EFFECT OF PASTURE AND LIPID SUPPLEMENTATION ON PRODUCTION PERFORMANCE, CARCASS QUALITY, FATTY ACID COMPOSITION AND LIPID METABOLISM OF LAMB

by

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DEDICATION PAGE

I would like to dedicate this thesis to my family. Although you may not fully understand the content of this work, you have been a crucial part of its production. Thank-you for the support, the love, the advice and the confidence you have given me.

"Families are the compass that guides us. They are the inspiration to reach great height, and our comfort when we occasionally falter" – Brad Henry

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ABSTRACT

The significance of diet during the growing and finishing of lambs on production and lipid metabolism was studied. Thirty-two intact 5-6 month old Suffolk cross ram lambs that had previously grazed either red clover (RC) or tall fescue (TF) were finished on a concentrate diet containing isolipidic supplements of either fish oil (FO), soybean oil (SBO) or a 30:70 mixture of CLA isomers and SBO (CLA^{+}). When compared to TF, RC increased carcass weight (1.1-fold) and content of muscle ALA, subcutaneous adipose ALA and total PUFA (1.2-fold), and decreased D9D expression in liver (1.6-fold). FO decreased carcass weight (1.1-fold) and increased muscle and subcutaneous adipose EPA (5.6- and 10.8-fold), DHA (5.8- and 13-fold) and total PUFA (1.4- and 1.2-fold) while decreasing SREBP (1.6-fold) expression in liver and SPOT14 and leptin expression in enteric adipose. CLA^{+} increased subcutaneous adipose CLA isomers (1.8-fold) with no effect on carcass weight or gene expression.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ACC	Acetyl-CoA carboxylase
ALA	Alpha-linolenic acid
BHBA	Betahydroxybutyrate
cDNA	Complimentary deoxyribonucleic acid
CLA	Conjugated linoleic acid
CoA	Coenzyme A
CP	Crude protein
df	Degrees freedom
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
D9D	Delta-9-desaturase
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
FAS	Fatty acid synthase
FO	Fish oil
HDL	High-density lipoprotein
HL	Hepatic lipase
LA	Linoleic acid
LD	Longissimus dorsi
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LSD	Least significant difference
MUFA	Monounsaturated fatty acid
NEFA	Non-esterified fatty acid
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
RT-PCR	Real time polymerase chain reaction
SBO	Soybean oil
SCAP	Sterol element binding protein cleavage activating protein
SEM	Standard error of the mean
SFA	Saturated fatty acid
SPOT14	Thyroid hormone responsive spot 14
SREBP	Sterol response element binding protein
TAG	Triacylglycerol
USFA	Unsaturated fatty acid
VA	Vaccenic acid
α	Alpha
β	Beta
δ	Delta
γ	Gamma
n	Omega

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CHAPTER 1 INTRODUCTION

The lipid composition of ruminant meat contains a high amount of saturated fatty acids (SFA) which has caused concern about whether including too much ruminant meat in the human diet is detrimental to health (Ladeira *et al*., 2012). In light of this concern, the potential health-promoting effects of the unsaturated fatty acid (USFA) composition of ruminant meat have been overlooked. Ruminant meat, in fact, is a natural source of USFA including oleic acid (C18:1, *cis*-9) and conjugated linoleic acid (CLA) isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12; Palmquist and Jenkins, 1980). Research has demonstrated that the lipid profile of ruminant meat can be manipulated by diet. Decreasing the concentration of SFA while elevating the USFA content of lamb tissues is a goal of the present thesis work.

1.2 THE FATTY ACID CONTENT OF RUMINANT MEAT

Saturated fat consumption in general has been found to raise total and LDL cholesterol. Elevated LDL cholesterol is associated with increased risk of atherosclerosis and coronary heart disease. However, individual SFA have been found to differ in respect to contributing negatively to health. In lamb meat, approximately 44% of total fatty acids are saturated (Table 1-1). Of the 44%, approximately 23% is palmitic acid (C16:0); 18.5% is stearic acid (C18:0); 3.2% is myristic acid (C14:0) and 0.3% is lauric acid (C12:0). According to Moloney *et al*. (2002) the two major SFA in ruminant meat, palmitic and stearic acid, do not contribute to elevated LDL cholesterol and associated coronary heart disease. Some studies suggest palmitic acid raises LDL cholesterol (Temme *et al*., 1996), however, these results are not consistently found (Higgs *et al*., 2002). Stearic acid has long been found to have no effect on plasma cholesterol, considered a neutral SFA (Bonanome and Grundy, 1988). With this in mind, the majority of SFA in ruminant meat should not be termed ‘unhealthy’ and gives a better outlook regarding its SFA content. Conversely, myristic and lauric acid have more potent effects on raising LDL cholesterol (Zock *et al*., 1994). Myristic acid has been found to raise cholesterol four-fold when compared to palmitic (Higgs *et al*., 2002). However, myristic and lauric acid are found in very small quantities in ruminant meat.

Oleic acid is the predominant fatty acid found in ruminant meat (Table 1-1). It originates primarily from endogeneous tissue desaturation [action of delta-9-desaturase (D9D); Corl *et al*., 2001]. Oleic acid has been linked to reducing LDL cholesterol as well as triacylglycerol (TAG) concentrations in plasma (Mills *et al*., 1992). Study by Feldman (2002) suggests that the monounsaturated fatty acid (MUFA) content of the diet is important in inhibiting platelet aggregation. Increased dietary MUFA has also been found to increase HDL cholesterol (Mills *et al*., 1992).

Table 1- 1 Fatty acid content of lamb, beef and pork

Fatty acid (mg/100 g)	Lamb	Beef	Pork
lauric (C12:0)	13.8	2.9	2.6
myristic (C14:0)	155	103	30
palmitic (C16:0)	1101	962	526
stearic (C18:0)	898	507	278
elaidic (C18:1 trans-9)	231	104	-
oleic (C18:1 cis-9)	1625	1395	759
vaccenic (C18:1 trans-11)	71.7	91.6	92.3
linoleic (C18:2n-6)	125	89	302
alpha linolenic (C18:3n-3)	66	26	21
arachidonic (C20:4n-6)	29	22	46
eicosapentaenoic (C20:5n-3)	21	10	6
docosapentaenoic (C22:5n-3)	24	16	13
docosahexaenoic (C22:6n-3)	7	2	8
Total	4934	3835	2255
Total PUFA	275	177	503
Total SFA	2167	1575	837
PUFA:SFA	0.15	0.11	0.58
n-6:n-3	1.32	2.11	7.22

Adapted from Enser *et al.* (1996). n=omega.

Meat and fish are the primary sources of long-chain PUFAs in the human diet; meat being consumed more regularly in the Western diet (Higgs *et al*., 2002). Trimmed lamb and beef have omega-6 fatty acid:omega-3 fatty acid (n-6:n-3) ratios of 1.3 and 2.2, respectively (Higgs *et al*., 2002). Isomers of CLA (mainly *cis*-9, *trans*-11 and *trans*-10, *cis*-12) have been studied in many animal models and findings include the ability to inhibit cancer, prevent cardiovascular diseases, reduce body fat (Mir *et al*., 2003; Park, 2009), improve immune response (Cook *et al*., 1999) and reduce the risk of developing diabetes (Belury and VandenHuevel, 1999). The North American CLA daily intake was

estimated to be 52-137 mg CLA (Ritzenthaler, 1998); animal studies suggest that in order to see health effects of CLA, humans must consume 3 g daily (Song and Kennelly, 2003). The CLA content of lamb is typically 5.6 mg/g of fat (see Table 1-2). Altering the fatty acid content of ruminant meat and products to increase the PUFA:SFA ratio, maintain (or lowering further) the n-6:n-3 ratio and increase CLA is current research where scientists are looking to manipulate the type of fat in ruminant meat for human benefits. Various studies have shown that ruminant food products can be altered by via diet – both through forage grazing as well as lipid supplementation.

Table 1- 2 CLA content of animal products

Product	CLA content (mg/g fat)
Ruminant products:	
Homogenized milk	5.5
Butter fat	4.7
Mozzarella cheese	4.9
Yogurt	4.8
Ice cream	3.6
Beef	2.9-4.3
Lamb	5.6
Non-ruminant products:	
Pork	0.6
Chicken	0.9
Salmon	0.3

Adapted from Chin *et al.* (1992).

Although fat from meat consumption has been considered to contribute negatively to human health by increasing the saturated fat content of the human diet, meat lipid is now being recognized as an important constituent of the nutritive value of meat. The intramuscular and subcutaneous adipose and muscle tissue itself are sources of important fatty acids. The lipid in adipose tissue is primarily TAG (>90%) whereas the lipid content of muscle tissue contains a significant portion of phospholipids (Wood *et al.*, 2008). While TAG are important in storing fat for energy reservation, phospholipids are crucial to membrane structure and preferentially uptake PUFA where they contribute membrane fluidity and function.

The fatty acid content of ruminant muscle and adipose tissue is heavily influenced by rumen fermentation. The action of biohydrogenation of dietary PUFA by microbes within the rumen results in the saturation of fatty acids to mainly stearic acid. This is a natural process that partially protects the rumen microbe population from toxic effects of USFA and is the major contributor to the fully and partially hydrogenated fatty acid content of ruminant meat and tissues. While biohydrogenation of dietary USFA contributes to stearic acid production, it also results in the production of beneficial fatty acids such as CLA. Conjugated linoleic acid isomers are products of incomplete biohydrogenation of linoleic acid (LA; C18:2 n-6) and alpha-linolenic acid (ALA; C18:3 n-3). Increasing CLA content of ruminant meat is a focus of many ruminant fatty acid studies due to its potential beneficial effects on human health.

In addition, vaccenic acid (VA; C18:1 *trans*-11), also an intermediate of ruminal biohydrogenation of LA and ALA can be converted to *cis*-9, *trans*-11 CLA through the action of D9D in ruminant tissues (Griinari *et al*., 2000). Therefore strategies to increase VA production in the rumen are of interest as the conversion of VA to *cis*-9, *trans*-11 CLA in ruminant tissues by action of D9D is responsible for the majority of the CLA content of ruminant products (Kim *et al*., 2009). As mentioned previously, D9D is also responsible for the conversion of stearic acid to oleic acid (a major MUFA in ruminant adipose tissue and crucial fatty acid in lipogenesis) supporting the lipogenic capacity of adipose tissue (being a major site of lipogenesis in the ruminant).

Although genotype contributes to some degree to the fatty acid composition of an animal's tissues, studies have found that diet is considerably more important in determining the fatty acid profile of ruminant tissues (De Smet *et al*., 2004).

1.2.1 Effect of Forage

Grazing legume-based pastures is an attractive option for sheep producers as it is a low input system that can provide feed with good nutritive value including a high crude protein concentration. Compared to a grass, legume-based pasture is not only higher in

crude protein but can be associated with higher dry matter intake (reduced ruminal retention time due to quick breakdown and digestibility of legume leaves) which translates into a forage with greater performance potential (Speijers *et al*., 2004). Forage legumes in general have a high amount of rumen-degradable protein which can be a source of protein and energy for rumen microbes. In addition, red clover legume has a natural protein protection as a result of greater polyphenol oxidase, an enzyme that reduces proteolysis (Wilkins and Jones, 2000) which allows protein to escape the rumen un-degraded, improving protein utilization (Turner *et al*., 2011; Jones *et al*., 1995a & b).

Forages are high in ALA compromising 50-75% of total fatty acids (Dewhurst *et al*., 2009). The increase in tissue PUFA content with ruminants that graze legumes as compared to grasses results from the dietary PUFA content of the legumes, higher intake and reduced biohydrogenation in the rumen due to high passage rate as well as protective effects of polyphenol oxidase in the case of red clover (Lee *et al*., 2009). Regardless, forages, in particular fresh grasses and legumes, provide the ruminant diet with a beneficial PUFA profile resulting in higher PUFA and CLA tissue content [as reviewed by Dewhurst *et al*. (2009) and Nieto and Ros (2012)].

Table 1- 3 Fatty acid composition of forages (% of total fatty acids)

Forage[A]	Oil content (g/kg DM)	16:0	18:0	18:1*cis* n-9	18:2 n-6	18:3 n-3	Reference
Grass	29	20.8	3.29	5.74	14.0	49.2	French *et al.* (2000)
RC (spring)	27	24.2	4.35	5.21	19.1	45.9	Loor *et al*. (2003)
RC (fall)	23	31.1	4.81	8.00	21.4	33.6	Loor *et al*. (2003)

[A]RC= red clover-based forage. Adapted from Woods and Fearon (2009).

French *et al.* (2000) studied the effects of increasing the amount of forage in ruminant diets on the fatty acid profile of muscle. They found that when steers grazed only grass (22 kg DM/head/d), the concentration of PUFA, ALA and total CLA in intramuscular adipose was significantly increased while SFA was decreased compared to treatments which contained some proportion of concentrate ($P<0.05$). Decreasing the concentrate, in turn increasing the amount of grass intake was found to cause a linear decrease in SFA ($P<0.001$) and n-6:n-3 ratio ($P<0.01$) in addition to a linear increase in PUFA:SFA

(P<0.01), ALA (P<0.001) and CLA (P<0.001). The decrease in SFA was attributed to decreases in C10:0 and C16:0 with increasing level of grass fed (P<0.05).

Realinia *et al*. (2004) studying effects of pasture vs. concentrate during finishing on fatty acid composition of muscle lipid in beef also suggest that pasture grazing increases LA, ALA, eicosapentaenoic acid (EPA; C20:5 n-3), docosapentaenoic acid (DPA; C22:5 n-3), *cis*-9, *trans*-11 CLA and total CLA, total PUFA and PUFA:SFA ratio (P<0.01) while decreases C14:0, C16:0, and n-6:n-3 ratio (P<0.01) of intramuscular adipose when compared to concentrate-fed steers. A similar study with lamb found comparable results where lambs that were fed vetch (*Vicia sativa*) had increased n-3 PUFA (P<0.05) and decreased amount of muscle total fat, C14:0 (P<0.05), C16:0 (P<0.01), C18:0 (P<0.01), total SFA and n-6 (P<0.01) resulting in a reduced n-6:n-3 ratio (P=0.001) when compared to concentrate-fed lambs (Vasta *et al*., 2009).

Additional studies have found that grazing cattle on grass or feeding grass silage, as opposed to concentrate feeding results in increased concentration of n-3 PUFA within the phospholipid and TAG portions of muscle lipid (Dannenberger *et al*., 2005; Warren *et al.*, 2002). Warren *et al.* (2002) and Warren *et al.* (2003) showed significant (P<0.001) increases in muscle ALA, EPA and DHA with cattle fed grass silage or grass when compared to concentrate fed cattle. Warren *et al.* (2003) also reported a significant (P<0.001) decrease in the n-6:n-3 ratio in grass-fed cattle.

Scollan *et al*. (2002) found that cattle grazing white clover-rich pasture had an increase in PUFA content of muscle lipids, contributing to an increase in the PUFA:SFA ratio when compared to cattle that had grazed grass. Similarly, sheep that grazed legume-based pastures had higher concentration of PUFA in total muscle lipid, contributing to a higher PUFA:SFA ratio when compared to sheep that grazed grass (Vipond *et al*., 1993; Fraser *et al*., 2004; and Lourenço *et al*., 2007).

When red clover is added to the diet of grass-grazed finishing beef, increases in muscle content of ALA is prominent and is greater as the proportion of red clover increases from

50:50 to 100% of DMI (Scollan *et al*., 2006b; P<0.001). In addition, a decrease in n-6:n-3 (P<0.001) and increase in the PUFA:SFA ratio (P<0.05) was found with red clover. In addition, Lee *et al.* (2008) found that red clover silage reduced the biohydrogenation of LA and ALA in the rumen and increased rate of flow into the duodenum. As mentioned previously, the response from red clover grazing and/or red clover silage-fed cattle has been linked to a reduction in partial hydrogenation in the rumen which is thought to be linked to the protective actions of enzyme polyphenol oxidase. Polyphenol oxidase protects glycerol-based lipid in the forage from plant lipases during ensiling as well as from microbial lipases in the rumen (Lee *et al*., 2007). As a result, cattle that consume red clover have more PUFA exiting the rumen (both dietary and produced by ruminal biohydrogenation) and therefore a greater PUFA:SFA content in tissues (Lee *et al*., 2008). In addition, polyphenol oxidase has been found to have greater protective effect on highly USFA [as seen in steers Lee *et al.* (2003b) and Lee *et al.* (2006)] which helps explain why red clover grazed ruminants have greater amounts of muscle n-3 compared with grass grazed ruminants. Cull dairy cows fed red clover silage had greater muscle ALA (P<0.001) and total n-3 fatty acids (P<0.01) with a tendency to have increased PUFA:SFA and decreased n-6:n-3 (P<0.10) compared to grass silage-fed cattle (Lee *et al.*, 2009).

Study by Fraser *et al*. (2004) found significant increases in LA and ALA percent of total fatty acids in muscle tissue when lambs grazed red clover (LA: 4.47 and ALA: 2.86), and alfalfa (LA: 4.02 and ALA: 2.72) when compared to perennial ryegrass (LA: 2.91 and ALA: 2.07; P≤0.01; Table 1-4). As a result of high tissue LA and ALA, grazing legume forage increased muscle PUFA:SFA content (0.19 and 1.16 vs. 0.12, respectively; P≤0.01). However, legume forage (red clover and alfalfa) grazed lambs had a greater n-6:n-3 ratio of muscle tissue when compared perennial ryegrass (1.13 and 1.08 vs. 0.98, respectively; P≤0.01). This is likely due to the higher intake and increased rate of passage seen with legume forages, resulting in greater amount of LA and ALA being absorbed (Scollan *et al.*, 2002). Although legume forages increased the n-6:n-3, the values are substantially lower than the recommended (Department of Health and Social Security, 1994) value of 4.

Daniel *et al*. (2004) also looked at forage (alfalfa pellets) vs. concentrate on lamb muscle fatty acid profile. They found that oleic acid and C18:1 *trans*-10 were decreased while VA, ALA and *cis*-9, *trans*-11 CLA were increased in the forage-based system when compared to concentrate ($P<0.001$). In addition, they analyzed the fatty acid content of adipose depots. Within subcutaneous adipose, VA, ALA and *cis*-9, *trans*-11 CLA were increased while oleic acid and C18:1 *trans*-10 were decreased with the forage-based system when compared to concentrate based ($P<0.001$).

Dervishi *et al*. (2010) showed that grazing ewes with nursing lambs on legume-based pasture with or without concentrate supplementation to lambs increased muscle content of ALA ($P<0.05$) and *cis*-9, *trans*-11 CLA ($P<0.001$). This is in agreement with Dhiman *et al*. (2006) whose study demonstrated in beef that pasture increased *trans*-10, *cis*-12 CLA, *cis*-9, *trans*-11 CLA and ALA ($P<0.01$). In addition, Dervishi *et al.* (2010) found increased muscle content of n-3 PUFA ($P<0.05$) with legume-based pasture when compared to concentrate-based systems (Table 1-4). With strictly grazed lambs, oleic acid and n-6 PUFA content of muscle ($P<0.05$) as well as n-6:n-3 ratio ($P<0.001$) were decreased compared to all other feeding systems which contained concentrate. They found that feeding system was a large contributor to lamb muscle fatty acid composition.

Companion studies found similar results demonstrating effects of pasture on lamb intramuscular fatty acid profile (Scerra *et al*., 2007; Dervishi *et al*., 2012). Both studies found that pasture grazed lambs had higher n-3 PUFA when compared to concentrate-based diet (Scerra *et al*., 2007; $P<0.05$) and when compare to hay-based diet (Dervishi *et al*., 2012; $P<0.05$) which is in agreement with results found by Dervishi *et al.* (2010). In addition, Dervishi *et al*. (2012) found an increase in intramuscular VA and CLA ($P<0.05$) content with pasture grazed lambs while Scerra *et al.* (2007) found a trend for pasture to increase CLA ($P<0.07$). Various ruminant studies beyond the ones mentioned above have found that grazing pasture promotes CLA formation in muscle tissue (French *et al*., 2000 – steers; Aurousseau *et al*., 2004 - lambs; Daniel *et al*., 2004 - sheep; Kay *et al*., 2004 – dairy cattle; Dannenberger *et al*., 2005 – beef cattle; Aurousseau *et al*., 2007 –

lambs). In summary, this research suggests that the fatty acid profile of ruminant meat can be enhanced with higher n-3 PUFA and CLA by pasture grazing.

Pasture grazing having many benefits to the fatty acid composition of ruminant meat can be limited by environmental conditions. Because fresh forage is often not available year-round, many producers incorporate concentrates into the diet of finishing animals. However, because concentrates are higher in LA and lower in ALA, alteration of muscle fatty acid profile will occur and may deflate or even diminish the effect of pasture grazing (Scerra *et al*., 2011). Scerra *et al*. (2011) studied the effects of adding a concentrate finishing period (either 14 days or 37 days before slaughter) to pasture grazed lambs. They found that lambs raised and finished on pasture had reduced proportions of C12:0 ($P<0.05$), C14:0 ($P<0.01$) and C16:0 ($P<0.01$) fatty acids when compared to all other treatments which included concentrate; these SFA are thought to be involved with health risks associated with raising plasma LDL and total cholesterol (Scollan *et al*., 2006a). As concentrate inclusion increased, the percentage of these fatty acids increased. Conversely, VA was highest in lambs that grazed pasture continuously, coinciding with results from Realini *et al.* (2004) in beef and Vasta *et al.* (2009) in lambs. In addition, *cis*-9, *trans*-11 CLA was highest in lambs that had solely grazed pasture and decreased as the time on concentrate increased ($P<0.01$). Linolenic acid, EPA and DHA were found to be significantly higher in pasture grazed lambs when compared to diets with any level of concentrate-inclusion ($P<0.01$), decreasing with time on concentrate. As a result, the muscle content of total n-3 PUFA followed the same pattern ($P<0.01$). When comparing the treatments where grazing lambs were finished on concentrate, the fewer days on concentrate resulted in higher ALA, EPA and DHA ($P<0.05$) with 37 days on concentrate being no different than strict concentrate rearing of lamb. The n-6:n-3 ratio was lower for grazing lambs ($P<0.01$) and increased with time on concentrate.

Table 1- 4 Studies in lambs showing effects of forage on percent fatty acid content of muscle

	16:0	18:0	18:1n-9	18:2n-6	18:3n-3	20:5n-3	22:6n-3	P:S[A]	n-6:n-3	VA	CLA[B]
Aurousseau *et al*. (2004)											
Grass low	20.0	22.3	26.3	3.9	1.9	0.64	0.35	0.23	2.0	6.4	1.2
Grass high	20.6	19.9	27.8	3.6	1.6	0.10	0.21	0.20	2.0	6.2	1.6
Concentrates low	24.8	17.5	31.1	5.1	0.7	0.12	0.21	0.19	6.0	3.5	0.7
Concentrates high	24.7	16.7	31.6	4.9	0.7	0.11	0.15	0.20	5.7	3.6	0.6
Aurousseau *et al.* (2007)											
Grass growth/finishing	20.7	15.8	23.4	5.8	2.6	1.8	0.6	0.7	1.3	4.4	1.1
Grass then 22d concentrate	23.2	16.3	25.8	5.7	1.7	1.3	0.5	0.8	1.7	4.7	1.0
Grass then 41d concentrate	22.5	15.3	29.9	5.7	1.2	1.2	0.5	1.2	2.2	2.0	0.9
Concentrate growth/finishing	23.9	13.8	31.4	6.4	1.3	1.0	0.5	1.2	2.4	1.6	0.7
Dervishi *et al.* (2010)											
Alfalfa grazed	22.6	11.4	34.1	6.43	2.56	1.30	0.86	0.37	1.4	3.94	1.17
Alfalfa grazed + concentrate	22.7	12.1	33.6	6.89	2.58	1.16	0.73	0.36	1.6	4.18	1.14
Concentrate	22.5	11.7	39.7	7.35	0.65	0.54	0.52	0.42	3.9	3.48	0.43
Dervishi *et al.* (2012)											
Grass	19.8	14.2	36.2	5.50	2.03	1.08	0.70	0.36	1.5	4.76	1.70
Hay	20.6	13.9	38.7	4.79	1.62	0.77	0.52	0.29	1.7	3.63	1.38
Daniel *et al.* (2004)											
Grass pellets	24.82	15.79	32.40	4.04	4.04	-	-	-	-	2.25	2.25
Concentrate similar growth rate	25.70	16.63	33.07	5.99	5.99	-	-	-	-	1.39	1.39
Concentrate ad lib.	25.96	14.06	36.93	5.46	5.46	-	-	-	-	0.85	0.85
Fraser *et al.* (2004)											
Alfalfa	22.5	16.5	32.0	4.02	2.72	0.93	0.25	0.16	1.08	3.10	1.09
Red clover	20.5	16.1	32.5	4.47	2.86	1.03	0.27	0.19	1.13	3.71	1.33
Perennial ryegrass	21.4	18.5	32.8	2.91	2.07	0.90	0.24	0.12	0.98	3.65	1.23
Scerra *et al.* (2011)											
Pasture	18.65	14.76	31.62	9.06	3.13	1.25	1.12	0.67	3.03	1.48	1.38
75d pasture then 14d concentrate	19.53	14.70	30.10	10.55	1.59	0.80	0.43	0.63	6.17	1.25	1.07
52 pasture then 37d concentrate	20.85	14.90	28.99	12.15	0.55	0.61	0.18	0.59	12.28	1.11	0.98
Concentrate	22.82	15.22	27.53	12.55	0.51	0.38	0.28	0.55	12.70	0.42	0.59
Vasta *et al*. (2009)											
Herbage	23.4	20.2	36.3	12.6	2.81	1.49	0.477	0.54	0.30	3.14	0.805
Concentrate	43.6	28.8	67.8	19.0	2.12	1.07	0.482	0.41	0.54	7.94	1.28

[A]P:S= polyunsaturated to saturated fatty acid ratio; [B]CLA = *cis*-9, *trans*-11 CLA

1.2.2 Effect of Lipid Supplementation

The majority of studies in lamb looking at altering and improving the fatty acid composition of muscle (and adipose) have focused on the addition of a lipid supplement to the lamb diet. Studies involving the addition of a lipid source rich in LA, such as soybean, safflower or rapeseed oil, have found increased concentrations of total fatty acid content of muscle tissue, in general (as reviewed by Sinclair, 2007). As one would expect, muscle LA content increases. Often, there is no effect on total SFA (slight decreases in C16:0 accompanied with slight increases in C18:0). Scientists have also found a decrease in oleic acid.

Wachira *et al.* (2002) demonstrated the benefits of feeding n-3 PUFA to lambs. Using linseed they found a 2.2-fold increase in ALA content of lamb muscle (Suffolk lambs). Cooper *et al.* (2004) found a similar response where a source of ALA increased in muscle lipid when linseed oil was fed. However, feeding ALA to increase the EPA and DHA content of lamb meat may not be effective. Both Wachira *et al.* (2002) and Demirel *et al.* (2004) found the conversion rate of ALA to EPA and DHA to be low in lamb muscle and unchanged by dietary ALA supplementation when compared with the control, confirming results by Scollan et al. (2001) who showed there was limited conversion of ALA to EPA and DHA in beef. As reviewed by Sinclair (2007) there is a poor relationship between ALA duodenal outflow and EPA and DHA content in the muscle. In addition, Raes *et al.* (2004a) found that the n-6:n-3 content of the diet affects the EPA and DHA concentration in lamb muscle, whereby diets high in n-6 had lower EPA and DHA muscle content. These studies suggest that supplementing a source of EPA and DHA is a more effective way to increase these fatty acids in muscle. When fish oil is supplemented into the lamb diet (as a source of EPA and DHA) muscle EPA increased 1.5 to 3.4-fold while DHA increased 1.3 to 2.8-fold (Wachira *et al.,* 2002; Copper *et al*., 2004; Demirel *et al.,* 2004; Table 1-5).

Table 1- 5 Studies in lambs showing effects of lipid supplementation on percent fatty acid content of muscle

	TFA[A]	16:0	18:0	18:1n-9	18:2n-6	18:3n-3	20:5n-3	22:6n-3	P:S[B]	n-6:n-3	VA	CLA
Wachira *et al*. (2002)												
Dried grass + saturated fat	32.3	25.4	14.5	34.1	4.9	1.40	0.68	0.28	0.15	2.08	3.83	0.99 *
Dried grass + whole linseed	30.3	21.8	14.3	30.9	4.0	3.10	1.03	0.40	0.18	0.94	6.61	1.55 *
Dried grass + fish oil (36 g/kg)	34.6	25.0	11.9	25.9	3.4	1.40	2.32	0.79	0.12	0.68	7.06	1.10 *
Dried grass + linseed + fish oil (18 g/kg)	32.7	23.9	12.2	27.4	3.5	1.99	1.61	0.63	0.14	0.78	8.59	1.66 *
Bessa *et al.* (2005) €												
No lipid supplement	10.5	24.4	14.1	35.6	6.4	0.36	0.15	0.17	0.23	7.7	0.96	0.55 ζ
Soybean oil (100g/kg DM)	10.1	22.7	14.2	26.0	9.5	0.61	0.23	0.17	0.35	7.5	0.55	0.44ζ
Copper *et al.* (2004) €												
Linseed oil (43 g/kg)	34.0	20.7	17.5	31.6	4.8	2.70	0.71	0.22	0.26	1.37	5.17	1.09ζ
Fish oil (43 g/kg)	37.0	24.9	13.6	28.5	3.3	1.54	1.29	0.61	0.19	1.10	4.79	0.739 ζ
Protected linseed/soybean	38.2	20.3	15.7	29.5	14.5	3.68	0.56	0.14	0.57	3.15	2.78	0.679 ζ
Fish oil/algae	36.7	24.3	13.6	27.1	4.1	0.79	2.33	2.55	0.30	0.68	4.89	0.752 ζ
Protected linseed/soybean/fish	39.3	23.0	13.9	28.1	10.1	2.50	1.24	2.20	0.46	1.70	5.50	0.815 ζ
Demirel *et al*. (2004)												
Dried grass + saturated fat	24.5	23.1	12.8	38.6	4.1	2.01	0.98	0.29	0.17	2.11	-	-
Dried grass + protected linseed	31.6	22.9	13.6	37.3	3.3	3.66	1.11	0.22	0.19	0.93	-	-
Dried grass + protected linseed and fish oil	31.4	23.5	13.5	33.0	3.5	2.41	1.51	0.37	0.15	1.54	-	-
Radunz *et al.* (2009) €												
No lipid supplement	-	23.6	9.64	0.27	6.17	4.18	0.07	0.31	0.33	-	0.25	0.80 ζ
2 soybean: 1 linseed oil (~5% DM)	-	24.1	9.38	0.33	6.85	4.90	0.04	0.32	0.38	-	0.29	0.92 ζ

[A]TFA= total fatty acids (g/kg)

[B]P:S= polyunsaturated to saturated fatty acid ratio

€ = concentrate-based diet

ζ = *cis*-9, *trans*-11 CLA

* = total CLA

Conjugated linoleic acid has been studied in many animal models to determine its effects on lipid metabolism, as *trans*-10, *cis*-12 is known to reduce adipose deposition. In the mouse model, CLA in the diet has been found to increase metabolic rate and fatty acid oxidation while reducing fatty acid synthesis, lipoprotein lipase activity and division and differentiation of adipocytes (Wahle *et al*., 2004). Study by Wynn *et al*. (2006) found a 36-fold increase in muscle *trans*-10, *cis*-12 CLA when a source of CLA (containing similar amounts of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) was fed to growing lambs at 100g/kg DM (approximately 50 g/kg DM of each isomer; Table 1-6). They also found a 2 to 5-fold increase in *cis*-9*, trans*-11 CLA and a 3 to 20-fold increase of *trans*-10, *cis*-12 CLA in liver (not shown) and adipose tissues (Table 1-6).

Table 1- 6 Fatty acid composition (percent total fatty acids) of muscle and adipose tissue of lambs supplemented with a source of CLA

Fatty acid	Control	CLA (25g/kg DM)	CLA (50g/kg DM)	CLA (100g/kg DM)
Muscle tissue				
Vaccenic acid	1.42	1.40	1.39	2.08
CLA cis-9, trans-11	1.01	1.15	1.30	1.53
CLA trans-10, cis-12	0.01	0.05	0.14	0.36
Subcutaneous adipose				
Vaccenic acid	1.11	1.42	1.73	2.72
CLA cis-9, trans-11	0.82	1.09	1.34	1.95
CLA trans-10, cis-12	0.04	0.18	0.38	0.76
Visceral adipose				
Vaccenic acid	1.05	1.38	1.88	2.72
CLA cis-9, trans-11	0.67	0.95	1.23	1.77
CLA trans-10, cis-12	0.05	0.19	0.36	0.75

CLA = 80% pure isomers – similar proportions of *cis*-9, *trans*-11 and *trans*-10*, cis*-12
From Wynn *et al*. (2006).

1.3 NUTRIGENOMICS AND LIPID METABOLISM

Changes in animal tissue fatty acid profile are associated to the activity of lipogenic enzymes (Ladeira *et al*., 2012). Abundance of these enzymes is regulated by genes. As mentioned previously, diet can affect the tissue lipid content. Study in nutrigenomics, looking at effects of nutrition on genes involved in fatty acid synthesis within tissues, will help us better understand the mechanisms behind changes in tissue fatty acid content by type.

The process of lipogenesis is defined by an animal's genotype, environment, as well as gene × environment interactions whereby genes are activated or deactivated in response to environmental conditions (Laliotis *et al*., 2010). Differences in lipid metabolism (metabolic rate and fat distribution) have been attributed to breed (dairy vs. meat breeds; Kempster, 1983) related to genetic make-up and the regulation of lipogenesis (Belk *et al*., 1993). According to Laliotis *et al*. (2010), an animal's lipid metabolism is controlled at three levels in response to environmental conditions for maintaining homeostasis: 1) acute; 2) chronic and 3) autonomic. Acute (rapid response to environmental changes) refers to the actions of hormones (insulin and glucagon) and endogenously produced mediators of physiological response (prostaglandins). Chronic (long-lasting or extended response to environment) refers to lipid metabolism at tissue-level to meet the changing needs of a physiological, nutritional or disease-state. Lastly, autonomic control refers to the functions of adipose tissue, whereby it produces hormone-like compounds (leptin and adiponectin) that control adipose-mass (i.e. lipogenesis).

In order to maintain homeostasis, genes involved in lipid metabolism must be regulated (be turn-on or shut-off). Measuring gene expression of proteins and enzymes involved in lipid metabolism helps us understand lipid metabolism. Expression of genes involved in lipogenesis, β-oxidation, lipid transport and uptake as well as transcription factors in response to controlled environmental challenges (via dietary fatty acid manipulation) will be a main objective of this thesis.

In mammals, PUFA play a role in membrane structure and signal transduction. More recently, with increased interest in dietary PUFA and health, the role of PUFA in metabolism have been studied extensively. The mammalian body has the capability, through altering gene expression, to adapt to changing diets and environments (Nakamura *et al*., 2004). Through the use of rodent models it was first discovered that diets containing high levels of PUFA decreased the activity of acetyl-CoA carboxylase (ACC; Salati and Clark, 1986) and fatty acid synthase (FAS) in the liver (Clark *et al*., 1990). These initial studies established the potential importance of PUFA in mammalian lipid

metabolism (Sessler and Ntambi, 1998). Aside from liver, enzymes involved in lipid metabolism have been found to be regulated by dietary PUFA in adipose tissue with differing effects dependent on deposition site. In a study by Raclot *et al*. (1997), dietary n-3 PUFA influenced the gene expression of FAS and LPL in rat internal adipose while having no effect on subcutaneous adipose.

1.3.1 Enzymes in Lipid Metabolism

Enzymes are specialized molecules that accelerate chemical reactions. The regulation of enzyme production and activation or deactivation is complex. Looking at mRNA abundance has been a chosen method to study enzymes as research shows that expression of mRNA is a good indication of protein expression (often being correlated). One must keep in mind that many factors affect enzyme activity including substrate availability, cofactors, allosteric and covalent modulation, hormones and dietary nutrients.

Fatty acid synthesis is tightly controlled by dietary nutrients and hormones (Sul *et al*., 2000). When conditions of lipogenesis are favorable the gene expression of lipogenic enzymes and their tissue concentrations increase while the opposite is true when conditions of lipogenesis are unfavorable (i.e. fasting). This is thought to occur due to dietary nutrients and hormones directly, and through nutrient-hormone interaction. Changes in energy intake result in changes of circulating glucose which in turn modulates the release of insulin. Insulin induces enzymes involved in fatty acid synthesis. The opposite is true for glucagon which, during starvation, suppresses enzymes involved in fatty acid synthesis (Sul *et al*., 2000).

Acetyl-CoA carboxylase is the first enzyme of the *de novo* synthesis of fatty acids. It generates malonyl-coA from acetyl-coA and is a major regulatory step in fatty acid synthesis (Stipanuk, 2006). This enzyme exists in the cytosol and is found to be expressed in all tissues with greatest abundance in lipogenic tissues (Lopez-Casillas *et al*., 1991). Acetyl-CoA carboxylase activity is very complex and is regulated through both allosteric (positively by citrate; negatively by malony-CoA and long-chain acyl-

CoA) and covalent (under hormonal control) modulation (Bernard *et al*., 2008; Brownsey *et al*., 2006).

Fatty acid synthase is the enzyme responsible for the synthesis of medium-chain fatty acids (C6-C16). It is responsible for the condensation, reduction, dehydration and further reduction of the malonyl and an acetyl group resulting in a saturated acyl group lengthened by two carbons. After each two carbon addition, reductions occur and convert the enlarging chain to a SFA of four to 16 carbons in length; the final product being palmitate (C16:0), although in the mammary gland C4:0-C16:0 can be formed. In the ruminant, it is a multi-complex enzyme of 2513 amino acids having 6 enzyme activity domains and is synthesized from an mRNA sequence ranging from 8.4-9.3 kb (Bernard *et al*., 2008). Palmitate is a negative feedback for ACC reducing the conversion of acetyl-CoA to malonyl-CoA thus decreasing further palmitate generation. Fatty acid synthase is regulated by dietary fat and plane of nutrition (Salati and Goodridge, 1996). Dietary PUFA decreased FAS activity in lipogenic tissues by reducing gene transcription and mRNA stability (Sul *et al*., 2000).

Within the mammalian body, desaturase enzymes function to desaturate fatty acids. They introduce a carbon-carbon double bond by removing two hydrogen atoms of a long-chain fatty acid. The importance of these enzymes lies with the desaturation of fatty acids that determines the physical properties of phospholipids (membranes) and TAGs (stored lipid) as well as in cell-signaling (Nakamura and Nara, 2004) where PUFA and PUFA-metabolite production is important. Delta-9-desaturase, also known as stearoyl-CoA desaturase-1, catalyses the removal of hydrogen from saturated fatty acyl chains, creating a double bond in the delta-9 position. In ruminants, it has two key functions: 1) as in other mammals, D9D catalyzes the endogenous formation of MUFA from SFA, most commonly oleic acid from stearic acid where oleic acid is the most predominant fatty acid in TAG stored in adipose (Nakamura and Nara, 2004); 2) D9D is the enzyme responsible for the synthesis of *cis*-9, *trans*-11 CLA from VA in ruminant tissues (Herdmann *et al*., 2010).

Thyroid hormone responsive spot 14 (Spot14) is a protein that is speculated to be involved in lipogenesis (Colbert *et al.,* 2010; Harvatine and Bauman, 2006). Although the exact function of Spot14 is not concrete, it has been found to be involved in the regulation of lipogenesis where it responds to sterol regulatory element binding protein (SREBP; Matel *et al*., 2006). In the mammary gland, along with SREBP, Spot14 is decreased during milk fat depression induced by *trans*-10, *cis*-12 CLA (Harvatine and Bauman, 2006) and aids in the regulation of mammary gland fatty acid synthesis through transcription regulation of lipogenic genes (Cunningham *et al*., 1998). Harvatine and Bauman (2006) found that Spot14 is highly expressed in bovine liver and adipose tissue and moderately in the mammary gland.

Lipoprotein lipase (LPL) is an important enzyme involved in the lipolysis of TAGs. Lipoprotein lipase is responsible for the breakdown of TAGs of chylomicrons and very low density lipoprotein (VLDL), providing non-esterified fatty acids (NEFA) to adipose tissue (Auwerx *et al*., 1992). Lipoprotein lipase also plays a vast role in lipoprotein uptake and transport by promoting the exchange of lipids between lipoproteins (Medh *et al*., 2000).

Hepatic lipase (HL), a member of the lipase family and expressed in the liver, hydrolyses phospholipids and TAGs in plasma lipoproteins. Essentially, HL enables hepatocytes to take up fatty acids. Another function of HL is the ability to convert intermediate density lipoprotein to low density lipoprotein and convert TAG-rich high density lipoprotein (HDL) to TAG-poor HDL (Connelly, 1999).

1.3.2 Adipokines

Leptin is a cytokine (cell-to-cell signaling protein) that is produced by adipocytes and is responsible for regulating energy intake and energy expenditure (Jéquier, 2002). Leptin inhibits lipogenesis while stimulating energy expenditure and fat break-down (Havel, 2004). Leptin expression is correlated to amount of adipose tissue. It is thought to control energy homeostasis by acting as a negative feedback signal to the brain when

adiposity is on the rise. In animal studies, leptin has been directly related to altering satiety and amount of energy burned by targeting TAG stores in adipose tissue as a source of energy (Hynes and Jones, 2001). Dietary fat has been linked to leptin expression and action; dietary PUFAs influence leptin circulation in the blood and may be the primary link to PUFAs and weight management (Hynes and Jones, 2001). Study in the ruminant (cattle; Gillis *et al*., 2004a) as well as the non-ruminant (rabbit; McCullough *et al*., 2011) has shown that feeding LA or ALA, increases leptin expression in adipose tissue. Also, study in ruminants (specifically cattle) has also found that visceral adipose has greater leptin expression than subcutaneous adipose (Chilliard *et al*., 2001; Ren *et al*., 2002).

Another adipocyte derived hormone, adiponectin, has been found to regulate energy homeostasis and is involved in the regulation of glucose and lipid metabolism. Circulating levels of adiponectin are inversely related to adiposity where lean individuals have higher circulating adiponectin than obese. Adiponectin influences the body's response to insulin and has been linked to decreasing insulin resistance by increasing fatty acid oxidation and inhibiting glucose production in the liver (Lihn *et al*., 2005). Insulin resistance is induced by high fat diets having a link to obesity. In obese mice, adiponectin is responsible for decreasing TAG content of muscle and liver which in turn decreased insulin resistance (Yamauchi *et al*., 2001). Adiponectin has been found to activate peroxisome proliferator-activated receptor alpha (PPARα) in muscle cells (Yoon *et al*., 2006) which is responsible for up-regulating genes involved in fatty acid oxidation.

1.3.3 Transcription Factors

In order to adapt to environmental changes, the mammalian body is equipped with mechanisms at the gene expression level that alter metabolism of cells for the changing situations. It is believed that the mechanism behind dietary PUFA regulation of genes involved in lipid and carbohydrate metabolism includes a PUFA responsive element (PUFA-RE) within the promoter region of such regulated genes and involves the binding (to activate or block transcription) of transcription factors (Sessler and Ntambi, 1998).

Included in these transcription factors are PPAR and SREBP which moderate gene expression in response to diet as well as environment (Ntambi, 1999). Much of the information and studies involving gene expression and regulation of lipid metabolism-related gene have been in monogastric species. Knowing there are prominent differences in physiology, study in the ruminant may be more challenging and less defined.

Sterol regulatory element binding proteins are a family of transcription factors or transcription regulatory proteins that regulate the expression of genes involved in the synthesis and uptake of cholesterol, fatty acids and TAGs. They are essential for lipid homeostasis within cells being heavily involved in insulin-induction of lipogenesis (Chatterjee *et al*., 2009). Two genes (SREBP-1 and SREBP-2) form three isoforms: the SREBP-1 gene produces isoforms SREBP-1a and SREBP-1c that are formed from different transcription start sites on the SREBP-1 gene, and the SREBP-2 gene produces the transcription factor SREBP-2. SREBP-2 is involved in regulation of cholesterol metabolism (Horton *et al*., 2002; Pai *et al*., 1998). SREBP-2 and SREBP-1a are regulated by cholesterol concentrations. When cholesterol levels are low, inactive SREBP 2 and SREBP-1a gene transcription is increased.

SREBP-1c plays an important role in fatty acid and TAG synthesis and is primarily expressed in lipogenic tissues (Nakamura *et al*., 2004). SREBP-1c activates the transcription of genes involved in fatty acid and TAG synthesis, such as ACC and FAS (Gondret *et al*., 2001; Shimano, 2008). SREBP-1c is predominantly found in lipogenic tissues; primarily the liver as well as in adipose tissue. SREBP-1c is suppressed in the fasted state but induced by high carbohydrates in the diet (Stipanuk, 2006) or over-consumption of sugars and SFA causing increased SREBP-1c transcription and results in high levels of SREBP protein which upregulates fatty synthesis and TAG synthesis in liver and adipose tissue (Shimano, 2008). Regulation of SREBP-1c is primarily transcription-induced (Eberlé *et al*., 2004), where it responds to insulin and converts carbohydrates to TAG (Ferré and Foufelle, 2007). An example includes the induction of SREBP transcription by insulin in adipocytes (Bizeau *et al*., 2003) and in liver cells (Foretz *et al*., 1999) and inhibition by glucagon (Eberlé *et al*., 2004). Therefore, in a

fasting state, SREBP-1c is depressed. SREBP-1c also responds to Liver X Receptor (LXR), a nuclear hormone receptor that is expressed in the liver and activated by a derivative of cholesterol (Lehmann *et al*., 1997). LXR is thought to induce SREBP-1c for fatty acid synthesis needed for cholesterol ester formation (Tontonoz and Mangellsdorf, 2003). LXR is inhibited by PUFA.

SREBPs are unique transcription factors because they are synthesized in an inactive form bound to the membrane of endoplasmic reticulum (ER), the site of their synthesis (Shimano, 2008). SREBP cleavage activating protein (SCAP) binds to SREBP and forms complexes in the ER which have two functions: 1) as an escort protein and 2) as a sterol synthesis regulator (Radhakrishnan *et al*., 2004). When stimulated (by cellular decline of cholesterol levels in the case of SREBP-2 and by insulin in the case of SREBP-1c), SCAP is activated and cleaves SREBP from the ER membrane and escorts it to the Golgi apparatus. In the Golgi, SREBP are again cleaved by Site-1 and Site-2 proteases which produces active transcription factors (Wang *et al*., 1994). These active transcription factors are transported to the nucleus where they bind to sterol regulatory element (SRE) of the promoter regions of lipogenic genes (Horton *et al*., 2002). When cells become depleted in cholesterol, SCAP binds to Insigs (ER retention proteins) which block transport (Radhakrishnan *et al*., 2004).

Normally, SREBP-1c is activated in response to high insulin levels (Gurr *et al*., 2002). Activated SREBP-1c increases the gene transcription of lipogenic enzymes. Dietary fatty acids induce changes in the nuclear abundance of SREBP (Bernard *et al.,* 2001). Specifically, PUFA prevent SREBP-1c activation at the mRNA level (Jump *et al*., 2005; Nakamura *et al*., 2004), halting lipogenesis in tissues (Shimano, 2008). However, when PUFA was incorporated into the diet of rats at low levels, it suppressed genes involved in lipogenesis in the liver even when re-feeding after fasting occurred (Clark *et al*., 1976). PUFAs interact with SREBP-1c at the molecular levels inhibiting SREBP-1c transcription and increasing SREBP-1c mRNA degradation (Jump *et al*., 2005). PUFAs also disrupt SREBP-1c processing by inhibiting cleavage (Shimano, 2008) and altering cell membranes, decreasing the cell's ability to transport SREBP-1c from the ER to the

Golgi attenuating the concentration of "active" SREBP-1c (Deckelbaum, 2006). SREBPs are key regulators of lipid metabolism.

Peroxisome proliferator activated receptors are three nuclear hormone receptors found to regulate genes involved in lipid metabolism pathways including the transport of plasma TAG, cellular fatty acid uptake, and β-oxidation in peroxisomes and mitochondria (Schoonjans *et al*., 1996). They are activated by peroxisome proliferators, thiazolidinedione, and fatty acids including PUFA and associated metabolites (Bernard *et al*., 2001). PPARs heterodimerize with *cis*-9 retinoic acid receptor (RXR) allowing them to bind to a peroxisome proliferator response element (PPRE) in the promoter regions of target genes. There are three isoforms: PPARα, PPARβ/δ, and PPARγ, each encoded by separate genes. Long-chain fatty acids activate these isoforms (Nakamura *et al*., 2004); PPARα is expressed in tissues that oxidize fatty acids for energy; mainly the liver where it controls the breakdown of fatty acids, PPARβ/δ is ubiquitously expressed and PPARγ is expressed in adipose tissue (Bionaz *et al*., 2013; Lee *et al*., 2003a; Schoonjans *et al*., 1996) where it influences the storage of fatty acids into the adipose tissue.

In ruminant studies, PPARγ has been the most abundantly studied isotype. It stimulates the differentiation of adipose cells and production of mature adipocytes (Kersten *et al*., 2000) and is highly expressed in all ruminant adipose tissues (including subcutaneous, omental and perirenal) with very low expression in liver, kidney and intestine (similar to the non-ruminant; Bionaz *et al*., 2013). They play a critical role in adipogenesis in cattle and sheep (Garcia-Rojas *et al*., 2010), responding directly to energy level of diet (Schoenberg and Overton, 2011; Loor *et al*., 2011; Ji, 2011). Study has linked PPARγ with marbling in beef (Lim *et al*., 2011).

The PPARα isotype has been less studied in the ruminant with highest expression in the kidney, followed by the liver (Bionaz *et al*., 2013). Studies have consistently found PPARα expression in the bovine liver (Bionaz *et al*., 2007a and 2007b; Loor *et al.,* 2007; Loor *et al.,* 2006; Selberg *et al*., 2005). In other mammalian species, mRNA has been found in the liver, kidney, heart, intestinal mucosa, and adipose tissue and induces the

expression of genes involved in fatty acid oxidation. It does this by regulating the expression of enzymes involved in β-oxidation (Ferré, 2004). PPARα is essential during fasting, as it regulates fatty acid oxidation of fat stores. Activation of PPARα in goat liver has shown increased fatty acid oxidation in the liver (Cappon *et al*., 2002) and as in non-ruminants, PPARα controls the breakdown of fatty acids (Bionaz *et al*., 2013).

Long-chain fatty acids stimulate the expression of PPARα, acting as ligands (Nakamura *et al*., 2004), activating it and increasing mRNA expression of fatty acid oxidative enzymes. Omega-3 PUFAs have the ability to bind directly to PPARα and PPARγ (Deckelbaum *et al.*, 2006). Binding to PPARα, n-3 PUFA promotes β-oxidation in the liver and adipose, while binding to PPARγ induces adipocyte differentiation in adipose tissue.

The PPARβ/δ is the least studied isotype in this family of regulators in both the ruminant and non-ruminant with limited published data available. As mentioned above, PPARβ/δ is more ubiquitously expressed where greatest expression is found in kidney and placenta followed by adipose tissues with low expression in liver (Bionaz *et al*., 2013).

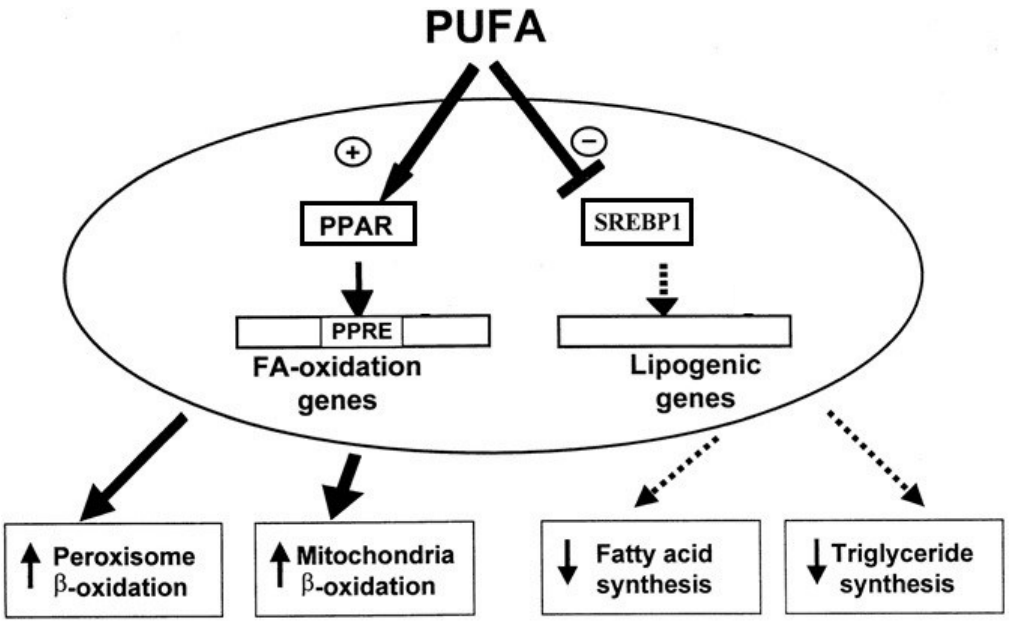

Figure 1: Adapted from Clark (2001). PUFA interaction with nuclear receptors PPAR and SREBP-1 and resultant effects on gene expression

1.3.4 Effect of Forage

Study has shown that changes in tissue fatty acid profile caused by dietary manipulation occur due to effects of dietary fatty acids on the gene expression of enzymes related to lipid metabolism (Dervishi *et al*., 2012). Therefore, it is expected that different diets will have differing effects on gene expression levels of such genes. Study in ruminants has found differences between diets including forage type where alteration of gene expression is evident (Daniel *et al*., 2004; Bernard *et al.,* 2008; Vasta *et al*., 2009; Dervishi *et al*., 2010, Dervishi *et al*., 2011; Dervishi *et al*., 2012).

Daniel *et al*. (2004) studied the effects of a forage diet (alfalfa pellets) vs. a concentrate-based diet in lambs on the fatty acid composition of tissue and gene expression levels of lipogenic enzymes ACC and D9D. In the adipose depots (subcutaneous and omental) of forage fed lambs, D9D expression was down regulated (P=0.001) 3.95-fold in subcutaneous adipose and 3.94-fold in omental while ACC was down regulated (P=0.003) by 2.01-fold in subcutaneous adipose and 2.78-fold in omental when compared to concentrate fed lambs. In the liver, D9D was down regulated with forage fed lambs by 2.7-fold while there was no difference detected with ACC expression. The down regulation of lipogenic genes may be due to the PUFA content of the forage where the alfalfa pellets contained higher levels of ALA which has a greater inhibition of D9D than LA (Sessler *et al*., 1996); the concentrate diet having predominantly LA. Increased oleic acid in the concentrate fed lambs suggests that increased D9D expression increases oleic acid in the tissues of these lambs. However, although D9D expression was decreased in forage fed lambs, *cis*-9, *trans*-11 CLA increased in their tissues. The authors suggest that substrate availability (finding increased VA in rumen fluid) overweighs the decrease in D9D expression.

The effect of feeding systems on gene expression involved in CLA production in muscle tissue of unweaned lambs was explored by Dervishi *et al*. (2010). A significant decrease (8.4-fold) in D9D gene expression was evident in lambs that grazed pasture when compared to strict concentrate system ($P<0.05$). Similar patterns were observed for SREBP1, PPARγ and PPARα where expression was 2.78-fold, 2.21-fold and 1.29-fold

lower, respectively, in grazed lambs although it was not found significantly different. Similar results were found in beef muscle were D9D was decreased by a diet high in n-3 PUFA, while PPARα was not affected (Waters *et al*., 2009). However, they did find significance with SREBP1 which authors suggest may be due to differences in the diet. Expression of D9D was found to reflect fatty acid composition: increased expression of D9D and increased oleic in muscle tissue which reflects findings of Daniel *et al*. (2004). A negative correlation between oleic and VA (-0.531; P=0.000) and positive correlations between oleic acid fatty acid content and D9D gene expression (0.345; P=0.024) and n-6:n-3 (0.708; P=0.000) as well as VA to CLA (0.479; P=0.001) were observed.

A companion study to Dervishi *et al*. (2010) explored the effects of similar treatments on additional lipogenic genes ACC, FAS, D9D and fatty acid uptake enzyme LPL as well as associated transcription factors SREBP1, PPARγ and PARRα in lamb muscle lipid. Significant effects were found among four feeding systems: grazing alfalfa, grazing alfalfa with supplement for lambs, indoor lambs on concentrate with grazing ewes and drylot. Genes related to adipogenesis (ACC, FAS, D9D and LPL) were upregulated with concentrate feeding which may be due to higher energy intake. They suggest that feeding system is an important tool to altering the fatty acid profile of lamb muscle through manipulation of gene expression of enzymes relating to lipid metabolism.

The effect of different forage types on lipogenic gene expression in the muscle was studied by Dervishi *et al.* (2012) in unweaned light lambs. Differences between male and female lambs were evident. In female lambs, SREBP1 expression was upregulated by 1.47-fold with pasture grazing when compared to hay based diets (P=0.03) while there was a tendency of PARRγ (P=0.08) and PARRα (P=0.06) upregulation. However, no relationships between fatty acid muscle content or indicators (SFA, MUFA, PUFA, CLA, n-6, n-3, SFA:PUFA, n-6:n-3) or of gene expression were found. In the male lambs, a hay based diet increased D9D expression (P<0.05) when compared to grazing grass and the increased D9D expression was related to n-6:n-3 and SFA levels. According to Waters *et al*. (2009) and Herdmann *et al*. (2010) D9D expression is regulated in the

muscle of cattle by the n-6:n-3 content of the diet through SREBP1 where SREBP1 is down-regulated by dietary n-3.

1.3.5 Effect of Lipid Supplementation

It is well known that PUFA-rich diets (both n-3 and n-6) reduce hepatic mRNA abundance of genes involved in lipid synthesis including ACC, FAS, D9D and SPOT14 and in adipose tissue, PUFA-rich diets upregulate genes of enzymes and proteins involved in lipid transport, oxidation and adipogenesis and storage are up-regulated in the non-ruminant (as reviewed by Wahle *et al*., 2003). These effects of dietary PUFA on gene expression are mediated by transcription factors (mainly SREBP-1 and PARR; Salter and Tarling, 2007). While DHA is found to be a potent inhibitor of SREBP-1 (and therefore lipid synthesis), CLA (mix of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) has been found to upregulate SREBP-1 in hamsters due to the stimulation of the SREBP promoter by *cis*-9, *trans*-11 CLA (Salter and Tarling, 2007). Conversely, study feeding only *trans*-10, *cis*-12 CLA was found to reduce SREBP-1 expression (Peterson *et al*., 2004).

In addition, adipokines (leptin and adiponectin) have been found to be regulated by dietary n-3 supplementation. Leptin and adiponectin are critical to the regulation of fat storage, energy metabolism and food intake (Moreno-Aliaga *et al*., 2010). Omega-3 PUFA seem to modulate leptin expression; however this modulation has been found to be dose dependent (EPA) as well as dependent on physiological and metabolical state and diet whereby n-3 dietary supplementation decreases circulating leptin in lean animals and increases leptin expression in overweight animals (Moreno-Aliaga *et al*., 2010). In addition, n-3 PUFA (EPA and DHA) increases expression of adiponectin and thus insulin sensitivity (Flachs *et al*., 2006). The action of n-3 PUFA EPA and DHA on leptin and adiponectin is thought to be through the action of PARRs: n-3 PUFA upregulating PPARs which activate the expression of leptin and adiponectin (Moreno-Aliaga *et al*., 2010).

While extensive study of dietary PUFA effects on lipogenic gene expression has been done in rodents and swine, little research has focused on the ruminant. Information on these nutrient-gene interactions in different tissues as well as the regulation of gene expression in the ruminant is inconsistent and lacking. Most of the study involving lipogenesis in the ruminant has linked n-3 PUFA dietary supplementation to decreased D9D expression and its association with reduced CLA and oleic acid tissue content (as in the study by Herdmann *et al*., 2010). Omega-3 PUFA reduce SREBP-1 in ruminants (as in other species) which are linked to reduced lipogenic gene expression (Waters *et al*., 2008).

Cherfaoui *et al.* (2012) recently demonstrated that the growing ruminant liver expresses all genes of transcription factors and enzymes required for n-3 long-chain PUFA synthesis and the tissue contains high amounts of corresponding n-3 PUFA. This study was performed on tissues from 15-18 month old Limousin bulls. They found that liver had the highest expression of key genes in long-chain PUFA metabolism supported with highest tissue long-chain PUFA content when compared to inter-muscular adipose (having 10-fold lower abundance than liver) and muscle (lower than the liver but higher than the inter-muscular adipose for most genes). They also found that the muscle was missing a key desaturase enzyme needed for long-chain PUFA synthesis and that its tissue content of long-chain PUFA was higher than what could be produced based on the amount of long-chain PUFA synthesis enzymes found. Their findings suggested that either the muscle takes up long-chain PUFA circulating from either the diet directly or synthesized by in the liver, packaged as VLDL in the circulation.

1.4 PRODUCTION ATTRIBUTES

1.4.1 Effect of Forage

For lambs in the finishing stages of production, grazing lambs on legume pasture is a natural system that can increase live weight gain and decrease time to slaughter (Fraser *et al*., 2004; Speijers *et al*., 2004). Besides the high crude protein content, most legumes

contain isoflavones or phytoestrogens that mimic the animal's natural estrogens (Pace *et al*., 2006). Red clover (*Trifolium pratense*), the legume in this experiment, contains the isoflavone formononetin which, after being metabolized to equol (an estrogen-like compound) in the rumen, can have deleterious effects when it comes to breeding flocks and reproduction (high estrogen-like compound causing temporary infertility in some cases) but beneficial when it looking at animal growth and finishing for market (anabolic action of estrogen-mimicking compounds). It has been found that phytoestrogens stimulate weight gains and growth rate in monogastrics and ruminants (Trenkle and Borroughs, 1978). Moorby *et al.* (2004) has linked the increase in weight gains to the equol formation from formononetin.

Grazing studies have consistently found greater weight gains when lambs graze legume pastures than grass pasture (Karnezos *et al*., 1994; Fraser *et al*., 2004; Speijers *et al*., 2004). In a two-year study by Karnezos and colleagues, lambs were grazed either on sainfoin (*Onobrychis viciifolia*), alfalfa (*Medicago sativa*), wheatgrass (*Triticum aestivum)* or wheatgrass and sainfoin mix for 85-92 days with equal herbage allowance per lamb across treatments. Lambs that had grazed either of the forage legume pastures had on average 1.6-fold greater cumulative weight gain (with no difference between sainfoin and alfalfa means for each year) than lambs that had grazed the grass pasture for both years of study ($P \leq 0.05$).

Fraser *et al*. (2004) compared the effects of grazing red clover, alfalfa or perennial ryegrass (*Lolium perenne*) on lamb production performance. This trial began in the month of July and finished 15 weeks later in October. Lambs were not restricted on herbage allowance and as a result lambs that grazed legumes had higher intakes. Red clover grazed lambs, when compared to alfalfa grazed lambs, had significantly greater gains (305 g d^{-1} vs. 243 g d^{-1}; $P \leq 0.001$) and as a result required significantly fewer days to finish or to achieve a body condition score of 3/UK fat score of 3L (38 d vs. 50 d; $P<0.01$). Both red clover and alfalfa pastures had significant increase in gain ($P \leq 0.001$) and less days to finish ($P<0.01$) when compared to perennial ryegrass (184 g d^{-1}; 66 d). In addition, the dressing percent of red clover grazed lambs was significantly higher than

alfalfa grazed lambs (48 vs. 46%; $P<0.05$) although no effect of pasture type (alfalfa, red clover or ryegrass) on carcass conformation (EUROP 15 point scale) or visual external fat cover was observed. Red clover being superior to alfalfa for most of the production aspects may be attributed to the polyphenol oxidase content of red clover resulting in more crude protein escaping from rumen degradation. Also, because alfalfa is so rapidly degraded in the rumen (Balde *et al*., 1993; Fraser *et al*., 2004) the amount of protein that escapes rumen degradation is deficient to supply a growing animal (Dhiman *et al*., 1993) further limiting alfalfa when compared with red clover pasture.

A similar study comparing the effects of forage legume [red clover, alfalfa and lotus/bird's-foot trefoil (*Lotus corniculatus*)] vs. grass (perennial ryegrass) found coinciding results for lamb gains on forage legumes when compared to grass (Speijers *et al*., 2004). This trial began later in the growing season, initiating in September and finishing 9 weeks later in November. Lambs were determined to be finished based on the BCS of 3 (as did Fraser *et al*., 2004). Lambs that grazed lotus gained at the greatest rate (278 g d^{-1}) with red clover producing the second greatest gains (228 g d^{-1}); both forage legumes producing gains statistically different from perennial ryegrass (182 g d^{-1}; $P<0.001$). Alfalfa (200 g d^{-1}) was found to be similar to red clover as well as perennial ryegrass. The number of days to finish drew a more concise picture; all three legumes had a similar number of days to finish (35, 40 and 37, respectively) while perennial ryegrass was significantly lower (49 days; $P<0.01$). However, red clover grazed lambs had greater dressing percent (51 %) when compared to all other pasture types which were found similar for dressing percent (lotus: 49; alfalfa: 48 and ryegrass: 48; $P<0.001$). The intakes for red clover and alfalfa were higher than ryegrass ($P<0.01$), however there was no difference found in digestibility between all pastures. Explanation for results of red clover effects on gain and dressing percent were similar to above studies.

Daniel *et al*. (2004) found decreased carcass weight when lambs were fed dried alfalfa pellets and concentrate at low levels when compared to lambs fed ad libitum concentrate ($P<0.001$). Perirenal adipose weight was greater for concentrate fed lambs when compared to alfalfa pellet-fed lambs ($P<0.001$). Alfalfa pellet fed lambs had the lowest

back fat depth ($P<0.05$). However, there was no effect of diet on depth ($P=0.24$) and width ($P=0.29$) of *Longissimus dorsi* (LD) muscle.

Using ultrasound scanning, Speijers *et al.* (2004) also found no effect of forage legumes (lotus, alfalfa, red cover) or ryegrass on depth of the LD muscle or depth of subcutaneous adipose along the back 10 mm from the spine. Lee *et al.* (2009) found no effect on carcass weight, back fat thickness, carcass conformation or fat grade of cull dairy cattle fed red clover silage when compared to grass silage fed for 12 week period.

Measuring the concentrations of key metabolic intermediates can be an effective tool for determining metabolic status. Glucose, beta-hydroxybutyrate (BHBH), NEFA, total protein (TP) and urea are commonly measured in animal nutrition studies for this purpose. Glucose, BHBH and NEFA serum concentrations can be used to indicate energy status of an animal; normal ranges indicating that energy requirement was met by diet (Kaneko *et al*., 2008). The TP can reflect the protein content of diet. The urea content is an indication of ammonia absorption in the rumen which is influenced by nitrogen and energy availability in the diet (Speijers *et al*., 2004).

In pasture studies comparing the effects of forage legumes vs. grasses, all of the above metabolic parameters are important to consider inorder to determine that the animal is receiving adequate energy requirements. Study by Fraser *et al*. (2004) found that, while glucose and BHBH were not affected by pasture type (NEFA was not studied), TP and urea serum concentrations were significantly ($P<0.001$) elevated in lambs that grazed forage legumes (alfalfa and red clover) as opposed to grass (perennial ryegrass) for a period of 4 weeks (TP: 70.5, 69.0 and. 67.0 g/L; urea: 12.5, 11.1 and 6.2 mmol/L, respectively). Between the legumes, alfalfa grazed lambs had higher serum urea than red clover grazed lambs (12.5 vs. 11.1 mmol/L; $P<0.001$). Similarly, urea drew significance in a study by Speijers *et al*. (2004) while TP and BHBH were not statistically altered by pasture-type. Serum urea was increased in lambs that had grazed forage legume (alfalfa or red clover) when compared to perennial ryegrass (12.3, 12.5 and 7.2 mmol/L, respectively; $P<0.001$). High serum urea reflects the high amount of rumen degradable

protein and nitrogen content of legumes. As a result excess nitrogen (in the form or urea) is lost and excreted. This may suggest that because of the loss in nitrogen, grass grazed lambs may more efficiently use dietary protein (Fraser *et al*., 2004). Elevated TP in legume grazed lambs is indicative of a high protein content of the legumes and thus greater amino acid absorption (Fraser *et al*., 2004).

1.4.2 Effect of Lipid Supplementation

Supplementing sheep diets with fish oil can cause reduced intake (Annett *et al*., 2008 – adult ewes; Wachira *et al*., 2002 – finishing lambs). Wachira *et al*. (2002) found a diet high in fiber (grass based) supplemented with fish oil at a 3.6 % DM basis caused reduced intakes. This reduction in intake has been attributed to reduced fiber digestion (Wachira *et al*., 2000). However, no effect on carcass parameters (with the exception of fat scores) was found between treatments (fish oil, linseed oil and fish oil/linseed oil; ($P<0.001$). Fat scores were higher for diets including fish oil ($P<0.05$) which agrees with Wachira *et al*. (2000). This is due to increased production of propionate with fish oil fed ruminants which favors lipogenesis (Solomon *et al*., 1986). A more recent study found similar results where including fish oil (5% DM basis) has been shown to reduce live weight gain and cause excessive fat deposition in the carcass (Annett *et al*., 2011a). A companion study (Annett *et al*., 2011b) showed that reducing the level of fish oil in the diet to 3.5 % DM diminished the negative effects on intake and animal performance with a low-fiber diet. The same study showed that including fish oil into a grass based (with fish oil included at 14 g/d) or concentrate-based diet (ad libitum intake of 3.5 % DM giving them ~3.125 times more) had no effect on lamb performance. However, fish oil with concentrate resulted in higher dressing percent than grazed lambs with and without fish oil ($P<0.001$). In addition, they found that fish oil had no effect on carcass fat and carcass characteristics.

Studies suggest that supplementation of LA (as oil or seed) has little or no effect on live animal performance. Supplementing soybean and linseed oil had no effect on production or carcass characteristics in lambs (Radunz *et al.,* 2009). Supplementing safflower oil as

a source of LA at 3 and 6% (as fed basis) had no effects on lamb intake, gain or carcass characteristics (Boles *et al*., 2005). Kott *et al*. (2003) reported an increase in daily gain when safflower seeds were supplemented into the diet to supply the lamb with 6% dietary oil. However, no effects on carcass characteristics were reported. Similarly, Bolte *et al*. (2002) in lambs and Hristov *et al.* (2005) in cattle found no effect of high-oleate or high-linoleate safflower seeds on growth of carcass. Study by Sampelayo *et al*. (2006) found that glucose, NEFA as well as TAG and cholesterol were reduced in the serum of goat kids whose dam was supplemented with dietary lipid (50 g/kg protected PUFA; $P<0.05$). Reduced cholesterol and NEFA in blood with the consumption of n-3 lipid (found in many animal models: Noakes *et al*, 1996; Gaiva *et al*., 2001) is due to reduced lipolysis (Sampelayo *et al*., 2006). Reduced glucose is thought to be caused by the increased insulin sensitivity stimulating the transport and utilization of blood glucose (Clark, 2000).

1.5 PROJECT HYPOTHESIS

Pasture type in the growing phase and lipid supplementation during finishing affects production performance, carcass quality, fatty acid tissue composition, and lipid metabolism of the ram lamb.

The purpose of this experiment is to compare the effects of supplementing the finishing lamb with a source of EPA and DHA or CLA isomers for 5 weeks before slaughter on lamb performance and product quality as well as lipid metabolism of the lamb. Effect of pasture type grazed before lipid supplementation will also be explored to determine differences between red clover and grass-grown lambs.

OBJECTIVES:

- Increase beneficial fatty acids (CLA, EPA and DHA) in meat and fat tissues
- Evaluate the effects of pasture type (legume vs. grass) and lipid supplement type (n-3 or CLA) on production performance and carcass quality
- Evaluate the effects of pasture type (legume vs. grass) and lipid supplement type (n-3 or CLA) on lipid metabolism through gene expression analysis and tissue fatty acid profile

CHAPTER 2 PRODUCTION PERFORMANCE AND TISSUE FATTY ACID COMPOSITION OF GRAZED LAMBS FINISHED WITH N-3 OR CLA LIPID SUPPLEMENTATION

2.1 ABSTRACT

The objective of this study was to determine the effects of finishing intact Suffolk cross ram lambs that had previously grazed either red clover (RC) or tall fescue (TF), on concentrate supplemented for 2 weeks with 200 g/d of an isolipidic oil supplement: fish oil (FO); soybean oil (SBO) or a 30:70 mixture of CLA isomers and SBO (CLA^{+}). Production parameters, carcass characteristics and fatty acid composition of muscle and subcutaneous adipose tissues were evaluated. RC increased carcass weight (1.1-fold), fat depth as well as content of muscle ALA (1.2-fold), ALA (1.6-fold) and total PUFA (1.2-fold) content of subcutaneous adipose when compared to TF. FO reduced carcass weight (1.1-fold), but had no effect on dressing percent or percent lipid in muscle. FO increased muscle and subcutaneous adipose EPA (5.6- and 10.8-fold), DHA (5.8- and 13-fold) and total PUFA (1.4- and 1.2-fold). CLA supplementation increased all CLA isomers in the subcutaneous adipose (1.8-fold).

2.2 BACKGROUND

It is recommended that the polyunsaturated to saturated fatty acid ratio (PUFA:SFA) of the human diet be 4 or above (Department of Health, 1994). Within dietary PUFA the omega-6 to omega-3 ratio (n-6:n-3) should lie below 4 (Department of Health, 1994). Altering the dietary fat in such a manner has been shown to decrease the risk of developing cardio-vascular disease and inflammatory diseases (Simopoulos, 2000; Belluzzi, 2002; Simopoulos, 2002). Ruminant meat contains a higher amount of SFA as compared to non-ruminant animals. Due to the negative association of SFA and heart disease, ruminant meat is deemed as a less-healthy meat for human consumption. However, ruminant products are good sources of protein, iron, copper, magnesium, cobalt, phosphorous, chromium, nickel, zinc, selenium, vitamin A, vitamin D and vitamin B (Health Canada, 2010; Higgs, 2000). Relative to poultry, lamb (similar to beef) is lower in cholesterol but higher in SFA (Health Canada, 2010). Also, ruminant meat is a natural source of conjugated linoleic acid (CLA) in the human diet. Conjugated linoleic acid has been found to have many benefits to health including ability to inhibit cancer, prevent cardiovascular diseases, reduce body fat (Mir *et al*., 2003; Park, 2009), improve immune response (Cook *et al*., 1999) and reduce risk of developing diabetes (Belury and VandenHuevel, 1999). Lamb, when compared with beef, has a more favorable PUFA:SFA (0.15 vs. 0.11, respectively) and n-6:n-3 (1.32 vs. 2.11, respectively; Enser *et al*., 1996).

Both lamb and beef production systems have influenced the current fatty acid profiles; the trend to grow and finish meat production animals with concentrates (high in linoleic acid; LA) as opposed to forage based systems has led to food products higher in SFA and n-6. Pasture grazing of ruminants is known to increase n-3 PUFA in the meat due to its higher content of alpha-linolenic acid (ALA). However, this system is very dependent on weather conditions and growing seasons which often limit the use of pasture to finish production animals. While nutrition studies show that the ruminant fatty acid profile can be manipulated with pasture grazing or lipid supplementation in concentrate based systems, no study to date has explored combining the two systems: growing lambs on

pasture with a concentrate based lipid supplemented finishing phase. The focus of this paper will be on the finishing portion of the experiment and will address effects of finishing lambs with lipid supplementation that had previously grazed either a legume based or grass based pasture looking at animal performance, growth, carcass quality and tissue fatty acid composition. Increasing the content of n-3 PUFAs and CLA isomers in lamb meat and tissues will create a more desirable product for health-conscious consumers which may create niche marketing opportunities for enriched lamb products with the potential to draw premium prices for the producer.

2.3 Materials and Methods

All animal procedures were reviewed and approved by the Canadian Council of Animal Care (2009).

2.3.1 Animals, Experimental Design and Measurements

Thirty-two 2-3 month old Suffolk cross ram lambs were used for this study which was conducted from late July to early December 2010 at the Agriculture and Agri-Food Canada Nappan Experimental Farm (Nappan, Nova Scotia Canada; lat. 45°N, long. 64°W, 20 m above sea level). Lambs were blocked by weight and randomly allocated to one of 2 pasture replicates: a pure stand of tall fescue (TF) or a tall fescue/red clover mix (RC; minimum of 30% red clover). Lambs were rotationally grazed on pasture for approximately 3 months. At 6-7 months of age (36$\pm$6 kg), the lambs were placed in a feedlot system, and using a Latin square design were assigned randomly within weight block to 1 of 4 lipid treatments: 1) no lipid supplementation (control; CON); 2) enriched fish oil (FO; Epax 5500 EE® containing 55% EPA and DHA, Epax, Norway); 3) soybean oil (SBO; Co-op Atlantic, Truro, Nova Scotia); 4) CLA oil containing 40% *cis*-9, *trans*-11 and 40% *trans*-10, *cis*-12 isomers (Triple action CLA liquid CLA MAXX®, Supplement Sources, Ontario, Canada) mixed with SBO to provide approximately 30% CLA isomers (CLA^{+}). Treatments were designed to provide a non-isolipidic treatment comparison of an oil high in EPA and DHA to the non-supplemented, basic lamb ration

(FO vs. CON); an isolipidic treatment comparison of an oil high in EPA and DHA to an oil without EPA or DHA (FO vs. SBO) and an isolipidic treatment comparison of an oil containing LA to one with LA and CLA (SBO vs. CLA^{+}). Lambs were placed in a feedlot and adapted to the basal diet of ad libitum 15% lamb finisher ration (Co-op 15% lamb grower mash®) and second cut tall fescue hay over one week. Beginning at week 2 in the feedlot, lambs were introduced to the lipid supplements via drench. Lipid supplementation levels began at 15 mL/d and were increased over a two week transitional period to a maximum level of 50 mL/d given as 25 mL twice daily to provide 200 g/d lipid calculated as 5% DMI for an additional two weeks. All lipid supplements were given at 0900h along with the basal concentrate and hay and again at 1700h when at the maximum dose. Concentrate was fed to lambs at 110% consumption measured by daily feed weigh-backs. Drinking water was available at all times. Every two weeks throughout the trial, the lambs were weighed and body condition scored (BCS) using a scale of 0-5.

Over the 5-week experimental trial, samples of hay and feed were collected daily, pooled by week and stored at -20°C for fatty acid composition analysis. Oil samples were collected (~7 mL) biweekly throughout the 5 weeks, placed in 10 mL sealed glass vials, and stored at -80°C until extracted for fatty acid analysis (Table 2-1).

2.3.2 Slaughter Procedure and Collection of Samples

When lambs had received full dietary lipid treatment (200 g/d) for two weeks they were slaughtered by stunning and exsanguination at Brookside Abattoir in Truro, NS. Slaughter procedure and carcass inspection were performed according to the Nova Scotia Provincial Meat Inspection Act. Immediately after exsanguination, samples of neck muscle and subcutaneous adipose from the brisket area were taken, dissected into small cm^3 pieces, snap frozen in liquid nitrogen and stored at -80°C for fatty acid composition analysis. For half of the lambs, the visceral adipose tissues were dissected from the carcasses and weighed as the carcass was being processed: enteric (E) adipose was dissected from the rumen and intestines and peri-renal (PR) adipose was removed from

around the kidneys. Cold carcass weight was recorded after carcasses were chilled for 24 h post mortem. Carcass fat depth (total adipose tissue depth at the 12th rib, 110 mm from the midline) was taken and the associated chop was dissected, packaged and stored at -30°C until chop measurements were complete.

2.3.3 Chop Analysis

The rib chop of each carcass was weighed and dissected into chop loin (LD) muscle, other muscle, subcutaneous adipose and bone. Weight was recorded for the above measures and the bone width was measured. These parameters were corrected for chop thickness and expressed as a proportion of the carcass weight. Intramuscular fat content of the LD muscle was determined using Ankom^{XT15} fat extractor (Ankom Technology, Macedon, NY) according to the manufacture's protocol.

2.3.4 Fatty Acid Analysis

Lipids were extracted from basal diet, lipid treatments and lamb tissues in duplicate following methods of Folch *et al*. (1957) as modified by Budge *et al*. (2006). The extracted lipid was Hilditch acid methylated following procedures of Budge *et al.* (2006) in order to transesterify all lipid classes. Due to the isomerization of CLA that occurs from acid methylation, base methylation of the tissues and CLA supplement was preformed according to method of Cruz-Hernandez *et al.* (2006). The fatty acid methyl esters (FAME) were analyzed by gas chromatography using a short column (Varian 450 with 30m DB-23) as well as a long column (Varian CP 3800 with 100m SP2560) to separate the 18:1 region (18:1's, some 18:2's and 20:2n-6). Results for the long column 18:1 region replaced this region on the short column run. In addition, for the samples that were base methylated, the base results for the CLA isomers were applied to the acid run.

Table 2- 1 Fatty acid composition of basal diet and lipid supplements

	Basal diet		Lipid treatments[B]		
Fatty acids (%)[A]	Concentrate*	Hay*	FO	SBO	CLA$^+$
12:0	0.0320	0.548	0.0170	0.0210	0.0180
13:0	0.00500	0.109	0.00400	0	0
14:0	0.138	0.700	0.357	0.0850	0.143
14:1*cis*-5	0	0	0.0100	0	0
14:1*cis*-7	0	0.203	0.00900	0	0
14:1*cis*-9	0	0.768	0.00500	0	0.0100
15:0	0.0410	0.259	0.0490	0.0170	0.0245
16:0	13.2	18.7	3.22	9.43	8.61
16:1*cis*-9	0.212	2.15	1.19	0.0770	0.095
16:1*cis*-11	0.0200	0	0.0590	0	0.0500
17:0	0.0830	0.264	0.202	0.0920	0.0680
18:0	1.91	1.88	3.79	3.66	3.39
18:1*cis*-7	0	0	0.0870	0	0
18:1*cis*-9	25.1	3.56	8.32	20.9	18.8
18:1*cis*-11	1.79	0.58	2.42	1.19	0.976
18:1*cis*-13	0	0	0.0960	0.0380	0.131
18:1*cis*-17	0	0	0.0660	0	0
18:2n-6	50.5	16.4	0.956	52.7	26.5
***cis*-9, *trans*-11 CLA**	0	0	0	0	13.8
***trans*-10, *cis*-12 CLA**	0	0	0	0	13.3
***cis*-11, 13 CLA**	0	0	0	0	0.472
***cis*-10, 12 CLA**	0	0	0	0	0
***trans*-11, 13 CLA**	0	0	0	0	0.193
18:3n-3	4.51	33.7	0.655	9.70	4.88
20:0	0.333	1.15	1.02	0.332	0.341
20:1*cis*-9	0	0	0	0	4.84
20:1*cis*-11	0.429	0.246	3.39	0.153	0.329
20:1*cis*-13	0.0190	0	0.339	0	0.112
20:2n-6	0.0610	0	0.420	0.0510	0.0695
20:3n-6	0.0210	0	0.361	0.0280	0.0140
20:4n-6	0	0	1.60	0.118	0.590
20:3n-3	0	0	0.257	0	0
20:4n-3	0	0	1.94	0	0
20:5n-3	0.0140	0	29.1	0.0160	0.0350
21:5n-3	0	0	1.44	0.0420	0.0210
22:0	0.235	1.27	0.483	0.443	0.354
22:1*cis*-11	0.0230	0.604	0.0110	0.0120	0.0330
22:1*cis*-13	0.0650	0.272	1.90	0	0.0150
22:1*cis*-15	0.0780	0	0.632	0.0270	0.0440
22:2n-6	0	0	0.0300	0	0.0065
22:4n-6	0	0	0.0740	0	0
22:5n-6	0	0	0.587	0	0
22:4n-3	0	0	0.171	0	0
22:5n-3	0.281	1.85	3.98	0.124	0.0620
22:6n-3	0	0	22.4	0	0
24:1n-9	0.0530	0	0.800	0	0.0260
Unknowns	0.860	14.8	7.56	0.788	2.35
Total	100	100	100	100	100

[A]c=*cis*, t=*trans*, n=omega, CLA= conjugated linoleic acid; [B]FO = enriched fish oil; SBO = soybean oil; CLA$^+$ = 30% *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA with 70% soybean oil; *Misplaced samples: same brand feed and similar composition hay were used to estimate the fatty acid composition analysis.

2.3.5 Experimental Design and Statistical Analysis

The design of this experiment was a replicated split-plot with pasture type and pasture replicates as the main plots and lipid treatments assigned to individual lambs as the sub-plots. Lambs were balanced by weight within two categories and allocated to one of two pasture types (heavy >24 kg and light $\leq$24 kg weight blocks). Four lambs within each main plot were assigned to 1 of 4 lipid treatments using orthogonal Latin squares. Data were analyzed using GenStat®. Results for gain, carcass characteristics, chop analysis and fatty acid composition of muscle and subcutaneous adipose tissue was analyzed by ANOVA. Three contrasts compared the effects of lipid treatment: FO vs. control (a non-isolipidic treatment comparison of an oil high in EPA and DHA to the non-supplemented, basic lamb ration); FO vs. SBO (an isolipidic treatment comparison of an oil high in EPA and DHA to an oil without EPA or DHA) and SBO vs. CLA^{+} (an isolipidic treatment comparison of an oil containing LA to one with LA and CLA). Other means were compared using LSD, with $P<0.05$ considered statistically significant. Results are reported as means and pooled SEM values from the indicated number of samples used on each treatment analysis.

2.4 RESULTS

2.4.1 Production Performance and Carcass Characteristics

The type of pasture the lambs grazed prior to lipid supplementation affected off pasture weight (weight at the end of grazing/start of the finishing period); lambs that grazed RC pasture were 1.1-fold heavier than lambs that grazed TF ($P<0.05$; Table 2-2). After receiving five weeks of the grain/hay diet and lipid supplementation, differences between the weights (final wt; Table 2-2) of the RC and TF grazed lambs were not significantly different when considering the average of the four lipid treatments, although the RC grazed lambs remained empirically heavier. However, when CON lambs are singled out, final weight is greater for RC grazed lambs when compared with TF grazed lambs (52.0 and 45.3 kg, respectively; $P<0.05$; Table 2-2). Average daily gain (ADG) was not affected by pasture type. Differences between carcass weights of the RC and TF grazed

lambs were significant ($P<0.05$) where RC grazed lambs had 1.1-fold greater carcass weight when compared with TF grazed lambs when averaged over the four lipid treatments. Additionally, carcass weights were 1.1-fold greater for RC grazed lambs when compared with TF grazed lambs in the CON treatment (22.9 and 20.5 kg, respectively; $P<0.05$; Table 2-2). Red clover grazed lambs had 1.3-fold greater fat depth measures at the 12^{th} rib (11.9 vs. 9.4 mm, $P<0.01$; Table 2-2). Red clover grazed lambs had smaller amounts of internal fat (both PR and E) when compared with TF lambs (PR: 340 vs. 395 g; E: 722 vs. 786 g, respectively; Table 2-2). Effect of pasture type varied with lipid supplement and this is discussed in detail below.

Lipid treatment significantly affected ADG, final weight and carcass weight (Table 2-3). The lambs receiving FO had 1.3-fold reduced ADG (210 vs. 318 g/day), 1.1-fold smaller final weight (43.6 vs. 48.7 kg) and 1.1-fold reduced carcass weight (19.6 vs. 21.7 kg) when compared with lambs receiving the CON ($P<0.01$). When compared to the SBO treatment, FO supplemented lambs had 1.1-fold reduced final weight (43.6 vs. 46.1 kg) and carcass weight (19.6 vs. 20.8 kg; $P<0.05$; Table 2-3). In addition, FO lambs had the lowest fat depth measures, perirenal and enteric adipose weights, followed by SBO and CON with CLA^+ supplemented lambs having the greatest numerical carcass fat measures. The final weight, ADG and carcass weight were also affected by CLA^+ supplementation where lambs that received CLA^+ had 1.3-fold greater ADG (327 vs. 244g/day, $P<0.05$) and 1.1-fold greater final weight (49.4 vs. 46.1kg, $P<0.05$) when compared with lambs that received SBO. There was a tendency for the CLA^+ treatment to produce 1-fold greater carcass weight (21.6 vs. 20.8 kg, $P<0.10$) when compared with the SBO treatment. Although not compared as contrasts, the SBO treatment was similar to the FO treatment while the CLA^+ was similar to the CON treatment for ADG (244 vs. 210 and 327 vs. 318 g/day, respectively; SEM: 18.7), final weight (46.1 vs. 43.6 and 49.4 vs. 48.7 kg, respectively; SEM: 0.70) and carcass weight (20.8 vs. 19.6 and 21.6 vs. 21.7 kg, respectively; SEM: 0.30; Table 2-3).

Table 2- 2 Effect of pasture type as well as pasture type × lipid supplement interaction on lamb production attributes and chop composition

Attributes[A]	Pasture type[B]				Treatment[C]									*P* pasture × lipid[D]		
					RC				TF							
	RC	TF	SEM	*P*	CON	FO	SBO	CLA$^+$	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$ vs. SBO
Production																
Off Pasture wt (kg)	38.9	34.4	0.96	*	39.5	37.8	39.5	38.8	34.2	34.3	33.8	35.1	1.26	ns	ns	ns
Final wt (kg)	48.6	45.4	1.33	ns	52.0	43.6	49.1	49.4	45.3	43.5	43.2	49.4	1.58	*	*	*
ADG (g/d)	263	287	33.0	ns	338	164	255	296	298	257	233	358	40.2	*	10	ns
Carcass wt (kg)	21.8	20.0	0.47	*	22.9	19.8	22.0	22.6	20.5	19.3	19.5	20.6	0.60	*	*	ns
Dressing (%)	47.3	46.1	2.03	ns	48.5	45.5	49.4	45.6	45.2	44.3	47.7	47.1	2.88	ns	ns	ns
Fat depth (mm)	11.9	9.40	0.20	**	12.0	10.2	12.5	12.8	9.70	8.80	8.50	10.8	0.73	ns	ns	ns
PR adipose (g)	340	395	[E]	ns	325	255	396	385	402	383	291	506	[F]	ns	ns	ns
E adipose (g)	722	786	[E]	ns	772	629	687	798	814	640	852	837	[F]	ns	ns	ns
Chop composition																
Total chop wt [log$_{(g/kg)}$]	0.920	0.930	0.039	ns	0.880	0.930	0.930	0.950	0.910	0.930	0.940	0.940	0.041	ns	ns	ns
Chop bone wt [log$_{(g/kg)}$]	0.290	0.290	0.032	ns	0.260	0.370	0.320	0.320	0.170	0.370	0.320	0.320	0.037	*	ns	ns
Chop loin wt [log$_{(g/kg)}$]	0.390	0.390	0.041	ns	0.350	0.450	0.380	0.390	0.420	0.380	0.420	0.380	0.043	*	**	10
Total muscle wt [log$_{(g/kg)}$]	0.580	0.600	0.050	ns	0.560	0.550	0.610	0.610	0.610	0.570	0.640	0.580	0.051	ns	ns	ns
Chop fat wt [log$_{(g/kg)}$]	0.420	0.420	0.031	ns	0.370	0.400	0.460	0.450	0.400	0.440	0.370	0.460	0.062	ns	ns	ns
Loin muscle fat (%)[G]	12.3	11.3	0.65	ns	11.9	12.3	12.9	12.1	9.50	12.8	9.90	13.1	1.39	ns	ns	ns

[A]ADG = average daily gain; PR= perirenal; E= enteric; chop composition measures are presented as a fraction of total carcass weight and presented as log transformations with the exception of loin muscle percent fat.

[B]Pasture type effects where RC= red clover; TF= tall fescue and n = 16 lambs/pasture type

[C]Pasture × lipid treatment interaction; CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oil; n=4 lambs/pasture × lipid treatment, 12df

[D]Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$

[E]n=4 lambs/treatment due to missing data

[F]n=2 lambs/pasture x lipid treatment due to missing data

[G]Percent fat of chop loin muscle (*Longissimus dorsi*).

The response to lipid supplementation during finishing was found to differ with the type of pasture the lambs had grazed prior to receiving the supplement. With the CON treatment (no lipid supplementation during finishing), lambs that had previously grazed RC pastures had 1.2-fold greater final weight (52 vs. 45.3 kg; P<0.05) and 1.1-fold greater carcass weight (22.9 vs. 20.5 kg; P<0.05) than lambs that had grazed TF pasture. Soybean oil and CLA^+ supplemented lambs performed similarly to CON lambs except for CLA^+ supplemented lambs that had grazed TF; having higher final weight when compared to SBO (49.4 vs. 45.3 kg; P<0.05). However, the FO supplemented lambs did not follow this pattern and significant differences were found for the FO vs. CON and FO vs. SBO treatments (see Table 2-2). In contrast to the CON and SBO supplements, TF grazed lambs that received FO had similar ADG, final (43.5 vs. 43.6 kg) and carcass weight when compared to RC grazed lambs.

Table 2- 3 Effect of dietary lipid supplement on lamb production attributes

	Treatment[B]					*P*-value[C]		
Attributes[A]	CON	FO	SBO	CLA^+	SEM	FO vs. CON	FO vs. SBO	CLA^+ vs. SBO
ADG (g/d)	318	210	244	327	18.7	**	ns	*
Final wt (kg)	48.7	43.6	46.1	49.4	0.70	**	*	*
Carcass wt (kg)	21.7	19.6	20.8	21.6	0.30	**	*	10
Dressing (%)	46.9	44.9	48.5	46.4	1.67	ns	ns	ns
Fat depth (mm)	10.8	9.50	10.5	11.8	0.57	ns	ns	ns
PR adipose (g)	364	319	344	445	[D]	ns	ns	ns
E adipose (g)	793	635	770	818	[D]	ns	ns	ns

[A]ADG= average daily gain; PR= perirenal; E= enteric
[B]CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA^+= 30% CLA isomers mixed with soybean oil; n= 8 lambs/treatment, means are average of two pasture types; 12df.
[C]Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$
[D]n= 4 lambs/treatment due to missing data.

2.4.2 Chop Composition

Lipid supplementation increased chop loin muscle weight (Table 2-4): FO supplementation increased chop loin muscle weight when compared with CON (0.42 vs. 0.37 $\log_{(g/kg)}$, respectively; P<0.01) with no difference between FO, SBO or CLA (0.42, 0.40, 0.39 $\log_{(g/kg)}$, respectively; SEM: 0.011). Total chop muscle weight was increased by SBO when compared with FO (0.63 vs. 0.56 $\log_{(g/kg)}$, respectively; P<0.01) with a tendency to increase total chop weight when compared with CLA^+ (0.59 $\log_{(g/kg)}$;

P<0.10); there was no significant difference between FO, CON and CLA^+ (0.56, 0.58 and 0.59 $log_{(g/kg)}$; SEM: 0.011).

Interactions between pasture type and lipid supplement were detected for chop composition attributes (Table 2-2). Control lambs that had grazed RC pastures had 1.5-fold greater chop bone weight [0.26 vs. 0.17 $log_{(g/kg)}$; P<0.05] when compared with lambs that had grazed TF pastures while FO lambs, regardless of pasture type had a chop bone weight of 0.37 $log_{(g/kg)}$ which is 1.4-fold greater than CON lambs that grazed RC and 118% greater than CON lambs that grazed TF (P<0.05). When comparing chop loin weight in the CON and FO fed lambs, FO fed lambs had 1.3-fold greater chop loin weight when compared to CON [0.45 vs. 0.35 $log_{(g/kg)}$] while the opposite was true in TF grazed lambs where CON lambs had 1.1-fold greater chop loin weight than FO lambs [0.42 vs. 0.38 $log_{(g/kg)}$; P<0.05]. When FO was compared to SBO for both pasture types, FO supplemented lambs that grazed RC had 1.2-fold greater chop loin weight when compared to SBO [0.45 vs. 0.38 $log_{(g/kg)}$] while grazing TF had the opposite effect where SBO increased chop loin weight by 1.1-fold [0.42 vs. 0.38 $log_{(g/kg)}$; P<0.01]. For the CON treatment, TF grazed lambs had greater chop loin weight when compared to RC grazed lambs (0.42 vs. 0.35 $log_{(g/kg)}$; P<0.05). Fish oil and CLA^+ supplementation had opposing effects to the above scenarios where RC grazed lambs (0.450 and 0.390 $log_{(g/kg)}$, respectively) had greater chop loin weight than TF lambs (0.380 and 0.380 $log_{(g/kg)}$, respectively). Differences were detected when FO was contrasted to CON (P<0.05) and SBO (P<0.01) and when CLA^+ was contrasted to SBO (P<0.10).

2.4.3 Muscle Tissue Fatty Acid Composition

Red clover grazed lambs had increased muscle ALA and other C18:2 (*trans*-9-*trans*-12, *cis*-9-*trans*-13, *trans*-8-*cis*-13, *cis*-9-*trans*-12, *trans*-9-*cis*-12 & *trans*-11-*cis*-15) compared with TF grazed lambs when averaged over all lipid treatments (1.2- and 1.1-fold increases; P<0.05; Table 2-5). In addition, C15-24:1 was decreased by 2.3-fold in muscle of RC grazed lambs when compared with TF grazed lambs (P<0.05).

Table 2- 4 Effect of dietary lipid supplement on lamb chop measures as a proportion of carcass wt

	Treatment[A]					*P*-value[B]		
Attributes	CON	FO	SBO	CLA^+	SEM	FO vs. CON	FO vs. SBO	CLA^+ vs. SBO
Total chop wt [$log_{(g/kg)}$]	0.90	0.93	0.94	0.95	0.012	10	ns	ns
Chop bone wt [$log_{(g/kg)}$]	0.21	0.35	0.28	0.32	0.016	**	*	ns
Chop loin wt [$log_{(g/kg)}$]	0.37	0.42	0.40	0.39	0.011	**	ns	ns
Total muscle wt [$log_{(g/kg)}$]	0.58	0.56	0.63	0.59	0.011	ns	**	10
Chop fat wt [$log_{(g/kg)}$]	0.38	0.42	0.42	0.45	0.044	ns	ns	ns
Loin muscle fat (%)[C]	10.7	12.5	11.4	12.6	1.00	ns	ns	ns

[A]CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA^+= 30% CLA isomers mixed with soybean oil; n = 8 lambs/treatment, 11df
[B]Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$
[C]Percent fat of chop loin muscle (*Longissimus dorsi*).

Table 2- 5 Effect of pasture type on lamb muscle fatty acid composition

	Pasture type[A]			
Fatty acid (%)	RC	TF	SEM	*P*-value
18:3n-3	0.88	0.73	0.046	*
18:2other[C]	0.91	0.82	0.028	*
15-24:1	0.04	0.09	0.014	*

[A]RC= red clover; TF= tall fescue; n= 16 lambs/pasture type, means are average of 4 lipid treatments; 15 df
[B]Statistical probability: *=$P \leq 0.05$
[C]18:2t9-t12, c9-t13, t8-c13, c9-t12, t9-c12 & t11-c15.

Fish oil supplementation decreased oleic acid when compared to CON by 1.2-fold (26.2 vs. 32.2; P<0.05; Table 2-6) with a tendency to decrease (1.2-fold) oleic acid when compared with SBO (26.2 vs. 31.2; P<0.10). Soybean oil significantly increased vaccenic acid when compared to FO and CLA^+ (P<0.01). Fish oil and CLA^+ (both similar) increased C18:1 *trans*-10 when compared to SBO (P<0.05 and P<0.01, respectively) but there was no effect of FO relative to CON. The CLA^+ treatment increased *cis*-9, *trans*-11 CLA by 1.5-fold when compared with CON lambs (CLA^+: 0.55; CON: 0.38; P<0.05). Fish oil supplementation increased EPA when compared with all other treatments: by 4.3-fold when compared with CON (P<0.001); 6.9-fold when compared with SBO (P<0.001) and similarly 6.5-fold when compared with CLA^+ (3.99 vs. 0.61; P<0.05). Fish oil also increased DHA when compared to all other treatments: by 4.1-fold when compared with CON (P<0.001); 7.5-fold when compared with SBO (P<0.001) and similarly 7.2-fold when compared to CLA^+ (1.88 vs. 0.26; P<0.05).

Table 2- 6 Effect of dietary lipid supplement on lamb muscle fatty acid composition

	Treatment[A]					*P*-value		
Fatty acid (%)	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$vs SBO
14:0	1.59	1.67	1.74	1.86	0.150	ns	ns	ns
14:1 *cis*-9	0.100	0.0900	0.0900	0.130	0.015	ns	ns	ns
16:0	17.5	18.4	17.7	17.8	0.705	ns	ns	ns
16:1 *cis*-9	1.67	1.46	1.69	1.81	0.131	ns	ns	ns
16:1other[B]	0.700	0.540	0.740	0.630	0.139	ns	ns	ns
18:0	11.0	10.6	11.7	10.8	0.562	ns	ns	ns
18:1 *cis*-9	32.2	26.2	31.2	30.5	1.696	*	10	ns
18:1 *trans*-9	0.420	0.420	0.310	0.250	0.137	ns	ns	ns
18:1 *trans*-10	1.83	2.71	1.35	3.58	0.414	ns	*	**
18:1 *trans*-11	0.880	1.28	2.19	1.07	0.191	ns	**	**
18:1other[C]	3.14	3.31	3.33	3.43	0.159	ns	ns	ns
18:2n-6	8.48	7.74	8.65	8.07	1.124	ns	ns	ns
***cis*-9, *trans*-11 CLA**	0.380	0.360	0.460	0.550	0.041	ns	10	ns
CLAother[D]	0.0600	0.0800	0.0400	0.0700	0.023	ns	ns	ns
18:2other[E]	0.790	0.990	0.810	0.870	0.039	**	**	ns
18:3n-3	0.780	0.800	0.810	0.830	0.065	ns	ns	ns
18:3other[F]	0.120	0.120	0.100	0.100	0.017	ns	ns	ns
20:0	0.0600	0.0500	0.0700	0.0600	0.012	ns	ns	ns
20:1 *cis*-9	0.0800	0.130	0.0700	0.0500	0.013	*	**	ns
20:1other[G]	0.110	0.160	0.100	0.110	0.012	*	**	ns
20:4n-6	2.92	2.44	2.38	2.38	0.401	ns	ns	ns
20n-6 other[H]	0.440	0.470	0.360	0.320	0.063	ns	ns	ns
20:5n-3	0.940	3.99	0.580	0.610	0.290	**	**	ns
20n-3 other[I]	0.190	1.28	0.100	0.0800	0.092	**	**	ns
22n-6[J]	0.310	0.120	0.240	0.230	0.040	**	10	ns
22:6n-3	0.460	1.88	0.250	0.260	0.134	**	**	ns
22n-3 other[K]	0.00	0.00	0.0100	0.00	0.002	ns	ns	ns
15-24:1	0.0600	0.0700	0.0500	0.0700	0.020	ns	ns	ns
***Other*[L]**	5.21	5.58	5.01	5.18	0.329	ns	ns	ns
Unknown	8.07	7.77	7.77	8.27	0.873	ns	ns	ns
Total SFA	31.8	32.3	32.9	32.1	1.188	ns	ns	ns
Total MUFA	43.5	37.5	42.4	42.9	1.487	*	*	ns
Total PUFA	15.2	21.3	15.8	15.5	1.740	*	*	ns
n-6:n-3(log$_{10}$)[M]	0.510	0.100	0.630	0.590	0.060	**	**	ns
D9D index 1[N]	-0.0700	0.0300	-0.0400	-0.0500	0.016	**	**	ns
D9D index 2[O]	-0.0500	0.0500	-0.0200	-0.0300	0.015	**	**	ns
D9D index 3[P]	0.330	0.560	0.660	0.290	0.063	*	ns	**

[A]CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oil; n = 8 lambs/treatment, 15df; [B]16:1c5, c7 & c11; [C]18:1t4, t5, t6-t8, t12, t16, c6-c8, c7, c11, c12, c13 & c15; [D]CLAt11-t13, t8-t10 & t9-t11; [E]18:2t9-t12, c9-t13, t8-c13, c9-t12, t9-c12 & t11-c15; [F]18:3n-6 & c17; [G]20:1c11 & c13; [H]20:2n-6 & 20:3n-6;[I]20:3n-3 & 20:4n-3; [J]22:2n-6, 22:4n-6 & 22:5n-6; [K]22:4n-3; [L]C10:0, C12:0, C13:0, C14:1 c-5 and c-7, C15:0, C15:1(n-6), C17:1, C17:0, C17:1 c-9, C22:0, C24:0, isomers of C14:0, C15:0 and C17:0, and anti-isomer C17:0; [M]back-transformed means = 3.24, 1.26, 4.27, 3.89, respectively; [N]log$_{10}$(C16:0+C18:0)/(C16:1 c-9 + C:18:1 c-9); [O]log$_{10}$(C14:0+C16:0+C18:0+C20:0)/(C14:1 c-9 +C16:1 c-9 + C18:1 c-9 + C20:1 c-9); [P]log$_{10}$(C18:1t11/CLAc9-t11);
Statistical probability: ns= non-significant,10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$

Total PUFA was increased by FO when compared to all other treatments ($P<0.05$). Fish oil supplementation also decreased the n-6:n-3 ratio ($P<0.01$). In addition, FO increased the D9D indexes 1 and 2 when compared to CON and SBO ($P<0.01$) and index 3 when compared to CON ($P<0.05$).

2.4.4 Subcutaneous Adipose Fatty Acid Composition

Lipid supplementation had an effect on MUFA, PUFA and CLA content of subcutaneous adipose (Table 2-7). *Trans*-9 18:1 was decreased with FO supplementation when compared to CON (1.6-fold; $P<0.05$) and SBO (1.7-fold; $P<0.01$). Fish oil decreased the total 18:1 content of subcutaneous adipose when compared to SBO ($P<0.01$). *Trans*-10 18:1 was decreased in subcutaneous adipose by the FO treatment when compared with SBO (1.7-fold; $P<0.01$) and CON (1.6-fold; $P<0.05$; Table 2-7). *Cis*-9 20:1 was also reduced by FO when compared to CON (2.2-fold; $P<0.05$) and SBO (2.6-fold; $P<0.01$) but increased by CLA^+ when compared to SBO (1.4-fold; $P<0.05$). Other C20:1 (*cis*-11 and *cis*-13) were also decreased by the FO treatment when compared to SBO ($P<0.01$). Soybean oil increased subcutaneous adipose LA content by 1.4-fold when compared to FO ($P<0.05$). The CLA^+ treatment increased *trans*-10, *cis*-12 CLA (2.7-fold; $P<0.01$), other CLAs (1.4-fold; $P<0.01$) and *cis*-9, *trans*-11 CLA (1.2-fold; $P<0.05$) when compared to the SBO treatment. A trend for SBO (similar to CLA^+) to increase C15-24:1 when compared to FO (similar to CON) was apparent ($P<0.10$).

Fish oil had a dramatic effect on n-3 PUFAs when compared to CON where it increased EPA by 8.6-fold, DPA by 2.6-fold, DHA by 13-fold and other C20n-3 (C20:3 n-3 and C20:4 n-3) by 11.7-fold ($P<0.01$). When compared to SBO, FO increased the above fatty acids by 10.8-, 2.6-, 13- and 17.5-fold, respectively. As a result, FO shifted the n-6:n-3 from 3.47 (CON) and 3.80 (SBO) to 1.02 ($P<0.01$).

Table 2- 7 Effect of dietary lipid supplement on lamb subcutaneous adipose fatty acid composition

	Treatment[A]					*P*-value		
Fatty acid (%)	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$vs . SBO
14:0	2.38	2.33	2.62	2.60	0.126	ns	ns	ns
14:1 *cis*-9	0.230	0.200	0.150	0.170	0.050	ns	ns	ns
16:0	17.4	17.7	18.1	18.5	0.672	ns	ns	ns
16:1 *cis*-9	1.59	1.47	1.39	1.36	0.196	ns	ns	ns
16:1other[B]	0.870	0.820	0.700	0.700	0.091	ns	ns	ns
18:0	15.9	13.7	16.5	17.0	1.77	ns	ns	ns
18:1 *cis*-9	29.3	27.5	27.6	26.0	1.090	ns	ns	ns
18:1 *trans*-9	0.600	0.370	0.630	0.540	0.055	*	**	ns
18:1 *trans*-10	4.47	6.13	7.67	7.16	0.526	10	10	ns
18:1 *trans*-11	2.08	1.87	1.62	2.29	0.455	ns	ns	ns
18:1other[C]	3.31	3.29	3.62	3.83	0.17	ns	ns	ns
18:2n-6	2.77	2.30	3.13	2.75	0.212	ns	*	ns
***cis*-9, *trans*-11 CLA**	0.490	0.490	0.490	0.610	0.036	ns	ns	*
***trans*-10, *cis*-12 CLA**	0.0500	0.0400	0.0700	0.190	0.016	ns	ns	**
CLAother[D]	0.220	0.260	0.260	0.370	0.023	ns	ns	**
18:2other[E]	1.14	1.32	1.24	1.19	0.068	10	ns	ns
18:3n-3	0.600	0.540	0.620	0.560	0.051	ns	ns	ns
18:3other[F]	0.250	0.240	0.230	0.190	0.034	ns	ns	ns
20:0	0.0900	0.110	0.100	0.100	0.011	ns	ns	ns
20:1 *cis*-9	0.280	0.130	0.340	0.490	0.038	*	**	*
20:1other[G]	0.250	0.250	0.260	0.260	0.002	ns	**	ns
20:4n-6	0.140	0.130	0.130	0.130	0.013	ns	ns	ns
20n-6 other[H]	0.0800	0.110	0.0700	0.140	0.017	ns	10	**
20:5n-3	0.0500	0.430	0.0400	0.0700	0.031	**	**	ns
20n-3 other[I]	0.0600	0.700	0.0400	0.110	0.044	**	**	ns
22n-6[J]	0.0700	0.0800	0.0600	0.150	0.057	ns	ns	ns
22:5n-3	0.180	0.460	0.180	0.170	0.025	**	**	ns
22:6n-3	0.0500	0.650	0.0500	0.0600	0.048	**	**	ns
22n-3 other[K]	0.0100	0.0200	0.0100	0.0300	0.012	ns	ns	ns
15-24:1	0.240	0.200	0.430	0.430	0.078	ns	10	ns
***Other*[L]**	6.97	6.50	5.70	5.98	0.602	ns	ns	ns
Unknown	8.26	9.69	5.03	5.08	1.770	ns	10	ns
Total SFA	6.97	6.50	5.70	5.98	0.60	ns	ns	ns
Total 18:1	37.3	36.8	40.0	38.9	0.72	ns	**	ns
Total MUFA	44.8	44.3	45.3	44.7	0.809	ns	ns	ns
Total PUFA	6.19	7.71	6.84	6.31	0.362	*	ns	ns
n-6:n-3(log_{10})[M]	0.540	0.0100	0.580	0.540	0.040	**	**	ns
D9D index 1[N]	0.0200	0.0200	0.0900	0.100	0.038	ns	ns	ns
D9D index 2[O]	0.0500	0.0500	0.120	0.120	0.038	ns	ns	ns
D9D index 3[P]	0.600	0.580	0.520	0.510	0.087	ns	ns	ns

[A] CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oil; n = 8 lambs/treatment, 15df;
[B]16:1c5, c7 & c11; [C]18:1t4, t5, t6-t8, t12, t16,c6-c8, c7, c11, c12, c13 & c15; [D]CLAt11-t13, t8-t10 & t9-t11; [E]18:2t9-t12, c9-t13, t8-c13, c9-t12, t9-c12 & t11-c15; [F]18:3n-6 & c17; [G]20:1c11 & c13; [H]20:2n-6 & 20:3n-6;[I]20:3n-3 & 20:4n-3; [J]22:2n-6, 22:4n-6 & 22:5n-6; [K]22:4n-3; [L]C10:0, C12:0, C13:0, C14:1 c-5 and c-7, C15:0, C15:1(n-6), C17:1, C17:0, C17:1 c-9, C22:0, C24:0, isomers of C14:0, C15:0 and C17:0, and anti-isomer C17:0; [M]back-transformed means = 3.47, 1.02, 3.80, 3.47, respectively;
[N]log_{10}(C16:0+C18:0)/(C16:1 c-9 + C:18:1 c-9); [O]log_{10}(C14:0+C16:0+C18:0+C20:0)/(C14:1 c-9 +C16:1 c-9 + C18:1 c-9 + C20:1 c-9); [P]log_{10}(C18:1t11/CLAc9-t11).
Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$.

Pasture type had no effect on SFA content (other than a trend for RC to increase C16:0 when compared to TF; $P<0.10$; Table 2-8), CLA content, and D9D indexes in subcutaneous adipose. Red clover pasture increased other C18:1 (*trans*-4, *trans*-5, *trans*-6-*trans*-8, *trans*-12, *cis*-6-*cis*-8, *cis*-7, *cis*-11, *cis*-12, *cis*-13, *trans*-16 and *cis*-15) and C20:1 *cis*-9 when compared to TF pasture ($P<0.05$). Linolenic acid ($P<0.01$), LA ($P<0.05$) and other C18:2 (*trans*-9-*trans*-12, *cis*-9-*trans*-13, *trans*-8-*cis*-13, *cis*-9-*trans*-12, *trans*-9-*cis*-12 and *trans*-11-*cis*-15; $P<0.05$) were increased with RC pasture grazing (1.6-, 1.2-, 1.1-fold, respectively). In turn, total subcutaneous adipose PUFA content was 1.2-fold greater in lambs that had grazed RC as opposed to TF ($P<0.05$).

There were some significant pasture × lipid supplement interactions for the fatty acid composition of subcutaneous adipose tissues (Table 2-8). Red clover grazed lambs that received CLA^+ treatment had increased 18:1 (other) and C20n-6 when compared to RC × SBO, TF × SBO and TF × CLA^+ ($P<0.05$). Fish oil, regardless of pasture type grazed, had the greatest total PUFA. On RC, FO supplementation increased total PUFA slightly compared to CON (7.90 vs. 6.69; $P<0.05$) whereas on TF, FO significantly increased the total PUFA content (7.52 vs. 5.69; $P<0.05$) although not different from FO × RC or CON × RC ($P>0.05$). When compared to CON or SBO supplementation, regardless of pasture type, FO significantly reduced the n-6:n-3 ratio ($P<0.01$).

2.5 Discussion

2.5.1 Production Performance and Carcass Characteristics

Type of pasture in the growing phase and type of lipid supplement fed during the finishing phase interacted to affect lamb productivity and carcass characteristics. The RC pasture produced lambs with greater weight than the grass based pasture as legume based pasture has been shown previously to have higher intakes and gains, reducing number of days to finish (Sinclair, 2007). High crude protein content, formononetin (estrogen-like compound) content and polyphenol oxidase (protective enzyme that protects some protein and lipid from rumen degradation) content of red clover in particular might all have contributed to greater gains. Fraser *et al*. (2004) found

that lambs gained the most when grazing red clover pasture (305 g/d) compared to lucerne (243 g/d) and perennial ryegrass (184 g/d). In the present study, as a result of grazing red clover pasture, lambs that had grazed RC were heavier at the beginning of the lipid supplementation period. Also, lambs that received no lipid supplementation (CON) that grazed RC had higher final weights when compared to TF although there was no difference when averaged over the four lipid treatments. Also, fat depth of the RC grazed lambs (average over all lipid treatments) was more favorable suggesting that a lamb's initial grower diet is important to fat cover and 'finish' of the carcass. This effect may simply be due to the larger weight of the RC grazed lambs off pasture being closer to market weight and condition than the TF grazed lambs. Regardless, the beneficial effects of RC pasture were seen 5 weeks after removed from pasture and changed to a diet of ad libitium consumption of a basal-finisher ration.

Lipid treatment affected production parameters and carcass characteristics due to differences in fatty acid composition of the lipid treatments and the high inclusion rates of unprotected lipids. Lambs in the present study supplemented with FO experienced reduced gains [although still higher than 200 g/d; the recommended daily gain for lamb production (NRC, 2007)] and weights when compared to lambs that received SBO or no lipid supplement at all. Similar results were found by Wachira *et al*. (2002) where three different breeds of lamb fed fish oil had reduced daily gain and had lighter final weight. Reduced DMI is common with lambs that are supplemented with sources of long chain PUFA [fish oil: Gulati *et al*. (1999); algal biomass: Papadopoulos *et al*. (2002)] suggesting that lambs supplemented with FO treatment may have had reduced body weight and rate of gain due to: 1) negative effects of unprotected PUFA on the rumen environment; 2) decreased intake and 3) effects of long chain PUFA on lipid metabolism.

Table 2- 8 Effect of pasture type as well as pasture × lipid supplement interaction on lamb subcutaneous adipose fatty acid composition

Fatty Acid (%)	Pasture type[A]				Treatment[B]									*P* pasture × lipid[C]		
					RC				TF							
	RC	TF	SEM	*P*	CON	FO	SBO	CLA^{+}	CON	FO	SBO	CLA^{+}	SEM	FO vs. CON	FO vs. SBO	CLA^{+} vs. SBO
16:0	18.6	17.3	0.475	10	17.9	18.6	18.8	18.7	17.7	16.8	17.4	18.3	0.951	ns	ns	ns
18:0	16.6	15.0	1.25	ns	15.8	14.5	16.7	19.4	15.9	13.0	16.4	14.6	2.50	ns	ns	ns
18:1 *t*10	6.33	6.39	0.372	ns	4.27	5.10	7.48	8.47	4.68	7.16	7.86	5.86	0.744	ns	ns	10
18:1 *t*11	2.00	1.92	0.321	ns	1.87	2.03	1.93	2.18	2.28	1.71	1.30	2.40	0.643	ns	ns	ns
other 18:1[D]	3.72	3.30	0.120	*	3.42	3.40	3.66	4.41	3.20	3.18	3.59	3.25	0.240	ns	ns	*
18:2n-6	2.98	2.49	0.150	*	3.05	2.40	3.16	3.32	2.49	2.19	3.11	2.17	0.300	ns	ns	10
***cis*-9, *trans*-11 CLA**	0.55	0.49	0.026	ns	0.51	0.50	0.56	0.64	0.48	0.48	0.42	0.59	0.052	ns	ns	ns
***trans*-10, *cis*-12 CLA**	0.10	0.08	0.011	ns	0.04	0.05	0.07	0.23	0.05	0.03	0.08	0.16	0.023	ns	ns	ns
other CLA[E]	0.29	0.26	0.016	ns	0.23	0.27	0.26	0.41	0.21	0.25	0.26	0.32	0.033	ns	ns	ns
other 18:2[F]	1.30	1.15	0.048	*	1.23	1.34	1.31	1.33	1.04	1.30	1.18	1.06	0.096	ns	ns	ns
18:3n-3	0.71	0.45	0.036	**	0.72	0.68	0.72	0.71	0.48	0.40	0.51	0.41	0.073	ns	ns	ns
20:1 *c*9	0.35	0.27	0.027	*	0.30	0.15	0.35	0.60	0.26	0.11	0.33	0.37	0.053	ns	ns	10
other 20:1[G]	0.26	0.26	0.001	ns	0.25	0.25	0.26	0.27	0.25	0.25	0.26	0.26	0.003	ns	ns	10
20:4n-6	0.14	0.13	0.009	ns	0.15	0.13	0.12	0.14	0.13	0.13	0.14	0.11	0.019	ns	ns	ns
other 20n-6[H]	0.10	0.09	0.012	ns	0.06	0.08	0.07	0.20	0.10	0.14	0.06	0.07	0.023	ns	ns	*
20:5n-3	0.16	0.14	0.022	ns	0.06	0.43	0.04	0.09	0.03	0.43	0.05	0.04	0.043	ns	ns	ns
22:6n-3	0.20	0.21	0.034	ns	0.07	0.61	0.04	0.08	0.04	0.70	0.05	0.05	0.069	ns	ns	ns
15-24:1	0.32	0.34	0.078	ns	0.31	0.23	0.37	0.49	0.17	0.17	0.49	0.37	0.110	ns	10	ns
Total SFA	6.07	6.51	0.430	ns	6.51	6.14	6.23	5.40	7.43	6.87	5.17	6.56	0.850	ns	ns	ns
Total PUFA	7.27	6.25	0.256	*	6.69	7.90	7.26	7.24	5.69	7.52	6.42	5.39	0.512	*	ns	ns
n-6:n-3(log_{10})	0.39	0.45	0.028	ns	0.47	0.02	0.57	0.49	0.60	0.01	0.59	0.58	0.056	**	**	ns

[A]Pasture type effects where RC= red clover; TF= tall fescue and n = 16 lambs/pasture type

[B]Pasture × lipid treatment interaction; CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA^{+}= 30% CLA isomers mixed with soybean oil; n=4 lambs/pasture × lipid treatment, 12df; [C]Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$; [D]18:1t4, t5, t6-t8, t12, t16,c6-c8, c7, c11, c12, c13 & c15;[E]CLAt11-t13, t8-t10 & t9-t11;[F]18:2t9-t12, c9-t13, t8-c13, c9-t12, t9-c12 & t11-c15;[G]20:1c11 & c13;[H]20:2n-6 & 20:3n-6

Unprotected PUFA has been found to impede bacteria involved in the ruminal digestion of carbohydrates (Wonsil *et al*., 1994; Chilliard and Doreau, 1997; Sutten *et al*., 2003) as well as fiber digestibility (Wachira *et al*., 2000; Reynolds *et al*., 2006), and microbial protein synthesis (Gulati *et al*., 1999). Dry matter intake is affected by fiber digestion rate as reduced fiber digestion relates to increased rumen fill (Reynolds *et al*., 2006). Unfortunately, the current study's design of experiment did not facilitate intake measurement so this is strictly a possible explanation. A study by Kook *et al*. (2002) where fish oil supplement was fed to Korean steers and bulls at 5% dietary inclusion found that fish oil decreased intake from 10.9 kg/d (control) to 8.47 kg/d. It was also found that fish oil decreased rumen pH from 6.71 (control) to 6.46 which may contribute to acidosis and reduced intake. A study by Chilliard and Doreau (1997) also found reduced intake (by 1.6 kg DM daily) when fish oil was included in the corn based diets of dairy cattle. In addition to the foregoing, the results found may have an association to effects on lipid metabolism where fish oil has been found to down-regulate genes involved in lipid and fatty acid synthesis. Lastly, the ingestion of long chain PUFA (such as EPA and DHA) has been linked to increased fatty acid oxidation in mitochondria and peroxisomes (Takada *et al*., 1994; Power and Newsholme, 1997) and reduced *de novo* lipid synthesis (Schmitz and Ecker, 2008). Polyunsaturated fatty acids (both n-3 and n-6) achieve this through the regulation of transcription factors involved in lipid metabolism. Dietary PUFA have metabolic regulatory function and are not simply energy-providing molecules (reviewed by Benatti, 2004). Changes in lipogenic enzyme mRNA abundance have been detected within hours of PUFA ingestion (Jump *et al*., 1993; Jump *et al*., 1994). In the present study, reduced fat depth, as well as reduced enteric and perirenal adipose tissue with supplementation of FO and SBO suggests that both n-3 and n-6 sources of dietary PUFA reduce lipogenesis.

Supplementing a source of *trans*-10, *cis*-12 CLA into the diet of growing lambs was expected to reduce adipose storage and result in leaner carcasses. From studies with this isomer (as well as *cis*-9, *trans*-11) fed to mice, rats, pigs and humans, CLA has been found to manipulate body composition reducing adipose tissue (Dugan *et al.,* 1999; Park

et al., 1999; Sisk *et al*., 2001; Smedman and Vessby, 2001) and increasing lean (Park *et al.,* 1999). While the source of CLA in the current study was not-rumen protected, due to the high dietary inclusion rate, some of the supplemented CLA isomers were expected to escape rumen biohydrogenation. Regardless, when looking at the results of the current study, one can see that including CLA isomers at a rate of approximately 30% CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 (~60 g/d) with 70% SBO (140 g/d) significantly increased ADG when compared to 100% SBO (200 g/d). Study supplementing protected CLA at 1.5 and 3.8 g/d of *trans*-10, *cis*-12 CLA and equal amount of *cis*-9, *trans*-11 CLA for a 10 week period had no effect on carcass composition of lactating ewes (Sinclair *et al*., 2010). Study in growing lambs (Wynn *et al*., 2006) and growing cattle (Gillis *et al*., 2004b) found no effect of CLA supplementation on body fat levels.

A pasture type and lipid supplement additive affect was anticipated knowing the benefits of a legume-based pasture on production as well as possible negative effects of supplementing unprotected long-chain PUFA in the diet of ruminants on the rumen environment, fiber digestibility and intake. Red clover grazed lambs gained better and had larger off pasture weight before finishing. Tall fescue grazed lambs that received FO had greater ADG during finishing with similar final and carcass weights when compared to RC grazed lambs supplemented with FO. This may be an indicator of compensatory growth where TF grazed lambs grew faster when placed in the feedlot. This may also suggest that including FO at 5% DM has more detrimental effects to greater gaining larger lambs (RC grazed lambs). As mentioned above, high levels of PUFA are known to have toxic effects on the rumen environment, specifically microbe and protozoa populations. Decreased microbial activity results in decreases in microbial protein synthesis and fiber digestion (Gulati *et al*., 1999). Soybean oil supplementation to TF grazed lambs decreased final body weight when compared to RC grazed lambs supplemented SBO and CLA^{+} regardless of pasture type. This may demonstrate the effects of CLA supplementation on muscle production whereby CLA has been demonstrated to increase lean muscle in several animal models (Steck *et al*., 2007) which is thought to be due to increased fatty acid oxidation and energy expenditure in both

muscle and adipose tissue cells (West *et al*., 2000 and Park *et al*. 1999). Tall fescue grazed lambs that may not have grown as well as RC grazed lambs only caught the RC grazed lambs when supplemented with CLA isomers when compared to SBO.

Two interactions were found in subcutaneous adipose tissue fatty acid profile with respect to pasture type grazed and lipid supplement. Docosapentaenoic acid increased with FO supplementation regardless of pasture type; TF grazed lambs supplemented with FO had the highest DPA content. Levels of other n-3 PUFA were also increased with FO supplementation in both pasture types; however the increase was similar between pasture types so interactions were not found. Secondly, CLA^{+} supplementation increased the subcutaneous adipose content of other C20n-6 in RC grazed lamb only. An explanation of this interaction is unclear.

2.5.2 Chop Composition

Greater chop loin muscle weight but smaller total muscle weight in FO lambs when compared to SBO lambs may relate to effects of dietary PUFA (especially n-3 EPA and DHA) on the rumen environment, intake and lipid metabolism resulting in leaner carcasses. When carcass weight is considered, chop measures may more accurately portray carcass composition of smaller lambs. For example: FO lambs were had smaller carcass weights but also less adipose (possibly due to long-chain PUFAs inhibition of lipogenesis) resulting in leaner carcasses. Fish oil lambs have greater chop weight, loin muscle and bone weight as a proportion of the smaller carcass weight when compared to lambs that received no lipid supplementation. A trend for CLA^{+} lambs to have reduced total chop muscle weight is atypical to research findings with CLA supplementation. While the *cis*-9, *trans*-11 isomer shows positive effects on disease and cancer prevention (Kelly *et al*., 2007), the *trans*-10, *cis*-12 isomer may be responsible for increased lean mass and decreased fat deposition (Steck *et al*., 2007; Dugan *et al*., 1997 and Ostrowska *et al*., 1999). There was no difference between treatments for loin muscle percent fat which demonstrates that the percentage of lipid in FO lambs was not compromised by a possible reduction in lipogenesis by long-chain PUFAs EPA and DHA in muscle tissue.

Eicosapentaenoic acid and DHA are preferentially incorporated into muscle phospholipids (Sinclair, 2007; Wachira *et al.,* 2002; Copper *et al*., 2004; Demirel *et al.,* 2004) so FO supplementation was expected to increases muscle lipid EPA and DHA.

2.5.3 Tissue Fatty Acid Composition

The SFA content of lamb muscle and subcutaneous adipose tissue lipid was not affected by lipid supplementation. This is supported by Bessa *et al*., (2005) who found that the addition of PUFA to a concentrate diet had a neutral effect (slightly decreasing) on SFA content of muscle tissue (16:0 and 18:0). Oleic acid (*trans*-9 18:1) was reduced in muscle lipid content which was expected as oleic acid is often depressed in ruminant tissues that were supplemented with dietary PUFA (Bessa *et al*., 2005; Boles *et al*., 2005). Speculation for this effect is related to a reduction in tissue 18:0 (which we did not see) due to incomplete ruminal biohydrogenation and/or the impeding effects of PUFA on D9D activity (Sampath and Ntambi, 2005). Other results of the present study suggest that the second of the above speculations is true: that FO decreased the D9D index in muscle tissue suggesting that there was less product (MUFA or *cis*-9, *trans*-11 CLA) when compared to substrate (SFA or vaccenic acid) and therefore less D9D activity. This was not seen in subcutaneous adipose tissue. Elaidic acid (*trans*-9 18:1) was reduced in subcutaneous adipose with FO, while there was no effect in muscle tissue. A reduction in elaidic acid may be explained by a diet high in concentrates. High concentrate diets have be shown to alter biohydrogenation pathways and results in higher *trans*-10 18:1 and lower *trans*-9 18:1 (Griinari and Bauman, 1999). Also, inclusion of dietary PUFA has been found to increase *trans*-10 18:1 duodenal flow (Duckett *et al*., 2002) and lamb muscle content (Bessa *et al*., 2005). While the subcutaneous adipose content of vaccenic acid (*trans*-11 18:1) was unaffected by lipid supplementation, SBO increased vaccenic acid in lamb muscle when compared to FO and CLA^{+}. In addition, *cis*-9, *trans*-11 CLA tended to be increased in muscle tissue by SBO when compared to FO. Diets high in LA and ALA have been shown to increase the production of vaccenic acid and *cis*-9, *trans*-11 CLA (Wachira *et al*., 2002). This pattern was not observed in

subcutaneous adipose tissue; however, CLA^+ significantly increased the content of *trans*-10, *cis*-12 CLA, *cis*-9, *trans*-11 CLA and other isomers.

While it was first thought that the majority of CLA in ruminant products was a result of biohydrogenation of LA in the rumen, studies suggest that in the mammary gland (Griinari *et al*., 2000) and in subcutaneous adipose and intramuscular adipose (Raes *et al*., 2004b) the majority of CLA (in the form of *cis*-9, *trans*-11) is synthesized endogenously within the tissue from vaccenic acid and action of D9D. However, the *trans*-10, *cis*-12 isomer cannot be synthesized endogenously in animal tissues due to a lack of desaturase enzyme needed to insert a double bond at the C12 position of *trans*-10 18:1 (Raes *et al*., 2004a). Therefore, all of the *trans*-10, *cis*-12 CLA in ruminant tissue and products is formed in the rumen. Although there was detectible *trans*-10, *cis*-12 CLA in muscle and no effect of CLA^+ on any CLA isomer, CLA^+ supplementation resulted in an increase in the fatty acid content of *trans*-10, *cis*-12 CLA, *cis*-9*, trans*-11 CLA and other CLA in subcutaneous adipose tissue. These results are similar to findings by Sinclair *et al*. (2010) where protected CLA supplementation had little effect on muscle fatty acid content but increased CLA isomers in liver, heart muscle and perirenal adipose with lactating ewes. In growing lambs fed rumen-protected CLA, liver and adipose tissues performed similarly (Wynn *et al*., 2006). In the present study, vaccenic acid was decreased by CLA^+ in muscle lipid and unaffected in subcutaneous adipose tissue. This is not supported by other studies supplementing protected CLA to sheep where vaccenic acid is increased by CLA supplementation where incomplete biohydrogenation was the suggested cause (Sinclair *et al*., 2010; Wynn *et al*., 2006). Although the CLA in the present study was not rumen protected, the increase in CLA isomers detected in subcutaneous adipose as well as decrease in *trans*-18:1 tissue content suggests that biohydrogenation did not reduce the absorption of the dietary CLA isomers.

Feeding fish oil or fish meal increases the n-3 content of lamb tissues even when the supplemental lipid is not rumen-protected. This is supported by the results of the current study where FO increased n-3 content of muscle lipid and subcutaneous adipose, decreasing the n-6:n-3 ratio. *In vitro* studies show that the biohydrogenation of long-

chain PUFA EPA and DHA is limited (Fievez *et al*., 2000; Dohme *et al*., 2003). Feeding a source of long-chain PUFA EPA and DHA has proven to be a more effective way to improve n-3 long chain PUFA fatty acid content of lamb tissue than supplementation of ALA (Raes *et al*., 2004a; Ponnampalam *et al*., 2001; Wachira *et al*., 2002; Demirel *et al*., 2004; Cooper *et al*., 2004). Feeding ALA to increase n-3 content is limited due to the fact that the endogenous conversion of ALA to EPA and DHA is dependent on the n-6:n-3 content of the diet as ALA and LA compete for the same elongation and desaturation enzymes.

Red clover pasture was expected to increase ALA when compared to grass grazed lambs as red clover is typically higher in ALA and LA than grasses (Fraser *et al*., 2004). Lambs that graze pasture, as opposed to lambs that are fed concentrate, have greater muscle ALA but reduced LA (Aurousseau *et al*., 2004). The reductions of ALA tissue content with concentrate fed lambs were not seen in pasture grazed and concentrate finished lambs in the current study. The content of concentrates is generally high in LA lipid and for this reason, the n-6:n-3 ratio of lamb muscle is improved by pasture grazing (as reviewed by Sinclair *et al*., 2007). In addition, grazing pasture decreases 16:0 while increasing 18:0 (Nuernberg *et al*., 2005; Aurousseau *et al*., 2004; Fisher *et al*., 2000), although there was no effect of pasture on SFA content of lamb muscle lipid in the current study.

When lambs are grazed on RC and then finished with concentrate supplemented with FO, isomers of 18:1 in muscle lipid increases. Red clover pasture and FO are rich sources of n-3 PUFA. The effect of combining these two sources of PUFA simultaneously in lamb diets is unknown. A similar effect is gained when TF grazed lambs are supplemented with SBO as opposed to FO. Soybean oil would increase the LA content of the lamb diet. Interaction on rumen biohydrogenation pathways is expected by alteration of dietary PUFA via pasture and lipid supplementation.

Grazing lambs on fresh forages (high in ALA) as well as supplementing lambs with fish oil have been found to increase CLA content of tissues by increasing rumen output of

vaccenic acid (Wonsil *et al*., 1994; Palmquist, 2005). Both grazing lambs on fresh forage and supplementing long-chain PUFAs into the diet stimulate the ruminant to produce CLA endogenously in tissues, providing more *cis*-9, *trans*-11 CLA precursor: vaccenic acid. The biohydrogenation process in the rumen involves saturation of fatty acids. To increase vaccenic acid outflow, complete biohydrogenation must be avoided. Fish oil and supplements high in long-chain PUFA may increase this output by slowing the rate of biohydrogenation by negatively affecting the rumen microbes.

2.5.4 Concluding Remarks

Increasing the n-3 and CLA content of lamb tissues is achievable via dietary supplementation of fish oil and CLA. Although FO supplementation reduced the final weight and carcass weight of the lambs, FO supplementation had no effect on dressing percent and produced a leaner carcass with enriched lipid content: FO seemed to effect lipogenesis in adipose tissue (seen by reduced fat depth and visceral fat storage), however, the percentage of lipid in the muscle was not compromised. Conversely, there was no effect on C12-C16 in muscle or subcutaneous adipose suggesting that *de novo* lipogenesis was not affected by treatment in these tissues. Fish oil enhanced the fatty acid content of lamb tissues by increasing EPA, DHA and total PUFA in turn decreasing the n-6:n-3 ratio in both muscle and subcutaneous adipose tissue. Soybean oil increased vaccenic acid and tended to increase CLA in muscle tissue which suggests increased tissue CLA formation from LA biohydrogenation product, vaccenic acid.

Supplementation of CLA into the diet of the lamb increased carcass fat measures, gain, and final weight and tended to increase carcass weight. Carcass fat measures were highest for CLA supplemented lambs although not different from any other lipid treatment. The major effect of CLA supplementation on tissue fatty acid composition was an increase in *cis*-9, *trans*-11 CLA, *trans*-10 18:1 in the muscle and an increase in all CLA isomers in the subcutaneous adipose.

The type of pasture that lambs grazed before finishing had effects on animal performance and carcass attributes. Red clover grazed lambs that did not receive lipid supplementation had greater final live weight and carcass weight. When averaged over all lipid treatments, RC grazed lambs obtained greater carcass weights. In addition, RC grazed lamb carcasses had more fat cover but less internal adipose tissue. Linolenic acid as well 18:2 isomers were increased in muscle as well as an increase of LA, ALA, 18:2 isomers and total PUFA in subcutaneous adipose tissue with RC grazing. Pasture type grazed before finishing produced similar gains on concentrate and lipid supplementation, similar final live weights and chop composition

CHAPTER 3 EXPRESSION OF GENES INVOLVED IN LIPID METABOLISM IN LIVER AND ENTERIC ADIPOSE OF GRAZED LAMBS FINISHED WITH N-3 OR CLA SUPPLEMENTATION

3.1 ABSTRACT

The objective of this study was to determine the effects of finishing intact ram lambs that had previously grazed either red clover (RC) or tall fescue (TF), on concentrate supplemented with 200 g/d for 2 weeks of an isolipidic oil supplement: fish oil (FO); soybean oil (SBO) or a 30:70 mixture of CLA isomers and SBO (CLA^{+}). Blood metabolites as well as gene expression of enzymes, transcription factors and adipokines involved in lipid metabolism in liver and enteric adipose tissue of 32 Suffolk cross ram lambs were evaluated. Compared to TF, RC pasture decreased D9D in the liver (1.6-fold). FO decreased expression of SREBP-1, a key transcriptional activator of lipid synthesis, in the liver (1.6-fold). FO down-regulated Spot14 (3.4-fold) and leptin (3.6-fold), and upregulated D9D (1.3-fold) in enteric adipose tissue. Both RC (growing phase) and FO supplement (finishing phase) altered lipid metabolism and growth characteristics of ram lambs.

3.2 BACKGROUND

Long-chain polyunsaturated fatty acids (PUFA), in particular omega-3 (n-3) EPA and DHA, as well as ruminant-derived CLA, specifically *cis*-9, *trans*-11 which makes up ~75-90% of total ruminant CLA (Kim *et al*., 2009) have many known human health benefits when incorporated into the diet. Dietary fatty acids are important for proper mammalian growth, development and health. They are crucial to cell structure and function and are involved in cell-to-cell signaling. With the increased interest in dietary PUFAs and health, PUFAs have been discovered to have primary roles in the regulation of lipid metabolism. Polyunsaturated fatty acids have been found to alter gene expression of key enzymes of lipid metabolism as well as act as transcriptional regulators of lipid metabolism, having regulatory functions on cell metabolism through nutrient-gene interactions (Deckelbaum *et al*., 2006; Wahle *et al*., 2003). One of the ways the mammalian body adapts to a change in diet is through altering gene expression (Dervishi *et al*., 2011; Deckelbaum *et al*., 2006).

While ruminant products are considered the best natural source of *cis*-9, *trans*-11 CLA (Song and Kennelly, 2003), omega-3 (n-3) EPA and DHA are readily found in marine plants and animals (notably oily fish). Grazing lambs on pasture has the potential to increase n-3 PUFAs (Dervishi *et al.,* 2010) and CLA (Scerra *et al*., 2007) in lamb meat. Pasture is a good source of alpha-linolenic acid (ALA), an essential n-3 fatty acid that can be converted into long-chain PUFAs, EPA and DHA, endogenously. Also, the incorporation of n-3 EPA and DHA from marine sources into ruminant diets has shown positive results for drastically increasing n-3 long-chain PUFAs in ruminant meat (beef: Scollan *et al.,* 2001 and sheep: Cooper *et al*., 2004). Both fresh pasture and marine products (algae or fish oil) have been found to promote ruminal production of vaccenic acid (VA; Palmquist *et al.,* 2005), a precursor of endogenous CLA synthesis in ruminant tissues.

Dervishi *et al*. (2011) and Dervishi *et al*., 2012 studied the effect of feeding system on suckling lamb muscle fatty acid composition and gene expression of lipogenic and β-

oxidative enzymes and transcription factors. Dervishi *et al*. (2011) found that while concentrate feeding of ewe and lamb increased gene expression of lipogenic genes, pasture grazing upregulated genes involved in oxidative processes. A companion study by Dervishi *et al*. (2012) looked at the difference between pasture or hay raised lambs and found that pasture increases VA, CLA and n-3 PUFA and effect on lipid metabolism is mediated by regulation of lipogenic enzyme expression. Enzymes involved in fatty acid synthesis, transport and oxidation are found to be regulated at the transcription level both by transcription factors, peroxisome perliferator-activated receptor (PPAR) and sterol response element binding protein (SREBP). The activity of these transcription factors are regulated by nutrients and metabolites including PUFAs and PUFA-derivatives (Deckelbaum *et al*., 2006; Dentin *et al*., 2005; Jump and Clarke, 1999).

The goal of this study was to investigate the effects of growing weaned ram lambs on either a legume based or grass based pasture with a concentrate finishing period incorporating three different dietary lipid supplements: enriched fish oil (as a source of EPA and DHA); CLA supplement (as a source of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers); and soybean oil (as a source of linoleic acid). To date, no study has addressed the effects of pasture growing with concentrate/lipid supplementation finishing in lambs on lipid metabolism. Blood metabolites and gene expression analysis of lipogenic and oxidative genes as well as associated transcription factors in lamb liver and enteric adipose will be explored.

3.3 Materials and Methods

All animal procedures were reviewed and approved by the Canadian Council of Animal care.

3.3.1 Animals, Experimental Design and Measurements

Animals were maintained at the Agriculture and Agri-Food Canada Nappan Experimental Farm (Nappan, Nova Scotia Canada; lat. 45°N, long. 64°W, 20 m above mean sea level).

Experimentation ran from late July to early December, 2010. Thirty-two Suffolk cross ram lambs (2-3 months old) were blocked by weight and randomly assigned to graze one of 2 pasture replicates: tall fescue/red clover mix (RC; minimum of 30% red clover) or a pure stand of tall fescue (TF) for approximately 3 months. When lambs reached 36±6 kg in weight, lambs were placed in a feedlot and assigned 1 of 4 lipid treatments using a Latin square design. After a 7 day adaptation to the basal diet of ad libitum 15% lamb finisher ration (Co-op 15% lamb grower mash®) and second cut tall fescue hay, the four lipid treatment groups were introduced: 1) no lipid supplementation (control; CON); 2) enriched fish oil (FO) Epax 5500 EE® containing 55% EPA and DHA (Epax, Norway); 3) soybean oil (SBO; Co-op Atlantic, Truro, Nova Scotia); 4) liquid CLA oil (Triple action CLA liquid CLA MAXX®, Supplement Sources, Ontario, Canada) mixed with SBO to provide approximately 15% *cis*-9, *trans*-11 and 15 % *trans*-10, *cis*-11 CLA isomers (CLA^{+}). Treatments were designed to provide a non-isolipidic treatment comparison of a treatment high in EPA and DHA to the non-supplemented treatment (FO vs. CON); an isolipidic treatment comparison of a treatment containing EPA and DHA to a treatment without EPA and DHA (FO vs. SBO) and an isolipidic comparison of a treatment containing LA to one with LA and CLA (SBO vs. CLA^{+}). Lipid supplementation was delivered via drench beginning at 15 mL/day and increased over a two week transitional period to a maximum level of 50 mL/day (25 mL am and pm; ~200 g/d lipid; calculated as 5% DMI) which was continued for an additional two weeks. All lipid supplements were given at 0900h along with the basal concentrate and hay and again at 1700h when on the maximum dosage. Concentrate was fed to lambs at 110% consumption measured by daily feed weigh-backs. Drinking water was available at all times. Every two weeks throughout the trial, the lambs were weighed and body condition scored (BCS) using a scale of 0-5. At the end of the trial, animals were killed by stunning and exsanguination at Brookside Abattoir in Truro, NS. Samples of liver and enteric adipose were removed from the carcass, snap froze in liquid nitrogen and stored at -80°C.

3.3.2 Blood Serum Recovery and Metabolite Determination

Jugular blood samples (20 mL) were collected prior to trial initiation and every two weeks throughout the grazing trial. Blood samples were kept in the sealed vials at room temperature for 3 hours and allowed to clot. The serum was removed and stored at -80°C for later assessment of non-esterfied fatty acids (NEFA), glucose, urea and beta-hydroxybutyrate (BHBA). This analysis was conducted in the Animal Health Laboratory at the University of Guelph (Guelph, Ontario).

3.3.3 Tissue Collection and RNA Extraction and Purification

Immediately after exsanguinations, samples of liver and enteric adipose were dissected from the carcass, using sterilized surgical instruments treated with Eliminase (Decon Laboratories Inc., King of Prussia, PA), cut into small (<1 cm^3) pieces, snap frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated from frozen tissues using RNeasy® Midi Kit (Cat. No. 75144; Qiagen Inc., Mississauga, Ontario) following the manufactures protocol for liver tissue. For adipose tissue, Qiazol lysis reagent (Qiagen Inc.) and chloroform were used instead of the RLT lysis buffer during the first step of RNA extraction. Precipitation using isopropanol and consecutive steps in the procedure remained unchanged. The RNA quantity was determined by absorbance at 260 nm using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The purity was evaluated using the ratio of absorbance at 260 to that of 280 nm (A260/A280) and 230 nm (A260/A230). Both ratios ranged between 1.8 and 2.1 for all RNA samples. The integrity of the RNA samples were confirmed by visualization of the ribosomal RNA bands (18S and 28S) on a 1% agarose gel containing ethidium bromide.

3.3.4 Complementary DNA Synthesis

Extracted RNA (1 μg) was converted to complimentary strand DNA (cDNA) using the Quantitect Reverse Transcription Kit (Cat. No. 205313; Qiagen Inc.) with random hexamers, according to the manufacturer's instructions. The cDNA was diluted with

RNase/DNase free water to a concentration of 1:25 for working stock and was stored at -30°C.

3.3.5 Real-Time Quantitative Reserve Transcription PCR

Primers for real-time (RT) PCR were commercially synthesized (Sigma-Aldrich, Oakville Ontario; Invitrogen, Life Technologies Inc., Burlington, Ontario; MWG/Operon; Huntsville, AL; Table 3-1). To check that the sequences were a match to the ovine genome, sequences were run through a BLAST™ search (nucleotide blast; other databases). When ovine gene sequence was not available bovine gene sequence was used. Genes involved in lipid metabolism are highly conserved across species and the majority of the genes used in this study were 100% homologous when comparing ovine and bovine sequence. Two exceptions were the ovine HL gene which was 95% homologous to bovine sequence and the SCAP gene which was 99% homologous to bovine sequence.

In addition to the target genes, mRNA abundance was also determined for five housekeeping genes (HKG; Kadegowda et al, 2009) including mitochondrial ribosomal protein L39 (MRPL39), ubiquitously expressed transcript (UXT), eukaryotic translation initiation factor 3, subunit K (EIF3K), peptidylprolyl isomerase A (PPIA/ cyclophilin A; CYCLO) and ribosomal proteins 9 (RPS9). The three HKG genes that exhibited the greatest stability during RT-PCR across all 32 samples within each tissue were identified using an algorithm in Gen Stat (VSN International). From this, a gene expression normalization factor was calculated for each sample based on the geometric mean of the three most stable reference genes. For liver tissue, the program indicated that CYCLO, EIF3K and UXT had the most stable expression. For the enteric adipose, CYCLO, UXT and MRLP39 were found to be the most stable gene expression. The geometric mean of the three selected HKG for each tissue was used as a covariate in the statistical analysis of gene expression data (Harvatine et al, 2009).

Table 3- 1 GenBank accession number, gene symbol, primer sequence and source of primers used for real-time PCR

Gene	Accession #	Direction	Sequence 5' to 3'	Source
ACC	NM001009256.1	Sense Antisense	AGCACGCCAGGTTCTTATTG AAATCGACGTTTCGGACAAG	Invitrogen
Adipo-nectin	BC140488	Sense Antisense	ATCAAACTCTGGAACCTCCTATCTAC TTGCATTGCAGGCTCAAG	Sigma-Aldrich
CYCLO	NM173820	Sense Antisense	CTTTCACAGAATAATTCCGGGATT CAGTACCATTATGGCGTGTGAAG	Sigma-Aldrich
D9D	GQ904712.2	Sense Antisense	TACTCAAGCTTGGGCTTTGG ACCATCACAGCACCTCCTTC	MWG/ Operon
EIF3K	NM001034489	Sense Antisense	CCAGGCCCACCAAGAAGAA TTATACCTTCCAGGAGGTCGATG	Sigma-Aldrich
FAS	GQ150557	Sense Antisense	GTGTGGTACAGCCCCTCAAG ACGCACCTGAATGACCACTT	Sigma-Aldrich
HL	NM001035410	Sense Antisense	TCATTGACTCCTTGCTGCAC CTGGAGTCACCAGGAGAGG	Sigma-Aldrich
Leptin	JQ711179	Sense Antisense	TCACCAGGATCAATGACATCTCA ACCAGTGACCCTCTGTTTGGA	MWG/ Operon
LPL	XM004008038	Sense Antisense	TGGACGGTGACAGGAATGTA GTCCCACCAGCTTGGTGTAT	Invitrogen
MRLP39	NM001080730	Sense Antisense	AGGTTCTCTTTTGTTGGCATCC TTGGTCAGAGCCCCAGAAGT	Sigma-Aldrich
PPARα	AY369138	Sense Antisense	TGCCAAGATCTGAAAAAGCA CCTCTTGGCCAGAGACTTGA	Sigma-Aldrich
PPARδ/β	NM001083636	Sense Antisense	CACTCTCACTGCTGGACCAA CATCCCTCACAAGCGTGAA	Invitrogen
PPARγ	NM1810024	Sense Antisense	CCAAATATCGGTGGGAGTCG ACAGCGAAGGGCTCACTCTC	MWG/ Operon
SCAP	DV935188	Sense Antisense	CCATGTGCACTTCAAGGAGGA ATGTCGATCTTGCGTGTGGAG	Sigma-Aldrich
Spot14	AY656814	Sense Antisense	CTACCTTCCTCTGAGCACCAGTTC ACACACTGACCAGGTGACAGACA	Sigma-Aldrich
SREBP	XM004013336	Sense Antisense	CCAGCTGACAGCTCCATTGA TGCGCGCCACAAGGA	Sigma-Aldrich
RSP9	XM004015434	Sense Antisense	CCTCGACCAAGAGCTGAAG CCTCCAGACCTCACGTTTGTTC	Sigma-Aldrich
UXT	NM001037471	Sense Antisense	CAGCTGGCCAAATACCTTCAA GTGTCTGGGACCACTGTGTCAA	Sigma-Aldrich

Real time PCR was conducted using a relative standard curve method using a QuantiTech SYBR Green PCR Kit (Qiagen, Inc.) on an ABI Step-One-Plus real-time PCR system (Applied Biosystems). A 4-point 5-fold standard curve of pooled cDNA was generated (0.2, 0.04, 0.008 and 0.0016 ng/reaction) and run in triplicate along with samples at 0.04 ng per reaction run in duplicate in the presence of 100 pM of each primer combination in a 96-well MicroAmp Optical reaction plate (Applied Biosystems) following gene expression normalization using the geometric mean of the three housekeeping genes. The cycler was set-up to run an initial denaturing for 10 min at 95°C, followed by 40

cycles of amplification (15s at 95°C and 60s at 57.4 °C. Amplification was followed by dissociation curve analysis to verify the presence of a single PCR product. Each plate contained one gene of interest or HKG, a standard curve in triplicate and 32 samples in duplicate. The relative mRNA abundance of the target gene in each sample was calculated using the relative standard curve method (Step-One-Plus software version 2.1, Applied Biosystems). Data was expressed in arbitrary units representing sample mRNA content relative to the standard curve.

3.3.6 Experimental Design and Statistical Analysis

The design of this experiment was a replicated split-plot with pasture type and pasture replicate as the main plots and lipid treatments assigned to individual lambs as the sub-plot. Lambs were balanced by weight within two categories (heavy > 24 kg and light < 24 kg weight blocks) and allocated to one of two pasture types (red clover or tall fescue). Four lambs within each main plot were assigned to 1 of 4 lipid treatments using orthogonal Latin squares. Results for blood serum metabolites and gene expression were analyzed by ANOVA using GenStat®. Three contrasts compared the effects of lipid treatment: FO vs. control (a non-isolipidic treatment comparison of an oil high in EPA and DHA to the non-supplemented, basic lamb ration); FO vs. SBO (an isolipidic treatment comparison of an oil high in EPA and DHA to an oil without EPA or DHA and SBO vs. CLA^{+} (an isolipidic treatment comparison of an oil containing LA to one with LA and CLA). Other means were compared using LSD, with $P < 0.05$ considered statistically significant. Results are reported as means and pooled SEM values from the indicated number of samples used on each treatment analysis.

3.4 RESULTS

3.4.1 Blood Serum Metabolites

Treatment means [lipid supplement (Table 3-2) and pasture effects (Table 3-3)] for serum NEFA, urea, and BHBA were within the ranges considered normal for sheep (Animal Health Lab, Guelph, Ontario). However, mean serum glucose for CON, SBO and CLA^{+}

(4.53, 4.83 and 4.69 mmol/L, respectively; Table 3-2) were above the normal range (2.6-4.4 mmol/L).

Fish oil supplemented lambs (4.00 mmol/L) had reduced serum glucose when compared to CON (1.1-fold; CON: 4.53 mmol/L; P<0.05) and SBO (1.2-fold; 4.83 mmol/L; P<0.01; Table 3-2). Fish oil (8.26 mmol/L) tended to increase serum urea by 1.2-fold when compared to SBO (7.01 mmol/L; P<0.10) and CLA^+ lambs (370 µmol/L). A tendency for CLA^+ to reduce (1.2-fold) BHBA (370 µmol/L) when compared to SBO (446 µmol/L; P<0.10) was observed. Tall fescue grazed lambs (0.090 mmol/L) tended to have higher (1.3-fold) NEFA serum levels when compared to RC grazed lambs (0.071 mmol/L; P<0.10; Table 3-3).

Table 3- 2 Effect of dietary lipid supplement on lamb blood metabolites

	Treatment[B]					*P*-value[C]		
Blood metabolite[A]	CON	FO	SBO	CLA^+	SEM	FO vs. CON	FO vs. SBO	CLA^+vs. SBO
Glucose (mmol/L)	4.53	4.00	4.83	4.69	0.139	*	**	ns
NEFA (mmol/L)	0.073	0.063	0.086	0.099	0.0120	ns	ns	ns
Total Protein (g/L)	68.8	70.4	68.4	70.6	1.60	ns	ns	ns
Urea (mmol/L)	7.89	8.26	7.01	7.75	0.471	ns	10	ns
BHBA (µmol/L)	393	447	446	370	30.0	ns	ns	10

[A]NEFA = non-esterified fatty acids; BHBA = beta-hydroxybutyrate.
[B]CON= control, no lipid supplement; FO= enriched fish oil; SBO= soybean oil; CLA^+= 30% CLA isomers mixed with soybean oil; n = 8 lambs/treatment, 12df.
[C]Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$.

3.4.2 Effect of Pasture Type on Gene Expression

The type of pasture lambs grazed prior to dietary PUFA supplementation had an effect on D9D expression where TF grazed lambs had a 1.6-fold increase in gene expression when compared to RC grazed lambs ($P \leq 0.05$; Table 3-4). A pasture type by lipid supplement interaction was found with leptin ($P \leq 0.01$; Table 3-4): RC grazed lambs that received SBO supplementation had a 1.5-fold increase in leptin expression when compared to CLA^+ supplemented lambs whereas the opposite was true for TF grazed lambs where CLA^+ supplementation increased leptin expression by 2.9-fold when compared to SBO. A similar trend was found with Spot14 gene expression where RC grazed lambs had a 1.2-fold increase in gene expression when compared to CLA^+ and TF grazed lambs that

were supplemented with CLA^+ had a 1.8-fold increase in gene expression ($P \leq 0.10$). When looking at gene expression in the liver, a trend for FO to decrease SREBP-1c expression when compared to SBO was found where lambs that grazed RC pasture had a greater reduction (2.2-fold) when compared to lambs that had grazed TF (1.5-fold; $P \leq 0.10$).

3.4.3 Effect of Lipid Supplementation on Gene Expression

In the liver, mRNA encoding for lipogenic transcription factor SREBP-1 was down-regulated 1.7-fold by FO when compared to SBO ($P \leq 0.05$). However, enzymes regulated by SREBP-1, ACC, FAS and D9D as well as sterol regulator element binding protein-cleavage activating protein (SCAP) were not significantly altered by lipid treatment (Table 3-5). There was no effect of dietary PUFA treatment on gene expression of HL in liver tissue or LPL in enteric adipose (Table 3-4). There was no effect of dietary PUFA treatment on transcription factors PPARα or PPARδ in liver tissue (Table 3-5) or PPARγ in enteric adipose (Table 3-6).

In enteric adipose tissue Spot14 was down-regulated by 3.1-fold with FO when compared to CON and 3.6-fold with FO when compared to SBO ($P \leq 0.05$). Also, a tendency for FO to increase D9D expression was found where FO increased expression of D9D by 1.4-fold when compared to the CON ($P \leq 0.10$; see Table 3-6). Messenger RNA expression of adipose-derived hormones leptin and adiponectin was studied in enteric adipose. Fish oil significantly down-regulated leptin expression (by 4.3-fold) when compared to SBO ($P \leq 0.05$; Table 3-6). A trend for FO to decrease leptin expression compared to CON was found (3-fold; $P \leq 0.10$). A similar trend for FO to decrease adiponectin when compared to SBO was also found (1.6-fold decrease with FO; $P \leq 0.10$). Although there is no difference between CLA^+ and SBO for leptin expression, leptin expression was increased by CLA^+ (2-fold) when compared to CON (0.25 vs. 0.12; $P<0.05$) and had the greatest leptin expression of all dietary treatments.

Table 3- 3 Effect of pasture type as well as pasture type × lipid supplement interaction on lamb blood metabolite profile

Blood metabolite[A]	Pasture type[B]				Treatment[C]									*P*pasture × lipid[D]		
					RC				TF							
	RC	TF	SEM	*P*	CON	FO	SBO	CLA$^+$	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$ vs. SBO
Glucose (mmol/L)	4.36	4.67	0.191	ns	4.52	3.72	4.60	4.57	4.54	4.27	5.05	4.80	0.256	ns	ns	ns
NEFA (mmol/L)	0.071	0.090	0.0052	10	0.072	0.050	0.088	0.073	0.074	0.075	0.085	0.125	0.0156	ns	ns	ns
Total Protein (g/L)	70.9	68.1	1.02	ns	70.8	72.8	68.8	71.5	66.8	68.0	68.0	69.7	2.21	ns	ns	ns
Urea (mmol/L)	8.26	7.19	0.460	ns	8.95	8.60	7.17	8.32	6.83	7.93	6.85	7.18	0.738	ns	ns	ns
BHBA (µmol/L)	370	459	48.9	ns	289	431	450	308	497	464	443	432	61.1	ns	10	ns

[A]NEFA = non-esterified fatty acids; BHBA = beta-hydroxybutyrate.

[B]Pasture type effects where RC= red clover; TF= tall fescue; n = 16 lambs/pasture type

[C]Pasture x lipid treatment interaction; CON= control, no lipid supplement; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oil; n=4 lambs /pasture x oil treatment, 12df.

[D]Statistical probability: ns= non-significant, 10=$P \leq 0.10$.

Table 3- 4 Effect of pasture type as well as pasture type × lipid supplement interaction on gene expression in lamb liver and enteric adipose tissue

Gene[A]	Pasture type[B]				Treatment[C]									*P*pasture × lipid[D]		
					RC				TF							
	RC	TF	SEM	*P*	CON	FO	SBO	CLA$^+$	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$ vs. SBO
Liver																
SREBP-1	0.16	0.19	0.010	ns	0.15	0.090	0.20	0.19	0.20	0.13	0.19	0.22	0.029	ns	10	ns
SCAP	0.22	0.22	0.028	ns	0.16	0.22	0.24	0.25	0.21	0.32	0.18	0.18	0.056	ns	ns	ns
ACC	0.23	0.28	0.047	ns	0.22	0.21	0.20	0.30	0.20	0.34	0.31	0.27	0.068	ns	ns	ns
FAS	0.27	0.28	0.040	ns	0.30	0.15	0.26	0.36	0.22	0.25	0.30	0.33	0.085	ns	ns	ns
D9D	0.17	0.27	0.015	*	0.24	-0.040	0.20	0.27	0.24	0.16	0.22	0.44	0.085	ns	ns	ns
HL	0.21	0.23	0.016	ns	0.20	0.19	0.21	0.23	0.23	0.22	0.26	0.20	0.033	ns	ns	ns
PPARα	0.24	0.22	0.022	ns	0.20	0.24	0.26	0.27	0.22	0.25	0.21	0.19	0.029	ns	ns	ns
PPARδ	0.30	0.36	0.076	ns	0.19	0.25	0.32	0.45	0.53	0.56	0.18	0.16	0.142	ns	ns	ns
Enteric adipose																
D9D	0.14	0.21	0.038	ns	0.12	0.17	0.13	0.13	0.15	0.21	0.20	0.27	0.045	ns	ns	ns
Spot14	0.23	0.27	0.063	ns	0.24	0.090	0.32	0.26	0.27	0.070	0.26	0.47	0.085	ns	ns	10
PPARγ	0.21	0.22	0.004	ns	0.19	0.24	0.19	0.21	0.21	0.22	0.25	0.19	0.031	ns	ns	ns
LPL	0.21	0.22	0.043	ns	0.22	0.22	0.15	0.26	0.15	0.26	0.24	0.25	0.050	ns	ns	ns
Adiponectin	0.25	0.21	0.014	ns	0.24	0.23	0.31	0.22	0.20	0.14	0.26	0.25	0.041	ns	ns	ns
Leptin	0.13	0.16	0.051	ns	0.13	0.030	0.22	0.15	0.12	0.040	0.12	0.35	0.062	ns	ns	**

[A]SREBP-1 = sterol regulator element binding protein-1c; SCAP = sterol regulator element binding protein-cleavage activating protein; ACC = acetyl-CoA carboxylase; FAS = fatty acid synthase; D9D = delta-9-desaturase; HL = hepatic lipase; PPAR = peroxisome perliferator-activated receptor alpha (α) or delta (δ); Spot14 = thyroid responsive spot 14; PPARγ = peroxisome perliferator-activated receptor gamma; LPL = lipoprotein lipase.

[B]Pasture type effects where RC= red clover; TF= tall fescue and n = 16 lambs/pasture type.

[C]Pasture x lipid treatment interaction; CON= control, no lipid supplementation; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oil; n=4 lambs /pasture x oil treatment, 12df.

[D]Statistical probability: ns= non-significant, 10=$P \leq 0.10$, *=$P \leq 0.05$, **=$P \leq 0.01$.

Table 3- 5 Effect of dietary lipid supplement on gene expression in lamb liver

	Treatment[B]					*P*-value[C]		
Gene[A]	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$vs SBO
Lipogenesis								
SREBP-1	0.17	0.11	0.19	0.21	0.023	10	*	ns
SCAP	0.18	0.27	0.21	0.22	0.041	ns	ns	ns
ACC	0.21	0.28	0.25	0.28	0.040	ns	ns	ns
FAS	0.26	0.20	0.28	0.35	0.062	ns	ns	ns
D9D	0.24	0.060	0.21	0.36	0.071	ns	ns	ns
FA uptake								
HL	0.21	0.20	0.24	0.21	0.024	ns	ns	ns
β-Oxidation								
PPARα	0.21	0.24	0.23	0.23	0.015	ns	ns	ns
PPARδ	0.36	0.41	0.25	0.31	0.100	ns	ns	ns

[A]SREBP-1 = sterol regulator element binding protein-1c; SCAP = sterol regulator element binding protein-cleavage activating protein; ACC = acetyl-CoA carboxylase; FAS = fatty acid synthase; D9D = delta-9-desaturase; HL = hepatic lipase; PPAR = peroxisome perliferator-activated receptor alpha (α) or delta (δ).
[B]CON= control, no lipid supplement; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oil; n = 8 lambs/treatment, n = 8 lambs/treatment, 12df.
[C]Statistical probability: ns=non-significant, 10=P= 0.108, *=$P\leq$ 0.05.

Table 3- 6 Effect of dietary lipid supplement on gene expression in lamb enteric adipose

	Treatment[B]					*P*-value[C]		
Gene[A]	CON	FO	SBO	CLA$^+$	SEM	FO vs. CON	FO vs. SBO	CLA$^+$vs. SBO
Lipogenesis								
D9D	0.14	0.19	0.16	0.20	0.023	10	ns	ns
Spot14	0.25	0.080	0.29	0.36	0.051	*	*	ns
FA uptake								
PPARγ	0.20	0.23	0.22	0.20	0.026	ns	ns	ns
LPL	0.18	0.24	0.19	0.25	0.025	ns	ns	ns
Adipose-derived hormones								
Adiponectin	0.22	0.18	0.29	0.23	0.032	ns	10	ns
Leptin	0.12	0.040	0.17	0.25	0.034	10	*	ns

[A]D9D = delta-9-desaturase; Spot14 = thyroid responsive spot 14; PPARγ = peroxisome perliferator-activated receptor gamma; LPL = lipoprotein lipase.
[B]CON= control, no lipid supplement; FO= enriched fish oil; SBO= soybean oil; CLA$^+$= 30% CLA isomers mixed with soybean oiln = 8 lambs/treatment, 12df.
[C]Statistical probability of non-orthogonal contrasts important to study: ns= non-significant, 10=$P\leq$0.10, *=$P\leq$ 0.05.

3.5 Discussion

3.5.1 Blood Serum Metabolites

Glucose, ketone bodies, and urea are common serum biochemical parameters analyzed in the ruminant to determine health status (Ramin et al., 2005). In the ruminant, glucose is needed as a source of energy. The ruminant stores glycogen (glucose reservoir) which is used to maintain serum glucose levels (Phiri et al., 2007). In the absence of glucose or with a poorly functioning liver (i.e. parasite damage so limited glycogen stores), ketone bodies (from the catabolism of fat) and urea (from the catabolism of protein) increase in circulating blood. From the metabolite results of the present study, lambs received adequate glucose from the concentrate and levels of BHBA and urea remained normal. When energy requirements are not satisfactory, ketones are produced in the liver from mobilized NEFA. Because we did not see an increase of NEFA or BHBA in any treatment or treatment combination, it can be assumed that dietary energy requirements were met. The reduction of serum glucose with FO supplemented lambs may be indicative of a reduced intake, although the serum glucose level for FO lambs was within the normal range. In the present study feed intake was not monitored by treatment.

3.5.2 Effect of Pasture Type on Gene Expression

Pasture type had a significant effect on D9D gene expression in the liver, with lambs having grazed RC exhibiting lower D9D gene expression in comparison to the TF grazed lambs. When considering enteric adipose tissue the D9D gene expression was also numerically lower in the lambs that had grazed RC although this was not statistically significant. When comparing pasture grazing to concentrate feeding, D9D gene expression was down-regulated in adipose depots (subcutaneous and omental; Daniel *et al.*, 2004) and muscle (Dervishi *et al.* (2010) and was numerically reduced in liver (Daniel *et al.* (2004) of the forage fed lambs. Delta 9 desaturase gene expression is known to be reduced by PUFA (Nakamura *et al*., 2004). Forages in general are high in ALA (50-75% total fatty acids; Dewhurst *et al.,* 2009). Scollan *et al.* (2006b) found that when red clover is substituted into the diet of grass grazed finishing beef, increases in

muscle content of ALA are observed and are greater as the proportion of red clover increases from 50:50 to 100%. Increases in muscle and subcutaneous adipose ALA with red clover grazing was also observed in the present study (Table 2-5 and Table 2-8). The polyphenol oxidase content of the RC pasture along with potentially higher intake and passage rates may have resulted in more ALA by-passing rumen biohydrogenation. Higher dietary PUFA being absorbed through the intestine would be expected to decrease D9D gene expression and activity. No studies were found comparing different pasture forages for effects on D9D gene expression. More research in this area is warranted particularly as the effect of pasture type in this study was observed five weeks after removing the animals from pasture.

Interestingly, a pasture type by dietary PUFA supplement interaction was evident where RC + SBO and TF + CLA^+ had heightened enteric adipose leptin expression when compared to RC + CLA^+ or TF + SBO, respectively. The increase in leptin expression with RC grazed, SBO supplemented lambs may be due to the phytoestrogen content of the red clover and soybean oil. Phytoestrogens have been shown to increase adipose-derived hormones, both leptin (Llaneza *et al.,* 2010 and Weickert *et al.,* 2006) and adiponectin (Charles *et al*., 2009 and Llaneza *et al.,* 2010) in the human. Another possible explanation for the increased leptin expression with this treatment combination is diets high in ALA are found to increase leptin expression in adipose tissue, as seen in retroperitoneal adipose tissue in rabbits fed flaxseed (McCullough *et al.,* 2011). As discussed above, red clover is high in ALA content and the SBO treatment contained the greatest amount of ALA when compared to all other treatments (Table 2-1). However, studies on the effect of PUFA on leptin gene expression are equivocal.

The reason for the increase in leptin expression with CLA^+ supplemented lambs that had grazed TF as opposed to RC is not understood. In general, the CLA^+ treatment seemed to increase leptin mRNA abundance in enteric adipose. The effects of the lipid supplements on gene expression of adipose derived hormones are examined further, later in the discussion.

3.5.2 Effect of Lipid Supplementation on Gene Expression

Transcription factor, SREBP-1 regulates the expression of genes involved in the synthesis and uptake of fatty acids, cholesterol and TAG upon binding to sterol regulatory elements in the promoter regions of lipogenic genes (Horton *et al.,* 2002). Sterol response element binding protein-1 is predominantly found in lipogenic tissues such as the liver and adipose tissue and is directly regulated by PUFA. It is known that longer chain n-3 PUFAs (such as DHA and EPA) have great inhibitory effects on SREBP-1 expression in the non-ruminant (Worgall *et al.,* 1998) whereby EPA and DHA decrease SREBP-1 mRNA abundance (Jump *et al.,* 2005; Nakamura *et al.,* 2004). In this study ram lambs fed a fish oil enriched with EPA and DHA had significantly reduced levels of SREBP mRNA. Research reporting the effect of PUFA on ruminant liver lipid metabolism is limited. In a recently reported study by Hiller *et al.* (2012) addition of n-3 and n-6 dietary PUFA significantly reduced SREBP-1 expression in the liver of lactating dairy cattle. In an earlier experiment also conducted by Hiller *et al*. (2011), dietary n-3 and n-6 PUFA supplementation in young growing Holstein bulls decreased SREBP-1 gene expression in muscle and subcutaneous adipose tissue; however effects on liver were not determined.

In addition to the reduction of SREBP gene expression in the liver found by Hiller *et al.* (2012) when feeding a plant oil high in ALA with an algae high in DHA to lactating cows, they also observed a reduction in the expression of genes positively regulated by SREBP including ACC, FAS and D9D. No effect on ACC or FAS gene expression in the liver was observed when feeding FO to the rams lambs in the present study, however D9D was numerically reduced (25% of the control). Had larger numbers of animals been available for analysis in this research this effect would be anticipated to be statistically significant. In addition to ruminant liver, diets rich in n-3 PUFA are known to decrease D9D gene expression in muscle and subcutaneous adipose tissue (Herdmann et al., 2010; Hiller *et al.,* 2011) which is consistent with studies in other species (Flowers and Ntambi, 2008)].

A reduction in ACC and FAS gene expression in response to lowered SREBP expression would be expected. Interestingly, Invernizzi *et al.* (2010) reported a decrease in ACC and FAS gene expression in lactating mammary tissue of the cow following seven days of lipid supplementation containing fish oil, but at 21 days there were no differences from the control diet. Adaptation to the source of dietary fatty acid was suggested, thus supporting continued milk fat synthesis. However, milk fat depression was still observed at 21 days and SREBP gene expression levels were numerically lower for the fish oil supplemented cows. In the experimentation conducted by Hiller *et al.* (2012) discussed above, cows were supplemented for ten weeks prior to collection of liver tissue, and a decrease in lipogenic gene expression was still observed. It is important to consider that both of these studies are using mature, lactating dairy cows. The effect of PUFA on regulation of lipid synthesis in the liver of a young growing ruminant lamb has not been studied and results obtained in this research warrant further investigation.

In contrast to the effects of PUFA on gene expression in liver, muscle and subcutaneous adipose tissue discussed above, Hiller *et al.,* 2012 observed an increase in lipogenic gene expression in omental adipose tissue of lactating cows supplemented with sunflower oil and DHA-containing algae, including SREBP, D9D, ACC and FAS as well as PPARγ (a lipid storage-related transcription factor) and LPL. In the present study, FO supplementation up-regulated D9D expression in enteric adipose tissue ($P \leq 0.10$) of the ram lambs and while PPARγ and LPL gene expression were numerically increased. These results need to be confirmed using a larger number of animals. Interestingly, this effect of dietary PUFA supplementation on visceral adipose tissue has been proposed (Hiller *et al.,* 2012) to benefit ruminant energy homeostasis whereby dietary PUFA decreases lipogenesis in the liver allowing for the liver to focus on gluconeogenesis (see Figure 3-1 showing how dietary PUFA may affect energy homeostasis) while increasing fatty acid synthesis in adipose tissue. Dietary n-3 PUFA may benefit energy homeostasis of growing ram lambs as well.

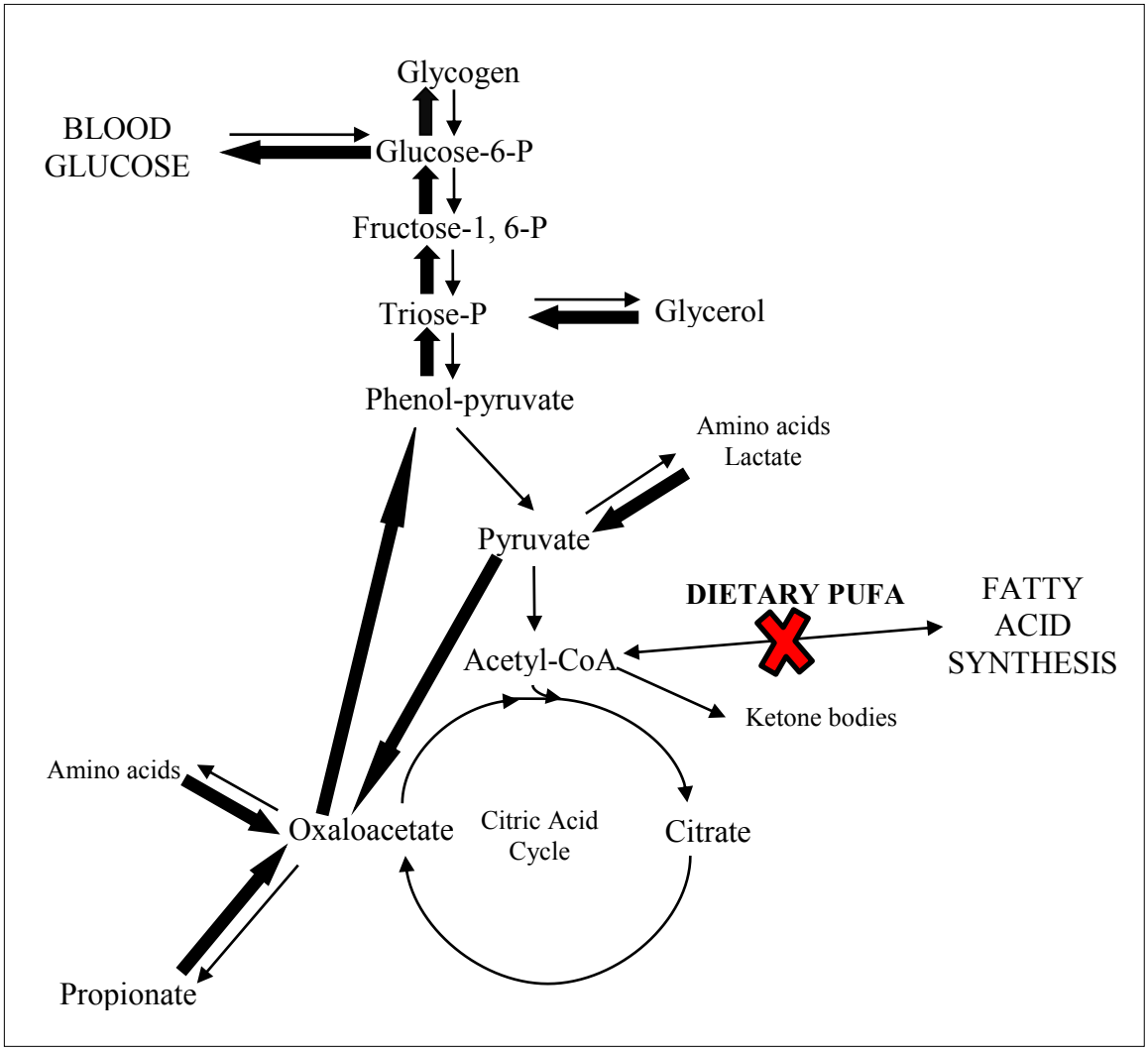

Figure 2: Major metabolic pathways of the ruminant liver; bold arrows showing gluconeogenesis; X indicating effect of dietary PUFA. Adapted from Reece (2009)

Conjugated linoleic acid is produced primarily within ruminant tissues endogenously by the action of D9D. It is also formed in the rumen during the biohydrogenation of unsaturated fatty acids. Interest in increasing the CLA content of ruminant tissues is prominent whereby researchers look at increasing D9D activity and/or by increasing CLA escape from the rumen degradation. However, confounding effects are seen when CLA is supplemented in the diet. While CLA content of adipose can be increased when CLA is fed to ruminants (Song and Kennelly, 2003) or abomasally infused (Loor and Herbein, 1998; Chouinard *et al*., 1999) studies reviewed by Hur *et al.* (2007; primarily non-ruminant animal models) and Bauman *et al.* (1999; ruminant studies) showed a

reduction in D9D expression when diets were supplemented with CLA. When considering the effect of lipid treatment only, no significant differences in lipogenic gene expression were detected between CLA and SBO supplementation, in the present study. However an interaction was observed between pasture type grazed and CLA supplementation. The TF grazed lambs that were supplemented with CLA had increased Spot14 expression in enteric adipose tissue when compared to SBO supplemented lambs ($P \leq 0.10$, Table 3-5). Thyroid hormone responsive spot 14 is a protein thought to be involved in lipogenesis (Colbert *et al.,* 2010; Harvatine and Bauman, 2006). Although the exact function of Spot14 has not been discerned, it has been related to regulation of lipid synthesis in mammary (Harvatine and Bauman, 2006). The TF grazed lambs were found to gain more when switched to concentrate and lipid supplementation. They may have been experiencing compensatory growth compared to RC grazed lambs due to the differing nutritional composition of the two pasture types. A lamb experiencing compensatory growth is transitioning from negative to positive energy balance whereby lipogenesis is active. Therefore, increased activity of lipogenic enzymes at this time might be expected.

In the present study, FO supplementation was found to down-regulate Spot14 in enteric adipose of growing ram lambs. The results achieved with FO down-regulation of Spot14 in enteric adipose may reflect reduced lipogenesis as is found in bovine mammary (Harvatine and Bauman, 2006). However reduced expression of Spot14 in mouse liver does not reduce ACC gene expression or lipogenesis (Colbert *et al*., 2010). Measurement of other lipogenic genes in enteric adipose tissue, such as ACC and FAS, is warranted.

A study with the muscle tissue of young crossbred bulls by Waters *et al.* (2008) demonstrated that dietary incorporation of FO decreased SREBP and D9D activity in muscle tissue while PPARα was not affected (which corresponds to findings of present study). Peroxisome proliferator activated receptors are a group of transcription factors involved in maintaining lipid homeostasis (Deckelbaum *et al.,* 2006) by regulating genes involved in the transport of plasma triglycerides, cellular fatty acid uptake, β-oxidation (Schoonjans *et al*., 1996) and adipocyte differentiation (Kersten *et al.,* 2000). There are

three isoforms: PPARα PPARβ/δ, and PPARγ. PPARα is expressed in tissues that oxidize fatty acids for energy; mainly the liver where it controls the breakdown of fatty acids, PPARβ/δ is ubiquitously expressed and PPARγ is primarily found in adipose tissue (Lee *et al*., 2003a; Schoonjans *et al.,* 1996) where it influences the uptake and storage of fatty acids into the adipose tissue and adipocyte differentiation (Kersten *et al*., 2000). It is known that long-chain PUFA affects the activity of PPARs in ruminants (Bionaz *et al*., 2012) and non-ruminants (Nakamura *et al*., 2004). Studies in non-ruminants have found differences among specific long-chain PUFA (Bragt and Popeijus, 2008) and amount of long-chain PUFA (Wolfrum *et al*., 2001) needed to activate transcription of PPARs. A recent *in vitro* study in ruminants using bovine kidneys cells, showed that PPARα expression is related to the expression of genes involved in β-oxidation as has been observed with ruminants (Bionaz *et al*., 2012). Their findings also indicate that EPA is a strong activator of PPARα with *cis*-9, *trans*-11 CLA having moderate induction. It was expected that FO, being high in long-chain PUFA, would increase liver mRNA abundance of PPAR as it is known in non-ruminants that n-3 PUFA bind directly to PPAR and are potent activators (Bragt and Popeijus, 2008). We did not see this relationship in ram lamb liver. A similar outcome was obtained in a study of muscle tissue in young crossbred bulls (Waters *et al.* 2008) where dietary incorporation of fish oil decreased SREBP and D9D activity in muscle tissue, while PPARα was not affected. In omental adipose tissue of lactating dairy cows, Hiller *et al*. (2012) found that PPARγ was up-regulated in dairy cattle that were fed PUFA supplements. This was not observed in enteric adipose tissue of the ram lambs fed FO in this study.

Adipose tissue derived hormones aid in the regulation of energy homeostasis. Leptin, one of the two adipose-derived hormones analyzed in this study, is involved in regulation of appetite, energy metabolism, growth, reproduction, and body composition and is being recognized in ruminant studies as an indicator of growth, where hypoleptinemia improves energetic efficiency (Agarwal *et al.,* 2009). Leptin stimulates energy expenditure and fat mobilization and inhibits lipogenesis in the liver and adipose tissue in non-ruminants (Havel, 2004). Research involving dietary PUFA supplementation to ruminants and effects on leptin expression is limited. Study in the ruminant (cattle; Gillis *et al*., 2004a)

as well as a non-ruminant hind-gut fermenter (rabbit; McCullough *et al*., 2011) has shown that dietary supplementation of LA and ALA increases leptin expression in adipose tissue. Supplementation of CLA in the ruminant diet (as well as LA-rich diets) had no effect on circulating leptin in beef heifers (Gillis *et al*., 2004a; Garcia *et al.,* 2003) however, LA-rich diets increased leptin concentration in adipose tissue (Gillis *et al*., 2004a). Baumgard *et al*. (2002) found no effect on circulating leptin concentrations when CLA isomers *cis*-9, *trans*-11 or *trans*-10, *cis*-12 were given to dairy cattle in late lactation through abomasal infusion. Also, studies in cattle have found that visceral adipose has greater leptin expression than subcutaneous adipose (Chilliard *et al*., 2001; Ren *et al*., 2002). In the present study when comparing CLA^{+} to SBO supplemented lambs, leptin gene expression was numerically elevated but the effect was not statistically significant. However, when comparing CLA^{+} supplemented lambs to the control lambs, leptin gene expression was significantly increased by 2-fold. Enteric adipose tissue leptin gene expression in ram lambs was significantly down-regulated by FO when compared to SBO ($P \leq 0.05$) and when compared to CON ($P \leq 0.10$; Table 3-4).

The majority of study with n-3 dietary PUFA supplementation on leptin expression has focused on the non-ruminant although the effect of lipid supplementation and associated metabolic interactions are still unknown. In the non-ruminant, the actions of n-3 dietary PUFA on leptin expression are suggested to be dependent on the physiological state of the animal. Reseland *et al*. (2001) found that prolonged (3 weeks) of n-3 PUFA resulted in lowered leptin plasma levels and reduced leptin expression in adipose tissue. However, when physiological state is considered, the effects of n-3 PUFA on leptin differ where lean rats have reduced plasma leptin and overweight rats have increased plasma leptin expression (Moreno-Aliaga *et al*., 2010).

Another adipocyte derived hormone, adiponectin, has been found to regulate glucose and lipid metabolism (Fruebis *et al.,* 2001), regulate cell differentiation (Luo *et al.,* 2005) and improve insulin sensitivity (Lihn *et al*., 2005). In contrast to leptin, adiponectin has been found to stimulate food intake and led to the hypothesis that suggests energy reserves are maintained by opposing actions of leptin and adiponectin (Kubota *et al*., 2007).

Adiponectin is thought to be transcriptionally activated by SREBP as the adiponectin gene contains a sterol response element in its promoter region (Shehzad *et al.,* 2012) although the regulation of adiponectin by SREBP is not understood. Diet has been found to affect adiponectin with n-3 PUFA (EPA and DHA) increasing expression of adiponectin and thus insulin sensitivity in the non-ruminant (Flachs *et al*., 2006; Duda *et al.*, 2007; Reis *et al*., 2010). Information concerning the effect of adiponectin in the growing ruminant (or ruminants in general) is minute with only a few studies involving lactating dairy cattle. In ruminant studies, adiponectin expression was found to increase during adipocyte differentiation (Roh *et al*., 2006). Adiponectin mRNA abundance in ram lamb enteric adipose tissue had a tendency to be up-regulated by SBO when compared to FO ($P \leq 0.10$); Table 3-4). This may correspond with the phytoestrogens in soybean oil where soy-derived phytoestrogens have been shown to increase adiponectin in the human (Charles *et al*., 2009; Llanneza *et al.,* 2010).

Leptin and adiponectin are critical to the regulation of fat storage, energy metabolism and food intake (Moreno-Aliaga *et al*., 2010). Given the effects of the dietary lipid supplements on leptin and adiponectin gene expression observed in this study and the potential significance of these adipokines to animal production, the role of PUFA in modulating adipokine production in growing ruminants must be studied further.

3.5.3 Concluding Remarks

In conclusion, RC pasture (rich in n-3 PUFA) decreased expression of lipogenic D9D in liver tissue 5 weeks after being removed from pasture with a dietary change to concentrate and lipid supplementation demonstrating long-term effects of the PUFA content of grower diets. Supplementing the finishing diet with FO decreased expression of lipogenic transcription factor SREBP-1 in liver and also lipogenic Spot14 in enteric adipose tissue suggesting that lipogenesis was decreased in both metabolically active tissues. Delta-9-desaturase was numerically decreased with FO supplementation indicating that the n-3 PUFA content of FO (EPA and DHA) decreased D9D in the liver.

Decreased D9D in liver from FO supplementation corresponds with decreased SREBP1 expression in the liver. However, a tendency for increased D9D expression in enteric adipose was observed. Leptin expression in enteric adipose was decreased by FO supplementation with a tendency for adiponectin to be decreased as well. Furthermore, leptin expression in enteric adipose is altered by pasture and lipid treatment where RC×SBO and TF×CLA^{+} had increased leptin expression. Further work is needed to elucidate the balance of regulatory mechanisms such as decreased leptin, adiponectin and Spot14 in enteric adipose tissue.

More study is needed considering that many lamb production systems are a combination of grazing followed by finishing on a concentrate based diet, understanding the differential effects of pasture type and the longevity of the effect may be important to maximizing productivity as well as improving carcass composition for the health conscious consumer. Monitoring nutrient-gene interactions provides insight into the effects of dietary manipulation on lipid metabolism in the growing lamb and points to the need for further work to better understand the effects of dietary manipulation in production animals.

CHAPTER 4 GENERAL CONCLUSION

This research has shown that there is potential to improve the fatty acid profile of lamb through altering the species composition of the pasture grazed during the growth phase and through limited lipid supplementation during a 5 week finishing phase on a concentrate-based diet. The longevity of the effect of pasture species grazed was demonstrated by a reduction in D9D gene expression in the liver of RC grazed lambs as well as changes in muscle and subcutaneous adipose tissue fatty acid composition whereby RC pasture during the growth increased ALA content 21 and 60 % in muscle and subcutaneous adipose tissue respectively and total PUFA in subcutaneous adipose tissue 17 %, measured after a 5 week dietary change to a concentrate-based diet. These are significant findings as no other studies have looked at this type of lamb production system (which is typical in Atlantic Canada) and long term effects of pasture type. As seen in this study, the growth-promoting effects of RC pasture seemed to diminish as lambs were finished on concentrate; however, effects on metabolism were maintained whereby the sustained elevation in PUFA muscle and subcutaneous adipose content of the RC pasture depressed D9D expression despite a 5 week concentrate based finishing diet.

Supplementing FO into the finishing diet has the potential to significantly affect the metabolism of the finishing lamb. Limited fish oil supplementation (200 g/d for 2 weeks) dramatically enhanced the n-3 PUFA concentration of lamb meat (295 %) and adipose tissue (196 %) while those of EPA and DHA were enhanced in muscle tissue by 460 % and 580 %, respectively and in subcutaneous adipose by 970 % and 1200 %, respectively. These health-promoting PUFA were substantially increased in the meat and fat tissues of FO supplemented lambs significantly beyond the increases observed by pasture type. In addition, FO supplementation decreased lipogenic transcription factor SREBP1 in the liver, and Spot14 in enteric adipose suggesting that lipogenesis was decreased in the lamb by FO supplementation. These results as well as a leaner carcass suggest that lipogenesis in the lamb was decreased with FO supplementation. However, the percent lipid in the

muscle (intramuscular fat) was not different between lipid treatments and *de novo* lipogenesis in muscle and subcutaneous adipose tissues, as measured by the percent of C14:0 and C16:0, was also unchanged by FO supplementation. Furthermore, lipid treatments were similar for percent lipid and tissue fatty acid content of fatty acids that result from *de novo* lipogenesis. While these results suggest that lambs may not be have been experiencing reduced lipogenesis as indicated by the reduction of SREBP1 gene expression in the liver and reduced Spot14 gene expression in the enteric adipose tissue, they may also indicate a tissue specific response to the FO supplementation. More detailed analysis of FO fed lambs is required including determination of feed intake and lipogenic gene expression in muscle and subcutaneous adipose tissues.

Studies of gene expression analysis in the growing ruminant are limited, especially for leptin, adiponectin and Spot14. The reported effects of dietary PUFA on leptin are often contradictory. This study is no exception where leptin was reduced by FO supplementation. As leptin decreases appetite and lipogenesis and increases oxidation of fatty acids, one might expect leptin to be increased with FO supplementation, supporting the established roles of PUFA and leptin in fat reduction and weight loss. However, leptin serum levels are most strongly associated with energy balance and body fat stores. In this study leptin gene expression may reflect the leaner carcasses of the FO supplemented lambs.

Ruminant animal products are a good source of CLA isomers because ruminants effectively convert LA to CLA. This research demonstrated that supplementing the growing lamb diet with a source of CLA has the potential to increase all CLA isomers in the subcutaneous adipose above levels obtained with SBO, a good source of LA. More specifically, levels of the cis-9, trans-11 CLA isomer increased by 25 % while the *trans*-10*, cis*-12 CLA isomer increased by 172 %. There was no evidence of decreased subcutaneous fat and lipogenesis or increased lean muscle accumulation in the ram lamb with CLA supplementation.

Enriching lamb with beneficial fatty acids has the potential to create niche marketing opportunities. Considering that many lamb production systems are a combination of grazing followed by finishing on a concentrate based diet, understanding the differential effects of pasture type, the longevity of the effect and the potential interaction of pasture type with lipid supplementation during finishing, are important to maximizing productivity as well as improving carcass composition for the health conscious consumer.

Further work is needed to elucidate the differences found between gene expression analysis in metabolically active tissues and tissue fatty acid composition of tissues consumed by human. No distinct relationships between the gene expression analysis in liver and enteric adipose tissues and fatty acid composition analysis of the muscle and subcutaneous adipose tissue were observed. Therefore, fatty acid composition analysis in liver and enteric adipose tissue and gene expression analysis in muscle and subcutaneous adipose tissue may help in determining direct links between gene expression and fatty acid composition. Evaluating more lipogenic genes (such as SREBP1, ACC and FAS) in in both enteric and subcutaneous adipose tissues would aid in establishing the relationship between the dietary effects of pasture species and lipid supplementation on lipid metabolism in the growing ruminant. From a commercial perspective future studies should consider the amount of lipid supplementation and the length of time required to most economically elicit beneficial improvement in the fatty acid composition of the lamb meat.

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