All diets were formulated to be isonitrogenous (14 % CP in total diet).

Each morning at 0900 h heifers received their entire daily allocation of supplementary lipid as a 1.0 kg DM bolus feed mixed with 1.5 kg DM of a 24 % CP ration to balance the low CP of the bolus diet. At 1200 h, heifers were offered the remainder of their daily concentrate allocation in the form of 3.50 kg DM of a second balancer ration together with 1.50 kg DM straw. All heifers were individually fed their respective diets for a 91-d period (minimum 42 d for DM intake (**DMI**) and 70 d for average daily gain (**ADG**) recording; Ahlberg et al., 2018) using an electronic feeding system (Calan Inc., Northwood, New Hampshire 03261, USA).

Dry matter intake and animal performance

Daily intake of straw and concentrate was measured for each heifer during the experimental period. All heifers had continuous free access to clean drinking water. Heifers were weighed weekly and their BCS assessed every 2 weeks on a scale of one (thin) to five (fat). Body condition scoring was performed by the same trained technician on each occasion (Childs et al., 2008b). Initial and final live weights were calculated from the mean of two weights taken on successive days. Live weight during the experimental period was recorded as a single measurement on a weekly basis. The difference between initial and final live weight divided by the intervening number of days was used to determine ADG.

Feed sampling and analysis

Weekly samples of straw and concentrates were stored at –20 °C until analysed. Samples were milled through a 1 mm screen using a Christy and Norris hammer mill (Christy and Norris Process Engineers Ltd., Chelmsford, England). Dry matter, CP, crude fibre, ether extract, crude oil, ash and gross energy were determined as previously described by Childs et al. (2008c).

Plasma and tissue sampling

Blood samples were collected by jugular venipuncture on day 0, to establish baseline concentrations of the fatty acids and on days 10, 35 and 91 of the supplementation period. On collection, samples were immediately stored in ice-water and centrifuged at 1 500 ×g at 4 °C for 15 min. Plasma was harvested and stored at –20 °C as described by Childs et al. (2008b).

On days 0, 10, 35 and 91 of the experimental period, samples of adipose tissue were taken via tissue biopsies from the tailhead (approx 200 mg) using the method described by Huerta-Leidenz et al. (1993). Tissue samples (2 g) from the liver and muscle tissue (*longissimus dorsi*) were taken at slaughter. Heifers were slaughtered in an EU licensed abattoir (Martin Jennings Wholesale Ltd., Neale Road, Ballinrobe, Mayo, Ireland) following 91 d on trial. All tissue samples were collected into sterile containers within 20 min of slaughter, flash frozen in liquid nitrogen and stored at –80 °C pending fatty acid analysis.

Fatty acid analysis of feed, plasma and tissue samples

The weight of fat in the samples was determined by Soxhlet method (AOAC, 2006). Total lipids (for fatty acid analysis) were extracted from 6 g of feed, 1 ml of plasma, 1 g of liver or muscle tissue and from 200 mg of adipose tissue, using chloroform methanol (2:1, v/v) as described previously, and dried at 45 °C under a steady flow of nitrogen (Folch et al., 1957). Methylation of all samples was carried out by *in situ* trans-esterification with 0.5 N sodium methoxide (Sigma Aldrich, St. Louis, MO, USA) followed by 14 % (v/v) boron trifluoride in methanol (Sigma Aldrich) as described by Park and Goins (1994). The fatty acid methyl esters were separated using a CP Sil 88 column (100 m × 0.25 mm i.d., 0.20 mm film thickness; Chrompack, Middleburg, The Netherlands) and on column injection, and quantified using GLC (3400; Varian, Harbor City, CA, USA). The GLC was calibrated using a range of commercial fatty acid standards (Sigma Aldrich). The GLC was fitted with a flame

ionisation detector and helium (37 psi) was used as the carrier gas. The injector temperature was held isothermally at 225 °C for 10 min and the detector temperature was 250 °C. The column oven was held at an initial temperature of 140 °C for 8 min and then programmed to increase at a rate of 8.5 °C/min to a final temperature of 200 °C, which was held for 41 min, previously described by Childs et al. (2008b).

Statistical analysis

All data were checked for normality of distribution (PROC UNIVARIATE, SAS v9.1, 2002). Data were analysed using two-way ANOVA with terms included for treatment and block. Variables having more than one observation per subject such as DMI, ADG, plasma and adipose fatty acids were analysed using repeated measures ANOVA (PROC MIXED) with terms for treatment, time period and their interaction included in the statistical model. The type of variance–covariance structure used was chosen depending on the magnitude of the Akaike criterion for models run under compound symmetry, unstructured, autoregressive or Toeplitz variance–covariance structures. The model with the lowest Akaike criterion was chosen. The CONTRAST (for orthogonal contrasts) statement was used to test for linear, quadratic and cubic effects of time of sampling on fatty acid concentrations of plasma and adipose tissue. The Tukey test was applied to evaluate pairwise comparisons of treatment means. Linear and stepwise multiple regression procedures (PROC REG and PROC STEPWISE) were also used as appropriate for univariate and multiple regression analyses, respectively. Regression analysis included tissue concentrations (%) of total fatty acids and plasma fatty acid concentrations (%) as independent and dependent concentrations, respectively. *P* values <0.05 were accepted as being statistically significant.

Results

Dry matter intake and animal performance

The DMI and performance of heifers in this study have been reported by Childs et al. (2008c). Briefly, heifers consistently consumed their entire daily dietary allocation of feed and consequently, there was no effect of diet on DMI ($P > 0.05$). Similarly, diet did not affect ADG, with heifers on the control and n-3 LC-PUFA diet gaining 0.81 ± 0.03 kg and 0.79 ± 0.03 kg, $P > 0.05$, respectively. There was also no effect of diet on BCS ($P > 0.05$).

Temporal fatty acid composition of plasma and adipose tissue

The fatty acid profile and fatty acid content of plasma and adipose tissue are presented in Tables 1 and 2, respectively. Heifers receiving the n-3 LC-PUFA diet had lower concentrations of palmitic, stearic, eicosatrienoic (n-6) and arachidonic acids (n-6) in their plasma relative to heifers on the control diet (Table 1, $P < 0.05$). Additionally, there were increases in the concentration of *t*10, *c*12 CLA, EPA (n-3), docosapentaenoic acid **(DPA)** (n-3) and DHA (n-3) occurred in the n-3 LC-PUFA group compared to the control ($P < 0.05$). Provision of the n-3 LC-PUFA diet also resulted in diet × day of sampling interactions for palmitic ($P < 0.01$), stearic and linoleic acids ($P < 0.05$). Temporally, supplementation with n-3 LC-PUFA diet reduced the concentration of eicosatrienoic acid (n-6) ($P < 0.001$) and arachidonic acid ($P < 0.01$) after 10 days, while the concentration of these fatty acids in the control increased over the 91-day period (Fig. 1). There were diet × day of sampling interactions observed in the concentrations of EPA (n-3) ($P < 0.001$), DPA (n-3) ($P < 0.01$) and DHA (n-3) ($P < 0.001$), whose

Table 1
Mean fatty acid composition (g/100 g FAME) of plasma from Charolais crossbred heifers receiving the control ($n = 10$) or n-3 LC-PUFA diet ($n = 10$), collected at days 0, 10, 35 and 91.

Fatty acid	Diet (mean over 91 days)			Days on control or n-3 LC-PUFA diet					*P* value		
	Control	n-3 LC-PUFA	SED	Day 0	Day 10	Day 35	Day 91	SEM	Diet	Day	Diet × day
C11:0	0.15	0.16	0.03	0.09	0.11	0.16	0.29	0.02	N.S.	<0.001	<0.05
C12:0	0.32	0.31	0.03	0.50	0.30	0.17	0.29	0.05	N.S.	<0.001	N.S.
C12:1n-3	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.01	N.S.	N.S.	N.S.
C13:0	0.09	0.09	0.02	0.18	0.08	0.01	0.10	0.03	N.S.	<0.001	N.S.
C14:0	4.54	4.27	0.32	3.11	5.09	3.67	5.20	0.47	N.S.	<0.001	N.S.
*t*C14:1n-5	0.56	0.58	0.08	0.65	0.60	0.74	0.33	0.11	N.S.	<0.001	N.S.
*c*C14:1n-5	0.44	0.42	0.04	0.51	0.31	0.35	0.54	0.04	N.S.	<0.001	<0.01
C16:0	13.08	11.69	0.38	11.64	10.34	12.01	12.76	0.53	<0.01	<0.01	<0.01
C16:1n-9	0.02	0.06	0.05	0.01	0.06	0.06	0.14	0.05	N.S.	<0.001	<0.01
C16:1n-7	0.76	0.79	0.05	0.96	0.67	0.69	0.85	0.06	N.S.	<0.01	N.S.
C17:1n-9	0.53	0.46	0.04	0.27	0.20	0.18	1.19	0.05	N.S.	<0.001	N.S.
C18:0	18.02	16.59	0.46	17.43	13.25	15.30	20.37	0.69	<0.01	<0.001	<0.05
Total C18:1	7.96	7.69	0.57	10.73	4.95	5.34	9.75	0.64	N.S.	<0.001	N.S.
C18:2n-6	21.41	19.57	1.15	20.68	18.31	18.38	20.93	1.13	N.S.	<0.001	0.02
γ-C18:3n-6	0.00	0.01	0.01	0.00	0.03	0.00	0.00	0.00	N.S.	N.S.	N.S.
α-C18:3n-3	1.43	1.64	0.13	2.36	1.27	1.56	1.39	0.12	N.S.	<0.001	N.S.
*c*9, *t*11/7*t*, 9*c* CLA	0.10	0.11	0.02	0.15	0.10	0.13	0.06	0.01	N.S.	<0.01	N.S.
*t*10, *c*12 CLA	0.09	0.13	0.01	0.13	0.15	0.15	0.10	0.03	<0.05	N.S.	N.S.
C20:3n-6	1.82	0.67	0.12	1.79	0.25	0.24	0.42	0.12	<0.001	<0.001	<0.001
C20:4n-6	2.44	2.12	0.12	2.23	1.98	2.03	2.25	0.11	<0.05	<0.001	<0.01
C20:5n-3	0.77	9.53	0.44	0.91	9.98	12.73	14.50	0.46	<0.001	<0.001	<0.001
C22:5n-3	0.86	1.23	0.10	1.13	1.15	1.22	1.42	0.09	<0.01	<0.01	<0.01
C22:6n-3	0.34	1.13	0.06	0.33	1.04	1.40	1.75	0.06	<0.001	<0.001	<0.001
Total CLA	0.19	0.24	0.04	0.28	0.24	0.27	0.16	0.04	N.S.	<0.05	N.S.
Total MUFA	10.56	9.91	0.58	13.19	6.85	7.39	12.19	0.49	N.S.	<0.001	N.S.
Total PUFA	29.40	36.17	1.50	29.70	34.24	37.84	42.88	1.44	<0.001	<0.001	<0.05
Total SFA	40.29	37.56	0.75	38.62	35.21	34.86	41.53	1.49	<0.01	<0.01	N.S.
Ratio PUFA to SFA	0.72	0.96	0.04	0.77	0.98	1.09	1.02	0.05	<0.001	<0.05	<0.001
n-6 PUFA	25.71	22.30	1.15	24.69	20.54	20.66	23.33	1.15	<0.05	<0.001	<0.01
n-3 PUFA	3.38	13.45	0.57	4.73	13.44	16.92	18.71	0.54	<0.001	<0.001	<0.001
Ratio n-6 to n-3 PUFA	8.10	2.27	0.54	5.30	1.55	1.24	1.01	0.53	<0.001	<0.05	<0.001

FAME = fatty acid methyl esters; PUFA = polyunsaturated fatty acid; CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid; N.S. = not significant.

Table 2
Mean fatty acid composition (g/100 g FAME) of adipose tissue from Charolais crossbred heifers receiving the control ($n = 10$) or n-3 LC-PUFA diet ($n = 10$), collected at days 0, 10, 35 and 91.

Fatty acid	Diet (mean over 91 days)			Days on control or n-3 LC-PUFA diet					*P* value		
	Control	n-3 LC-PUFA	SED	Day 0	Day 10	Day 35	Day 91	Pooled SEM	Diet	Day	Diet × day
C12:0	0.10	0.12	0.009	0.11	0.12	0.12	0.12	0.01	N.S.	N.S.	N.S.
C14:0	2.91	3.21	0.170	2.87	3.25	3.39	3.34	0.16	N.S.	<0.05	N.S.
*t*C14:1n-5	0.30	0.29	0.020	0.29	0.27	0.30	0.32	0.02	N.S.	N.S.	N.S.
*c*C14:1n-5	1.48	1.78	0.158	1.58	1.85	1.88	1.81	0.14	N.S.	N.S.	N.S.
C15:0	0.56	0.59	0.034	0.57	0.53	0.61	0.64	0.03	N.S.	<0.01	N.S.
C16:0	24.83	24.61	0.778	24.70	25.35	24.43	23.95	0.64	N.S.	<0001	N.S.
C16:1n-9	6.92	7.25	0.485	7.75	7.24	7.06	6.94	0.43	N.S.	N.S.	N.S.
C16:1n-7	0.24	0.32	0.038	0.24	0.34	0.44	0.26	0.04	N.S.	N.S.	N.S.
C17:0	0.84	0.82	0.040	0.80	0.75	0.81	0.93	0.04	N.S.	<0.05	N.S.
C17:1n-9	1.34	1.31	0.064	1.38	1.26	1.30	1.30	0.05	N.S.	N.S.	N.S.
C18:0	8.69	7.88	0.661	7.53	7.14	8.06	8.80	0.61	N.S.	<0.05	N.S.
Total C18:1	43.76	42.71	2.29	43.91	41.88	42.13	42.92	2.39	N.S.	N.S.	N.S.
C18:2n-6	1.13	1.16	0.084	1.10	1.35	1.17	1.02	0.30	N.S.	<0.001	N.S.
γ-C18:3n-6	0.01	0.01	0.004	0.26	0.30	0.33	0.34	0.02	N.S.	<0.001	N.S.
α- C18:3n-3	0.27	0.31	0.028	0.00	0.00	0.02	0.03	0.01	N.S.	<0.001	N.S.
C20:0	0.09	0.11	0.011	0.06	0.16	0.11	0.11	0.01	N.S.	N.S.	<0.01
*c*9, *t*11/*t*7, *c*9 CLA	0.96	1.20	0.130	1.03	1.29	1.29	1.20	0.10	N.S.	<0.001	<0.001
C20:1	0.14	0.15	0.013	0.14	0.14	0.15	0.17	0.01	N.S.	<0.001	N.S.
C20:3n-6	0.06	0.04	0.011	0.04	0.04	0.04	0.05	0.01	N.S.	N.S.	N.S.
C20:4n-6	0.02	0.06	0.010	0.03	0.11	0.09	0.02	0.01	<0.05	<0.01	N.S.
C20:5n-3	0.02	0.10	0.011	0.00	0.21	0.13	0.07	0.01	<0.001	<0.001	<0.001
C22:5n-3	0.02	0.11	0.014	0.03	0.21	0.10	0.09	0.02	<0.001	<0.001	<0.001
C22:6n-3	0.01	0.05	0.007	0.00	0.08	0.06	0.07	0.01	<0.001	<0.001	<0.001
Total CLA	0.96	1.20	0.130	0.97	1.08	1.17	1.11	0.07	N.S.	<0.001	<0.001
Total MUFA	54.41	54.04	1.268	55.53	53.20	53.50	53.93	1.09	N.S.	N.S.	N.S.
Total PUFA	2.50	3.05	0.147	2.50	3.59	3.22	2.88	0.12	<0.05	<0.001	<0.001
Total SFA	38.18	37.47	1.434	36.76	37.45	37.66	38.01	1.23	N.S.	N.S.	N.S.
Ratio PUFA to SFA	0.07	0.08	0.007	0.07	0.10	0.09	0.08	0.01	<0.05	<0.01	<0.01
n-6 PUFA	1.24	1.29	0.090	1.17	1.50	1.31	1.16	0.08	N.S.	<0.001	N.S.
n-3 PUFA	0.31	0.56	0.040	0.29	0.80	0.61	0.52	0.04	<0.001	<0.001	<0.001
Ratio n-6 to n-3 PUFA	4.50	2.65	0.552	4.26	1.92	2.16	2.26	0.48	<0.01	<0.05	N.S.

FAME = fatty acid methyl esters; PUFA = polyunsaturated fatty acid; CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid; N.S. = not significant.

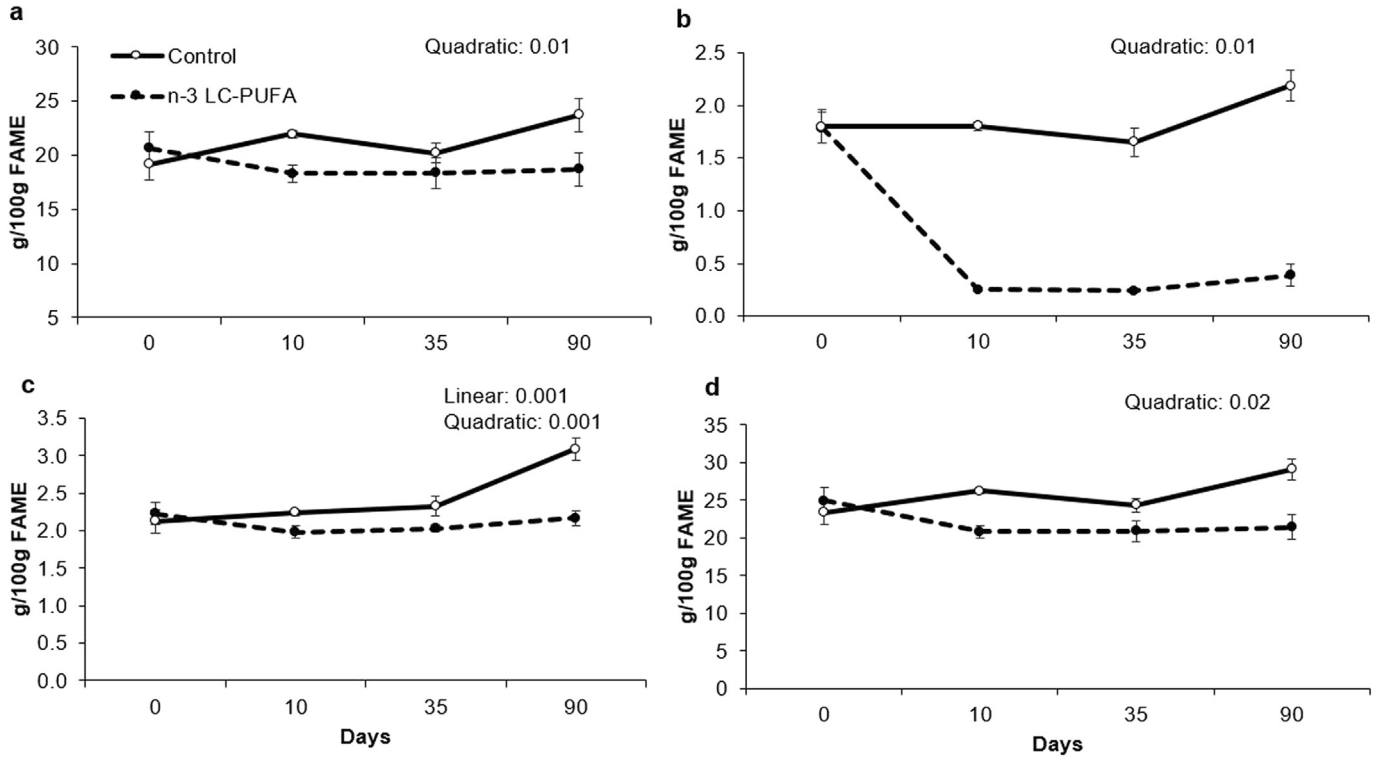

Fig. 1. Temporal changes in the concentration of a) linoleic acid, b) eicosatrienoic acid, c) arachidonic acid and d) total n-6 polyunsaturated fatty acid (PUFA) in the plasma of beef heifers receiving the control or n-3 LC-PUFA diet. FAME = fatty acid methyl esters.

concentrations increased temporally following supplementation with the n-3 LC-PUFA diet over the 91-day period (Fig. 2).

Plasma from heifers receiving the n-3 LC-PUFA diet had greater concentrations of total PUFA and total n-3 PUFA ($P < 0.001$), while simultaneously having lower concentrations of total SFA and total n-6 PUFA than heifers on the control diet (Table 1; $P < 0.05$). Indeed, diet × day of sampling interactions were observed for the concentration of total PUFA ($P < 0.05$) and total n-3 PUFA ($P < 0.001$), which increased with the duration of supplementation with the n-3 LC-PUFA diet compared to the control (Fig. 2). Diet × day of sampling interactions were also observed in the concentration of total n-6 PUFA ($P < 0.01$), which decreased after 10 days on the n-3 LC-PUFA diet (Fig. 1). These changes in fatty acid composition resulted in a diet × day of sampling increase in the ratio of PUFA to SFA ($P < 0.001$), and a diet × day of sampling decrease in the ratio of n-6 to n-3 PUFA (Table 1; $P < 0.001$).

In adipose tissue, the most abundant fatty acids identified were the SFA palmitic and stearic acid (Table 2). The provision of the n-3 LC-PUFA diet resulted in increases in the concentration of arachidonic acid (n-6), EPA (n-3), DPA (n-3) and DHA (n-3) ($P < 0.01$) compared to the control diet. Moreover, increases in the concentration of total PUFA and total n-3 PUFA were observed in the adipose tissue of heifers on the n-3 LC-PUFA diet ($P < 0.01$).

There were diet × day of sampling interactions for arachidic acid ($P < 0.01$), EPA (n-3) ($P < 0.001$), DPA (n-3) ($P < 0.001$), DHA (n-3) ($P < 0.001$) and in the concentrations of total PUFA ($P < 0.001$) and total n-3 PUFA ($P < 0.001$) in the adipose tissue of heifers on the n-3 LC-PUFA relative to the control diet (Table 2). The most substantial increase in the concentrations of these fatty acids occurred 10 days after the animal's introduction to the n-3 LC-PUFA diet (Fig. 3). A diet × day of sampling interaction was also observed for the *c*9, *t*11/*t*7, *c*9 CLA ($P < 0.001$), which increased in heifers following 10 days of supplementation with the n-3 LC-PUFA diet. This resulted in an increase in the ratio of PUFA to SFA ($P < 0.05$) and a decrease in the ratio of n-6 to n-3 PUFA in heifers on the n-3 LC-PUFA diet ($P < 0.05$) over time, but no effect was observed in the control heifers. Indeed, there was a diet × day of sampling increase in the ratio of PUFA to SFA in heifers on the n-3 LC-PUFA diet ($P < 0.01$).

Fatty acid composition of liver and muscle

The fatty acid composition of liver and muscle tissue from heifers receiving the control or n-3 LC-PUFA diets is presented in Tables 3 and 4, respectively. Compared to the control, heifers receiving the n-3 LC-PUFA diet displayed increased concentrations of palmitelaidic acid, α-linolenic acid (n-3), arachidic acid, *c*9, *t*11/*t*7, *c*9 CLA, EPA (n-3), DPA (n-3) and DHA (n-3) in their liver tissue (Table 3; $P < 0.05$). Additionally, the concentrations of stearic, linoleic (n-6), γ-linolenic (n-6), eicosatrienoic (n-6) and arachidonic (n-6) acids were reduced in the liver tissue of heifers consuming the n-3 LC-PUFA diet relative to those on the control diet ($P < 0.05$). In addition to changes in the concentration of the individual fatty acids, heifers on the n-3 LC-PUFA diet also had increased concentrations of total monounsaturated fatty acids, total PUFA and total n-3 PUFA ($P < 0.001$), with decreases in the concentration of total SFA and total n-6 PUFA ($P < 0.001$). The above alterations resulted in an overall increase in the ratio of PUFA to SFA ($P < 0.001$) along with a decrease in the ratio of n-6 to n-3 PUFA in heifers receiving the n-3 PUFA diet compared to the control ($P < 0.001$).

Concentrations of stearic and eicosatrienoic (n-6) acids strongly tended to be reduced in muscle of heifers fed the n-3 LC-PUFA diet relative to heifers on the control diet (Table 4; $P = 0.05$). Moreover, the muscle of the heifers on the n-3 LC-PUFA diet had increased concentrations of palmitoleic acid, EPA (n-3), DPA (n-3) and DHA (n-3) compared to muscle of heifers receiving the control diet ($P < 0.01$). Overall, heifers supplemented with n-3 LC-PUFA had lower total SFA concentrations and lower ratio of n-6 to n-3 PUFA than heifers receiving the control diet ($P < 0.05$), while increases in the concentration of total

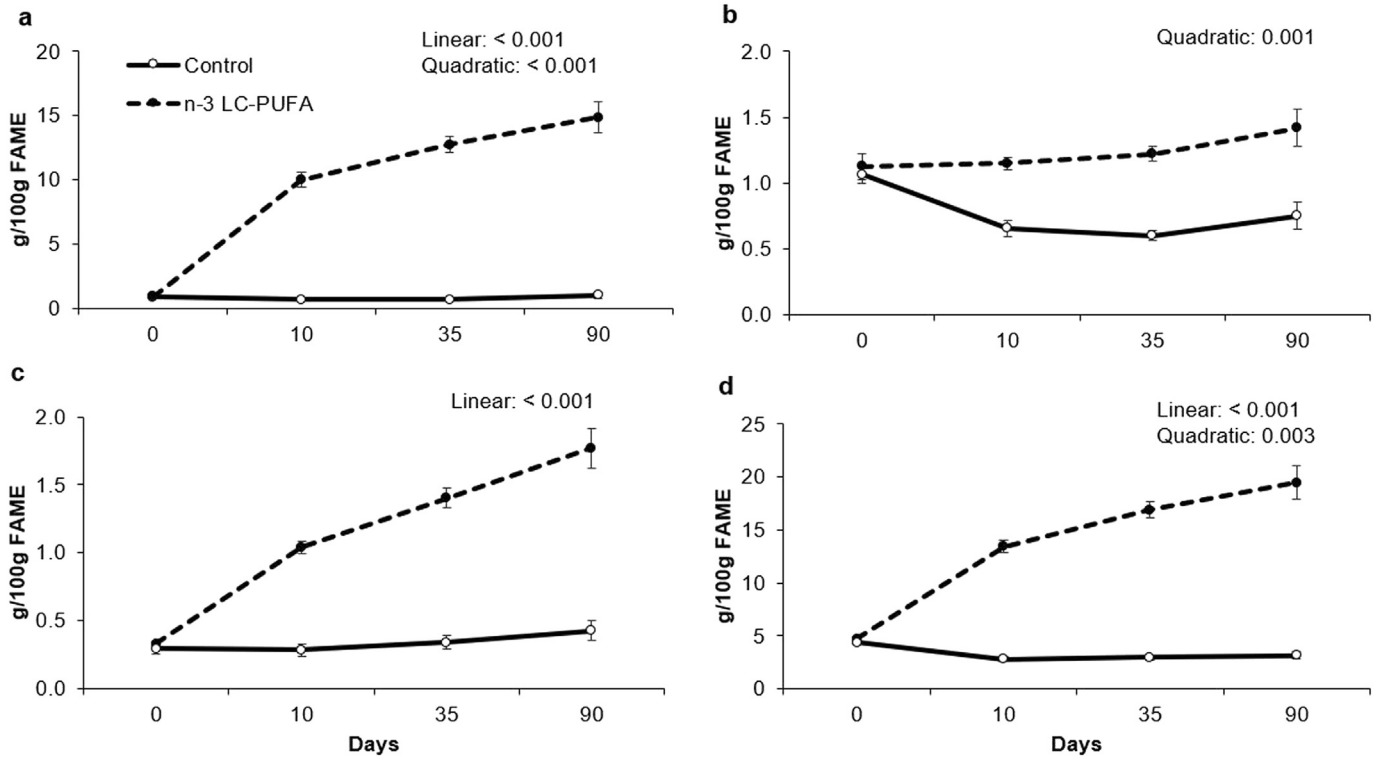

Fig. 2. Temporal changes in the concentration of a) eicosapentaenoic acid (EPA), b) docosapentaenoic acid (DPA), c) docosahexaenoic acid (DHA) and d) total n-3 polyunsaturated fatty acid (PUFA) in the plasma of beef heifers receiving the control or n-3 LC-PUFA diet. FAME = fatty acid methyl esters.

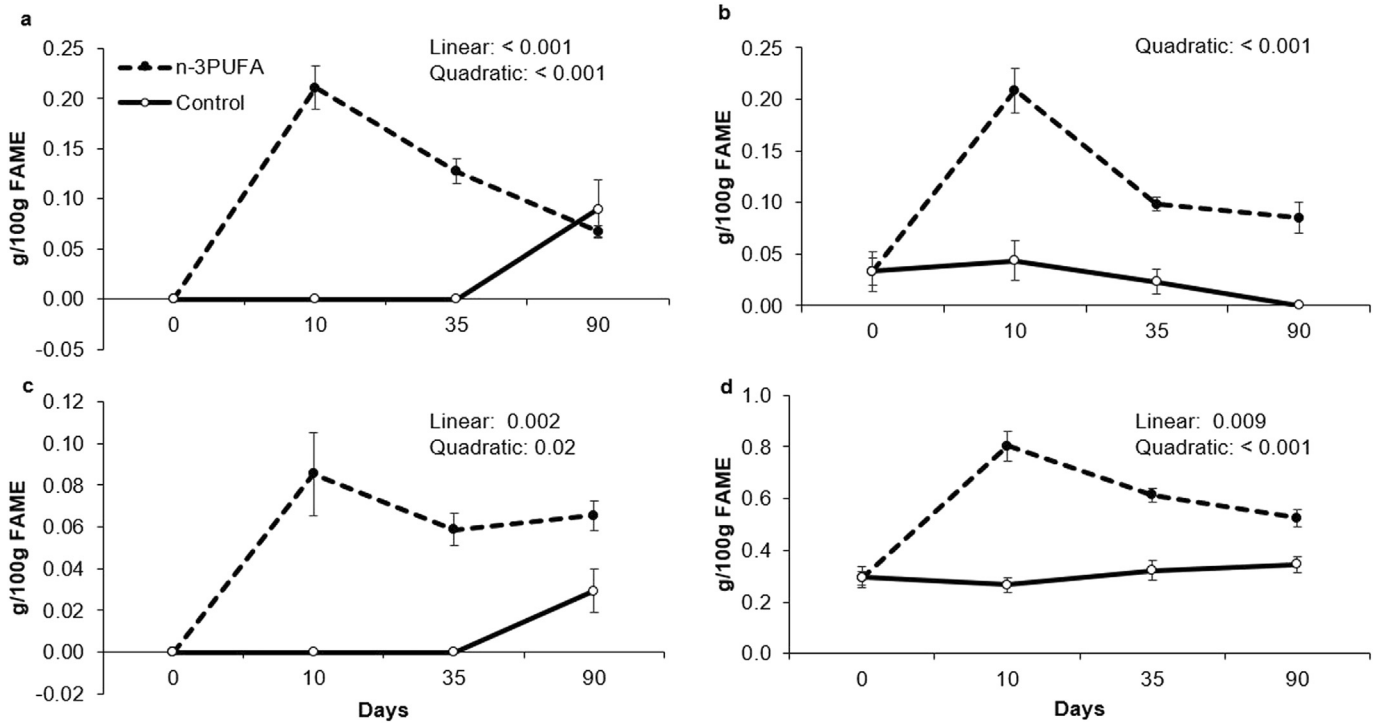

Fig. 3. Temporal changes in the concentration of a) eicosapentaenoic acid (EPA), b) docosapentaenoic acid (DPA), c) docosahexaenoic acid (DHA), and d) total n-3 polyunsaturated fatty acid (PUFA) in the adipose tissue of beef heifers receiving the control or n-3 LC-PUFA diet. FAME = fatty acid methyl esters.

PUFA, total n-3 PUFA and in the ratio of PUFA to SFA were also detected ($P < 0.05$). Interestingly, the concentration of total n-6 PUFA was not affected by the provision of the n-3 LC-PUFA diet ($P > 0.05$).

Table 3
Mean fatty acid composition (g/100 g FAME) of liver tissue from Charolais crossbred heifers receiving the control ($n = 10$) or n-3 LC-PUFA diet ($n = 10$), at slaughter following 91 days of feeding.

Fatty acid	Diet (g/100 g FAME)			*P* value
	Control	n-3 LC-PUFA	SED	Diet
C14:0	0.99	1.16	0.190	N.S.
C14:1	0.42	0.48	0.104	N.S.
C16:0	14.02	12.63	0.761	N.S.
C16:1n-9	0.50	0.64	0.061	<0.05
C16:1n-7	0.84	0.92	0.156	N.S.
C17:0	1.25	1.38	0.077	N.S.
C17:1n-9	0.10	0.06	0.083	N.S.
C18:0	28.03	21.37	1.007	<0.001
Total C18:1	11.75	12.25	1.716	N.S.
C18:2n-6	7.31	4.45	0.384	<0.001
γ-C18:3n-6	0.27	0.00	0.101	<0.05
α- C18:3n-3	0.37	0.70	0.090	<0.01
C20:0	0.00	0.49	0.039	<0.001
*c*9, *t*11/*t*7, *c*9 CLA	0.30	0.55	0.075	<0.01
C20:3n-6	6.50	0.46	0.459	<0.001
C20:4n-6	9.57	3.77	0.350	<0.001
C20:5n-3	1.84	13.44	0.688	<0.001
C22:5n-3	5.78	10.36	0.597	<0.001
C22:6n-3	4.08	10.27	0.773	<0.001
Total CLA	0.30	0.55	0.075	<0.01
Total MUFA	13.60	14.34	2.12	<0.001
Total PUFA	36.03	44.01	1.413	<0.001
Total SFA	44.71	37.49	0.734	<0.001
Ratio PUFA to SFA	0.81	1.17	0.033	<0.001
n-6 PUFA	23.66	8.68	0.787	<0.001
n-3 PUFA	12.07	34.77	1.757	<0.001
Ratio n-6 to n-3 PUFA	1.96	0.25	0.249	<0.001

FAME = fatty acid methyl esters; PUFA = polyunsaturated fatty acid; CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid; N.S. = not significant.

Fatty acid content of meat and offal tissues

Provision of the n-3 LC-PUFA diet did not result in substantial changes in the fatty acid content of the liver tissue which contained 52.07 mg of fat/g of tissue, compared to the control which contained 52.82 mg of fat/g of tissue. The n-3 LC-PUFA diet did alter the FA content of specific FA, with the same relative differences observed between concentration and content between diets. The n-3 LC-PUFA diet resulted in reductions in the amount of stearic acid (from 14.81 mg/g vs 11.29 mg/g; $P < 0.001$), and total n-6 PUFA (from 12.55 mg/g vs 4.95 mg/g; $P < 0.001$) in the liver tissue. Additionally, the n-3 LC-PUFA diet resulted in increases in the amount of the n-3 LC-PUFA, EPA (from 0.97 mg/g vs 7.10 mg/g; $P < 0.001$), DPA (from 3.05 mg/g vs 5.47 mg/g; $P < 0.001$) and DHA (from 2.16 mg/g vs 5.42 mg/g; $P < 0.001$).

Provision of the n-3 LC-PUFA diet yielded muscle tissue containing 52.10 mg of fat/g of tissue, compared to the control which contained 52.72 mg of fat/g of tissue. The notable changes in the FA composition were reductions in the amount of total SFA and in particular stearic acid (from 11.98 mg/g vs 8.83 mg/g; $P < 0.01$), and small increases in the amount of the n-3 LC-PUFA, EPA (from 0.05 mg/g of tissue vs 0.83 mg/g of tissue: $P < 0.001$), DPA (from 0.19 mg/g of tissue vs 0.40 mg/g of tissue; $P < 0.01$) and DHA (from 0.03 mg/g of tissue vs 0.29 mg/g of tissue; $P < 0.001$).

Regression analysis

The relationship between the concentration of stearic and vaccenic acid in plasma and that of muscle, adipose and liver tissue of heifers was weak. Similarly, the relationship between the concentration of arachidonic acid in plasma and in muscle, and adipose tissue was also weak (Table 5). However, a strong positive relationship between the concentration of arachidonic acid (n-6) was detected in plasma relative to liver tissue ($\beta_1 = 5.32 \pm 0.934$, $R^2 = 0.82$; $P < 0.001$). Strong-to-medium positive relationships were also detected between the concentration of EPA (n-3) and DHA (n-3) in plasma and those of muscle, adipose and liver tissue of heifers. Liver tissue had the strongest relationships

Table 4
Mean fatty acid composition (g/100 g FAME) of muscle tissue from Charolais crossbred heifers receiving the control ($n = 10$) or n-3 LC-PUFA diet ($n = 10$), at slaughter following 91 days of feeding.

Fatty acid	Diet (g/100 g FAME)			*P* value
	Control	n-3 LC-PUFA	SED	Diet
C12:0	0.04	0.02	0.031	N.S.
C14:0	2.40	2.43	0.245	N.S.
*t*C14:1n-5	0.23	0.18	0.051	N.S.
*c*C14:1n-5	0.26	0.33	0.070	N.S.
C15:0	0.58	0.56	0.045	N.S.
C16:0	24.44	23.98	1.208	N.S.
C16:1n-9	0.81	0.69	0.120	N.S.
C16:1n-7	1.62	2.38	0.251	<0.05
C17:0	1.25	1.14	0.133	N.S.
C17:1n-9	0.94	0.92	0.111	N.S.
C18:0	22.68	16.95	1.547	<0.01
Total C18:1	32.07	35.56	4.316	N.S.
C18:2n-6	3.42	4.07	0.727	N.S.
γ-C18:3 n-6	2.77	0.65	1.712	N.S.
α-C18:3n-3	0.46	0.53	0.055	N.S.
C20:0	0.15	0.18	0.076	N.S.
*c*9, *t*11/*t*7, *c*9 CLA	0.42	0.56	0.078	N.S.
C20:1	0.08	0.04	0.046	N.S.
C20:3n-6	0.27	0.08	0.087	0.05
C20:4n-6	0.78	0.80	0.282	N.S.
C20:5n-3	0.10	1.60	0.350	<0.001
C22:5n-3	0.36	0.77	0.103	<0.01
C22:6n-3	0.06	0.55	0.083	<0.001
Total CLA	0.42	0.56	0.078	N.S.
Total MUFA	36.01	40.08	2.232	N.S.
Total PUFA	5.86	8.97	1.369	<0.05
Total SFA	51.54	45.27	2.235	<0.05
Ratio PUFA to SFA	0.12	0.21	0.039	<0.05
n-6 PUFA	4.46	4.95	1.067	N.S.
n-3 PUFA	0.98	3.45	0.506	<0.001
Ratio n-3 to n-6 PUFA	4.60	1.48	0.639	<0.001

FAME = fatty acid methyl esters; PUFA = polyunsaturated fatty acid; CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid; N.S. = not significant.

with plasma, displaying strong-to-medium positive relationships in terms of the concentration of total SFA, total n-6 PUFA and total n-3 PUFA. Furthermore, this trend also extended to both the ratio of PUFA to SFA ($\beta_1 = 0.56 \pm 0.167$, $\beta_0 = 0.56$, $R^2 = 0.61$; $P < 0.05$) and to the ratio of n-6 to n-3 PUFA ($\beta_1 = 0.15 \pm 0.054$, $\beta_0 = 0.24$, $R^2 = 0.52$; $P < 0.05$), both important indices of the fatty acid quality of beef. Significant relationships were also observed between the concentration of total n-3 PUFA in plasma and that of the muscle and adipose tissue of heifers (muscle: $\beta_1 = 0.12 \pm 0.013$, $\beta_0 = 0.60$, $R^2 = 0.91$; $P < 0.001$, adipose: $\beta_1 = 0.01 \pm 0.003$, $\beta_0 = 0.30$, $R^2 = 0.64$; $P < 0.01$). A strong correlation was also detected in the ratio of n-6 to n-3 in plasma and muscle tissue of heifers ($\beta_1 = 0.53 \pm 0.089$, $\beta_0 = -0.31$, $R^2 = 0.83$; $P < 0.001$).

Table 5
Regression coefficients (β_1) ± SEM for the fatty acid composition of plasma relative to that of muscle tissue, adipose tissue and liver tissue for Charolais crossbred heifers receiving the control ($n = 10$) or n-3 LC-PUFA ($n = 10$) diets.

Fatty acid	Muscle	Adipose	Liver
C18:0	−0.55 ± −0.439	−0.09 ± 0.213	−0.83 ± 0.393[a]
C20:4n-6	0.11 ± 0.369	0.07 ± 0.041	5.32 ± 0.934***
C20:5n-3	0.08 ± 0.011***	0.01 ± 0.001***	0.75 ± 0.095***
C22:5n-3	0.35 ± 0.183[a]	0.04 ± 0.019[a]	3.05 ± 1.984
C22:6n-3	3.12 ± 0.832**	0.02 ± 0.004**	3.67 ± 0.841**
Total MUFA	1.09 ± 0.783	0.02 ± 0.414	−0.51 ± 0.300
Total PUFA	0.14 ± 0.131	−0.02 ± 0.024	0.31 ± 0.183
Total SFA	0.32 ± 0.307	−0.31 ± 0.229	0.56 ± 0.156**
Ratio PUFA to SFA	0.08 ± 0.095	−0.03 ± 0.028	0.56 ± 0.167*
n-6 PUFA	0.09 ± 0.156	0.01 ± 0.035	1.16 ± 0.407*
n-3 PUFA	0.12 ± 0.013***	0.01 ± 0.003**	1.18 ± 0.203***
Ratio n-6 to n-3 PUFA	0.53 ± 0.089***	0.19 ± 0.149	0.15 ± 0.054*

PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid.
$P < 0.05$*, $P < 0.01$**, $P < 0.001$***.
[a] Approaching significance $P = 0.05$–0.10.

Discussion

If sufficient n-3 LC-PUFA is supplied in the diet, such as when supplementary FO is included, a proportion will escape ruminal hydrogenation, but if substantial quantities are to be delivered to tissues, protection is required. Indeed, the extent to which unprotected EPA and DHA from FO have undergone ruminal hydrogenation have been reported at 0.92 and 0.91, respectively, in steers (Scollan et al., 2001). A potential limitation in the design of the current study was the omission of an unprotected FO treatment group. While this may be a valid criticism given the resources available and our prior knowledge of the effect of the unencapsulated (muscle; Kenny et al., 2007) and encapsulated (reproductive tissues; Childs et al., 2008b) on certain tissue fatty acid concentrations, we decided to use the encapsulated product to explore the questions of interest to the current study. Needless to say that the muscle concentrations of n-3 LC-PUFA generated using the encapsulated product in the current study were significantly higher than we attained using the same unencapsulated FO in previous work (Kenny et al., 2007).

It has been reported that supplementation with a ruminally protected form of n-3 LC-PUFA did not affect DMI or ADG (Childs et al., 2008a; Childs et al., 2008b). Previous investigations have reported that the inclusion of FO in the ruminant diet at concentrations in excess of 2 % DM can result in a reduction in DMI and ADG (Mattos et al., 2002). As inclusion rate was at 2 % DM, in the current study, our findings agree with those of Mattos et al.

Provision of the n-3 LC-PUFA supplement resulted in significant increases in the concentration of total PUFA found in plasma, adipose, liver and muscle tissue. These increases were mediated through increases in the concentration of total n-3 PUFA and in particular the n-3 LC-PUFA, EPA and DHA, although increases in the concentration of CLA also contributed. The increases in total n-3 PUFA recorded here correspond well with other studies where fish meal or FO was supplied in the diet of cattle (Childs et al., 2008b; Ballou et al., 2009). It is currently estimated that in Westernised society the approximate intake of the n-3 LC-PUFA is approximately 0.1–0.5 g/d. This is lower than the 0.85–0.9 g/d intakes which prevention trials and dietary studies have estimated might be required for n-3 LC-PUFA to mediate any potential beneficial effects (Williams and Burdge, 2006). The results of our study suggest that the *Longissimus dorsi* muscle and liver from heifers receiving the n-3 LC-PUFA diet would achieve the levels of PUFA intake required to promote health benefits, according to Williams and Burdge (2006). Indeed, in a 100 g serving of uncooked liver from heifers receiving the n-3 LC-PUFA, approximately 0.7 g of EPA and 0.5 g of DHA would be provided, relative to heifers on the control diet whose liver contained only 0.1 g and 0.2 g of the respective n-3 LC-PUFA. A similar trend was also observed in muscle of heifers receiving n-3 LC-PUFA with 0.1 g of EPA and 0.03 g of DHA provided by an uncooked 100 g serving compared to the control with only trace amounts of each n-3 LC-PUFA. Although small amounts, the 0.1 g of EPA and 0.03 g of DHA would provide 53 % of the recommended daily intake of EPA and DHA according to Givens and Gibbs (2006).

The n-3 LC-PUFA diet also reduced the SFA content of the plasma, liver and muscle tissues. These observations correspond well with those of others who reported reductions in the SFA content (primarily palmitic and stearic acid) when n-3 PUFA was included in bovine diets (Ballou et al., 2009). These reductions in SFA have been attributed to the increased competition of PUFA with SFA for uptake in the intestine and into the membrane phospholipids, or as a result of their suppressive

effects on lipogenesis in tissues such as liver (Mashek et al., 2002; Wortman et al., 2009).

The respective increase and decrease in total PUFA and total SFA concentrations, caused by the n-3 LC-PUFA diet, significantly altered the ratio of PUFA to SFA in the plasma, adipose, muscle and liver. Indeed, the ratio of PUFA to SFA was increased by 1.14-fold in the adipose tissue and by 1.75-fold in the muscle tissue of heifers receiving the n-3 LC-PUFA diet. In humans, low ratios of PUFA to SFA in the diet have been associated with increased atherosclerotic risk (Muller et al., 2003). As a result, the ratio of PUFA to SFA has become one of the most important indices of the fatty acid quality of human diet, with the British Department of Health (1994) recommending a ratio of PUFA to SFA in the human diet of 0.45. Of the tissues assayed in the current study, only the liver tissue displayed a PUFA to SFA ratio which was greater than the 0.45 recommendation; however, significant improvements were also observed in the muscle tissue (0.12 vs 0.21 for control vs LC-PUFA diets, respectively).

In addition to the changes which the n-3 LC-PUFA diet evoked on the concentrations of total PUFA, n-3 PUFA and total SFA, and on the ratio of PUFA to SFA, significant reductions in the concentration of total n-6 PUFA were also observed in the plasma and liver tissue. Such reductions are attributed to the competition between n-3 LC-PUFA and n-6 PUFA for intestinal absorption and incorporation into the plasma and tissues, or alternatively to the interference of n-3 PUFA with the endogenous synthesis of eicosatrienoic (n-6) and arachidonic (n-6) acids (Li et al., 2003).

The increases in the concentration of n-3 LC-PUFA coupled with the reductions in n-6 PUFA as a result of provision of the n-3 LC-PUFA diet led to significant reductions in the ratio of n-6 to n-3 PUFA. In humans, reducing the ratio of dietary n-6 to n-3 PUFA has been associated with reducing the pathogenesis of diseases such as cancer, cardiovascular disease and diabetes (Simopoulos, 2006). In the current study, the ratio n-6 to n-3 PUFA in the muscle and liver tissue was reduced from 4.6:1 to 1.48:1 and 1.96:1 to 0.25:1, respectively. These ratios are significantly lower than the ratio of 4:1 associated with a 70 % reduction on total mortality from cardiovascular disease, the ratio of 2.5:1 associated with the reduced proliferation of colorectal cancer, the ratio of 2:1 associated with suppression of inflammatory rheumatoid arthritis and the ratio of 5:1 which beneficially affected asthma sufferers (Simopoulos, 2002).

In addition to the direct transfer of dietary n-3 LC-PUFA to the plasma and tissues of beef heifers, the diet also impacted on the endogenous production of bioactive *c*9, *t*11/*t*7, *c*9 CLA. Inclusion of n-3 LC-PUFA in the diet of ruminants increases the concentration of CLA in the plasma and milk of cows as recently reviewed by Moallem (2018). Such increases are related to the increased concentration of long-chain PUFA which modify the microbial population within the rumen and which inhibit the reductase enzymes that catalyse the conversion of vaccenic acid to stearic acid (Loor et al., 2005). This vaccenic acid is subsequently absorbed and converted to CLA at a tissue level via the activity of the enzyme Δ^9-desaturase. As the concentration of this enzyme naturally found in the adipose tissue is high, it is possible that the increased concentration of CLA and low concentration of vaccenic acid found in adipose tissue during the current study is a result of this endogenous CLA production system (Hennessy et al., 2007). The increase in arachidonic acid on the n-3 LC-PUFA diet compared to the control is in contrast to the previous reports that dietary FO reduces n-6 fatty acids and that arachidonic acid is reduced due to competition from EPA and DHA (Raes et al., 2004). Both EPA and DHA were also increased on the n-3 LC-PUFA diet so it remains unclear what promoted such an increase in arachidonic acid.

The impact of the n-3 LC-PUFA diet on the fatty acid composition of the plasma and adipose tissue was at its greatest following 10 days provision of diet. Thereafter, the effect of dietary supplementation plateaued or was reduced. This observation would appear to suggest that the provision of an n-3 LC-PUFA supplement in the diet 10 days before slaughter yields the highest possible reward in terms of improving the fatty acid composition or at least in adipose tissue. Wistuba et al. (2007) reported similar increases in PUFA composition of subcutaneous adipose when steers were supplemented with FO, from samples collected at day 71 or 72. There are few studies that examine the minimum supplementation period necessary for appreciable changes to the fatty acid profile. In the adipose tissue, the decrease in PUFA after 10 days of supplementation was unexpected. Indeed, it has been reported that EPA and DPA decreased following supplementation of steers with soya bean and linseed oil compared to a control (Carvalho et al., 2017); however, these effects were observed in muscle and not in the adipose tissue. The decrease in adipose PUFA observed in the current study requires further investigation to ascertain the cause. Typically, the supplementation period lasts between 70 and 90 days (Carvalho et al., 2017; Marino et al., 2019) which resembles the length of a typical finishing period for beef cattle.

Direct determination of the impact of diet on the quality of the fatty acids found in beef can be difficult due to the invasiveness of biopsies, which can also reduce the marketability of meat. As a potential mitigation strategy to the above, we have highlighted the strong positive relationship between the concentration of the n-3 LC-PUFA, EPA (n-3) and DHA (n-3), in the plasma with that found in the muscle, adipose and liver tissue of heifers in this study. This association was further reflected in the strong relationship between the concentration of total n-3 PUFA in plasma and in muscle, adipose and liver tissues. These data suggest that plasma concentrations can successfully be used to predict the concentration of n-3 PUFA in *longissimus dorsi* and other tissues in a simple non-invasive manner and is consistent with another study from our laboratory (Childs et al., 2008b). Similarly, plasma may also prove a valuable tool in determining the ratio of n-6 to n-3 PUFA in the muscle and liver and the ratio of PUFA to SFA found in the liver of heifers receiving the n-3 PUFA diet, with strong relationships observed between the tissues and the plasma samples. As these ratios are important indicators of the overall fatty acid quality of meat, such data may be extremely important for the early assessment and monitoring of nutritional strategies to improve the nutraceutical composition of beef.

In conclusion, the inclusion of a ruminally protected n-3 LC-PUFA supplement in the diet of beef cattle can be utilised to improve the overall fatty acid composition of beef. These improvements were seen in the plasma, adipose, liver and *Longissimus dorsi* of heifers receiving the n-3 PUFA diets, where two of the indices of the fatty acid quality of meat, namely the ratio of PUFA to SFA and the ratio of n-6 to n-3 PUFA, were increased and decreased, respectively. In the plasma and tissues, provision of the n-3 PUFA diet resulted in significant increases in the concentration of the health promoting fatty acids EPA and DHA. This study has also demonstrated how plasma fatty acid measurements can assist in predicting PUFA to SFA and n-6 to n-3 PUFA ratios in important bovine tissues.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100039

Ethics approval

The work described in this manuscript was carried out in accordance with local and national animal ethics recommendations.

Data and model availability statement

The data presented in this manuscript are not deposited in an official repository. Data are available from the corresponding author upon reasonable request.

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Author contributions

DAK and CS conceived the study and oversaw statistical analysis and manuscript preparation. AH conducted the laboratory analysis. CJB assisted with statistical analysis and manuscript preparation. PR and RD assisted with manuscript preparation.

Declaration of interest

The authors have no conflicts of interest to disclose.

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