Chapter 7

Increasing the Concentration of ω-3 Fatty Acids in the Meat and Mammary Tissue of Beef Heifers

Abstract

The objective of this study was to investigate whether the fatty acid composition of beef could be improved by the provision of a ruminally protected ω-3 PUFA supplement in the animals diet pre-slaughter. Twenty Holstein Friesian heifers were blocked by live weight and body condition score and randomly assigned to one of two isolipid dietary treatments (n = 10 per diet) supplemented with either palmitic acid as a control or a ω-3 PUFA supplement for a 91 day period. Animal feed intake and liveweights were recorded on a weekly basis, while body condition score was recorded fortnightly. Plasma sampling and adipose biopsies were undertaken on days 0, 10, 35 and 91 of the supplementation period, while samples of muscle, liver and mammary tissue were taken following the slaughter of the animals on day 91. The fatty acid composition of the feed, plasma and tissue samples were determined by gas liquid chromatography. Dietary lipid source did not affect average daily gain or body condition score. Diet x day of sampling interactions were observed in the ratio of ω-6 to ω-3 PUFA ($P < 0.001$) and increases in the ratio of PUFA to saturated fatty acids (SFA) ($P < 0.01$) in the plasma of heifers on the ω-3 PUFA enriched diet, while treatment diet x day of sampling interactions were observed in the ratio of PUFA to SFA in the adipose tissue ($P < 0.01$). Additionally, the ω-3 PUFA enriched diet also resulted in reductions in the ratio of ω-6 to ω-3 PUFA in the liver ($P < 0.001$), muscle ($P < 0.001$) and mammary tissue ($P < 0.001$) and significant increases in the ratio of PUFA to SFA in the liver ($P < 0.001$), muscle ($P < 0.05$) and mammary tissue ($P < 0.001$). There were positive relationships for ω-6 to ω-3 PUFA ratio between plasma and liver ($\beta_1 = 0.15 \pm 0.054$, $R^2 = 0.52$, $P < 0.01$) and also between plasma and muscle ($\beta_1 = 0.53 \pm 0.089$, $P < 0.001$).

7.1 Introduction

There is clear evidence to suggest that consumption of ω-3 polyunsaturated fatty acids (PUFA) by humans has the potential to positively influence health, with direct relationships observed between increased ω-3 PUFA intake and reduced prevalence of cardiovascular disease, inflammatory diseases, osteoporosis and cancer (Breslow, 2006; Chapkin *et al.*, 2008; Colussi *et al.*, 2007; Fernandes *et al.*, 2008). Furthermore, it has been reported that these fatty acids play an important role in brain development, vision and the prevention of physiological disorders (Li *et al.*, 2003). The predominant sources of ω-3 PUFA are α-linolenic acid, from green leafy plants and oilseeds such as linseed, rapeseed and soybean, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), from fish and marine algae (Li *et al.*, 2003). However, in recent years, as a result of the increased "Westernisation" of the human diet, the dietary intake of these fatty acids has fallen dramatically, resulting in the emergence of a ratio of ω-6 to ω-3 PUFA in the diet, which is considered detrimental to human health (Simopoulos, 2003; Simopoulos, 2008). Indeed, it is estimated that in Western society the ratio of ω-6 to ω-3 PUFA is currently 15-20:1, a value which is substantially greater than that consumed by our paleolithic ancestors (1:1) and the 4:1 ratio which is currently believed to be optimal (Simopoulos, 2003; Simopoulos, 2008). This trend of increased ω-6 PUFA consumption and reduced ω-3 PUFA consumption has been directly associated with the increased risk of cancer, diabetes, and cardiovascular disease (Bagga *et al.*, 1997; Simopoulos, 2006; Simopoulos, 2008).

In a recent Australian study, it was reported that 28.2% of that population's long chain ω-3 PUFA intake was derived from beef and lamb products (Howe *et al.*, 2006; Howe *et al.*, 2003). These observations are reflective of the increased

importance of red meat as a contributor to the ω-3 PUFA intake in Western society. While red meat may play an important role in the dietary intake of health promoting ω-3 PUFA, it has also been associated with the increased pathogenesis of diseases such as colorectal cancers, diabetes and cardiovascular disease (Fung *et al.*, 2004; Tavani *et al.*, 2000; van Dam *et al.*, 2003). The reasons behind the increased risk of disease are multifaceted; however, the high concentration of saturated fatty acids (SFA) and *trans* fatty acids found in red meat have in particular been associated with the increased prevalence of these conditions (Cross *et al.*, 2008; Hu *et al.*, 1999; Woodside *et al.*, 2008). Indeed, the associated risk of dietary SFA intake and certain diseases have led organisations such as the British Department of Health to recommend a dietary intake ratio of PUFA to SFA of 0.45 for the British diet as a whole (DepartmentofHealth, 1994).

A potential answer to reduce the negative aspects of the fatty acid composition of red meat may lie in its enrichment with ω-3 PUFA of marine origin such as EPA and DHA. Indeed, such strategies have been successfully used to reduce the ratio of ω-6 to ω-3 PUFA and the ratio of PUFA to SFA of red meat and poultry, along with increasing the concentration of health promoting long chain PUFA and conjugated linoleic acids (CLA) (Dhiman *et al.*, 2005; Mach *et al.*, 2006; Rymer & Givens, 2005). In one such study, the concentration of EPA and DHA found in the phospholipids of steers was increased two-fold by the inclusion of fish oil in their diet without significantly impacting on the animals feed intake, growth rate, cold carcass weight or carcass fatness (Scollan *et al.*, 2001). In addition to the potential health benefits which an EPA/DHA enriched red meat may offer the consumer, the inclusion of ω-3 PUFA in the animal's diet has also been reported to benefit the overall health and reproductive performance of the animal (Ambrose *et al.*, 2006; Lessard *et al.*, 2003; Mattos *et al.*, 2001).

To successfully enrich beef with ω-3 PUFA such as EPA and DHA, the supply of ω-3 PUFA must first escape ruminal biohydrogenation. Indeed, without protection these PUFA would undergo conversion to SFA, which following absorption in the intestine, would be undesirably deposited in the meat and adipose tissue of the animal (Hennessy *et al.*, 2007; Mach *et al.*, 2006). Using ruminally-protected oils, this process can, in part, be overcome resulting in much lower bioconversion of PUFA to SFA and thus greater concentrations of PUFA in the meat and adipose tissue.

The objective of the current study was to evaluate the potential of enriching the ω-3 PUFA content of the meat and adipose tissue of Holstein Friesian heifers by supplementing the animal's diet with the fatty acids EPA and DHA delivered in the form of a ruminally protected ω-3 PUFA dietary supplement. The ratio of ω-6 to ω-3 PUFA, and the ratio of PUFA to SFA in the meat and adipose tissue was also assessed following dietary supplementation with ruminally protected ω-3 PUFA. Finally, using regression analysis we attempted to determine if the plasma of Holstein Friesian heifers receiving the ω-3 PUFA enriched diet could be successfully utilised to predict the fatty acid composition of the adipose, liver muscle and mammary tissues.

7.2 Materials and methods

7.2.1 Animals, diets and feeding regime

Reproductively normal crossbreed heifers (n = 20) with a mean ± S.E.M. live weight of 473.14 ± 5.41 kg and a body condition score (BCS) 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 animals were individually fed a barley straw (1.50 kg dry matter, DM) and concentrate (6 kg DM) based ration, supplemented with either palmitic acid (Palmit 801; SFA 151 g) as a control or with 334 g of a partially rumen protected, ω-3 PUFA supplement. Both supplements were provided by Trouw Nutrition, Belfast, Northern Ireland. The ω-3 PUFA supplement was estimated to provide approximately 140 g/(head/day), of the long chain ω-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) based on the results of an earlier study (Childs *et al.*, 2008b). The ingredient composition and chemical analysis of the concentrates and straw are presented in **Table 7.1**. The fatty acid compositions of the diets are presented in **Table 7.2**. All diets were formulated to be isonitrogenous (14% crude protein in total diet) and isolipid.

Each morning at 09:00 h the animals received their entire daily allocation of supplementary lipid in the form of a 1.0 kg DM bolus feed mixed with 1.5 kg DM of a 24% crude protein ration to balance the low crude protein of the bolus diet. At 12:00 h, animals were offered the remainder of their daily concentrate allocation in the form of 3.50 kg DM of a second balancer ration (13.08% crude protein; **Table 7.1**) together with 1.50 kg DM straw. All animals were individually fed using an electronic feeding system (Calan Inc., Northwood, New Hampshire 03261, USA).

Table 7.1 Ingredient composition (g/kg as fed) and chemical analysis (expressed as g/kg of DM unless otherwise stated) of the experimental rations (control and ω-3 PUFA) and the balancer rations (Bal 1 and Bal 2) and straw which were common to both experimental groups.

	Control	ω-3 PUFA	Bal-1	Bal-2	Straw
Barley	430	330	225	420	-
Soya Bean Meal (48%)	4	56	430	110	-
Molassed Sugar Beet Pulp	415	280	230	421	-
Molasses (Cane)	-	-	45	20	-
Ground Limestone	-	-	15	7	
Vitamin Mineral Mix [a,b]	-	-	50	20	-
Di-calcium Phosphate	-	-	4	1	-
Salt	-	-	1	1	-
Palmit 80 Prills [c]	151	-	-	-	-
ω-3 PUFA Supplement	-	334	-	-	-
DM (g/kg)	880	893	878	890	917
Crude Protein	86.6	86.2	237.9	130.8	43.4
Crude Fibre	86.3	74.8	63.7	85.1	406.8
Ash	45.7	175.2	96.5	73.6	38.6
Ether Extract	10.86	14.78	1.26	1.14	0.81
Gross Energy (MJ/kg DM)	17.03	15.52	15.23	15.29	16.13

[a] Premix supplied per kilogram of supplement: 22,400 IU of vitamin A; 5600 IU of vitamin D3; and 700 mg of vitamin E as alpha tocopherol.

[b] Premix supplied per kilogram of supplement: 2.1 mg selenium as sodium selenite; 22.4 mg iodine as calcium iodate; 87.5 mg copper as cupric sulphate; 280 mg zinc as zinc oxide; 175 mg manganese as manganese oxide; and 7 mg cobalt as cobalt carbonate.

[c] Minimum content of palmitic acid (C16:0) = 80%.

7.2.2 Dry matter intake and animal performance

Daily intake of straw and concentrate was measured for each heifer during the experimental period. All animals had continuous free access to clean drinking water. Animals were weighed weekly and their BCS assessed fortnightly on a scale of 1–5. 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 was used to determine average daily gain.

7.2.3 Feed sampling and analysis

Weekly samples of straw and concentrates were stored at -20^{o}C until analysed for DM, crude protein, crude fibre, ether extract, crude oil, ash and gross energy. Samples were milled through a 1 mm screen using a Christy and Norris hammer mill (Christy and Norris Process Engineers Ltd., Chelmsford, England). DM was determined by oven drying at 104^{o}C for a minimum of 16 h. Ash was determined on all materials after ignition of a known weight of ground material in a muffle furnace (Nabertherm, Bremen, Germany) at 550^{o}C for 4 h. Crude fibre was determined on all samples using a Fibertec extraction unit (Tecator, Hoganas, Sweden) according to the method of Van Soest *et al.* (1991). Crude protein (total nitrogen x 6.25) was determined using the method of Sweeney (1989), using a Leco FP 528 nitrogen analyser (Leco Instruments, UK Ltd., Newby Road, Hazel Grove, Stockport, SK7 5DA Cheshire). Ether extract was determined using a Sortex instrument (Tecator, Hoganas, Sweden), while the gross energy of the samples was determined using a Parr 1201 oxygen bomb calorimeter (Parr, Moline, IL, USA).

Table 7.2 Relative fatty acid concentration of diets fed (mean ± S.E.M).

	Diet			
Fatty acid	Control	ω-3 PUFA	Bal-1	Bal-2
Myristic (C14:0)	3.87 ± 0.21	0.88 ± 0.06	0.46 ± 0.02	0.39 ± 0.05
Palmitic (C16:0)	78.35 ± 0.80	8.71 ± 0.63	20.75 ± 0.10	22.09 ± 0.16
Stearic (C18:0)	1.06 ± 0.03	4.85 ± 0.02	2.76 ± 0.07	2.65 ± 0.02
Oleic (C18:1)	6.97 ± 0.33	11.02 ± 0.11	11.39 ± 0.13	10.89 ± 0.20
Linoleic (C18:2)	6.95 ± 0.70	7.18 ± 0.44	49.46 ± 0.13	48.98 ± 0.01
α-linolenic (C18:3)	0.63 ± 0.07	1.87 ± 0.12	6.16 ± 0.03	6.15 ± 0.04
Eicosatrienoic (C20:3)	-	0.19 ± 0.06	-	-
Arachidonic (C20:4)	-	1.85 ± 0.07	-	-
EPA (C20:5)	-	24.99 ± 0.41	0.10 ± 0.07	0.03 ± 0.02
DPA (C22:5)	-	2.96 ± 0.09	-	-
DHA (C22:6)	-	11.93 ± 0.34	-	-
ω-3 family	0.63 ± 0.07	41.76 ± 0.66	6.26 ± 0.01	6.18 ± 0.01
ω-6 family	6.95 ± 0.70	9.22 ± 0.42	49.46 ± 0.13	48.98 ± 0.01
Ratio ω-6 to ω-3	11.18 ± 0.19	0.22 ± 0.01	7.91 ± 0.10	7.92 ± 0.02

[a] C18:3 + C20:5 + C22:5 + C22:6.

[b] C18:2 + C20:3 +C20:4.

7.2.4 Plasma sampling

Plasma was collected by jugular venipuncture under license in accordance with the European Community Directive, 86-609-EC. Plasma sampling was performed on day 0, to establish baseline concentrations of the fatty acids and on days 10, 35 and 91 of the supplementation period. Sampling took place in the morning immediately prior to the commencement of the daily feeding protocol. On collection, samples were immediately stored in ice-water and centrifuged at 1500 x g at 4^{o}C for 15 min. Plasma was harvested and stored at -20^{o}C as described by Childs *et al.* (2008b).

7.2.5 Tissue sampling

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 (1993). Tissue samples (2 g) from the liver, mammary gland and muscle tissue were taken at slaughter. Heifers were slaughtered in an EU licensed abattoir (Martin Jennings Wholesale Ltd., Neale Road, Ballinrobe, Co. Mayo, Ireland) on day 91. All tissue samples were collected into sterile containers within 20 min of slaughter, flash frozen in liquid nitrogen and stored at -80^{o}C pending fatty acid analysis.

7.2.6 Fatty acid analysis of feeds, plasma and tissue samples

Total lipids were extracted from 6 g of feed sample, 1 ml of plasma, 1 g of liver, muscle or mammary tissue and from 200 mg of adipose tissue, using chloroform methanol (2:1, v/v) as described previously (Folch *et al.*, 1957). The samples were then heated at 45^{o}C under a steady flow of nitrogen to evaporate the remaining chloroform. The remaining lipid was then stored at -20^{o}C until methylated. Methylation of all samples was carried out by *in situ* trans-esterification with 0.5 N

sodium methoxide followed by 14% boron trifluoride in methanol as described by Park & Goins (1994). The fatty acid methyl esters (FAME) were separated by gas liquid chromatography (GLC) as described previously (Childs *et al.*, 2008b).

7.2.7 Statistical analysis

All data were checked for adherence to a normal 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 dry matter intake (DMI), average daily gain, plasma and adipose fatty acids were analysed using repeated measures ANOVA (PROC MIXED, SAS v9.1, 2002) 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 (AIC) for models run under compound symmetry, unstructured, autoregressive or Toeplitz variance-covariance structures. The model with the lowest AIC was chosen. The CONTRAST (for orthogonal contrasts) statements of SAS (v9.1, 2002) were 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, SAS) were also used as appropriate for univariate and multiple regression analyses respectively. P values < 0.05 were accepted as being statistically significant while those < 0.10 were considered to indicate a tendency towards statistical significance.

7.3 Results

7.3.1 Dry matter intake and animal performance

All animals consistently consumed their entire daily dietary allocation of feed and consequently there was no effect of diet on DMI. Similarly, diet did not affect average daily gain, with animals on the control and ω-3 PUFA enriched diet gaining 0.81 ± 0.03 kg and 0.79 ± 0.03 kg, respectively, as per formulation objectives. There was also no effect of diet on BCS.

7.3.2 Temporal fatty acid composition of plasma and adipose tissue

Fatty acid profile and fatty acid content of plasma and adipose tissue are presented in **Tables 7.3** and **Table 7.4**, respectively**.** The most abundant fatty acids identified in the plasma were palmitic acid (13.21-11.55%), stearic acid (14.17-22.09%), oleic acid (4.50-9.61%), and linoleic acid (19.31-22.59%), with EPA constituting a considerable concentration (9.53%) of the plasma fatty acids from animals receiving the ω-3 PUFA enriched diet **(Table 7.3)**. Animals receiving the ω-3 PUFA enriched diet were found to have lower concentrations of palmitic acid ($P < 0.01$), stearic acid ($P < 0.01$), oleic acid ($P < 0.001$), eicosatrienoic acid ($P < 0.001$), and arachidonic acid ($P < 0.001$) in their plasma relative to animals on the control diet. Increases in the concentration of vaccenic acid ($P < 0.01$), *t*10, *c*12 CLA ($P < 0.05$), EPA ($P < 0.001$), docosapentaenoic acid (DPA) ($P < 0.01$), and DHA ($P < 0.001$) were found in the plasma of animals receiving the ω-3 PUFA enriched diet relative to those on the control diet. Significant diet x day of sampling interactions were observed for palmitic acid ($P < 0.01$), stearic acid ($P < 0.05$) and linoleic acid ($P < 0.05$) whose concentrations were reduced following 10 days exposure to the ω-3 PUFA enriched diet, subsequently increasing thereafter **(Figure 7.1)**.

Table 7.3 Fatty acid composition (g/100g FAME) of plasma from Holstein Friesian heifers fed an ω-3 PUFA enriched or control diet

	Diet			Day					*P* value		
Fatty acid	Control	ω-3 PUFA	SED	Day 0	Day 10	Day 35	Day 91	SEM	DIET	DAY	DIET x DAY
Undecanoic (C11:0)	0.15	0.16	0.026	0.09	0.10	0.13	0.31	0.017	N.S.	<.0001	0.0307
Lauric (C12:0)	0.32	0.31	0.031	0.47	0.28	0.19	0.32	0.035	N.S.	0.0005	N.S.
Lauroleic (C12:1)	0.01	0.00	0.005	0.02	0.00	0.00	0.00	0.005	N.S.	N.S.	N.S.
Tridecanoic (C13:0)	0.09	0.09	0.023	0.17	0.07	0.00	0.13	0.018	N.S.	<.0001	N.S.
Myristic (C14:0)	4.54	4.27	0.316	3.20	5.12	3.80	5.50	0.019	N.S.	0.0002	N.S.
Myristelaidic (C14:1 *t*)	0.56	0.58	0.081	0.66	0.66	0.66	0.31	0.080	N.S.	<.0001	N.S.
Myristoleic (C14:1 *c*)	0.44	0.42	0.043	0.48	0.36	0.40	0.49	0.031	N.S.	0.0005	0.0017
Palmitic (C16:0)	13.08	11.69	0.375	11.56	11.82	12.95	13.22	0.379	0.0023	0.0030	0.0016
Palmitelaidic (C16:1 *t*)	0.02	0.06	0.048	0.01	0.04	0.07	0.05	0.035	N.S.	0.0003	0.0055
Palmitoleic (C16:1 *c*)	0.76	0.79	0.046	0.93	0.75	0.72	0.71	0.044	N.S.	0.0073	N.S.
Heptadecenoic (C17:1)	0.53	0.46	0.038	0.26	0.20	0.18	1.34	0.034	N.S.	<.0001	N.S.
Stearic (C18:0)	18.02	16.59	0.456	17.07	14.17	15.89	22.09	0.491	0.0071	<.0001	0.0309
Vaccenic (C18:1 *t*11)	0.79	2.16	0.173	1.08	1.15	1.30	2.37	0.138	<.0001	0.0068	<.0001
Oleic (C18:1)	7.17	5.53	0.406	9.61	4.50	4.54	6.76	0.324	0.0012	<.0001	N.S.
Linoelaidic (C18:2)	0.00	0.01	0.007	0.00	0.01	0.00	0.00	0.000	N.S.	N.S.	N.S.
Linoleic (C18:2)	21.41	19.57	1.146	19.93	20.14	19.31	22.59	0.800	N.S.	<.0001	0.0238
α-linolenic (C18:3)	1.43	1.64	0.126	2.26	1.23	1.44	1.22	0.082	N.S.	<.0001	N.S.
c 9, *t* 11 CLA	0.10	0.11	0.022	0.15	0.09	0.11	0.06	0.015	N.S.	0.0011	N.S.
t 10, *c* 12 CLA	0.09	0.13	0.014	0.13	0.15	0.11	0.06	0.022	0.0133	N.S.	N.S.
Eicosatrienoic (C20:3)	1.82	0.67	0.118	1.79	1.03	0.94	1.23	0.083	<.0001	<.0001	<.0001
Arachidonic (C20:4)	2.44	2.12	0.117	2.18	2.11	2.18	2.66	0.078	0.0158	0.0001	0.0019
EPA (C20:5)	0.77	9.53	0.436	0.88	5.31	6.71	7.71	0.327	<.0001	<.0001	<.0001
DPA (C22:5)	0.86	1.23	0.105	1.10	0.90	0.91	1.26	0.067	0.0030	0.0094	0.0031
DHA (C22:6)	0.34	1.13	0.058	0.31	0.66	0.87	1.11	0.044	<.0001	<.0001	<.0001
Total CLA	0.19	0.24	0.037	0.27	0.23	0.22	0.13	0.031	N.S.	0.05	N.S.
Total monounsaturates	10.56	9.91	0.577	13.09	7.74	7.91	12.19	0.489	N.S.	<.0001	N.S.
Total *trans* fatty acids	1.81	2.96	0.209	2.02	2.09	2.25	3.18	0.190	<.0001	0.0184	0.0132
Total PUFA	29.40	36.17	1.504	28.72	31.63	32.58	38.20	1.020	0.0005	0.0001	0.03
Total SFA	40.29	37.56	0.755	38.35	37.26	36.58	43.51	1.060	0.0027	0.0039	N.S.
Ratio PUFA to SFA	0.72	0.96	0.039	0.75	0.86	0.91	0.85	0.033	<.0001	0.0025	0.002
ω-6 fatty acids	25.71	22.30	1.153	23.90	23.27	22.43	26.41	0.818	0.0105	<.0001	0.0045
ω-3 fatty acids	3.38	13.45	0.568	4.55	8.10	9.93	11.07	0.387	<.0001	<.0001	<.0001
Ratio ω-6 to ω-3	8.10	2.27	0.539	5.29	5.68	4.87	4.90	0.373	<.0001	0.0286	<.0001

Table 7.4 Fatty acid composition (g/100g FAME) of adipose tissue from Holstein Friesian heifers fed an ω-3 PUFA enriched or control diet

	Diet			Day					*P* value		
Fatty acid	Control	ω-3 PUFA	SED	Day 0	Day 10	Day 35	Day 91	SEM	DIET	DAY	DIET x DAY
Lauric (C12:0)	0.10	0.12	0.009	0.10	0.11	0.11	0.12	0.006	N.S.	N.S.	N.S.
Myristic (C14:0)	2.91	3.21	0.170	2.85	2.96	3.21	3.23	0.006	N.S.	0.0274	N.S.
Myristelaidic (C14:1 *t*)	0.30	0.29	0.020	0.29	0.28	0.30	0.31	0.012	N.S.	N.S.	N.S.
Myristoleic (C14:1 *c*)	1.48	1.78	0.158	1.51	1.62	1.75	1.65	0.098	N.S.	N.S.	N.S.
Pentadecanoic (C15:0)	0.56	0.59	0.034	0.55	0.52	0.60	0.63	0.023	N.S.	0.0094	N.S.
Palmitic (C16:0)	24.83	24.61	0.778	24.95	25.42	24.43	24.08	0.451	N.S.	0.0008	N.S.
Palmitoleic (C16:1 *c*)	6.92	7.25	0.485	7.25	7.23	7.17	6.69	0.303	N.S.	N.S.	N.S.
Palmitelaidic (C16:1 *t*)	0.24	0.32	0.038	0.23	0.27	0.35	0.28	0.030	N.S.	N.S.	N.S.
Heptadecanoic (C17:0)	0.84	0.82	0.040	0.82	0.78	0.83	0.90	0.031	N.S.	0.0270	N.S.
Heptadecenoic (C17:1)	1.34	1.31	0.064	1.36	1.32	1.33	1.29	0.038	N.S.	N.S.	N.S.
Stearic (C18:0)	8.69	7.88	0.661	8.25	7.61	8.24	9.04	0.429	N.S.	0.0133	N.S.
Vaccenic (C18:1 *t* 11)	11.19	10.66	1.249	10.62	10.72	10.50	11.86	0.859	N.S.	N.S.	N.S.
Oleic (C18:1)	32.57	32.05	1.034	33.19	32.31	32.28	31.44	0.831	N.S.	N.S.	N.S.
Linoleic (C18:2)	1.13	1.16	0.084	1.12	1.29	1.10	1.07	0.539	N.S.	<.0001	N.S.
γ-linolenic (C18:3)	0.01	0.01	0.004	0.00	0.00	0.01	0.02	0.004	N.S.	0.0002	N.S.
α-linolenic (C18:3)	0.27	0.31	0.028	0.26	0.26	0.31	0.32	0.016	N.S.	0.0007	N.S.
Arachidic (C20:0)	0.09	0.11	0.011	0.08	0.12	0.10	0.10	0.009	N.S.	N.S.	0.0039
c 9, *t* 11 CLA	0.96	1.20	0.130	0.97	1.08	1.17	1.11	0.068	N.S.	0.0002	0.0002
Eicosenoic (C20:1 *t* 11)	0.14	0.15	0.013	0.13	0.13	0.15	0.16	0.008	N.S.	0.0057	N.S.
Eicosatrienoic (C20:3)	0.06	0.04	0.011	0.05	0.06	0.03	0.05	0.009	N.S.	N.S.	N.S.
Arachidonic (C20:4)	0.02	0.06	0.010	0.03	0.07	0.06	0.02	0.009	0.0018	0.0092	N.S.
EPA (C20:5)	0.02	0.10	0.011	0.00	0.11	0.06	0.08	0.009	<.0001	<.0001	<.0001
DPA (C22:5)	0.02	0.11	0.014	0.03	0.13	0.06	0.04	0.010	<.0001	<.0001	0.0005
DHA (C22:6)	0.01	0.05	0.007	0.00	0.04	0.03	0.05	0.006	<.0001	<.0001	<.0001
Total CLA	0.96	1.20	0.130	0.97	1.08	1.17	1.11	0.068	N.S.	0.0002	0.0002
Total monounsaturates	54.41	54.04	1.268	54.82	54.12	54.05	53.91	0.770	N.S.	N.S.	N.S.
Total *trans* fatty acids	12.69	12.48	1.272	12.11	12.36	12.32	13.55	0.860	N.S.	N.S.	N.S.
Total PUFA	2.50	3.05	0.147	2.47	3.03	2.84	2.76	0.122	0.0025	<.0001	<.0001
Total SFA	38.18	37.47	1.434	37.73	37.64	37.65	38.28	0.868	N.S.	N.S.	N.S.
Ratio PUFA to SFA	0.07	0.08	0.007	0.07	0.08	0.08	0.07	0.004	0.0247	0.0019	0.0035
ω-6 fatty acids	1.24	1.29	0.090	1.21	1.42	1.20	1.22	0.059	N.S.	0.0002	N.S.
ω-3 fatty acids	0.31	0.56	0.040	0.29	0.53	0.47	0.43	0.026	<.0001	<.0001	<.0001
Ratio ω-6 to ω-3	4.50	2.65	0.552	4.53	3.63	2.94	3.19	0.342	0.0050	0.0373	N.S.

Furthermore, the ω-3 PUFA enriched diet also reduced the concentration of eicosatrienoic acid ($P < 0.001$) and arachidonic acid ($P < 0.01$) after 10 days exposure to the ω-3 PUFA enriched diet, while the concentration of these fatty acids in the control increased over the 91 day period **(Figure 7.1)**. Significant diet x day of sampling interactions were observed in the concentrations of vaccenic acid ($P < 0.001$), EPA ($P < 0.001$), DPA ($P < 0.01$) and DHA ($P < 0.001$) which increased with the duration of exposure to the ω-3 PUFA enriched diet over the 91 day period **(Figure 7.2)**.

Overall, animals receiving the ω-3 PUFA enriched diet had a higher concentrations of *trans* fatty acids ($P < 0.001$), total PUFA ($P < 0.001$) and total ω-3 PUFA ($P < 0.001$) and lower concentrations of total SFA ($P < 0.01$) and total ω-6 PUFA ($P < 0.05$) in their plasma than animals on the control diet **(Table 7.3)**. Indeed, diet x day of sampling interactions were observed for the concentration of *trans* fatty acids ($P < 0.05$), total PUFA ($P < 0.05$), and total ω-3 PUFA ($P < 0.001$) which increased with the duration of provision of the ω-3 PUFA enriched diet **(Figure 7.1)**, while diet x day of sampling interactions were also observed in the concentration of total ω-6 PUFA ($P < 0.01$) which decreased after 10 days on the ω-3 PUFA enriched diet, remaining relatively constant thereafter **(Figure 7.2)**. These changes in fatty acid composition resulted in a diet x day of sampling increase in the ratio of PUFA to SFA ($P < 0.01$) and a diet x day of sampling decrease in the ratio of ω-6 to ω-3 PUFA ($P < 0.001$) **(Table 7.3)**.

In adipose tissue, the most abundant fatty acids identified were the SFA, palmitic acid (24.08-25.42%) and stearic acid (7.61-9.03%), along with the monounsaturated fatty acids, vaccenic acid (10.50-11.86%) and oleic acid (31.44-33.19%) **(Table 7.4)**. The provision of the ω-3 PUFA enriched diet resulted in

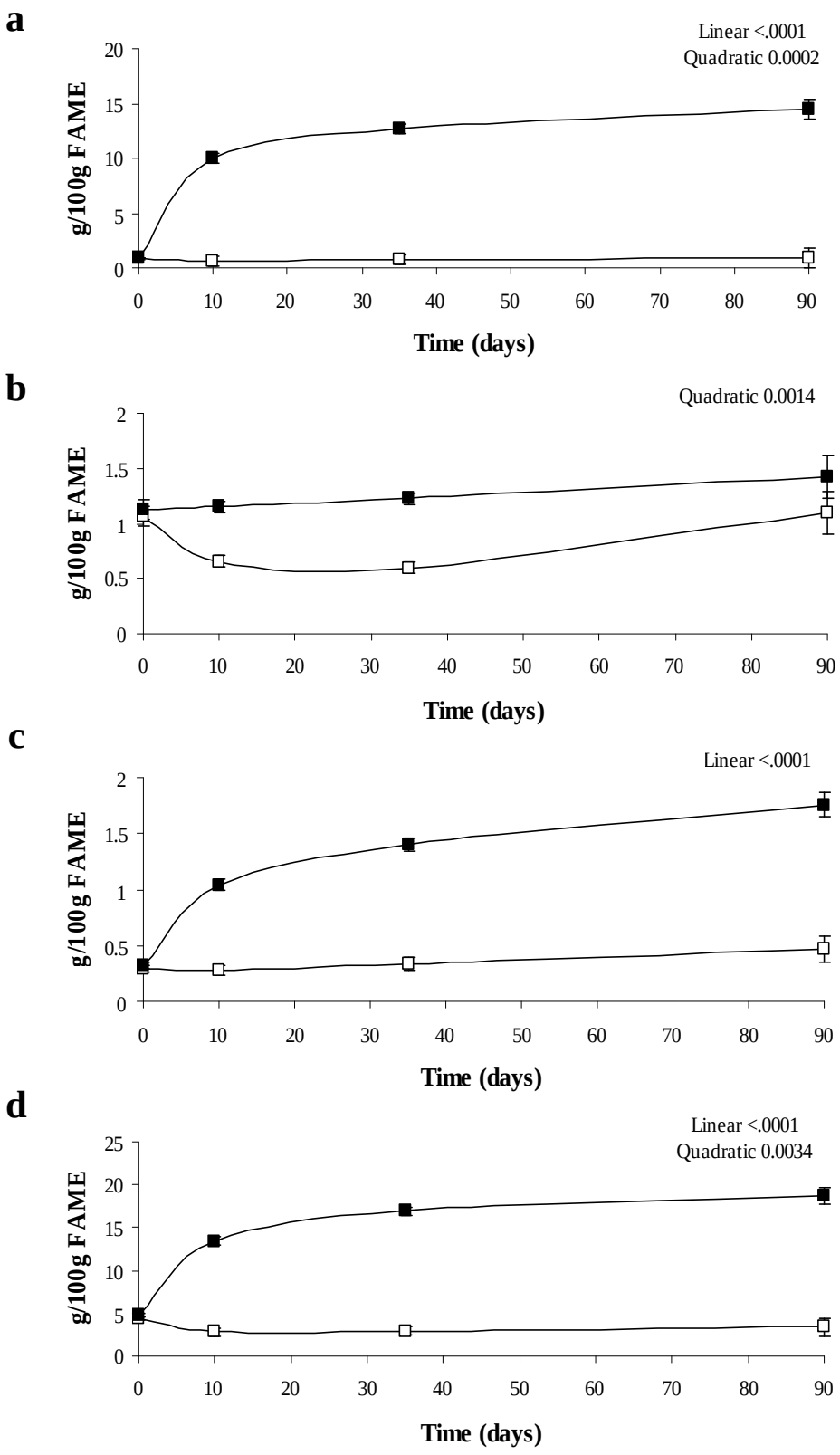

Figure 7.1 Temporal changes in the concentration of **a)** EPA, **b)** DPA, **c)** DHA, and **d)** total ω-3 PUFA in the plasma of Holstein Friesian heifers receiving the control (□) or ω-3 PUFA enriched diet (■).

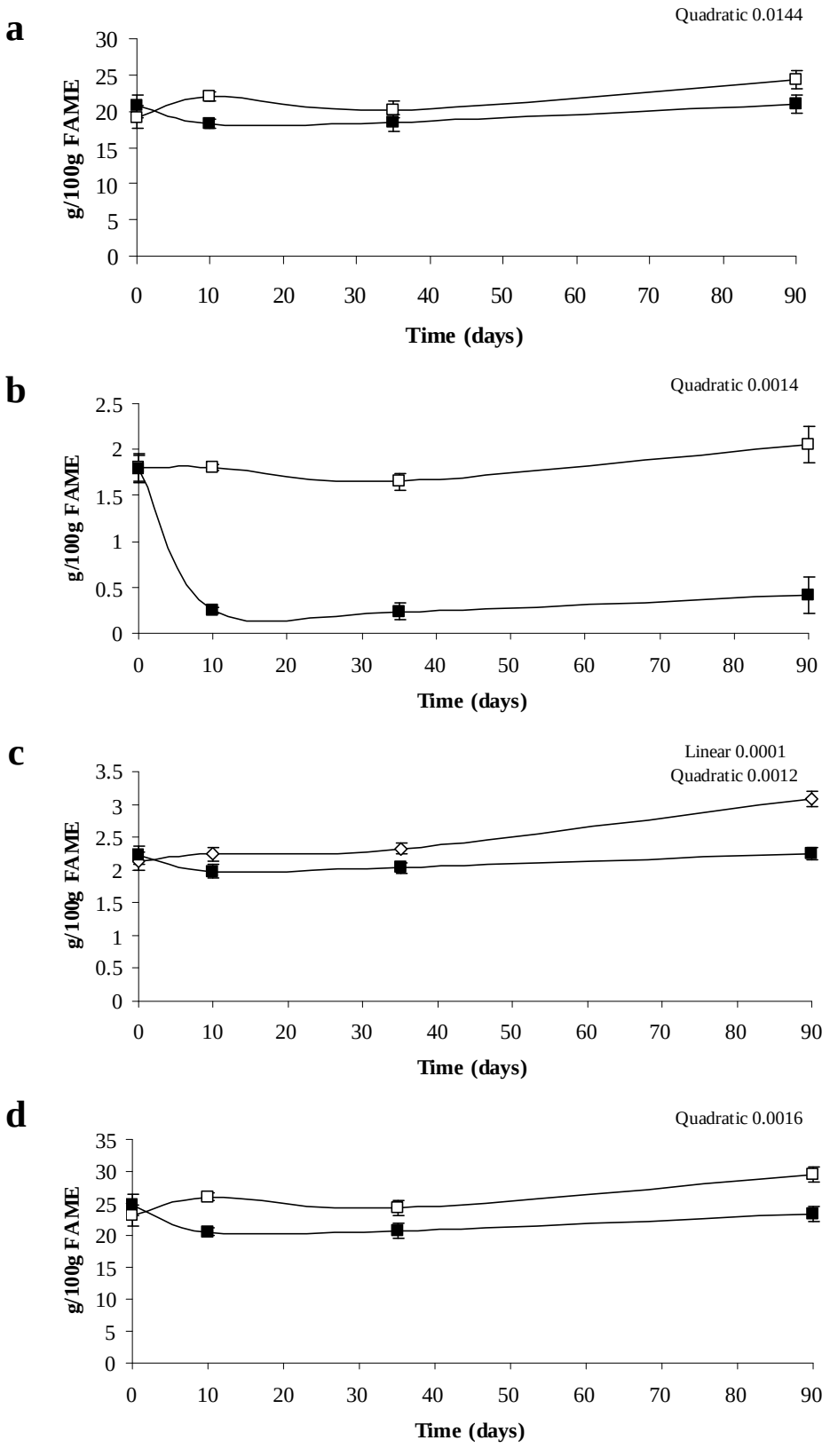

Figure 7.2 Temporal changes in the concentration of **a)** linoleic acid, **b)** eicosatrienoic acid, **c)** arachidonic acid, and **d)** total ω-6 PUFA in the plasma of Holstein Friesian heifers receiving the control (□) or ω-3 PUFA enriched diet (■).

statistically significant increases in the concentration of arachidonic acid ($P < 0.01$), EPA ($P < 0.001$), DPA ($P < 0.001$), and DHA ($P < 0.001$) in heifers fed the ω-3 PUFA enriched diet. Moreover, significant increases in the concentration of total PUFA ($P < 0.001$) and total ω-3 PUFA ($P < 0.01$) were observed in the adipose tissue of animals on the diet enriched in ω-3 PUFA **(Table 7.4)**.

Significant diet x day of sampling interactions were observed for arachidic acid ($P < 0.01$), EPA ($P < 0.001$), DPA ($P < 0.001$), DHA ($P < 0.001$) and in the concentrations of total PUFA ($P < 0.001$) and total ω-3 PUFA ($P < 0.001$) in the adipose tissue of animals on the ω-3 PUFA enriched diet. The greatest increases in the concentrations of these fatty acids occurred 10 days after the animals introduction to the ω-3 PUFA enriched diet, declining thereafter but remaining substantially higher than the control **(Figure 7.3)**. A significant diet x day of sampling interaction was also observed for the *c*9, *t*11 CLA isomer ($P < 0.001$), which increased in animals following 10 days exposure to the ω-3 PUFA enriched diet, while a similar smaller increase was also observed in the control following 35 days. The overall impact of these changes in fatty acid composition were a significant increase in the ratio of PUFA to SFA ($P < 0.05$) and decrease in the ratio of ω-6 to ω-3 PUFA ($P < 0.01$) in animals on the ω-3 PUFA enriched diet overtime, but no effect in the control animals. Indeed, a significant diet x day of sampling increase in the ratio of PUFA to SFA ($P < 0.01$) was observed in animals on the ω-3 PUFA enriched diet **(Table 7.4)**.

7.3.3 Fatty acid composition of other tissues

The fatty acid compositions of the liver, muscle and mammary tissues from animals receiving the control or ω-3 PUFA enriched diets are presented in **Tables 7.5**, **Table 7.6**, and **Table 7.7**, respectively. In liver tissue, the most abundant fatty acids

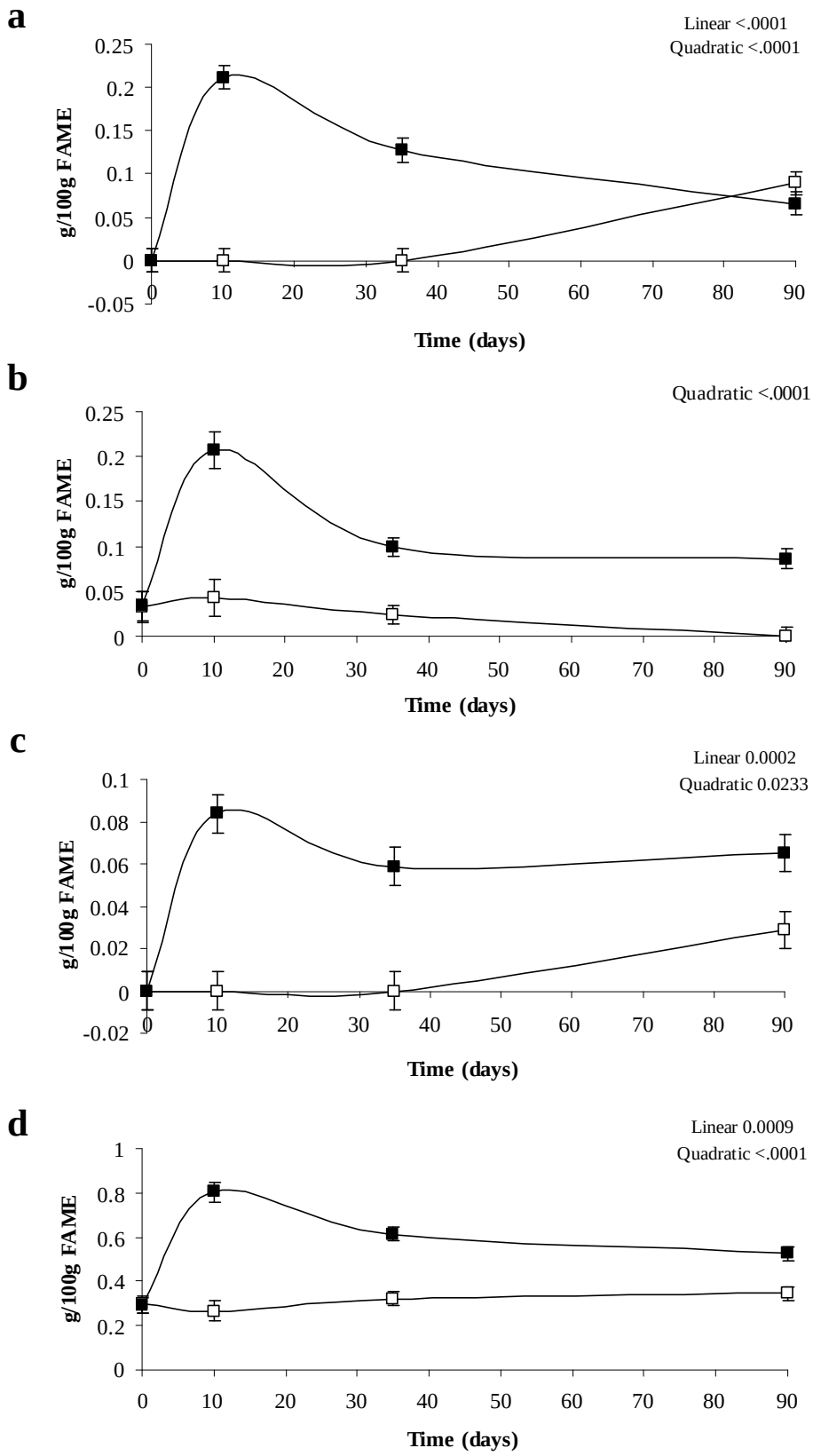

Figure 7.3 Temporal changes in the concentration of **a)** EPA, **b)** DPA, **c)** DHA, and **d)** total ω-3 PUFA in the adipose tissue of Holstein Friesian heifers receiving the control (□) or ω-3 PUFA enriched diet (■)

identified were palmitic acid (12.63-14.02%), stearic acid (21.36-28.03%), oleic acid (7.33-10.07%), linoleic acid (4.44-7.31%), eicosatrienoic acid (0.46-6.50%), arachidonic acid (3.77-9.57%), EPA (1.84-13.44%), DPA (5.78-10.35%), and DHA (4.08-10.27%) **(Table 7.5)**. Animals receiving the ω-3 PUFA enriched diet displayed increased concentrations of palmitelaidic acid ($P < 0.05$), vaccenic acid ($P < 0.001$), C18:1 *t*13 ($P < 0.001$), α-linolenic acid ($P < 0.01$), arachidic acid ($P < 0.001$), the *c*9, *t*11 CLA isomer ($P < 0.01$), EPA ($P < 0.001$), DPA ($P < 0.001$), and DHA ($P < 0.001$) in their liver tissue **(Table 7.5)**. Reductions in the concentration of stearic acid ($P < 0.001$), oleic acid ($P < 0.05$), linoleic acid ($P < 0.001$), γ-linolenic acid ($P < 0.01$), eicosatrienoic acid ($P < 0.001$), and arachidonic acid ($P < 0.001$) were also observed in the liver tissue of heifers consuming the ω-3 PUFA enriched diet relative to those on the control diet. In addition to changes in the concentration of the individual fatty acids, animals on the ω-3 PUFA enriched diet also had increased concentrations of total monounsaturated fatty acids ($P < 0.001$), total *trans* fatty acids ($P < 0.001$), total PUFA ($P < 0.001$), and total ω-3 PUFA ($P < 0.001$). Furthermore, decreases in the concentration of total SFA ($P < 0.001$), and total ω-6 PUFA ($P < 0.001$) were also observed. The impact that these changes had on the fatty acid composition was an overall increase in the ratio of PUFA to SFA ($P < 0.001$) along with a significant decrease in the ratio of ω-6 to ω-3 PUFA ($P < 0.001$) in animals receiving the ω-3 PUFA enriched diet **(Table 7.5)**.

In muscle tissue, the most abundant fatty acids were palmitic acid (23.98-24.44%), stearic acid (16.95-22.68%) and oleic acid (26.40-32.18%) **(Table 7.6)**. Decreases in the concentration of stearic acid ($P < 0.01$) and eicosatrienoic acid ($P < 0.05$) were found in muscle tissue of animals fed the diet enriched in ω-3 PUFA relative to heifers on the control diet. Moreover, statistically significant increases in the concentration of the monounsaturated fatty acids, palmitoleic acid ($P < 0.05$)

Table 7.5 Fatty acid composition (g/100g FAME) of the liver tissue of Holstein Friesian heifers fed an ω-3 PUFA enriched or control diet.

	Diet			*P* value
Fatty acid	Control	ω-3 PUFA	SED	DIET
Myristic (C14:0)	0.99	1.16	0.190	N.S.
Myristelaidic (C14:1 *t*)	0.42	0.48	0.104	N.S.
Palmitic (C16:0)	14.02	12.63	0.761	N.S.
Palmitelaidic (C16:1 *t*)	0.50	0.64	0.061	0.0388
Palmitoleic (C16:1 *c*)	0.84	0.92	0.156	N.S.
Heptadecanoic (C17:0)	1.25	1.38	0.077	N.S.
Heptadecenoic (C17:1)	0.10	0.06	0.083	N.S.
Stearic (C18:0)	28.03	21.37	1.007	<.0001
Vaccenic (C18:1 *t* 11)	1.59	4.08	0.528	0.0004
C18:1 *t*13	0.09	0.84	0.100	<.0001
Oleic (C18:1)	10.07	7.33	1.088	0.0259
Linoleic (C18:2)	7.31	4.45	0.384	<.0001
γ-linolenic (C18:3)	0.27	0.00	0.101	0.0184
α-linolenic (C18:3)	0.37	0.70	0.090	0.0025
Arachidic (C20:0)	0.00	0.49	0.039	<.0001
c 9, *t*11 CLA	0.30	0.55	0.075	0.0055
Eicosatrienoic (C20:3)	6.50	0.46	0.459	<.0001
Arachidonic (C20:4)	9.57	3.77	0.350	<.0001
EPA (C20:5)	1.84	13.44	0.688	<.0001
DPA (C22:5)	5.78	10.36	0.597	<.0001
DHA (C22:6)	4.08	10.27	0.773	<.0001
Total CLA	0.30	0.55	0.075	0.0055
Total monounsaturates	3.53	7.01	1.031	<.0001
Total *trans* fatty acids	2.47	6.11	0.633	<.0001
Total PUFA	36.03	44.01	1.413	<.0001
Total SFA	44.71	37.49	0.734	<.0001
Ratio PUFA to SFA	0.81	1.17	0.033	<.0001
ω-6 fatty acids	23.66	8.68	0.787	<.0001
ω-3 fatty acids	12.07	34.77	1.757	<.0001
Ratio ω-6 to ω-3	2.17	0.25	0.249	<.0001

Table 7.6 Fatty acid composition (g/100g FAME) of the muscle tissue of Holstein Friesian heifers fed an ω-3 PUFA enriched or control diet.

	Diet			*P* value
Fatty acid	Control	ω-3 PUFA	SED	DIET
Lauric (C12:0)	0.04	0.02	0.031	N.S.
Myristic (C14:0)	2.40	2.43	0.245	N.S.
Myristelaidic (C14:1 *t*)	0.23	0.18	0.051	N.S.
Myristoleic (C14:1 *c*)	0.26	0.33	0.070	N.S.
Pentadecdanoic (C15:0)	0.58	0.56	0.045	N.S.
Palmitic (C16:0)	24.44	23.98	1.208	N.S.
Palmitelaidic (C16:1 *t*)	0.81	0.69	0.120	N.S.
Palmitoleic (C16:1 *c*)	1.62	2.38	0.251	0.0102
Heptadecanoic (C17:0)	1.25	1.14	0.133	N.S.
Heptadecenoic (C17:1)	0.94	0.92	0.111	N.S.
Stearic (C18:0)	22.68	16.95	1.547	0.0027
Vaccenic (C18:1 *t*11)	5.67	3.38	1.916	N.S.
Oleic (C18:1)	26.40	32.18	2.400	0.0316
Linoleic (C18:2)	3.42	4.07	0.727	N.S.
Arachidic (C20:0)	0.15	0.18	0.076	N.S.
*c*9, *t*11 CLA	0.42	0.56	0.078	N.S.
Eicosaenoic (C20:1)	0.08	0.04	0.046	N.S.
Eicosatrienoic (C20:3)	0.27	0.08	0.087	0.0468
Arachidonic (C20:4)	0.78	0.80	0.282	N.S.
EPA (C20:5)	0.10	1.60	0.350	0.0009
DPA (C22:5)	0.36	0.77	0.103	0.0015
DHA (C22:6)	0.06	0.55	0.083	<.0001
Total CLA	0.42	0.56	0.078	N.S.
Total monounsaturates	36.01	40.08	2.232	N.S.
Total *trans* fatty acids	7.12	4.80	1.984	N.S.
Total PUFA	5.86	8.97	1.369	0.0409
Total SFA	51.54	45.27	2.235	0.0149
Ratio PUFA to SFA	0.12	0.21	0.039	0.0446
ω-6 fatty acids	4.46	4.95	1.067	N.S.
ω-3 fatty acids	0.98	3.45	0.506	0.0003
Ratio ω-3 to ω-6	4.60	1.48	0.639	0.0003

and oleic acid ($P < 0.05$), and the ω-3 PUFA, EPA ($P < 0.001$), DPA ($P < 0.01$) and DHA ($P < 0.001$) were also obtained in the muscle tissue of the heifers on the ω-3 PUFA enriched diet relative to those consuming the control diet **(Table 7.6)**. Overall, heifers supplemented with ω-3 PUFA had lower total SFA concentrations ($P < 0.05$) and lower ratio of ω-6 to ω-3 PUFA ($P < 0.001$), while the increases in the concentration of total PUFA ($P < 0.05$), total ω-3 PUFA ($P < 0.001$) and in the ratio of PUFA to SFA ($P < 0.05$) were also observed. Interestingly, the concentration of total ω-6 PUFA was not affected by the provision of the ω-3 PUFA enriched diet **(Table 7.6)**.

The most abundant fatty acids identified in mammary tissue were the SFA palmitic acid (25.63-27.18%) and stearic acid (13.80-17.18%), and the monounsaturated fatty acids, vaccenic acid (14.53-15.74%) and oleic acid (24.68-25.62%) **(Table 7.7)**. Heifers offered the ω-3 PUFA enriched diet had higher concentrations of myristoleic acid ($P < 0.01$) and palmitoleic acid ($P < 0.05$), *c*9, *t*11 CLA ($P < 0.01$), and the ω-3 PUFA, EPA ($P < 0.001$), DPA ($P < 0.001$) and DHA ($P < 0.001$) in mammary tissue relative to those on the control diet **(Table 7.7)**. Reductions in the concentration of stearic acid ($P < 0.01$) and eicosatrienoic acid ($P < 0.05$) were also observed in the mammary tissue of animals on the ω-3 PUFA enriched diet. The overall impact of these changes were reductions in the concentration of total SFA ($P < 0.05$) and total ω-6 PUFA ($P < 0.05$) and an increase in the concentration of total PUFA ($P < 0.01$) and total ω-3 PUFA ($P < 0.01$) in the mammary tissue of animals offered the diet enriched in ω-3 PUFA **(Table 7.7)**. These changes in the fatty acid composition resulted in a significant increase in the ratio of PUFA to SFA ($P < 0.001$) and a decrease in the ratio of ω-6 to ω-3 PUFA ($P < 0.001$) **(Table 7.7)**.

Table 7.7 Fatty acid composition (g/100g FAME) of the mammary tissue of Holstein Friesian heifers fed an ω-3 PUFA enriched or control diet.

	Diet			*P* value
Fatty acid	Control	ω-3 PUFA	SED	DIET
Lauric (C12:0)	0.10	0.09	0.013	N.S.
Myristic (C14:0)	3.31	3.47	0.283	N.S.
Myristelaidic (C14:1 *t*)	0.28	0.27	0.022	N.S.
Myristoleic (C14:1 *c*)	0.58	0.89	0.104	0.0095
Pentadecanoic (C15:0)	0.61	0.56	0.049	N.S.
Palmitic (C16:0)	27.18	25.63	1.070	N.S.
Palmitelaidic (C16:1 *t*)	0.57	0.68	0.238	N.S.
Palmitoleic (C16:1 *c*)	2.63	3.45	0.308	0.0184
Heptadecanoic (C17:0)	1.21	1.11	0.062	N.S.
Heptadecenoic (C17:1)	0.92	1.04	0.059	N.S.
Stearic (C18:0)	17.18	13.80	1.053	0.0063
Vaccenic (C18:1 *t*11)	14.53	15.74	2.300	N.S.
Oleic (C18:1)	24.68	25.62	2.649	N.S.
Linoleic (C18:2)	1.26	1.21	0.138	N.S.
γ-linolenic (C18:3)	0.00	0.01	0.009	N.S.
α-linolenic (C18:3)	0.30	0.33	0.022	N.S.
Arachidic (C20:0)	0.15	0.16	0.014	N.S.
*c*9, *t*11 CLA	0.71	1.06	0.114	0.0093
Eicosenoic (C20:1 *t* 11)	0.08	0.08	0.023	N.S.
Eicosatrienoic (C20:3)	0.07	0.04	0.011	0.0201
Arachidonic (C20:4)	0.08	0.10	0.016	N.S.
EPA (C20:5)	0.00	0.14	0.013	<.0001
DPA (C22:5)	0.10	0.19	0.023	0.0008
DHA (C22:6)	0.00	0.08	0.009	<.0001
Total CLA	0.71	1.06	0.114	0.0093
Total monounsaturates	44.26	47.77	1.817	N.S.
Total *trans* fatty acids	16.09	17.75	2.555	N.S.
Total PUFA	2.53	3.15	0.156	0.0013
Total SFA	49.72	44.82	1.823	0.0176
Ratio PUFA to SFA	0.05	0.07	0.004	0.0003
ω-6 fatty acids	1.41	1.36	0.174	N.S.
ω-3 fatty acids	0.40	0.74	0.066	0.0058
Ratio ω-6 to ω-3	3.55	1.84	0.003	<.0001

7.3.4 Regression analysis

Regression analysis was used to establish the relationship between concentrations of selected fatty acids groups and ratio of plasma fatty acids relative to their respective concentrations in muscle, adipose, mammary and liver tissues **(Table 7.8)**. The relationship between the concentration of stearic and vaccenic acid in plasma and that of muscle, adipose, mammary, and liver tissue of animals on the ω-3 PUFA diet was weak. Similarly, the relationship between the concentration of arachidonic acid in plasma and in muscle, adipose and mammary tissue was also weak **(Table 7.8)**. However a strong positive relationship between the concentration of arachidonic acid was found 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 obtained between the concentration of EPA and DHA in plasma and those of the muscle, adipose, mammary, and liver tissue of animals on the ω-3 PUFA diet **(Table 7.8)**. Although not statistically significant, the relationship between the concentration of DPA in plasma and that of muscle, adipose and mammary tissue had a tendency towards significance **(Table 7.8)**. There was a positive relationship between the total PUFA concentration of plasma and that of mammary tissue ($\beta_1 = 0.04 \pm 0.017$, $R^2 = 0.44$, $P < 0.05$), however, this did not extend to the muscle, adipose or liver tissues. Liver tissue showed the strongest relationships with plasma, displaying strong to medium positive relationships in terms of the concentration of total SFA, total ω-6 PUFA and total ω-3 PUFA **(Table 7.8)**. Furthermore, this trend also extended to both the ratio of PUFA to SFA ($\beta_1 = 0.56 \pm 0.167$, $R^2 = 0.61$, $P < 0.05$) and to the ratio of ω-6 to ω-3 PUFA ($\beta_1 = 0.15 \pm 0.054$, $R^2 = 0.52$, $P < 0.05$), both important indices of the fatty acid quality of meat. Significant relationships were also observed between the concentration of total ω-3 PUFA in plasma and that of

the muscle and adipose tissue of animals fed the ω-3 PUFA enriched diet. Strong correlation was also found in the ratio of ω-6 to ω-3 in plasma and muscle tissue of animals fed the ω-3 PUFA enriched diet ($\beta_1 = 0.53 \pm 0.089$, $R^2 = 0.83$, $P < 0.001$) **(Table 7.8)**.

Fatty acid	Muscle	Adipose	Mammary	Liver
Stearic (C18:0)	-0.55 ± -0.439	-0.09 ± 0.213	0.54 ± 0.293	-0.83 ± 0.393 [a]
Vaccenic (C18:1 *t* 11)	-0.27 ± 0.372	0.43 ± 0.724	-0.56 ± 0.772	0.36 ± 0.235
Arachidonic (C20:4)	0.11 ± 0.369	0.07 ± 0.041	-0.02 ± 0.015	5.32 ± 0.934 ***
EPA (C20:5)	0.08 ± 0.011 ***	0.01 ± 0.001 ***	0.01 ± 0.001 ***	0.75 ± 0.095 ***
DPA (C22:5)	0.35 ± 0.183 [a]	0.04 ± 0.019 [a]	0.10 ± 0.049 [a]	3.05 ± 1.984
DHA (C22:6)	3.12 ± 0.832 **	0.02 ± 0.004 **	0.05 ± 0.014 **	3.67 ± 0.841 **
Total monounsaturates	1.09 ± 0.783	0.02 ± 0.414	0.11 ± 0.560	-0.51 ± 0.300
Total *trans* fatty acids	-0.33 ± 0.363	0.14 ± 0.761	-1.47 ± 0.920	0.64 ± 0.322 [a]
Total PUFA	0.14 ± 0.131	-0.02 ± 0.024	0.04 ± 0.017 *	0.31 ± 0.183
Total SFA	0.32 ± 0.307	-0.31 ± 0.229	0.32 ± 0.257	0.56 ± 0.156 **
Ratio PUFA to SFA	0.08 ± 0.095	-0.03 ± 0.028	-0.02 ± 0.022	0.56 ± 0.167 *
ω-6 fatty acids	0.09 ± 0.156	0.01 ± 0.035	-0.50 ± 0.239 [a]	1.16 ± 0.407 *
ω-3 fatty acids	0.12 ± 0.013 ***	0.01 ± 0.003 **	-0.30 ± 0.134 [a]	1.18 ± 0.203 ***
Ratio ω-6 to ω-3	0.53 ± 0.089 ***	0.19 ± 0.149	0.00 ± 0.000	0.15 ± 0.054 *

Table 7.8 Regression co-efficients (β_1) ± S.E.M. for the fatty acid composition of plasma relative to that of muscle tissue, adipose tissue, mammary tissue and liver tissue. ($P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***, [a] approaching significance $P = 0.1$-0.05

7.4 Discussion

Recent studies have confirmed the important role which red meat plays in the ω-3 PUFA intake of humans in Western society (Howe *et al.*, 2006; Howe *et al.*, 2003; Ponnampalam *et al.*, 2006). However, despite its ω-3 PUFA content the elevated concentrations of SFA, ω-6 PUFA and *trans* fatty acids found in red meat make its dietary intake undesirable due to the association of these fatty acids with the pathogenesis of a number of diseases in humans (Fung *et al.*, 2004; Giovannucci *et al.*, 1994; Gonzalez *et al.*, 2006; Hu *et al.*, 1999; Stender *et al.*, 2008). The aim of the current study was to improve the fatty acid composition of beef via the inclusion of a ruminally protected ω-3 PUFA supplement in the diet of Holstein Friesian heifers. It was expected that through the use of this strategy the nutritional quality of the fatty acids in beef could be improved significantly.

In the current study, supplementation with a ruminally protected form of ω-3 PUFA did not affect DMI or average daily gain, and is consistent with our previous observations (Childs *et al.*, 2008a; Childs *et al.*, 2008b). Feeding heifers a diet containing a ruminally protected ω-3 PUFA supplement resulted in a significant increase in the concentration of total PUFA found in the plasma, adipose, liver, muscle and mammary tissues. These increases are greater than those achieved in previous studies were non-ruminally protected ω-3 PUFA supplements were included in the bovine diet (Childs *et al.*, 2008c; Mach *et al.*, 2006; Noci *et al.*, 2007; Scislowski *et al.*, 2005). Elevations in the concentration of total PUFA in the plasma, adipose, liver, muscle and mammary tissues were primarily mediated through increases in the concentration of total ω-3 PUFA, although increases in the concentration of CLA also contributed. The increases in the concentration of total ω-3 PUFA correspond well with other studies where fish meal or fish oil were

supplied in the diet of cattle (Ballou *et al.,* 2009; Cant *et al.*, 1997; Childs *et al.*, 2008b; Scollan *et al.*, 2001). The inclusion of the ω-3 PUFA supplement in the heifers diet significantly reduced the SFA content of the plasma, liver, muscle and mammary tissues primarily through decreases in the concentration of palmitic acid and stearic acid. These observations correspond well with those of others who reported reductions in the SFA content (primarily palmitic and stearic acid) of plasma (Ashes *et al.,* 1992), liver tissue (Ballou *et al.*, 2009) and muscle tissue (Ashes *et al.*, 1992) when ω-3 PUFA were included in the bovine diet. The reductions in SFA concentrations may be a result of the increased competition provided by the higher total ω-3 PUFA from the diet or as a result of the potent suppressive effects of ω-3 PUFA on lipogenesis in tissues such as the liver (Jump *et al.*, 1994; Jump *et al.*, 1996; Jump *et al.*, 1999). The ω-3 PUFA enriched diet also resulted in a significant increase in the ratio of PUFA to SFA in the plasma and tissues. 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 animals receiving the ω-3 PUFA enriched diet. These results correlate well with similar studies such as those conducted by Scollan *et al.* (2001a) where a 1.25 fold increase in the ratio of PUFA to SFA was observed in the adipose tissue of Charlois steers, and by Mach *et al.* (2006) where a 1.19 fold increase in the ratio of PUFA to SFA was observed in the muscle tissue of Holstein bulls when provided with a diet enriched in ω-3 PUFA. The relationship between the ratio of PUFA to SFA and the risk of diseases such as those of the cardiovascular system has been substantially investigated. These studies have in particular highlighted the correlation between the higher dietary intake of SFA and increased atherosclerotic risk (Grundy & Denke, 1990; Hegsted *et al.*, 1965; Hu *et al.*, 1999; Keys *et al.*, 1965; Muller *et al.*, 2003). As a result,

groups such as the Department of Health (1994) have recommended a ratio of PUFA to SFA in the human diet of 0.45. Of the tissues assayed, only the liver tissue displayed a PUFA to SFA ratio which was greater than the 0.45 recommended by the Department of Health (1994), however, significant improvements were also observed in the adipose and muscle tissue. Thus, the consumption of the adipose, muscle and liver tissue from Holstein Friesian heifers receiving a ω-3 PUFA enriched diet might be beneficial in improving the overall ratio of PUFA to SFA in the human diet.

The ω-3 PUFA rich diet also resulted in significant reductions in the concentration of total ω-6 PUFA (in particular eicosatrienoic and arachidonic acids) in the plasma and liver tissue and in the concentration of eicosatrienoic acid in the muscle and mammary tissues of Holstein Friesian heifers. Such reductions are attributed to the competition provided by ω-3 PUFA with ω-6 PUFA for intestinal absorption and incorporation into the plasma and tissues, or alternatively to the interference of the ω-3 PUFA with the endogenous synthesis of eicosatrienoic and arachidonic acids (Li *et al.*, 2003; Ratnayake *et al.*, 1989; Scollan *et al.*, 2001). An overall increase in the concentration of total ω-6 PUFA was observed in the adipose tissue of animals fed the ω-3 PUFA enriched diet prompted by significant increases in the concentration of arachidonic acid. Such an increase is likely a result of the higher availability of dietary arachidonic acid in ω-3 PUFA enriched diet. Moreover the increase in the arachidonic acid content of the adipose tissue may go some way to explaining the reductions in arachidonic acid seen in the plasma and liver, with the adipose tissue serving as a sink for the fatty acid. Provision of the ω-3 PUFA enriched diet also significantly reduced the ratio of ω-6 to ω-3 PUFA in heifers via significant increases in the concentration of ω-3 PUFA. In humans

reducing the ratio of ω-6 to ω-3 PUFA in cells has been associated with reducing the pathogenesis of diseases such as cancer, cardiovascular diseases and diabetes, which have become associated with the increased prevalence of ω-6 PUFA in the human diet (Astorg, 2004; Bagga *et al.*, 1997; Simopoulos, 2006; Simopoulos, 2008). In the current study, the ratio ω-6 to ω-3 PUFA in the muscle and liver tissue were reduced to levels which 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 2-3:1 which suppressed inflammatory rheumatoid arthritis, and the ratio of 5:1 which had a beneficial impact on asthma sufferers (Simopoulos, 2002). In addition to improvements in the ratio of ω-6 to ω-3 PUFA, the meat from animals fed the ω-3 PUFA enriched diet contained significantly higher concentrations of the health promoting fatty acids EPA and DHA. These ω-3 PUFA are regularly associated with the prevention or alleviation of a number of diseases prevalent in Western society including cancer, osteoporosis, cardiovascular disease, inflammatory diseases, and diabetes along with improving brain function (Berquin *et al.*, 2008; Fernandes *et al.*, 2008; Innis, 2008; Li *et al.*, 2003; Simopoulos, 2008).

Provision of the ω-3 PUFA enriched diet also resulted in a significant increase in the concentration of CLA and in particular the *c*9, *t*11 CLA isomer found in the adipose, mammary and liver tissues of heifers, which is consistent with other studies (Dhiman *et al.*, 2005; Griinari & Bauman, 1999; Hennessy *et al.*, 2007). Approximately 70% of ruminant CLA is endogenously produced via the action of the enzyme Δ^9-desaturase present in the mammary and adipose tissue on vaccenic acid adsorbed from the rumen. Thus, it is likely this endogenous CLA production which is responsible for increased concentration of CLA and relative

absence of vaccenic acid in the mammary and adipose tissues (Griinari & Bauman, 1999; Hennessy *et al.*, 2007; Kepler *et al.*, 1966). Furthermore, as significant concentrations of endogenously produced CLA have also been found in the liver endogenous CLA production may also account for the higher CLA concentrations found in the liver tissue (Shen *et al.*, 2007). As the CLA isomers have been attributed with a range of health promoting activities in humans any increases in their concentration in meat are likely to be desirable from a nutritional standpoint (Belury, 2002; Bhattacharya *et al.*, 2006; Wahle *et al.*, 2004).

Importantly, the current study highlighted the strong positive relationship between the concentration of EPA and DHA, which constituted the major fatty acids in the ω-3 PUFA supplement, in the plasma with that found in the muscle, adipose, mammary, or liver tissue of animals on the ω-3 PUFA enriched diet. This correlation was further reflected in the strong relationship between the plasma total ω-3 PUFA concentration and that of the muscle, adipose and liver tissues. Thus, the current study would appear to demonstrate the plasma of animals receiving a ruminally protected ω-3 PUFA enriched supplement can successfully be used to predict the effect of such a diet on the ω-3 PUFA concentration of muscle, adipose, and liver tissues. Interestingly, the plasma may also prove a valuable tool in determining the ratio of ω-6 to ω-3 PUFA in the muscle and liver and the ratio of PUFA to SFA found in the liver of animals receiving the ω-3 PUFA enriched diet, with strong correlations seen between the tissues and the plasma samples. As these ratios are important indicators of the overall fatty acid quality of meat, such a correlations in muscle and liver tissue may be extremely important, permitting the determination of the fatty acid quality of two of the most important bovine tissues with regard to human dietary consumption from a plasma sample.

7.5 Conclusions

In conclusion it is evident that the inclusion of a ruminally protected ω-3 PUFA enriched supplement in the diet of Holstein Friesian heifers can be utilised as a successful strategy to improve the overall fatty acid composition of bovine meat. These improvements were best reflected in the changes observed in two of the indices of the fatty acid quality of meat, namely the ratio of PUFA to SFA and the ratio of ω-6 to ω-3 PUFA, which were increased and decreased, respectively, in the plasma and tissues of the heifers receiving the ω-3 PUFA enriched diets. In particular, the provision of the ω-3 PUFA enriched diet resulted in significant increases in the concentration of the health promoting fatty acids EPA and DHA in the plasma and tissue. This study has also demonstrated how the plasma from animals receiving the ω-3 PUFA enriched diet can assist in predicting the ratio of PUFA to SFA in the liver tissue and the ratio of ω-6 to ω-3 PUFA the muscle and liver tissue. Indeed, such correlations may be useful given the commercial value of both tissues. Overall, the study shows how a ruminally protected ω-3 PUFA dietary supplement can be employed to improve the fatty acid quality of the meat of Holstein Friesian heifers without impacting on animal performance.

7.6 References

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