MANIPULATION OF THE FATTY ACID COMPOSITION OF PORCINE TISSUES WITH RESPECT TO THE HUMAN DIET

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by

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ABSTRACT

An attempt was made to manipulate the fatty acid composition of porcine tissues in accordance with contemporary human dietary guidelines and to investigate various factors affecting such manipulation. A review of the literature was undertaken with respect to: i) the alterations of the fatty acid composition of porcine tissues and ii) the effect of dietary *n-3* polyunsaturated fatty acids on human health and disease.

The first experiment attempted to define the minimum period of time required to alter the fatty acid composition of porcine tissues in line with human dietary recommendations. A diet containing 50 g/kg soybean oil plus 10 g/kg refined fish oil (Seven Seas Ltd., Hull, England) was fed to Large White x Landrace pigs for two, four and six weeks immediately before slaughter. Growth performance and carcass characteristics, including penetrometer firmness, were recorded. The total lipid contents of the outer and inner backfats, *Semitendinosus*, *Longissimus dorsi* and liver were determined by gravimetry. The content of cholesterol ester, triacylglycerol, free fatty acid and phospholipid of the tissues and their constituent fatty acid compositions were determined by thin-layer and gas-liquid chromatographies. Neither pig growth performance nor carcass characteristics were adversely affected by feeding the soybean oil/fish oil diet over differing pre-slaughter periods. Whilst the total lipid contents were generally not affected by treatment, the fatty acid composition of the lipid fractions of the tissues was significantly affected by diet. Marked increases in the levels of eicosapentaenoic acid and docosahexaenoic acid were observed in the presence of reductions in the levels of linoleic and oleic acids. The levels of linoleic acid in the outer and inner backfats were approximately 30 % of total fatty acids. Alterations in the ratios of total *n-6* to *n-3* fatty acids and polyunsaturated to saturated fatty acids were achieved in line with dietary recommendations; these ratios were approximately 6 and 1, respectively. Feeding the modified diet for six weeks gave the closest approximation to the dietary recommendations.

The second experiment was an attempt to evaluate the effects of alterations in the levels of dietary copper and vitamin E on the fatty acid composition of tissues obtained after feeding the soybean oil/fish oil diet which had been fed in the first experiment. Copper (as copper (II) sulphate) at nominal levels of 20 or 100 mg/kg diet and vitamin E (as α-tocopheryl acetate) at 75 or 375 mg/kg diet were fed to Large White x Landrace entire males and females for six weeks pre-slaughter. The growth performance and carcass characteristics of the pigs were unaffected by diet. The fatty acid compositions of the muscle, backfats and liver were significantly affected by diet in accordance with the findings of the first experiment. Whereas tissue copper contents were not affected by diet, the contents of vitamin E in the tissues significantly increased as a result of dietary supplementation of α-tocopheryl acetate. The effect of vitamin E on fatty acid composition was more prominent than that of copper. Ratios of P:S and *n-6*:*n-3* were affected by both dietary copper and vitamin E. Olfactory sensory properties of samples of cooked outer backfat from two of the diets were determined by a trained taste panel. Whilst no marked effect of diet was observed, significant differences in fat flavour characteristics between two and six months of frozen storage were discerned.

The third experiment attempted to evaluate the effects of alterations in dietary soybean oil (SO) and fish oil (FO) on the distribution of triacylglycerol (TAG) molecular species in pig outer backfat. In attempting this, a specific methodology involving silver ion high performance liquid chromatography was optimised. Pigs were fed diets containing (per kg diet): 50g tallow; 25g SO; 25g SO plus 10g FO; 50g SO; 50g SO plus 10g FO; and 75g SO. A total of 14 TAG fractions was identified. Neither growth performance, carcass characteristics, fat thickness (P2) nor backfat total lipid content was affected by diet. Significant differences in the fatty acid content and TAG molecular species content of the outer backfat were observed between the diets. In spite of a similarity in linoleic acid content (approximately 25% of total fatty acids), shoulder fat from pigs fed 25g SO plus 10g FO was significantly firmer than that from pigs fed 25g SO. The difference was probably related to the alterations in the content of TAG species containing the long chain *n-3* polyunsaturated fatty acids.

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ABBREVIATIONS

IFOMA	International Fishmeal and Oil Manufacturers Association
NATO	North Atlantic Treaty Organisation
NDC	National Dairy Council
et al	and others
%	Percent(age)
r	Pearson correlation coefficient
MJ	MegaJoule(s)
α	Alpha
β	Beta
Δ	Delta
γ	Gamma
sn-1, *sn*-2, *sn*-3	Denotes stereospecific numbering of carbon atoms 1, 2 and 3, respectively, of the trihydric alcohol glycerol
cis-	Fatty acid groups on adjacent sides of the carbon - carbon double bond
trans-	Fatty acid groups on the same side of the carbon - carbon double bond
PUFA	Polyunsaturated fatty acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
EPO	Evening primrose oil
C18	Fatty acid with 18 carbons
C20	Fatty acid with 20 carbons
C22	Fatty acid with 22 carbons
BHT	Butylated hydroxytoluene
CHD	Coronary heart disease
HDL	High density lipoprotein
LDL	Low density lipoprotein
VLDL	Very low density lipoprotein
TAG	Triacylglycerol
TBARS	Thiobarbituric acid - reactive substances
TXA_2, TXA_3	Thromboxane A_2, Thromboxane A_3
PGI_2, PGI_3	Prostacyclin I_2, Prostacyclin I_3
PGE_2, PGE_3	Prostaglandin E_2, Prostaglandin E_3
LTB_4, LTB_5	Leukotriene B_4, Leukotriene B_5
HPLC	High performance liquid chromatography
L. dorsi	*Longissimus dorsi*
ml	Millilitre(s)
$mlmin^{-1}$	Millilitre(s) per minute
μg	Microgram(s)
μl	Microlitre(s)
v/v	Volume for volume
w/v	Weight for volume

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Trust in the Lord with all thine heart
and lean not unto thine own understanding.
In all thy ways acknowledge him,
and he shall direct thy paths
Be not wise in thine own eyes,
fear the Lord and depart from evil.
It shall be health to thy navel
and marrow to thy bones

Proverbs Chapter 3 Verses 5-8

God be merciful to me a sinner

Luke Chapter 18 Verse 13

For God so loved the world
that he gave his only begotten son,
that whosoever believeth in him should not perish,
but have everlasting life

John Chapter 3 Verse 16

He was wounded for our transgressions,
He was bruised for our iniquities,
the chastisement of our peace was upon him
and with his stripes we are healed

Isaiah Chapter 53 Verse 5

There is therefore now no condemnation
to them which are in Christ Jesus

Romans Chapter 8 Verse 1

INTRODUCTION

It is well recognised that the standard of health assumed by an individual or population is markedly dependent on the type of diet which is available. In order to obtain an optimum intake of the principal nutrients and therefore to attain an optimum health status, the importance of achieving a 'balance' in the intake of nutrients from a range of food groups has often found expression. However, in the case of fat consumption, a perceived imbalance in the intake of the various types of fatty acids has become apparent and has been a subject for discussion and pronouncement by a variety of international committees. Contemporarily, a large number of studies has observed specific and beneficial effects on human health and well-being through the consumption of the long chain n-3 *polyunsaturated fatty acids, in particular eicosapentaenoic acid and docosahexaenoic acid, which occur in high levels in fish oil. Not only have beneficial effects been observed in disease conditions, most notably cardiovascular disease, but these fatty acids have also been found to be important in the development of the neonate and during ageing. As a result of these observations, recommendations pertaining to the consumption of dietary fat have stressed the importance of consuming higher levels of the long chain* n-3 *polyunsaturated fatty acids as found in fish oil.*

The tissues of animals for food use have come to be associated with a predominance of saturated and monounsaturated fatty acids, the result of which has been to contribute to a perceived dietary imbalance in fatty acid composition and an unacceptability in terms of human health. In addition to an increase in the consumption of more oily fish which characteristically contain high levels of the long chain n-3 *polyunsaturates, a higher intake of these fatty acids could be achieved by the consumption of commonly-occurring non-fish foods containing elevated levels of these fatty acids. The fatty acid composition of pig tissues is generally closely dependent on the fatty acid composition of the diet. The potential therefore exists to manipulate the fatty acid composition of the pig diet to produce tissues containing fatty acids deemed most appropriate for human health requirements. The present study beginning October, 1990, sought to alter the fatty acid composition of porcine tissues in line with current human health requirements. Due attention was also taken of effects on growth performance parameters in line with the maintenance of commercial production requirements.*

In parallel with efforts to reduce the content of saturated fatty acids whilst increasing the content of polyunsaturated fatty acids in pig subcutaneous adipose tissue ('backfat') have been concerns over the development of unacceptable fat softness making meat processing difficult. The physical properties of porcine fat as indicated by its firmness or melting point are dependent almost entirely on fatty acid composition and structural characteristics. A major feature of the present study was to monitor any

changes in fat firmness arising as the result of alterations in fatty acid content. Consequential effects on molecular lipid speciation with respect to physical parameters were therefore of prime signficance.

Other aspects of the pig diet have to be taken into consideration. The feeding of dietary copper in pig diets at levels above that required for normal growth has been frequently undertaken due to the beneficial effects of this element on pig growth performance. However, the feeding of copper has also been associated with changes in the physical properties of pig backfat due to accompanying increases in the content of unsaturated fatty acids. Vitamin E is one of the principal biological antioxidants. Its level in tissues has been shown to be subject to alteration according to the amount of polyunsaturated fatty acids present. The feeding of fish oil containing highly polyunsaturated fatty acids could therefore be seen to compromise the content of vitamin E. Thus, consistent with its antioxidant effects, dietary vitamin E has been associated with a significant improvement in the storage stability of pig fat in terms of chemical, physical and sensory parameters. The present study has attempted to assess the effects of altering dietary copper and vitamin E on the fatty acid content and sensory characteristics.

The outcomes of the experiments undertaken to embrace these objectives will be interpreted in the light of contemporary information on human dietary intakes and requirements of lipids and their fatty acids.

1. Review of the literature

SECTION I

1.1. Fundamental characteristics of lipids in animals

1.1.1. Definition of lipid

Although in previous years there has been no specific, universally-accepted definition of the term lipid, the following broad definition has been used by those engaged in lipid research. The term 'lipid' embraces " a wide variety of natural products including fatty acids and their derivatives, steroids, terpenes, carotenoids and bile acids which have in common a ready solubility in organic solvents such as diethyl ether, hexane, benzene, chloroform or methanol " (Christie, 1982). More recently, however, Christie (1989) proposed a less all-encompassing definition: "lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds".

1.1.2. Characteristics of fatty acids

1.1.2.1. Nomenclature

Fatty acids are the major constituents of lipids. They are composed of a chain of methylene ($-CH_2$) groups, at one end of which is a carboxyl (-COOH) group. Fatty acids containing no double bonds are termed saturated and those containing one or more double bonds are termed unsaturated. Double bonds exist in one of two isomeric forms, *cis* or *trans*. Double bonds with the *cis* configuration possess hydrogen atoms on the same side of the double bond, thereby incurring a bend in the molecule. Those in the *trans* configuration contain hydrogen atoms on either side of the double bond. Unsaturated fatty acids are further distinguished by the position of the double bond closest to the methyl/terminal or omega (ω) end of the molecule. Thus, fatty acids with a double bond between carbon-6 and carbon-7 from the methyl end and those with a double bond between carbon-3 and carbon-4 from the methyl end, are termed *n-6* (or ω-6) and *n-3* (or ω-3) fatty acids, respectively. By similar reasoning, fatty acids with *n-9* and *n-7* structural characteristics may be identified. This distinction is of great importance in considering the differing physiological roles of fatty acids and has implications for health and disease as will be described later. The British Nutrition Foundation (1992), in line with the general consensus, has recommended the adoption of the shorthand chemical notation which recognises the position of the terminal methyl double bond. Thus, for example, identification of α-linolenic acid would be 18:3*n-3*, indicating a fatty acid with 18 carbon atoms and three double bonds (see Table 2.1).

Essential fatty acids are defined as those fatty acids which must be ingested due to the fact that they cannot be synthesised in the human body. Thus, both linoleic and α-

linolenic acids are considered as essential but from which other fatty acids can be synthesised. Due to their importance with respect to human health, it has been suggested that the long chain *n-3* polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid should also be considered as essential (British Nutrition Foundation, 1992; Linder, 1991; Neuringer *et al*, 1988).

1.1.2.2. Importance of fatty acids

Fatty acids are the major components of triacylglycerols and phospholipids, the functions of which are described below in Section 1.1.3, as well as a number of other lipid fractions. Fatty acids are involved in the formation of a wide range of metabolic regulators. The main regulators in this respect are prostaglandins, prostacyclins, thromboxanes and leukotrienes, all of which are hydroxylated derivatives of 20-carbon polyunsaturated fatty acids (Watkins, 1995). These affect blood pressure, clotting and the immune response, operating ubiquitously in the body at very low concentrations (10^{-13} to 10^{-10}M) and with very short half-lives (Sardesai, 1992). Prostacyclins and thromboxanes have essentially opposing physiological effects. Prostacyclins, formed in blood vessel walls, strongly inhibit platelet aggregation, relax arterial walls and tend to reduce blood pressure. However, thromboxanes, formed in platelets, stimulate platelet aggregation, contract arterial walls and increase blood pressure (Needleman *et al*, 1979). The formation of prostaglandins, thromboxanes and prostacyclins is catalysed by the cyclo-oxygenase enzyme whilst leukotrienes are derived from the lipoxygenase enzyme (Yamamoto, 1992). As will be described later in this review, arachidonic acid (20:4*n-6*) and eicosapentaenoic acid (20:5*n-3*) are central in the biosynthesis of these substances. Use of either fatty acid precursor by these enzyme systems produces prostanoids with different pharmacological properties. Further information regarding such effects is to be found in reviews by Watkins (1995), Sardesai (1992) and the British Nutrition Foundation (1992).

1.1.3. Functions of major lipid classes

1.1.3.1. Triacylglycerol

Triacylglycerols are composed of a glycerol molecule esterified with three fatty acids at the so-called *sn*-1, *sn*-2 and *sn*-3 positions, respectively. These compounds serve both as a valuable source of energy and as a reservoir of fatty acids for use in other metabolic processes. Unlike the limited storage capacity for carbohydrates and protein, triacylglycerol can be stored in almost unlimited amounts. The majority of triacylglycerol is found in adipose cells where it comprises over 95% of both the cell volume and the total lipid present (Gurr, 1981). In animals, fatty acids are not esterified at random to glycerol hydroxyl (-OH) groups. For example, in the adipose tissue of most species, the *sn*-2 position is occupied by an unsaturated fatty acid (Gunstone and Norris, 1983). However, exceptions to the rule are in pig tissues and human milk where

this position is occupied by a saturated fatty acid, mainly palmitic acid (Christie and Moore, 1970; Gurr, 1992, respectively). Furthermore, triacylglycerols are asymmetric in biochemical terms. Thus, positions *sn*-1 and *sn*-3 are distinguishable to the various enzyme systems (Brockerhoff, 1965).

The melting points of triacylglycerols depend both upon the fatty acids they contain and, to a lesser extent, upon the positional distribution of the fatty acids esterified to the glycerol moiety (Gunstone and Norris, 1983; Dziubajlo, 1991). Thus, triacylglycerols containing unsaturated fatty acids have lower melting points than those with corresponding saturated fatty acids. Melting point also depends on the configuration of the double bonds; *trans* fatty acids melt at higher temperatures than their *cis* counterparts as the molecular structure of the *trans* fatty acids resembles that of the corresponding saturated fatty acid.

1.1.3.2. Phospholipid

Phospholipids are diacylglycerols conjugated to a substituent grouping composed of phosphoric acid and a base. The commonest basic groups are choline, serine and ethanolamine with the phospholipids being named accordingly (i.e. phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine, respectively). Other phospholipids include sphingomyelin and cardiolipin. Phospholipids are involved primarily in the formation and maintenance of cell membrane integrity (Singer, 1972). In animal cell membranes, phosphatidylcholine is normally the most abundant lipid. Together with cholesterol, cholesterol esters and proteins, among others components, phospholipids provide a semi-fluid, selectively permeable boundary for cells. As in the case of triacylglycerols, fatty acids of phospholipids have distinctive positional distributions due to the specificity of the acyltransferases which are responsible for synthesising the phospholipids. In most cases, saturated fatty acids are concentrated in position *sn*-1 whilst unsaturated fatty acids are concentrated in position *sn*-2 (Gurr, 1992). Further to their structural role, phospholipids supply polyunsaturated fatty acids, most notably arachidonic acid and eicosapentaenoic acid, as substrates for the formation of prostaglandins, leukotrienes and thromboxanes which are important in a number of cell regulatory processes (Sanders, 1988).

1.2. Manipulation of the fatty acid composition of porcine tissues

Since the work of Ellis and Isbell (1922) in which pigs were fed a diet containing whale oil, a large number of studies has recorded the effects of the fatty acid composition of the pig diet on the fatty acid composition of the tissues. Such studies have been possible due largely to the fact that the pig is a monogastric animal and therefore incorporates digested fat into the tissues without significant changes to the

chemical properties of the fat (Kidder and Manners, 1978). This is in contrast to the situation in ruminants in which the presence of micro-organisms within the rumen causes marked changes to the fatty acid composition of the ingested food. Thus, up to 90% of the ingested unsaturated fatty acids in ruminants may become hydrogenated resulting in the formation, either directly or indirectly, of saturated fatty acids, branched chain fatty acids and/or *trans* fatty acids (Gurr, 1992).

The manipulation of pig fatty acid composition in accordance with human dietary recommendations has provided a means of contributing to the needs of various aspects of human health and disease including coronary heart disease (Morgan *et al*, 1992; Rhee *et al*, 1988a). In such cases, alterations in tissue levels of saturated, monounsaturated and polyunsaturated fatty acids which were perceived as beneficial were obtained after feeding diets containing different oils. In other studies, the needs of human health were secondary to the need to utilise an inexpensive by-product of the food or agriculture industry. For example, West and Myer (1987) observed the changes in pig fatty acid composition in pigs allowed to glean peanuts from a field after harvest.

1.2.1. Sources of oils in diets for pigs

A wide range of fats and oils has been utilised as a component of the pig diet based on by-products of the food or agriculture industry. Although fats have been included in pig diets as a valuable source of digestible energy at 36 MJ/kg dry matter (Shepperson, 1990), this review will concentrate only on the use of dietary fat as a means of altering pig tissue fatty acid composition. Amongst the vast range of products that has been used tallow (Koch *et al*, 1968), soybean oil (Christensen, 1964), maize oil (Leat *et al*, 1964), sunflower oil (Viljoen and Ras, 1991) and rapeseed/canola oil (Suomi *et al*, 1993; Rhee *et al*, 1988b) have been some of the most prominent. Oils have been included in the diet as a component of the respective oilseed meal, e.g. rapeseed meal (Hertzman *et al*, 1988) or as part of the whole seeds in pelleted form (Castell and Falk, 1980). Other additions have been via the extracted oil which may or may not have received additional refining or deodorising (Rhee *et al*, 1988a, 1988b). The oils have generally been used as substitutes for the commonly-utilised animal fats, e.g. tallow, although more recently diets have been fed to pigs which contained added fish oil (Morgan *et al*, 1992; Irie and Sakimoto, 1992). The oils have been introduced into the compound 'grower/finisher' feed which has been prepared in the form of pellets of varying sizes or in loose meal form. The addition of oil to pig diets may be subject to various constraints set by the particular feed mill which is preparing the diet (Shepperson, 1990). Additionally, the feeding of high levels of highly polyunsaturated oils has been associated with the development of unacceptable pig backfat firmness (Whittington *et al*, 1986). For these reasons, levels of oils added to diets have usually not exceeded the level of 10% of the weight of the diet. The Meat and Livestock

Commission's *Blueprint for Lean and Tender Pork* has recommended that inclusion of oils in the diet may not be more than 3.5% by weight of the diet.

1.2.2. Alterations in tissue fatty acid composition achieved by changes in dietary oil content

In pigs fed a conventional diet, the fatty acid composition of the muscle and fat tissues, as determined by standard gas-liquid chromatography, is characterised by a high level of oleic acid in the approximate range 35 to 45% of total fatty acids (MLC, 1991). Palmitic acid is present in the range 20 to 25% whilst stearic and linoleic acids are present at levels of 11 to 15%. The results of dietary oil manipulations in the diets of pigs have been marked changes in the fatty acid composition of the major tissues including muscle, adipose tissue and liver, with consequent alterations in the ratios of polyunsaturated to saturated and *n-6* to *n-3* fatty acids (Morgan *et al*, 1992; Leskanich *et al*, 1993). Not only have such changes been noted in the triacylglycerol (storage lipid) fraction, but also in the phospholipid (structural lipid) fraction of the tissues.

To widely varying degrees, all of the fatty acids occurring in pig tissues have been shown to be open to dietary manipulation - the direction and extent of change usually bearing close resemblance to the fatty acid composition of the specific oil added and its level in the diet. For example, the level of linoleic acid in pig backfat has been shown to be closely correlatable to the dietary level (Prescott and Wood, 1988; Whittington *et al*, 1986; Koch *et al*, 1968); this may be due to the fact that it is an essential fatty acid (Brooks, 1971). In contrast, the levels of a number of saturated fatty acids in the tissues have been shown to be relatively poorly related to those in the diet, including lauric and myristic acids (Christensen, 1964) and stearic acid (Roberts and Enser, 1988); when fed at high levels they do not increase beyond certain concentrations and are apparently catabolised for energy release. Christensen (1964) proposed that such 'selection' of fatty acids for deposition was a way in which the pig could prevent the formation of adipose tissue which would be overly firm. More recently, it has been demonstrated that by feeding long chain *n-3* polyunsaturated fatty acids to pigs, there is a dose-dependent incorporation of these fatty acids into both adipose tissues and intramuscular fat (Morgan *et al*, 1992; Irie and Sakimoto, 1992) with selection of these fatty acids occurring even in preference to linoleic acid, a fatty acid for which essentiality has long been established. The manipulation of the polyunsaturated fatty acid content of porcine tissue will be described further in Section 1.8.2. Where high levels of certain fatty acids have been fed, the increases in tissue levels of these fatty acid have been accompanied by proportional reductions in the levels of other fatty acids. Thus, for example, the increases in tissue levels of linoleic acid occurring as a result of feeding soybean oil have been accompanied by reductions in oleic and palmitic acids (Leat *et al*, 1964). The inclusion of a particular fatty acid may also enhance or suppress biosynthetic mechanisms which lead to the formation of other fatty acids. For example, the feeding

of diets containing eicosapentaenoic acid to pigs has resulted in a reduction in the level of arachidonic acid in the tissues (Irie and Sakimoto, 1992; Monahan *et al*, 1992) which is due in part to the inhibition by eicosapentaenoic acid of the conversion of linoleic to arachidonic acid (Hwang *et al*, 1988).

1.2.3. The effect of fatty acid manipulations on pig growth performance and carcass characteristics

As a result of altering the fatty acid composition of the diet the effects on the resulting pig growth performance have not been consistent. Thus, a number of workers has observed no effect on pig growth parameters, e.g. daily liveweight gain, of feeding diets containing different fatty acid compositions (Kemppinen *et al*, 1993; Valaja *et al*, 1992; Leszczynski *et al*, 1992a; Prescott and Wood, 1988; Whittington *et al*, 1986). However, other workers noted that by including more fat in the diet, particularly when the added fat was relatively unsaturated, a benefit in terms of growth performance was obtained (Suomi *et al*, 1993; Allee *et al*, 1972). With respect to carcass characteristics, an effect of more unsaturated fat has been observed on the thickness of backfat (Christensen, 1964). Although such an effect has been attributed to alterations in protein to energy ratios (Leszczynski *et al*, 1992a), the preferential deposition of unsaturated fatty acids in adipose tissue is likely to be the main cause (Leat *et al*, 1964). No differences in a number of carcass characteristics have been observed between pigs fed diets with differing oil contents. These have included carcass dressing percentage, carcass length, muscle area and muscle proportion (McDonald and Hamilton, 1976) and marbling scores (Miller *et al*, 1990). Effects on meat colour have been observed after feeding pigs a high level (10%) of canola oil (Miller *et al*, 1993) but not as a result of feeding a lower level of (flaxseed) oil (Specht-Overholt *et al*, 1993). Dramatic effects on various other carcass characteristics have been recorded. Thus, carcasses from pigs fed high levels of unsaturated fat appeared more 'oily' upon visual observation (Rhee *et al*, 1988a; St. John *et al*, 1987; Leat *et al*, 1964) due obviously to the lower melting point of such fat compared to a more saturated fat, such as tallow.

It has been widely reported that the inclusion of unsaturated fatty acids at high levels in the pig diet is able to significantly reduce the firmness of the subcutaneous fat (Suomi *et al*, 1993; West and Myer, 1987; Enser *et al*, 1984) and consequently to make it unacceptable for retail sale and meat product preparation. Whittington *et al* (1986) observed that the firmness of pig backfat (measured by mechanical probe) was closely correlated with the ratio of stearic acid to linoleic acid (r=0.78), the level of linoleic acid (r=-0.73) and the summated concentrations of palmitic and stearic acids (r=0.62). However, stearic acid has been described as an equally good predictor of carcass fat firmness (Enser, 1995). Enser (1995) outlined how the important effect of stearate could be mediated in terms of specific alterations in the concentrations of this and other fatty acids esterified to each position of the glycerol moiety. Thus, an increase in the

level of stearate in the fat would displace oleic and palmitoleic acids from position *sn*-1 of the triacylglycerols thereby creating more high melting, disaturated triacylglycerols as position *sn*-2 is occupied primarily by palmitic acid. On the other hand, if linoleic acid increases in the triacylglycerol, it will be placed in position *sn*-3, displacing primarily oleic acid rather than stearic acid, the latter of which is present at low levels in this position. Consequently, Enser argues, there would be less effect on consistency of increasing linoleate than of increasing stearate. The importance of stearic acid in relation to melting point is supported by the observation that the more saturated triacylglycerol species within pig backfat appear to have the greatest influence on melting point (Christie and Moore, 1969).

Due to concerns over the adverse effects of high levels of unsaturated fatty acids on the physical, chemical and sensory properties of pig backfat, several recommendations pertaining to the composition of the pig fat and the pig diet have been made. With respect to the level of linoleic acid, maximum recommended levels in the backfat of 15% (Whittington *et al*, 1986) and 13% of total fatty acids (Prescott and Wood, 1988) have been stated. Enser *et al* (1984) highlighted the fact that a stearic to linoleic acid ratio of less than 1.47 was representative of unacceptably soft fat. The iodine value provides a measure of the amount of total unsaturation in a substance and has been used as an indication of the acceptability or otherwise of pig feed or pig fat. Thus, an iodine value of 70 or higher in the fat could be used to identify unacceptable softness (Barton-Gade, 1987) and would arise with a diet supplying 160 or more 'iodine value product' per day (Madsen *et al*, 1992) where iodine value product was defined as the percentage of dietary fat × iodine value of dietary fat × 0.1. Similarly, in the pig diet maximum levels of linoleic acid have been stated at 1.4% by weight of diet (Whittington *et al*, 1986) and 0.04 MJ/MJ Digestible Energy (Prescott and Wood, 1988). Madsen *et al* (1992) stated that the pig diet should not contain more than 4% of rapeseed or sunflower seed which in terms of oil would supply approximately 1.6 and 1.2%, respectively.

1.3. The effect of changes in dietary pro-oxidant and antioxidant levels on porcine tissue composition and meat quality

As a result of increasing pressures to both maintain and enhance the eating quality of meat, it has been necessary for the meat producer to identify all potential areas along the production chain where meat quality could potentially be adversely affected. One such area of concern is that related to the process of oxidative deterioration of the lipids occurring in pig and other meats which can lead to the development of toxic compounds as well as rancid and 'off' flavours (Pearson *et al*, 1983). Fatty acids which are unsaturated are particularly susceptible to oxidation (Dahle *et al*, 1962). Therefore, where pigs are being fed diets containing high levels of unsaturated fatty acids the tendency for rancidity to develop will be increased (Rhee *et al*, 1988b). There has been

considerable interest in the use of vitamin E in the diets of pigs and other food animals due to the inherent ability of this vitamin to reduce the extent of oxidative decay of fatty acids and hence the formation of malodourous compounds (Monahan *et al*, 1992; Astrup, 1973).

1.3.1. The effect of vitamin E on lipid peroxidation in porcine tissues

Vitamin E is the generic name given to a range of fat-soluble compounds divided into two major forms, tocopherols and tocotrienols, each of which is further subdivided into four forms, namely alpha, beta, gamma and delta. Of these forms, the most biologically active form is alpha(α)-tocopherol. Vitamin E performs this vital function by acting as a free radical scavenger thereby obstructing the continuation of the autocatalytic peroxidation of polyunsaturated fatty acids. The mechanism of action of vitamin E in this process has been described in more detail in a review by Burton (1994). A number of studies has been conducted involving supplementation of grower pig diets with α-tocopherol, or more specifically, α-tocopheryl acetate, the more stable feed form of the vitamin. These studies were performed in order to test the antioxidant effects of the vitamin in the context of pig meat production. For example, Monahan *et al* (1992) observed that muscle from pigs fed a diet supplemented with 200 mg α-tocopheryl acetate/kg diet, compared to a basal level of 10-50 mg/kg diet, was significantly less prone to oxidative deterioration as determined by the relative formation of thiobarbituric-acid reactive substances (TBARS). The feeding of the α-tocopherol supplemented diet resulted in levels of α-tocopherol in plasma, muscle and adipose tissue which were 3.3, 2.8 and 2-times higher, respectively, than in pigs fed the basal level of α-tocopherol. In addition, it has also been observed that supplementation of pig diets with vitamin E positively influences the flavour/taste of the resulting meat (Astrup, 1973), the colour stability (Monahan *et al*, 1994) and reduces the amount of post-slaughter fluid losses from the meat (Monahan *et al*, 1991).

Contingent upon its antioxidant effects, vitamin E is itself reduced to the so-called α -tocopheroxyl radical, rendering it unable to further respond as a free-radical scavenger until it is regenerated to the oxidised form within the cells by, for example, ascorbic acid. As mentioned briefly above, several factors may increase the requirement for vitamin E in the pig diet by increasing the rate of utilisation of the vitamin. Increased oxidative challenge may arise from the consumption of more unsaturated fatty acids. In rats it has been observed (Meydani *et al*, 1987) that the level of vitamin E in the tissues was lower after feeding a diet containing fish oil (a highly unsaturated oil) than after feeding a diet containing coconut oil (a relatively saturated oil). An additional concern with regard to the adequacy of vitamin E in the diet is the reduced absorption of vitamin E which has been observed when feeding diets containing high levels of polyunsaturated fatty acids (Brin and Gallo-Torres, 1974). Nevertheless, Monahan *et al* (1992) showed that dietary supplementation with α-tocopherol was able to make a significant

contribution to neutralising the increased oxidative load resulting from feeding a more unsaturated fat (soybean oil).

1.3.2. The use of other naturally-occurring antioxidants in reducing lipid peroxidation

Besides vitamin E, a number of substances of natural origin has been shown to have marked effects on the oxidative stability of meat and meat products. Due to consumer concerns regarding the widespread use of additives in foods, there has been considerable interest in replacing the use of synthetic phenolic antioxidants (e.g. butylated hyroxytoluene, BHT) with naturally-occurring antioxidants (Pokorný, 1991). In this connection, the extracts of a number of naturally-occurring herbs and spices have been shown to possess strong antioxidant properties. In a study by Boyd *et al* (1993), the addition of rosemary extract either alone or in combination with a synthetic antioxidant (TBHQ) inhibited the extent of oxidative deterioration of frozen cooked fish flakes. In another study, an α-tocopherol/rosemary extract mixture was shown to behave synergistically in significantly reducing the peroxidation of long chain *n-3* polyunsaturated fatty acids in frozen and comminuted fish meat (Wada and Fang, 1992). It was observed that the ratio of (eicosapentaenoic acid + docosahexaenoic acid) to palmitic acid in the meat after frozen storage was 10% higher for the α-tocopherol/rosemary extract treatment than for the α-tocopherol treatment alone.

The inclusion of a high level of ascorbic acid with or without supplemental vitamin E in the pig diet has been investigated in an attempt to increase the oxidative stability of pork (Tsai *et al*, 1978). In accordance with other findings (see Section 1.3.1), the addition of vitamin E significantly increased the oxidative stability of the pork. However, there was no effect of ascorbic acid supplementation (at 2000 ppm) on the levels of α-tocopherol in the tissues or on tissue oxidative stability. The carotenoids, which include among many others, β-carotene, also have been demonstrated to possess antioxidant activity (Thurnam, 1994; Burton and Ingold, 1984). There exists the potential, in theory at least, of enhancing the oxidative stability of meat by feeding of carotenoids. However, information on such an effect is lacking.

Antioxidant effects have also been associated with the formation of the so-called Maillard reaction products which are formed in cooked meats and other foods by the nonenzymic reaction of amino acids with reducing sugars (Pokorný, 1991). Their antioxidant effects arise mostly from their ability to bind heavy metals, particularly iron, to form inactive complexes. Huang and Greene (1978) attributed the reduction in TBARS in meat which had been cooked to a high internal temperature or for an extended cooking period to the antioxidant effects of Maillard reaction products.

1.3.3. The effect of dietary copper on the fatty acid composition of pig backfat

An additional factor which has the potential to influence the level of vitamin E both in the diet before feeding and within the body of the pig is inorganic copper. This metal has been clearly demonstrated to act as a growth promoter when fed at levels up to 250 ppm in the pig diet (Bowland, 1990; Burnell *et al*, 1988; Braude, 1967). However, its presence at a high level has been associated with the degradation of vitamin E within the pig diet before being fed to the pig. Thus, in one study, the presence of 250 ppm copper in the diet caused the rapid oxidation of α-tocopherol and, to a lesser extent, α-tocopheryl acetate following periods of storage of the diet up to 12 weeks (Dove and Ewan, 1991). Copper has also exhibited noticeable effects on the lipid metabolism of the pig. In a study by Moore *et al* (1969), the addition of 250 ppm dietary copper was responsible for marked increases in the ratios of oleic acid to stearic acid in the inner and outer backfat layers compared to the control group. These differences in fatty acid composition were manifested in a 10 to 15 °C reduction in the melting points of the inner and outer backfats of the copper supplemented pigs compared to the inner backfat of the control. Such effects have been attributed to a copper-induced increase in the activity of microsomal stearoyl-CoA desaturase (Dziubajlo, 1991). Christie and Moore (1969) undertook analyses of the structural characteristics of triacylglycerols taken from the same control and copper-fed pigs as described in Moore *et al* (1969). Using argentation thin-layer chromatography, it was found that copper supplementation caused significant decreases in the proportions of two of the more saturated triacylglycerol species (SSS and SSM species, where S=saturated and M=monounsaturated fatty acid) and significant increases in two of the more unsaturated triacylglycerol species (MSM and MMM species). These effects were observed in both the inner and outer layers of the backfat. It was proposed that the differences in triacylglycerol species accounted for the differences in melting point. Analysis of the stereospecific positional distribution of fatty acids on the glycerol moiety of the triacylglycerols from the control and copper-fed animals revealed that dietary copper exerted no preferential effect on the fatty acids at any single position. The higher level of stearic acid in the inner backfat of the control pigs was distributed equally between the 1 and 3 positions.

1.3.4. The effect of iron on muscle lipid peroxidation

A range of other substances has been shown to affect the extent of peroxidative deterioration of meat, either in pork or in other meats such as beef or poultry. The presence of iron within haem pigments (e.g. haemoglobin, myoglobin ferritin, etc.) and in the free form or associated with other proteins (the so-called non-haem iron) has been associated with the catalysis of lipid peroxidation in muscle (Love, 1983). Although there remains considerable debate as to the relative importance of these forms of iron in

catalysing lipid peroxidation in muscle, an appreciable amount of evidence points to the greater pro-oxidant capacity of non-haem iron than haem iron (Ahn *et al*, 1993; M[ac]Donald *et al*, 1980; Igene *et al*, 1979). In the recent study by Ahn *et al* (1993), it was observed that bound iron present in haemoglobin, ferritin or transferrin had little effect on lipid peroxidation in turkey muscle homogenate whereas free iron had a significant effect on the rate of peroxidation. Furthermore, it was observed that the strong iron chelator DTPA, which chelates the free iron, virtually eliminated the formation of TBARS. However, in contrast to these results, Monahan *et al* (1993) observed that the pro-oxidant effects of haem proteins (haemoglobin and myoglobin) were greater than those of inorganic iron (as $FeSO_4$) in raw and heated pork muscle redsidue when these pro-oxidants were present at levels similar to those in red meats. Catalysis of lipid peroxidation by non-haem iron has been shown to proceed more rapidly at acidic pH values whereas pH appears to have less effect on lipid oxidation catalysed by haem iron (Wills, 1966). The presence of haem-associated iron in both the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states has been shown to be important in stimulating lipid peroxidation with ferric ions giving rise to a higher rate of catalysis (Greene and Price, 1975). However, with regard to non-haem iron, most workers have observed that ferrous ions possess greater pro-oxidant activity than ferric ions, as reviewed by Love (1983).

SECTION II

1.4. Epidemiology of coronary heart disease

Over the past several decades, there has been widespread concern regarding the extremely high incidence of cardiovascular disease in humans in Western society. The occurrence of this disease has been shown to be affected by a range of factors including the type of diet. Among various elements of the diet, the nature of the fat consumed has been regarded as an important determinant of the propensity to develop some form of cardiovascular disease. This section of the present review will attempt to consolidate the current state of knowledge related to: i) the incidence of cardiovascular disease in developed society, ii) the influence of dietary factors on the occurrence of the disease with particular emphasis on dietary fatty acids, iii) the importance of the *n-3* polyunsaturated fatty acids on aspects of human health and disease, including cardiovascular disease, and, iv) the contribution of non-fish foods to the consumption of *n-3* polyunsaturated fatty acids.

Cardiovascular disease is the major cause of death in Western society. It encompasses coronary heart disease (also called ischaemic heart disease or coronary artery disease), cerebrovascular disease and rheumatic heart disease. However, it is

coronary heart disease (CHD) which presents the greatest human health problem. In the United Kingdom, CHD is the major cause of death in middle and old age (British Nutrition Foundation, 1992), giving rise to approximately 300 deaths per 100 000 in men aged 35 to 64. In the United States (U.S.), it accounts for more than 50% of all deaths, totalling 600 000 deaths per year.

In the United Kingdom, death rates from CHD in both men and women exceed those in most other countries (NDC, 1993). In particular, Scotland and Northern Ireland are at the top of the international league for deaths from CHD. Rates in England and Wales are lower but are still higher than in countries such as Australia and the U.S.. Although CHD - along with other circulatory diseases and cancer - can be considered as a degenerative condition due to the fact that it increases with age, it is the most common cause of death in those who are middle-aged and therefore is also viewed as a cause of premature death.

1.4.1. Reduction in mortality from coronary heart disease

Coronary heart disease mortality showed a decreasing tendency between 1970 and 1980 in industrialised countries (World Health Organization, 1986). In the United Kingdom, CHD death rates for men and women in most age categories have returned to levels seen in the 1950s (NDC, 1991). In the U.S., CHD incidence reached a peak in 1970 and death rates have fallen since then by 25-30% (Rosenman, 1993 cited by Mann, 1994). This decline, which has been greatest in recent years, has occurred in all geographic areas of the U.S., in all age groups and in both men and women. The role or otherwise of a change in total fat consumption during this period in the U.S. has been debated. However, it is clear that the sources of fat changed (NDC, 1993; Szostak, 1990). The majority of fat is supplied by three food groups: meat, poultry, fish; dairy products; and fats and oils. During this period, there were shifts in consumption away from butter to margarine, from lard to vegetable shortenings and there was increased consumption of salad and cooking oils. These changes resulted in a rise in the ratio of polyunsaturated to saturated fatty acids (P:S ratio) in the diet from 0.24 in 1960-1969 to 0.46 in 1980-1985 (Stephen and Wald, 1990). In the U.K., a similar increase was observed from 0.19 in 1975 to 0.41 in 1991 (NDC, 1993). Although CHD incidence has decreased in many western countries, the situation is different in Eastern European countries where cardiovascular disease is accounting for an increasing proportion of deaths. For example, in Poland the frequency of deaths from cardiovascular disease increased almost threefold between 1960 and 1989 and, in 1989, this disease was associated with 50% of total deaths (Szostak and Sekula, 1991).

1.4.2. Causes of coronary heart disease

The causes of coronary heart disease are many and varied and have been individually termed risk factors. These are elements of life-style, blood and body characteristics which have been associated with an increased incidence or risk of the disease. Such risk factors include smoking, high blood pressure, obesity, stress, lack of exercise and elevated plasma levels of low-density lipoprotein (LDL) cholesterol, triacylglycerols and apolipoprotein B (apoB). These factors are believed to act synergistically in the promotion and progression of coronary heart disease. It would appear that previous explanations of CHD have been too restricted to considering intakes of total fat without due regard for other salient factors. This view has been clearly stated by NDC (1993), as follows:

> "... when considering heart disease, insufficient consideration is given to aspects other than dietary fat, such as the haemostatic factors and antioxidant status or the evidence concerning the balance between different types of polyunsaturates."

The influence of these other dietary factors on CHD incidence will be discussed further below.

1.5. Elements of the diet and coronary heart disease

1.5.1. Types of fat related to coronary heart disease

1.5.1.1. Saturated fatty acids

High intakes of saturated fats have been shown to increase plasma total and LDL cholesterol levels (British Nutrition Foundation, 1992) and to induce atherosclerosis in experimental animals (Sanders, 1991). Also, epidemiological studies indicate a positive association between saturated fat intake and CHD. Hence, dietary recommendations stress the need for a reduction in saturated fatty acid intake to 10% of dietary energy (DH, 1991) from the current value of approximately 17% (British Nutrition Foundation, 1992). The main saturated fatty acids in the diet are lauric, myristic, palmitic and stearic acids. However, it seems that, to varying degrees, only lauric, myristic and palmitic acids raise cholesterol. Stearic acid seems not to affect total cholesterol (Tholstrup *et al*, 1994; Ulbricht and Southgate, 1991). The longer chain saturates myristic, palmitic and stearic acids accelerate thrombus formation (Ulbricht and Southgate, 1991).

1.5.1.2. Cholesterol

The effect of cholesterol intake on CHD mortality has been the subject of widespread scientific research. From evidence indicating highly significant across-country associations between daily cholesterol intake and mortality from CHD it has

been postulated that cholesterol intake is a critical factor in the development of CHD. However, several observations have cast doubt on this theory. For example, certain subpopulations such as Greenland Eskimos (Bang and Dyerberg, 1972) and Masai herdsmen (Ho *et al*, 1971) consume large amounts of cholesterol and yet have low blood cholesterol and low incidence of atherosclerosis. Furthermore, altering cholesterol and saturated fat intake changes blood cholesterol only by a factor of 10-15% (Linder, 1991). Also, no significant difference in mortality was observed between human subjects who lowered cholesterol intake to 10% of calories per day and those who did not (Multiple Risk Factor Intervention Trial Research Group, 1982). It is plausible that cholesterol itself may not be so important as the oxidation products of cholesterol in development of cardiovascular disease. Coupled with this will be other factors, e.g. inflammation and antibody-antigen interactions, which determine an individual's propensity to oxidise cholesterol. More recently, evidence has been gathered to suggest that a person's 'early environment' - *in utero* and during the first years of life - may influence the subsequent development of a number of diseases including coronary heart disease (see NDC, 1993).

1.5.1.3. *Trans* fatty acids

The effect of *trans* fatty acid (TFA) consumption on human health has been reviewed by Sanders (1988). These fatty acids occur to variable degrees in both natural and 'man-made' food products, e.g. butter and margarine, respectively. There is considerable debate as to whether such fatty acids promote CHD. The effects of TFAs have been likened to those of saturated fatty acids but this would seem to be inappropriate as, unlike saturated fatty acids, *trans* monounsaturates have not been known to be either hypercholesterolaemic or atherogenic. Sanders (1988) concludes that consumption of TFA poses no risk to human health. The British Nutrition Foundation (1992) stated concerning TFA: "... current evidence could not be used to relate them conclusively to long-term health concerns". However, in a recent review by Mann (1994), it was hypothesised that *trans* fatty acids impair lipoprotein receptors on endothelial cells leading to hypercholesterolaemia and atherogenesis.

1.5.2. The effect of long chain *n-3* polyunsaturated fatty acid on the incidence of coronary heart disease

Interest in fish consumption as a possible means of preventing CHD stems largely from the classical work of Sinclair (1953) who investigated diet/health interactions among North American Inuits. The effect of fish consumption on the incidence of coronary heart disease is believed to be mediated primarily by the long-chain polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid.

The initial observation that fish consumption is one important way of reducing CHD risk has been supported by a large number of epidemiological studies, some of which are

referred to in Table 1.1. A more recent study showed a similar relationship between diet and factors related to CHD in Alaskan Eskimos (Parkinson *et al*, 1994). From these studies it is clear that the incidence of CHD is markedly affected by intake of fish oils. Furthermore, the effects of fish oil on CHD mortality have been observed not only at high, pharmacological doses but also at low to moderate intakes of fish oil. In the so-called Zutphen study (Kromhout *et al*, 1985), mortality from CHD was over 50% lower in those who consumed at least 30 g of fish per day than among those who did not consume fish. This level of fish consumption was markedly lower than the recorded intakes of 100 g among some Japanese (Kagawa *et al*, 1982) and 400 g among Greenland Eskimos (Bang and Dyerberg, 1972) who have been associated with a low incidence of CHD. Also, CHD mortality does not seem to vary with fish intakes of 30 to 150 g per day; above 150 g intake, a further reduction in CHD mortality occurs (Kromhout, 1989). The finding that a moderate fish consumption is effective is supported by the work of Burr *et al* (1989). In this study, subjects advised to eat fatty fish had a 29% reduction in all-cause mortality compared to those who did not receive this advice. This result was statistically significant ($P \leq 0.05$) and was not affected by adjusting for ten potential confounding factors which could have biased the results. Furthermore, a difference in mortality became apparent early in the trial, namely after 6 months. As viewed by NDC (1992), this suggested that there was an effect on blood clotting rather than on atherosclerosis. The amount of fish consumed by patients who were told to eat more fish was about 300 g fatty fish/week which provided 2.5 g eicosapentaenoic acid (i.e. 0.3 g/day); this involved no radical change of diet. Patients in the control group consumed approximately 0.1 g/day. Burr *et al* concluded that a modest intake of fatty fish (two or three portions per week) may reduce mortality from second occurrence of myocardial infarction.

The incidence of coronary heart disease is dependent on two different processes - atherosclerosis and thrombosis (Ulbricht, 1992) - where atherosclerosis relates to the thickening of the arterial wall and thrombosis relates to the formation of a clot which occludes the artery, causing a heart attack. Table 1.2 shows the observed effects of fish oil supplementation on factors which are implicated in both of these processes. As shown in the table, fish oil addition effects a number of changes in blood lipoprotein composition, platelet responsiveness and blood and circulatory parameters. Fish oil thus seems to exercise a beneficial influence via a number of mechanisms. Figure 1.1 shows the formation of different eicosanoids depending on the identity of the precursor fatty acid. Arachidonic acid, derived directly from the diet or via linoleic acid, gives rise to eicosanoids which are strongly pro-aggregatory and pro-inflammatory. On the other hand, eicosapentaenoic acid derived from the diet and from α-linolenic acid is metabolised to form eicosanoids (e.g. thromboxane A_3) which are only weakly pro-aggregatory and pro-inflammatory. As shown in Table 1.2, consumption of fish oil has caused formation of more of the weakly pro-aggregatory eicosanoids. This effect of

Observation	Reference
atherosclerosis rare among North American Inuits with high intake of fish	Sinclair (1953)
dose-dependent inverse relationship between CHD and fish oil consumption	Burr *et al* (1989); Dolecek *et al* (1989); Norell *et al* (1986); Kromhout *et al* (1985); Dolecek and Grandits (1991)
increased hardening of arteries in fish- than in non-fish eating healthy and diabetic humans	Wahlqvist *et al* (1989)
regression of atherosclerosis in pigs, dogs and primates in hypercholesterolaemic state with dietary *n-3* long chain fatty acids	Sassen *et al* (1989); Leaf and Webber (1988); Landymore *et al* (1985)
reduction in post-surgical re-narrowing of coronary arteries (restenosis) in patients given fish oil	Dehmer *et al* (1988)

Table 1.1 - Effect of *n-3* polyunsaturated fatty acids on the incidence of atherosclerosis

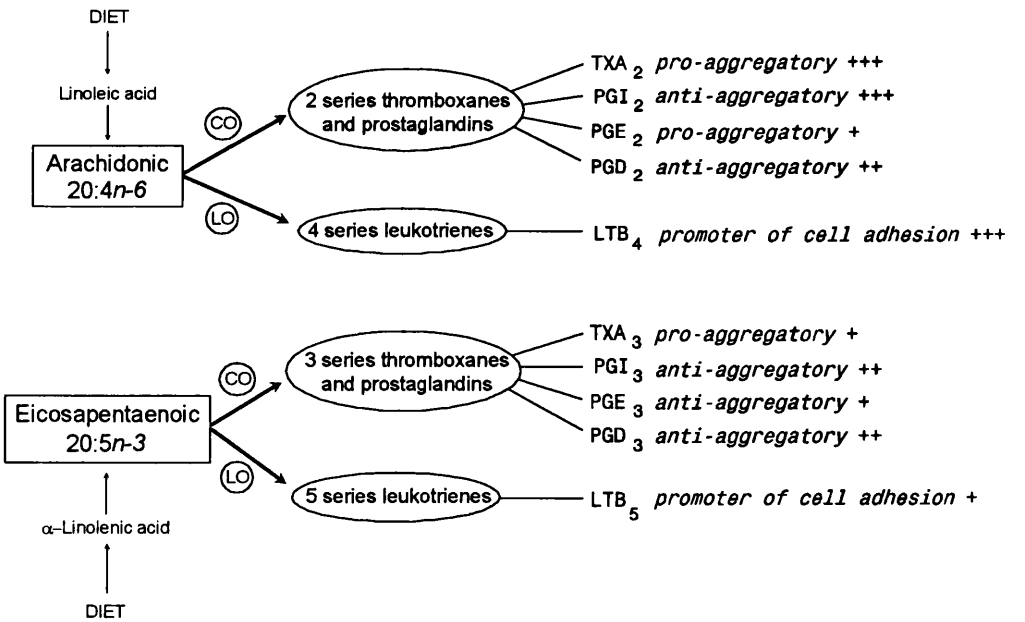


Figure 1.1 - Formation and effects of a number of important eicosanoids in platelets and blood vessels. (CO and LO = cyclooxygenase and lipoxygenase, respectively; TX = thromboxane; PGI = prostacyclin; PG = prostaglandin; LT = leukotriene; +++/++/+ indicate strong, moderate and weak effects, respectively) (from Linder, 1991)

Effect of *n-3* fatty acid consumption	Reference
EFFECTS ON BLOOD LIPOPROTEIN PROFILE	
plasma TAG and VLDL concentrations lowered	Sanders *et al* (1989); Molgaard *et al* (1990); Sanders and Hinds (1992)
3g EPA + DHA/da. reduces serum TAG in diabetic patients	Nestel *et al* (1984)
high fish oil intake (24g long chain *n-3* PUFA/da.) lowers concentration of LDL-cholesterol and apoprotein B	Harris (1989)
moderate fish oil intake increases HDL_2-cholesterol	Harris (1989); Sanders *et al* (1989)
increased chylomicron clearance	Harris (1989)
reduction in post-prandial hyperlipaemia compared with olive oil supplement	Sanders *et al* (1989); Weintraub *et al* (1988)
increased proportion of small VLDL in humans	Sullivan *et al* (1986)
lowering of TAG in type IV hyperlipo-proteinaemic patients	Saynor *et al* (1984)
reduction of Lipoprotein (a) in patients with elevated Lp(a)	Herrmann *et al* (1989)
inhibition of TAG synthesis and apoB synthesis and secretion	Wang *et al* (1989); Sanders *et al* (1985)
normalisation of plasma electrophoretic profile	Harris (1989); Molgaard *et al* (1990)
EFFECTS ON HAEMOSTATIC AND CIRCULATORY PARAMETERS	
consumption of 2-3g EPA/da. increased bleeding time in humans and rats	Atkinson *et al* (1987); Mark and Sanders (1994)
reduced platelet adhesiveness and blood fibrinogen	Li and Steiner (1990); Saynor and Gillot (1988)
increased PGI_3; decreased TXA_2 and TXA_3 in subjects with high basal levels	von Schacky *et al* (1985)
TXA_2 + TXA_3 : PGI_2 + PGI_3 ratio shifted in Inuit	Fischer and Weber (1986)
PGH_3 and TXA_3 produced from EPA are poor inducers of platelet activation	Needleman *et al* (1979)
EPA competes with 20:4 as a substrate for cyclo-oxygenase	Needleman *et al* (1979)
moderate intakes (~5g *n-3* PUFA/da.) reduce systolic and diastolic blood pressure 3-5 mmHg	British Nutrition Foundation (1992)
reduced risk of ventricular fibrillation and sudden cardiac death	Gudbjarnason (1989)
reduction in angina	Saynor *et al* (1984)
increased erythrocyte deformability	Saynor and Ryan (1990)

Table 1.2 - Possible means by which effects of *n-3* fatty acids on coronary heart disease are mediated; abbreviations as per Figure 1.1

eicosapentaenoic acid is believed to be important in the observed decreases in CHD mortality due to fish oil consumption.

For the prevention of CHD, Duthie and Barlow (1992) recommended the consumption of 0.3 to 1.0 g of long chain *n-3* polyunsaturated fatty acids per day as proposed by Harris (1989) in an extensive review of papers dealing with the subject of fish oil related to human health. From a review of a number of clinical trials on effects of fish oils on coronary heart disease, it was concluded that 2-5 g (eicosapentaenoic acid + docosahexaenoic acid) per day or more may be sufficient to generate positive effects on many of these variables in humans in the clinical situation (Barlow *et al*, 1990; Duthie and Barlow, 1992).

1.5.3. Antioxidants and coronary heart disease

Whilst the role of *n-3* polyunsaturated fatty acids in reducing CHD has been affirmed, there is increasing evidence for an active involvement of antioxidants in protection against CHD (Riemersma, 1994). Thus, a combination of the so-called 'lipid' and 'antioxidant' hypotheses can allow for a better understanding of the development of this disease and can provide explanations for observations which are not explained by the 'lipid hypothesis' alone (James *et al*, 1989). Interest in antioxidants has arisen due to the role of oxidative modification of low density lipoprotein in the development of atherosclerosis (Parthasarathy *et al*, 1990). Modification of LDL is inhibited by various antioxidants including vitamin E (Steinbrecher *et al*, 1984). Other antioxidants which could influence CHD incidence include vitamin C, β-carotene, vitamin A, selenium, manganese, zinc and copper.

An effect of antioxidant nutrients on CHD is also supported by epidemiological evidence (for a review see NDC, 1992). In the U.K., the distribution pattern of heart disease mortality mirrored a low consumption pattern of foods containing these nutrients more closely than consumption patterns of fat (NDC, 1993). For example, in Scotland and parts of Northern Ireland, where CHD mortality is highest, consumption of vegetable and fruit is relatively low but intake of saturates is similar to other parts of the U.K.. Recently it has been shown that the supply of antioxidant nutrients, derived largely from vegetable oils, was strongly and inversely correlated with CHD death rates in 19 European and 5 other developed countries (Bellizzi *et al*, 1994). The strongest inverse association was with α-tocopherol ($r = -0.75$; $P \leq 0.001$). In support of this, the work of Gey *et al* (1991) indicated a strong and highly significant ($P \leq 0.0003$) inverse correlation between lipid standardised plasma vitamin E and CHD risk.

1.6. Official recommendations for intake of polyunsaturated fatty acid

The most recent comprehensive report on the effects of unsaturated fatty acids on human health and disease is that of the British Nutrition Foundation (1992). Some of the salient recommendations of that Report will be discussed here. The Report recommended the abolition of the polyunsaturated:saturated fatty acid (P:S) ratio which it concluded to be outmoded. It stressed that not all polyunsaturated fatty acids have the same effects and not all saturated fatty acids are atherogenic; it is therefore unwise to group these classes of fatty acids. Instead, the Report advised that recognition should be given of the *n-6* or *n-3* nature of the fatty acids. The report of the British Nutrition Foundation (1992) recommended the consumption of *n-6* and *n-3* fatty acids in the ratio 6:1 and that the ratio of linoleic acid to α-linolenic acid should be used instead of total *n-6* and total *n-3*, respectively. However, the compilers of dietary guidelines established as a result of a prominent NATO scientific conference have used the ratio of total *n-6* to total *n-3* fatty acids (Galli and Simopoulos, 1989). No minimum requirement was specified by the British Nutrition Foundation for eicosapentaenoic acid and docosahexaenoic acid as it was felt that there was not enough evidence to support a minimum requirement. However, the NATO conference recommended an intake of 0.8 g (eicosapentaenoic acid + docosahexaenoic acid) per day for an average person living in the U.S.A.. In association with recommendations to increase the intake of *n-3* polyunsaturated fatty acids, emphasis has also been placed on the importace of consuming more antioxidants obtained from, for example, fruit and vegetables (British Nutrition Foundation, 1992). The Task Force recommended that the intake of vitamin E should be 0.4 mg/g linoleic acid with prudent daily ranges of 3.2-10.4 mg for men and 2.5-8 mg for women.

1.7. Effects of *n-3* fatty acids on aspects of human health other than coronary heart disease

1.7.1. *n-3* Polyunsaturated fatty acids and inflammatory diseases

As shown in Table 1.3, the *n-3* polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid have been implicated in the alleviation of symptoms of a number of diseases including cancer. Many of these diseases are associated with lipid peroxidation and the formation of oxygen radicals. It has been feared that provision of such polyunsaturates could have the effect of contributing to the formation of lipid peroxides which in turn can damage cell components. However, as shown by a number of workers, *n-3* polyunsaturated fatty acids have desirable rather than undesirable effects (see Table 1.3 and Bégin, 1990). Thus, supplements of *n-3* polyunsaturates have been

Observation	Reference
EFFECTS ON IMMUNE SYSTEM - MEDIATED INFLAMMATION	
in rheumatoid arthritis, anti- inflammatory effects (reduced LTB_4; increased LTB_5) with reduction of synovitis, stiffness and swelling	van der Tempel *et al* (1990)
EPA reduced LTB_4 production (pro-inflammatory)	Prescott (1984)
suppression of synthesis of tumour necrosis factor and interleukin-1 (important mediators of immune reactions)	Endres *et al* (1989)
small effect of EPA on systemic lupus erythematosus (SLE; 'lupus')	Westberg and Tarkowski (1990)
possible effect in slowing progression of multiple sclerosis	British Nutrition Foundation (1992)
EFFECTS ON CANCER DEVELOPMENT	
inhibitory effect on tumour induction in mammary gland and in rat pancreas	Braden and Carroll (1986); O'Connor *et al* (1985)
diets rich in *n-3* PUFA suppress, but *n-6* PUFA enhances, production of PGE_2 which accompanies colonic tumours	Minoura *et al* (1988)
expression of oncogenic *ras p*21 protein decreased after culturing murine hyperplastic alveolar nodules with EPA	Telang *et al* (1988)
protection by high dose of fish oil (25-50% total energy) against lethal loss of body weight and condition (cachexia) and reduced tumour size in experimental cancer model. Fish oil inhibited tumour growth more than 5-fluorouracil	Tisdale and Dhesi (1990)
EPA inhibits catabolic action of a tumour lipolytic factor by preventing cyclicAMP accumulation in fat cells	Tisdale and Beck (1991)
EFFECTS ON SKIN DISEASE	
reduction of skin redness and scaling in psoriatic patients	Maurice *et al* (1987)
possible reduction of acne	British Nutrition Foundation (1992)

Table 1.3 - Effects of *n-3* polyunsaturated fatty acid on aspects of human health not related to coronary heart disease; abbreviations: EPA = eicosapentaenoic acid, all other abbreviations as per Figure 1.1

shown to have effects on rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis, associated in part with a reduction in the formation of leukotriene B_4, a pro-inflammatory eicosanoid. In one study involving patients with mild rheumatoid arthritis, evening primrose oil (EPO)/fish oil and single EPO treatments were able to substitute for non-steroidal anti-inflammatory drug treatments (Belch *et al*, 1988). At high levels of intake, *n-3* polyunsaturates have shown anti-promotional effects on some types of cancer (e.g. breast and pancreatic cancer) in a number of human and animal studies (for a review of these studies see British Nutrition Foundation, 1992). The effects on cancer may be mediated partly through a reduction in the formation of certain prostaglandins and thromboxanes. At high doses, fish oil prevented the loss of body condition which normally occurs in cancer, as well as proving to be more effective than a conventional drug in inhibiting tumour growth. These effects have been shown to be mediated primarily by eicosapentaenoic acid. According to Sanders (1993), a mild improvement in psoriasis has been observed in the majority of studies in subjects given fish oil supplements containing eicosapentaenoic acid and docosahexaenoic acid. This is attributed to the anti-inflammatory effects of these fatty acids.

1.7.2. *n-3* Polyunsaturated fatty acids and early development

Docosahexaenoic acid occurs in high levels in the human brain and retina where it is found in its highest concentrations in the phospholipids of synaptic membranes and rod outer segments. Evidence from animal studies suggests that retinal function and learning ability are permanently impaired if there is a failure in the accumulation of sufficient docosahexaenoic acid during early development (Neuringer *et al*, 1988). Lucas *et al* (1992) observed an apparent effect of breast milk consumption by infants on subsequent intelligence quotient (IQ). In that study, children who consumed mother's milk in early life had an 8.3 point higher IQ ($P \leq 0.0001$) at age 7-8 even after adjusting for differences in mother's education and social class. Evidence of a dose-response relationship was also observed. The authors pointed to the difference in docosahexaenoic acid content between breast milk and formula milk and the role of this fatty acid in the development of the nervous system.

The brain utilises only C20-C22 fatty acids in any appreciable quantity and among these docosahexaenoic acid and arachidonic acid are of particular importance. The supply of these fatty acids in a preformed state is therefore of critical necessity and especially so during the last trimester of foetal development when there is rapid accumulation of docosahexaenoic acid in the brain. Conversion of α-linolenic acid to eicosapentaenoic acid can occur at a significant rate but synthesis of docosahexaenoic acid is not efficient in the neonate (British Nutrition Foundation, 1992). The human foetus obtains docosahexaenoic acid by selective placental transfer such that the level of docosahexaenoic acid is 6% in maternal plasma but 18% in the developing brain (British Nutrition Foundation, 1992).

Newborn babies exhibit slow body growth coupled with rapid neural and vascular development (British Nutrition Foundation, 1992). The content of preformed docosahexaenoic acid and other essential fatty acids in human milk reflects the need for appropriate substrates for neural development of the neonate. On the basis of this, the British Nutrition Foundation regarded eicosapentaenoic acid and docosahexaenoic acid as 'conditionally essential' and recommended that infant formula be adapted as far as possible to mimic the fatty acid composition of breast milk which generally contains *n-6* and *n-3* fatty acids in the ratio of 11:1. Human milk has been shown to display a wide variation in docosahexaenoic acid content, ranging from 0.04 to 3.3% of total fatty acids. In the U.K., a level of 0.4% has been stated (British Nutrition Foundation, 1992). Docosahexaenoic acid in breast milk is affected by the maternal diet: vegetarians and vegans generally have the lowest levels of docosahexaenoic acid (0.4%) and Inuits the highest levels (3.3%). Further to their effects on brain and neural development, effects of long chain *n-3* polyunsaturates on gestation time and birthweight have been observed. Faroe Islanders who have high birthweights and a long duration of pregnancy also consume a relatively large amount of fish. Olsen *et al* (1992) tested this apparent relationship in 533 Danish women who were given 2.7 g *n-3* polyunsaurates per day, olive oil or no supplement from the seventh month of gestation. Mean length of gestation was different between groups ($P \leq 0.006$) with the fish oil group having the longest, and the olive oil group the shortest, duration.

1.8. Meat consumption and coronary heart disease

In view of the relatively high content of saturated fatty acids in red meat, concerns have been expressed regarding the consumption of meat and meat products and the perceived risk from coronary heart disease. Vegetarians have been shown to have a lower risk of CHD than non-vegetarians but the reason for this cannot be attributed only to the absence of meat in the diet. Apart from not eating meat, vegetarians tend to be leaner, smoke less and consume more nuts/seeds, pulses and vitamins (British Nutrition Foundation, 1992).

The consumption of meat and meat products has been slowly declining during the past 15 years in the U.K. although due to the fact that meat has been sold increasingly with less fat and bone, the decline in consumption has been less for lean meat (see NDC, 1993). Over the past ten years, the consumption of pork, beef and lamb has decreased whilst there has been a marked increase in the consumption of poultry meat which is now the most-consumed meat. The consumption of meat products (bacon, ham and sausage) has also decreased over this time.

In the U.K., meat and meat products provide 24% of the total fat consumed (British Nutrition Foundation, 1992). Of this fat, approximately 40% is saturated, 40% is monounsaturated and the remainder is split between polyunsaturated and *trans* fatty

acids. Such a predominance of saturated and monounsaturated fatty acids within domesticated mammals has been shown to be in stark contrast to the situation in free-living mammals in which polyunsaturated fatty acids are present in much greater abundance in the tissues (Crawford *et al*, 1969). It has therefore been speculated that the high levels of saturated and monounsaturated fatty acids within the meat from domestic animals could make an important contribution to the development of atherosclerosis in humans (Crawford *et al*, 1989; Crawford, 1968). However, although a role of saturated fatty acids in the development of CHD has certainly been established (see Section 1.5.1.1), the consumption of meat has not been associated with CHD incidence (Ulbricht, 1992). Thus, Ulbricht and Southgate (1991) plotted consumption of total meat and red meat consumption in EC countries against CHD incidence and discerned no relationship. Furthermore, it was seen that the U.K., with the second lowest meat consumption, had the second highest CHD incidence whereas Greece, with the highest red meat consumption, had one of the lowest rates of CHD. Other workers have noted either no effect or a beneficial effect with respect to serum lipids resulting from inclusion of lean meat in the diet (Watts *et al*, 1988; Malmros and Wigand, 1957). There is therefore no concrete evidence supporting a relationship between meat or red meat consumption and CHD incidence. Although people in France exhibit a high intake of saturated fat from dairy and meat products, the population is characterised by a surprisingly low mortality from CHD, giving rise to the so-called 'French paradox'. This feature has been accounted for by the consumption of alcohol in the form of wine with its putative inhibitory effects on platelet reactivity (Renaud and de Lorgeril, 1992). In contrast to meat consumption, the consumption of dairy fat (excluding cheese) is closely related to CHD mortality ($r = 0.73$, Renaud and de Lorgeril, 1992; $r = 0.78$, Simons, 1986).

1.9. Food sources of *n-3* polyunsaturated fatty acid

1.9.1. *n-3* Polyunsaturated fatty acids and non-fish food

A major source of *n-3* polyunsaturates is oily fish such as sardine, menhaden and mackerel. Such fish absorb most of their *n-3* fatty acids from a diet of sea-borne zooplankton and show variations in the proportions of eicosapentaenoic acid and docosahexaenoic acid within the tissues depending on the species and the season of the year. Although the consumption of more oily fish provides a very effective way of increasing the intake of *n-3* fatty acids, consumption of fatty fish in the U.K. has fallen in the past few decades. In 1960, per capita consumption of herring was 0.6 g per day; in 1990, it was 0.1 g per day (Barlow and Pike, 1991). From National Food Survey data for weekly household consumption, Duthie and Barlow (1992) estimate that the consumption of *n-3* polyunsaturates (presumably total *n-3* polyunsaturates)

approximates to 0.06 g per day. Apart from increasing *n-3* polyunsaturate intake by consuming more oily fish, the possibility exists of incorporating fish-derived *n-3* fatty acids into non-fish foods. The more common a food is which can be supplemented, the greater the effectiveness of increasing the human intake for prophylactic and/or therapeutic purposes (Duthie and Barlow, 1992).

As reported by Nielsen (1992), white bread supplemented with microencapsulated fish oil was marketed in Denmark since 1990 under the name of 'Omega Bread'. By consuming 200 g of this bread daily, it was estimated that one could obtain 25-30% of the amount of *n-3* polyunsaturates recommended by Kromhout *et al* (1985) which would afford protection from coronary heart disease, i.e. 30 g white + oily fish/ day. Also, the *n-3* fatty acids in the bread dough were not destroyed under the heat conditions required for baking.

The International Fishmeal and Oil Manufacturers Association ('IFOMA'), in cooperation with Leatherhead Food Research Association, has undertaken trials to investigate this possibility (Barlow *et al*, 1990). Refined and deodorised fish oil was incorporated within a range of foods including pork sausages, pork and beef salami, yoghurt and margarine. Fish oil was included in pork sausages and salami at levels of 3% and 2% by weight, respectively. Fish oil thereby accounted for 17 and 10% of total fat in sausages and salami, respectively. It was calculated that a 100 g serving of sausages (i.e. two sausages) would provide approximately 3.3 g (eicosapentaenoic acid + docosahexaenoic acid), assuming that the fish oil contained 20% of (eicosapentaenoic acid + docosahexaenoic acid) of total fatty acids. Salami and sausages were submitted to taste panel analysis at 9 and 21 days after preparation, respectively, but no deleterious effect of fish oil on flavour characteristics was discernible.

1.9.2. *n-3* Polyunsaturated fatty acids and meat

Unsupplemented meat generally contains low levels of long chain *n-3* polyunsaturated fatty acids (Gurr, 1984). Thus, beef and lamb meat are characterised by a virtual absence of these fatty acids. Poultry meats, however, contain appreciably higher levels of long chain *n-3* fatty acids depending on the species being considered and the location of the meat in the carcass. Meat from pigs fed a diet with either no added fat or tallow contains only trace amounts of these fatty acids (Morgan *et al*, 1992). Due to the nature of the respective digestive systems, both poultry and pigs can be fed diets containing fish oils with a consequent dose-dependent increase in levels of *n-3* polyunsaturates in the tissues (see Section 1.2). Under normal circumstances, ruminants are able to deposit only minor amounts of *n-3* polyunsaturated fatty acids obtained from the diet. Morgan *et al* (1992) showed that the level of docosahexaenoic acid in pig outer backfat could be significantly increased from 0.06% of total fatty acids in tallow-fed pigs to 0.45% in pigs fed a diet containing 1% fish oil. In a study by Irie and Sakimoto (1992), pigs were fed diets containing 0, 2, 4 and 6% of dietary weight of fish

(sardine) oil. Levels of *n-3* fatty acids in the fat depots increased in proportion to the level of fish oil in the diet. In the outer backfat, the level of docosahexaenoic acid was 1.28% of total fatty acids in pigs fed the 6% fish oil diet.

Associated with feeding unsaturated fat diets to pigs are concerns over physical properties of the fat including firmness, melting point and colour (see Section 1.2.3). Morgan *et al* (1992) observed that there was no adverse effect of feeding a 4% soybean oil/1% fish oil diet on the firmness of pig fat; this was also noted by Leskanich *et al* (1993). Irie and Sakimoto (1992) observed that the colour of fat from pigs fed fish oil was white and showed no evidence of yellow fat syndrome, a disease characterised by deficiency of vitamin E in the presence of oxidative stress. Iodine numbers of the fat tissues were high due to the increased presence of polyunsaturates. Perhaps surprisingly, melting points of fat from all fat depots were not significantly different between pigs fed diets with 0, 2, 4 and 6% fish oil (overall range: 37.3 - 38.2 °C). Hardness of the fat decreased ($P \leq 0.05$) with increasing level of dietary fish oil. However, the authors stated that, judging from some of their previous work, even the fat from pigs fed the 6% fish oil diet was not classified as very soft fat. This study met with approval in a popular pig farming magazine as the means to a marketing advantage when directed at health-conscious consumers (Brooks, 1992). Although such manipulation of fatty acid composition of fats within pigs might appear to be 'unnatural' and therefore likely to adversely affect consumer opinion about pig meat, these changes represent a partial reversion to the fatty acid composition observed in 'free-living', undomesticated pigs. Such pigs have been shown to contain a greater diversity of polyunsaturated fatty acids than domesticated pigs, as well as showing marked differences in the overall distribution of the major lipid classes (Crawford *et al*, 1969).

In summary, an effect of dietary fatty acid has been indicated with regard to a number of disease states in humans, most notably coronary heart disease, which is a major cause of premature death in western countries. Most notable among the fatty acids are the long chain *n-3* polyunsaturated fatty acids which have recently been recognised for their involvement in the alleviation of coronary heart disease as well as other diseases and for their importance in early development. As a result of these observations made in a large number of studies, human dietary recommendations have stressed the need for alterations in the consumption of fatty acids with an emphasis being placed on the necessity of consuming more long chain *n-3* polyunsaturated fatty acids. Enhancement of the levels of eicosapentaenoic acid and docosahexaenoic acid in commonly-occurring non-fish foods is one way of increasing the consumption of these fatty acids. One such food in which there has been successful incorporation of these fatty acids has been pig meat. However, marked changes in the fatty acid composition of pig fat have been associated with unacceptable changes in physical properties and organoleptic characteristics. The present series of investigations was designed to extend

the limited information which currently exists regarding i) the time taken to bring about a reorientation of fatty acid composition in line with human dietary recommendations within a number of porcine tissues, ii) the degrees to which the major lipid classes are affected by the alterations of dietary fatty acids, iii) the extent to which alterations in dietary pro- and antioxidant substances interact with effects of dietary oil manipulation on pig tissue fatty acid composition and the organoleptic characteristics of the carcass, iv) the qualitative and quantitative changes to the structural characteristics of pig adipose tissue triacylglycerols following dietary manipulation and the related changes in physical properties of the fat.

2. Materials and methods

2.1. Pig growth trial

The diets were mixed in a vertical mixer of one tonne capacity at the Institute of Applied Physiology and Genetics Research, Roslin, Midlothian, pelleted (6mm diameter pellets) and then stored in multi-wall paper sacks. As sacks of feed were emptied into the hopper of the feed stations, samples were taken throughout the trial, bulked and the fatty acid composition and proximate analysis were determined on a subsample of the bulk.

One week was allowed for the pigs to become accustomed to the diets and the feed stations. The pigs were housed at Easter Howgate Pig Research Unit (Edinburgh School of Agriculture) in pens (3m x 7m) with access to a well-ventilated kennel (2.5m x 3m) with straw bedding. Pens were situated in a building which was open to the front with an adjustable wind-break. Water was available *ad libitum* via a bowl drinker and the diets were offered *ad libitum* through a computer-controlled feed station in each pen (Hunday Electronics Ltd., Newcastle Upon Tyne, England). Recordings of the food intake of the pigs occurred through the electronic interaction of individually-coded transponder ear tags with a detector in each feed station. Records of food intake for each pig were established using an IBM personal computer. This also allowed monitoring of both the feeding system and the eating behaviour of the pigs.

2.2. Carcass sampling procedure

Pigs from each treatment group were slaughtered at a commercial slaughter house. One half carcass, together with the liver, from each pig were delivered to the Edinburgh School of Agriculture Carcass Evaluation Unit for removal of tissues for carcass and lipid analysis.

The ham joint was prepared by removing the ham at the sixth lumbar vertebra and was then weighed. Following an incision, the *rectus femorus* was displaced to expose the *Semitendinosus*. A sample of this muscle was removed from its distal end at approximately a third of its length. The loin joint was prepared by removing the portion of the carcass between the third and fourth thoracic vertebrae to the sixth lumbar vertebra. The flank was removed by a ventral cut at the midway point to give equal separation into loin and flank. The loin joint was then weighed and divided between the 12th and 14th thoracic vertebrae to allow sampling of the *Longissimus dorsi* and the inner and outer backfat layers. The backfat thickness was determined using callipers at three sites on the carcass (shoulder, mid-back and lower-back). Measurements were made from beneath the skin to the surface of the muscle; only the averages have been presented. This method of quantifying backfat thickness was found to be more reliable than using the probe measurements from the abattoir. A sample was taken from the liver by removing a portion of the first anterior lobe.

All samples and joints were kept frozen at -20 °C in polythene bags to await determination of lipid content and their fatty acid profiles.

2.3. Measurement of backfat firmness

Measurements of backfat firmness were undertaken by the Division of Food Animal Science (University of Bristol) using an 'Instron' penetrometer according to standardised procedures. Backfat samples were taken from the shoulder region and measurements were obtained at approximately 0 °C and replicated four times. All values were corrected to 1 °C for comparison and subsequent statistical analysis using the following standard equation:

$$\text{Corrected Value} = \text{Measured Value} - (\text{Standard temperature } (^{\circ}\text{C}) - \text{Measured temperature } (^{\circ}\text{C}) \times 18)$$

2.4. Trace element determination

Feeds were prepared for analysis by dry ashing and mineralising in dilute hydrochloric acid and trace elements were measured using a Plasma 100 Inductively Coupled Plasma Atomic Emission Spectrophotometer (Thermoelectron, Warrington, England). For measurement of selenium, feeds were first digested with an acid mixture comprised of nitric, sulphuric and perchloric acids on a heating block (Perstorp Ltd., Bristol, England) and analysis was performed using a 251 Atomic Absorption Spectrometer (Thermoelectron, Warrington, England) by means of a 440 Hydride Generator.

2.5. Determination of vitamin E

The method of α-tocopherol analysis was derived from McMurray *et al* (1980). Samples were saponified in aqueous medium in order to hydrolyse the tissue and to convert α-tocopheryl acetate to the non-acetylated form thereby precluding the problem of both forms of tocopherol giving rise to two peaks in the chromatogram. Pyrogallol in ethanol was added as an antioxidant and α-tocopherol was extracted into hexane prior to determination by reversed-phase high performance liquid chromatography, as outlined below.

2.5.1. Feed

2.5.1.1. Sample preparation

Diets were milled to a fine powder in a Cyclotec 1092 Sample Mill (Perstorp Ltd., Bristol, England). 1.5g of the milled sample (in duplicate) were weighed into a 50 ml screw-cap polyethylene centrifuge bottle. 10 ml of a 10% (w/v) solution of 1,2,3-trihydroxybenzene (pyrogallol) in ethanol was added and, following shaking, the tube was placed in a water-bath at 70 °C for five minutes to allow temperature equilibration. 2.5 ml of 60% (w/v) aqueous potassium hydroxide was then added and the contents heated for 30 minutes at 70 °C for complete saponification.

Approximately 20 ml of deionised water together with 10 ml of hexane were added and the bottles were shaken vigorously to allow extraction of α-tocopherol into the hexane. The aqueous and hexane phases were allowed to separate over a five minute period and were centrifuged as necessary.

2 ml of the hexane layer were transferred to a 5 ml test-tube and evaporated to dryness at 50 °C under a stream of oxygen-free nitrogen. Immediately after drying, the residue was redissolved in 600 µl methanol, transferred to glass microvials and sealed.

2.5.1.2. High performance liquid chromatography (HPLC)

α-Tocopherol was separated from other components by reversed-phase HPLC and quantified by fluorimetry. The HPLC and fluorimeter conditions as follows:

HPLC	Column	15 cm x 4 mm 'Superspher' 4 µm RP18 with 'Lichrocart' 4-4 guard column (Merck Ltd., Lutterworth, England)
	Eluant	methanol: water (98.04: 1.96, v/v)
	Pump	dual-piston Spectra System P1000 (Thermo Separation Products, Stone, England) isocratic pump; flow rate: 1.5 $mlmin^{-1}$
	Injection	manual injection via Rheodyne valve with 20 µl injection loop; sample volume: 150 µl
Fluorimeter	Type	JASCO 821 - FP Spectrofluorometer (Mettler-Toledo Ltd., Beaumont Leys, England)
	Wavelength	excitation: 295 nm emission: 330 nm
Integrator	Type	SP 4400 Datajet Integrator (Thermo Separation Products, Stone, England)

The eluant was degassed using helium. A standard d-α-tocopherol (Eastman Kodak, Deeside, Wales) solution in methanol, containing 105 ppm α-tocopherol, was injected until consistent peak areas were obtained. The concentration of the α-tocopherol standard was verified by measuring its absorbance at 292 nm in a SP8-500 U.V./Visible Spectrophotometer (A.T.I. Unicam, Cambridge, England) and comparing the results with standards quoted in the literature. Comparison between results obtainable from the literature and the standard prepared was as follows:

Standard from literature:	$A^{100\ \mu g/ml}$ at 292 nm = 0.75 in ethanol
Prepared standard:	$A^{46.6\ \mu g/ml}$ at 292 nm = 0.337
	≡ 0.72 (for 100 μg/ml)

The prepared standard therefore compared favourably with the given figures in the literature for absorbance. Additionally, the same concentration of α-tocopherol was prepared in methanol in order to determine whether this solvent caused a difference in the spectrophotometric absorbance. The result was as follows:

$A^{46.6\ \mu g/ml}$ at 292 nm = 0.354 in methanol
≡ 0.71 (for 100 μg/ml)

Hence, the effect of methanol on the absorbance was negligible. The mean α-tocopherol standard peak area was used as an external standard for the calculation of vitamin E content of the feeds and tissues.

2.5.2. Tissue

Estimation of tissue α-tocopherol concentration was also based on the method of McMurray *et al* (1980) as described above. However, the method was further adapted to suit specifically the measurement of α-tocopherol in porcine liver and muscle tissue. Consequently, the reproducibility of determination and recovery of α-tocopherol in the modified method were investigated.

2.5.2.1. Sample preparation

In the first instance, the fresh tissue (muscle and liver) was cut into small pieces. This, however, resulted in the formation of an unbreakable emulsion during hexane extraction which gave rise to inconsistent results. As an alternative method, the frozen tissue was pulverised using an agate pestle and mortar in the presence of liquid nitrogen. However, emulsification problems still persisted; additionally, dark-brown particulate matter was formed after addition of methanol immediately before HPLC injection.

The method of sample preparation eventually chosen was as follows. The frozen sample of tissue was freeze-dried for 48 hours at -2 °C at a pressure of less than 4.7

mmHg. The dehydrated tissue was then pulverised in a pestle and mortar and the α-tocopherol content measured from a small subsample (0.1g) of this material. Freeze-drying resulted in several marked improvements over previous methodology, that included:

1. Sample homogeneity was achieved. Pulverisation of the freeze-dried material produced a fine powder which was easily mixed and weighed out.
2. Excellent separation of organic from aqueous phase was achieved, even after thorough mixing.
3. There was no appearance of any insoluble material upon addition of methanol immediately prior to injection onto the HPLC column.

A variety of indicators of the reliability of the complete methodology were investigated. Good consistency of results was achieved with coefficients of variation for standards (comprising 28 ppm α-tocopherol) and samples (freeze-dried liver tissue) of 1.0% and 4.9%, respectively, being obtained. Excellent recoveries were also obtained in experiments involving standard α-tocopherol additions. The α-tocopherol content of freeze-dried liver tissue to which had been added 0, 2.8, 5.6 and 8.4 μg α-tocopherol was determined with three replications at each level of addition. The results are illustrated in Figure 2.1 and displayed a regression coefficient of 0.999.

The method was also 'rugged' with respect to a number of other interfering parameters. There was no apparent individual or crossover effect on the estimation of α -tocopherol due to extraction period (20 or 60 minutes) and extraction temperature (10 °C or 20 °C). The effect of shaking on hexane extraction of the α-tocopherol was investigated. With the addition of a known quantity of α-tocopherol, tubes were exposed to 'mild' or 'vigorous' shaking after addition of hexane, followed by short (20 min.) or long (12 hr.) periods of separation of the organic and aqueous phases. No difference in α-tocopherol measurements were detectable. The final step of the procedure involved drying the hexane phase and redissolving the solute in methanol prior to HPLC injection. It was therefore necessary to determine whether heating at 60 °C under nitrogen and redissolving in methanol caused any loss of α-tocopherol. A comparison of results for an α-tocopherol standard with or without previous drying showed that α-tocopherol was not destroyed by the process and that following drying complete dissolution in methanol occurred. On the basis of the above findings, the following method was employed for all α-tocopherol quantifications of the muscle and liver tissues:

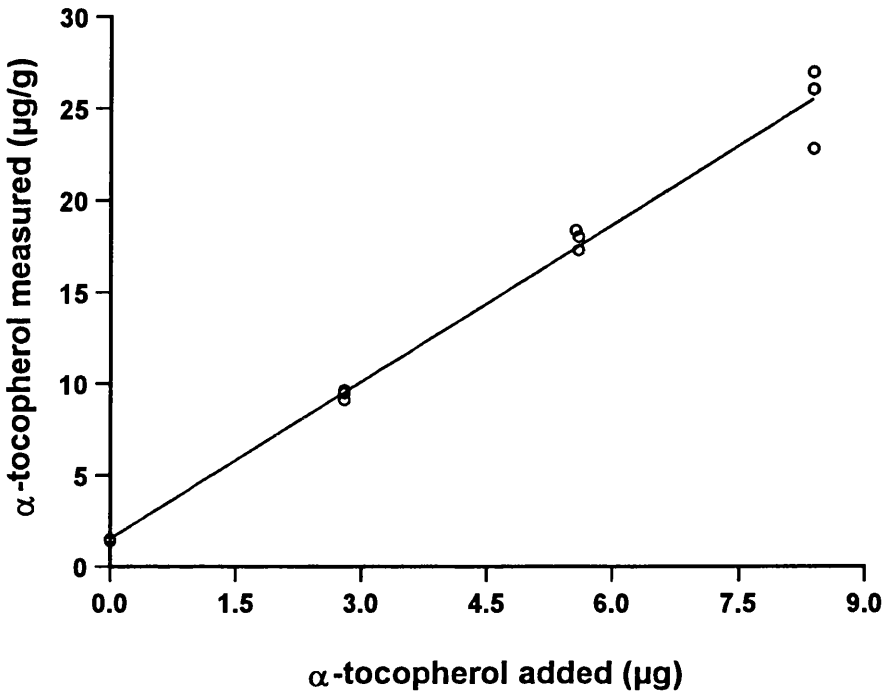


Figure 2.1 - Quantification of α-tocopherol following standard additions to freeze-dried liver tissue

2.5.2.2. Method employed

Approximately 5g of tissue was weighed into a polystyrene vial, frozen at -20 °C and placed in a freeze-dryer for 48 hours. The freeze-dryer was a 'Consol 12' (Virtis, Gardiner, New York, U.S.A.) with a chamber shelf temperature of 0-5°C and condenser temperature of -40 °C. Pressure was 30 mTorr (0.03 mmHg at 0 °C). The dehydrated tissue was pulverised to a powder in a pestle and mortar. 0.1g of the dried tissue was accurately weighed into a 13 ml screw-cap Pyrex glass test-tube complete with Teflon seal. 2 ml of a 50% (w/v) solution of pyrogallol in ethanol was added and the contents vigorously mixed in a vortex shaker. The tube was placed in an aluminium block heater at 70 °C. After five minutes, 1 ml of a 60% (w/v) solution of potassium hydroxide was added and the contents heated for a further 25 minutes at 70 °C to allow saponification to occur. At the end of the heating period, the tube was cooled under tap water. Deionised water (2 ml) followed by 3 ml hexane was added. The tube was capped and shaken vigorously for 30 seconds by hand and by vortex mixer. The tube was centrifuged at 300 Relative Centrifugal Force (g) for 15 minutes in a Sorvall T6000B centrifuge (Du Pont (U.K.) Ltd., Stevenage, England) enabling two distinct phases to form: an upper organic phase and a lower aqueous phase. Exactly 2 ml of the hexane layer were transferred to a 5 ml screw-cap test-tube using a positive displacement pipette. The contents were evaporated to dryness at 60 °C under nitrogen and the residue containing α-tocopherol was immediately redissolved in 200 µl methanol. The methanol solution was transferred to a 500 µl microvial with Teflon crimp cap.

HPLC analysis was performed as described in Section 2.4.1.2 with two exceptions. The column used was a 150 mm x 4.6 mm 'Spherisorb' 3µ S3 ODS2 (Phase Separations Ltd., Deeside, Wales) and the flow rate of the mobile phase was 1.0 $mlmin^{-1}$.

2.6. Determination of lipid and fatty acid compositions

2.6.1. Extraction of lipid

2.6.1.1. Tissues and dietary oil supplements

The total lipid associated with each sample was extracted with chloroform: methanol 2:1 (v/v) according to the method of Folch *et al* (1957). Chopped tissue was weighed (muscle and liver, approximately 5 g; adipose tissue, approximately 0.5 g; dietary oils, approximately 50 mg) into a ground-glass stoppered test-tube. 20 ml of methanol were added followed by 40 ml chloroform. Following complete homogenisation, the mixture was filtered using a fluted Whatman's Number 41 filter paper. To the filtrate was added 12 ml of 0.88% (w/v) potassium chloride and the solution shaken vigorously . After allowing separation of the aqueous and organic phases for 16 hours, the upper aqueous layer was siphoned off and discarded. The lower organic phase was freed of organic

solvent by evaporation under vacuum using a rotary film evaporator. Following dissolution in chloroform, the residual was transferred quantitatively to a 10 ml screw-cap vial which was then sealed under oxygen-free nitrogen and stored at -20 °C until analysis.

2.6.1.2. Feed

Samples of the diets in pelleted form were finely ground in a pestle and mortar and approximately 2 g were measured into 100 ml round-bottomed flasks. 20 ml of methanol were added and the contents refluxed for 20 minutes; this was followed by the addition of 40 ml of chloroform and the contents were refluxed for a further 20 minutes. Glass beads were added to prevent excessive 'bumping'. After cooling, the mixture was filtered via Whatman Number 41 filter paper into a measuring cylinder and the residue washed well with chloroform: methanol (2:1 v/v). To the filtrate was added 20% by volume of a 0.88% (w/v) solution of potassium chloride and the mixture vigorously shaken. Subsequent steps performed were as described above (Section 2.5.1.1).

2.6.2. Extraction and determination of total lipid content

The lipid extract (from tissues, diets and oils) in chloroform was transferred quantitatively to a 10 ml volumetric flask and made up to the mark with chloroform. Using a bulb pipette, 5 ml of the solution were transferred to a pre-weighed 25 ml round-bottomed flask. The chloroform was removed using a rotary film evaporator and the flask containing the lipid was heated in an oven at 110 °C for three hours to allow complete removal of the solvent. After cooling, the flask was weighed and the total lipid weight determined from the difference between the empty and lipid-containing flask. The lipid content of the tissues was expressed as g lipid per 100g fresh tissue.

Lipid extracts were freed of chloroform by heating at 50 °C on an aluminium block heater under a flow of oxygen-free nitrogen. 1.0 ml of chloroform was added and the extract stored at -20 °C under oxygen-free nitrogen until required for determination of the fatty acid composition.

2.6.3. Separation of major lipid classes

Lipids were fractionated into their major lipid classes on thin layer chromatoplates (20cm x 20cm) of silica gel G (Merck Ltd., Lutterworth, England), thickness 0.25 mm, using a solvent system of hexane:diethyl ether:formic acid, 80:20:1 (v/v/v). Using a glass syringe, a suitable aliquot of the lipid (between 5 and 10 mg) was applied as a band approximately 7 cm long to the origin. Following separation of the major lipid fractions, the plates were allowed to air dry and were then sprayed with a 0.1% (w/v) solution of 2,7-dichlorofluorescein in methanol. Bands corresponding to cholesterol ester, triacylglycerol, free fatty acid, free cholesterol and phospholipid were visualised under

ultraviolet light and scraped from the plate into centrifuge tubes. The phospholipid fraction was eluted from the silica gel by two washings with a suitable volume of methanol; all other fractions were eluted by washing with diethyl ether. In each case, the tubes were centrifuged to sediment the silica gel and the lipid-containing solvent was transferred to a 50 ml round-bottomed flask by decantation.

2.6.4. Determination of fatty acid composition

2.6.4.1. Transmethylation of fatty acids

Fatty acid methyl esters were generated by refluxing the lipid fractions with dry methanolic sulphuric acid. In all cases a pentadecanoic acid (15:0) internal standard in methanol was added to flasks containing the lipid fractions, 0.322 mg 15:0 being added to triacylglycerol and phospholipid and 0.0322 mg 15:0 being added to cholesterol ester and free fatty acid fractions to increase the accuracies of quantification. Following the addition of the internal standard, the lipid fractions were freed of solvent by rotary evaporation and 4 ml of a mixture of dried methanol:toluene:concentrated sulphuric acid, 20:10:1 (v/v/v), added and the mixture refluxed for 30 minutes. After refluxing, the flasks were allowed to cool and 10 ml each of deionised water and hexane were added. The flasks were then shaken vigorously to allow full extraction of fatty acid methyl esters into the hexane phase and the contents were transferred to a suitable test-tube and allowed to separate into two phases. The upper (hexane) layer was transferred to a further test-tube and residual water removed by the addition of approximately 10 g anhydrous sodium sulphate:sodium hydrogen carbonate, 4:1 w/w. The methyl ester-containing hexane was then transferred into a small test-tube and dried at 50 °C under a stream of nitrogen gas. The methyl esters were then taken up in volumes of hexane appropriate to their amount present.

2.6.4.2. Determination of fatty acids by gas-liquid chromatography

An AMS 94 Gas Chromatograph (Ai Cambridge Ltd., Cambridge, England) was used to separate the fatty acid methyl esters. The temperature of the injector, column and detector were 195, 225 and 225 °C, respectively. Analyses were performed on a packed column (1.8 m x 6 mm o.d. x 2 mm i.d.) with the following specifications as supplied commercially (Chrompack (U.K.) Ltd., London):

Liquid phase	CP-Sil 84
Liquid phase Conc^{n}	15%
Support/Adsorbent	Chromosorb W-HP
Mesh size	100/120

Nitrogen, hydrogen and air flows were adjusted to 20, 25 and 250 $mlmin^{-1}$, respectively, for the carrier and flame ionisation detector gases using a bubble manometer. Fatty acid peaks were identified by comparison with known standards and quantified using a SP4600 Electronic Integrator (Thermo Separation Products, Stone, England). Figure 2.2 shows a typical gas-liquid chromatographic separation obtained under such conditions. The systematic and common names of the fatty acids determined are as shown in Table 2.1. The double bond configuration of all the unsaturated fatty acids is *cis* and the position of the terminal double bond is expressed as *n-x* where *n* refers to the terminal methyl ($-CH_3$) carbon atom and *x* refers to the position of the first double bond numbered from that methyl group.

Systematic name	Common name	Terminal double bond	Shorthand designation
tetradecanoic	myristic	-	14:0
hexadecanoic	palmitic	-	16:0
9-hexadecenoic	palmitoleic	*n-7*	16:1*n-7*
octadecanoic	stearic	-	18:0
9-octadecenoic	oleic	*n-9*	18:1*n-9*
9,12-octadecadienoic	linoleic	*n-6*	18:2*n-6*
9,12,15-octadecatrienoic	α-linolenic	*n-3*	18:3*n-3*
8,11,14-eicosatrienoic	dihomo-γ-linolenic	*n-6*	20:3*n-6*
5,8,11,14-eicosatetraenoic	arachidonic	*n-6*	20:4*n-6*
5,8,11,14,17-eicosapentaenoic	eicosapentaenoic	*n-3*	20:5*n-3*
7,10,13,16,19-docosapentaenoic	docosapentaenoic	*n-3*	22:5*n-3*
4,7,10,13,16,19-docosahexaenoic	docosahexaenoic	*n-3*	22:6*n-3*

Table 2.1 - Nomenclature of fatty acids determined

Absolute amounts of cholesterol ester, triacylglycerol, free fatty acid and phospholipid were quantified by relating the total amount of fatty acids to the amount of the internal standard according to the method of Christie *et al* (1970). The weights of the lipid classes were obtained as the product of the weight of total fatty acids in each class and a factor calculated by dividing the molecular weight of the pentadecanoic acid derivative of the lipid class by the molecular weight of methyl pentadecanoate. Phospholipids were considered to be dipentadecanoyl-phosphatidylcholine.

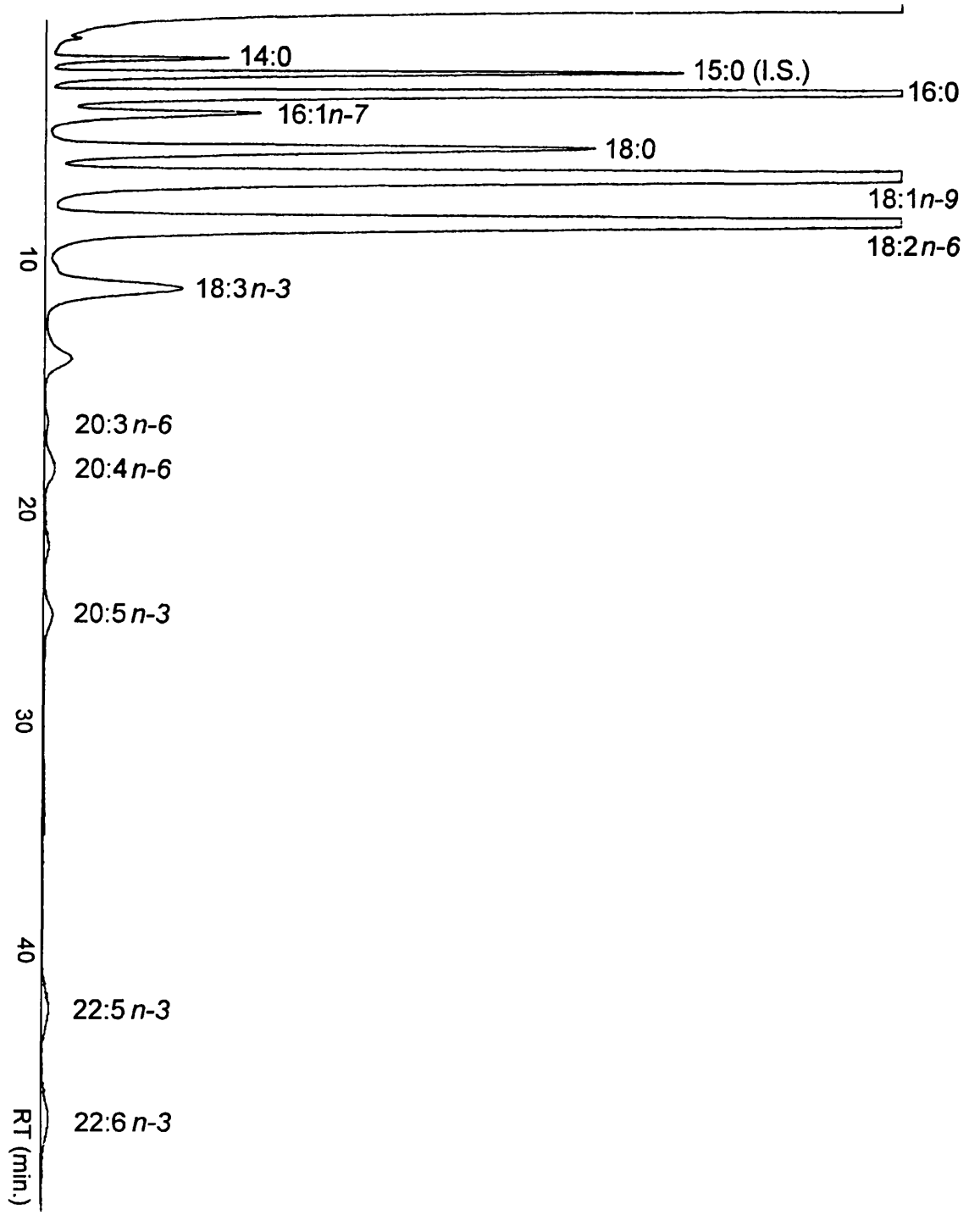


Figure 2.2 - Gas-liquid chromatographic trace of major fatty acids present in pig outer backfat triacylglycerol. Conditions of separation were as designated in Section 2.6.4.2.; I.S. = pentadecanoic acid internal standard; all other abbreviations as per Table 2.1.

2.7. Determination of free cholesterol

Following the separation of free cholesterol from other lipid components by thin-layer chromatography as described above (Section 2.6.3), the diethyl ether was removed by evaporation under a flow of nitrogen and the free cholesterol redissolved in 1 ml propan-2-ol and was quantified using a standard Cholesterol Test Kit (Boehringer Mannheim, Lewes, England) according to the approved protocol as described. The method is based on the colorimetric determination of the yellow lutidine dye, 3,5-diacetyl-1,4-dihydrolutidine, which is stoichiometric with respect to the amount of cholesterol present. A calibration curve of free cholesterol was prepared as follows.

[cholesterol] ($mgml^{-1}$)	Volume (ml) of 1.0 $mgml^{-1}$ cholesterol std.	Volume propan-2-ol (ml)
1.00	no dilution	
0.80	0.40	0.10
0.60	0.30	0.20
0.40	0.20	0.30
0.20	0.10	0.40
	Volume 0.6 $mgml^{-1}$	Volume propan-2-ol
0.10	0.10	0.50

In all cases, duplicates of standards and samples were added to the cells of a 96-cell MR5000 microplate reader (Dynatech Laboratories Ltd., Billingshurst, England) and absorbance measured at 405 nm. The standard curve was plotted (typically $r^2 = 0.999$) and free cholesterol was expressed either in absolute amounts or in conjunction with the other lipid fractions in relative terms per unit weight of lipid.

2.8. Specialist chromatographic methodologies

These are described in detail in appropriate sections.

2.9. Solvents, reagents and gases

Where necessary, all reagents, chemicals and gases were of the highest purity obtainable. In all cases, the products were obtained from reputable sources. The major suppliers were:

Solvents

Rathburn Chemicals Ltd., Walkerburn, Scotland

Fisons Scientific Equipment, Loughborough, England

BDH, Lutterworth, England

Hayman Ltd., Witham, England

Reagents

Sigma Chemical, Poole, England

Gases

BOC, Glasgow, Scotland

3. The effect of dietary inclusion of long-chain polyunsaturated fatty acids on pig performance and tissue fatty acid composition

3.1. INTRODUCTION

The chemical characteristics of fat in the pig are able to be influenced quite considerably by the composition of fat in the diet. The pig, being a monogastric animal, absorbs the fatty acids from the diet in a relatively unchanged state and is able to incorporate them directly into body tissues. Over the years, numerous studies have demonstrated the significant effects which commonly-occurring, natural foodstuffs in the diet may have on the chemical, and hence physical, characteristics of fat in the tissues of pigs (Hertzman *et al*, 1988; Brooks, 1971; Ellis and Isbell, 1926). Most recently, Morgan *et al* (1992) adapted this feature of the nutrition and fat metabolism of the pig to improve the "health value" of its meat appropriate to U.K. government recommendations regarding fat intake in relation to cardiovascular and related circulatory diseases (British Nutrition Foundation, 1992). Feeding pigs a diet containing 50 g/kg soybean oil and a relatively low level of fish oil (9.5 g/kg) which was high in eicosapentaenoic acid and docosahexaenoic acid, the polyunsaturated fatty acid contents of muscle, liver and fat tissue were markedly increased above those of conventional tallow-fed pigs. The alterations achieved in the carcass lipid composition fulfilled the health recommendations in several respects. For example, the polyunsaturated to saturated fatty acid (P:S) ratio of the body lipid throughout was increased substantially to exceed in all cases the satisfactory level of 1.0 recommended by the Health Education Council of the United Kingdom (NACNE, 1983) whilst the percentage of saturated fatty acids in the meat from the pigs fed the modified diet was at or below the level recommended by the U.K. Department of Health (COMA, 1991). Furthermore, no adverse effects of this diet on backfat firmness were detected, in spite of the fact that in all previous studies the levels of linoleic acid attained would certainly have given fat which was unacceptably soft (Prescott and Wood, 1988; Whittington *et al*, 1986; Ellis and Isbell, 1926). Additionally, strictly controlled investigations failed to reveal any differences in taste between the meat from the pigs on the experimental diet and that from the pigs on the conventional diet.

In the experiment described, there was an attempt to define the minimum period over which the significant lipid changes would be elicited. Thus, in continuance of this work, the present investigation describes the feeding of a modified fish oil diet based on 50 g/kg soybean oil plus 10 g/kg of refined fish oil for two, four and six weeks to growing-finishing pigs prior to slaughter at approximately 70 kg, the precept being that such a feeding regime would indicate to some degree the optimum length of time required to significantly alter muscle and fat lipid compositions. Changes in the fatty acid composition of muscle and fat would be examined in detail, accompanied by the effect of the diet on pig performance and the associated physical properties of the resulting backfat.

3.2. MATERIALS AND METHODS

3.2.1. Composition of diets

The diets were based on barley and soya bean meal and comprised a basal 50 g/kg soybean oil diet and an experimental soybean oil/fish oil diet ('SFOD') containing 50 g/kg soybean oil and 10 g/kg of a commercial fish oil product, "Boost" oil (Seven Seas Ltd., Hull, England). The fatty acid compositions of the soybean and the fish oils are shown in Table 3.1. The major fatty acid component of the soybean oil was linoleic acid accounting for some 54% of the total fatty acids present whilst the fish oil contained as its major polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid. The declared peroxide and iodine values were 0.98 m.eq/kg and 185.0, respectively. A peroxide value of less than 3 has been considered acceptable (Vitec Manual, Roche Ltd.). The fish oil was derived from a selection of oily fish only and had been subject to refining, deodorisation and fractionation (Vas Dias, 1990, personal communication). Proximate and mineral analyses for the basal and experimental diets are shown in Table 3.2. As can be seen, the analyses were similar apart from their respective iron contents which were significantly higher in the experimental diet. Table 3.3 shows the total lipid content and composition of the basal and experimental diets; the fish oil diet was slightly higher, but not markedly so, in total lipid content (7% by weight) than the basal diet (6%). The overall fractional lipid composition was similar for both diets with the triacylglycerol being by far the major component present.

3.2.1.1. Fatty acid composition

Table 3.4 shows the fatty acid composition of the diets, expressed both as weight percentage of total fatty acids and as their concentration (g/kg) within the diet as fed. The major fatty acid in both diets was linoleic acid with 50% of total fatty acids in each. Levels of palmitic and stearic acids were also similar for the two diets. A reduced level of oleic acid within the experimental fish oil diet was balanced by increases in C18, C20 and C22 polyunsaturated fatty acids of the *n-3* series. In overall terms, the diets contained similar levels of total saturated, monounsaturated and polyunsaturated fatty acids and therefore displayed similar ratios of polyunsaturated to saturated (P:S) and unsaturated to saturated (U:S) fatty acids. The total *n-6* to *n-3* fatty acid ratio was markedly lower in the experimental diet due to inclusion of the fish oil.

3.2.1.2. α-Tocopherol content

The α-tocopherol content of the basal and experimental diets which was

Fatty acid	Soya Oil	'Boost' Oil
16:0	10.8	22.0
16:1*n-7*	-	13.6
18:0	3.5	5.6
18:1*n-9*	24.6	18.1
18:2*n-6*	54.1	4.3
18:3*n-3*	6.2	2.9
20:3*n-6*	0.4	-
20:4*n-6*	-	2.1
20:5*n-3*	0.2	18.8
22:5*n-3*	-	1.43
22:6*n-3*	-	11.3

Table 3.1 - The fatty acid composition of the oil supplements (major fatty acids, percentage of total present; shorthand designations as per Table 2.1)

Component	Soya Oil Diet	Soya/Fish Oil Diet
Dry matter	866	876
Digestible energy (MJ/kg dry matter)	16.2	16.3
Crude protein	245	241
Acid hydrolysed ether extract	78.8	91.6
Crude fibre	47.0	51.0
Neutral detergent fibre	167	191
Calcium	13.4	14.1
Phosphorus	8.6	8.6
Sodium	1.4	1.5
Copper (mg/kg)	22.0	18.7
Zinc (mg/kg)	158	165
Iron (mg/kg)	394	531

Table 3.2 - The proximate and mineral composition of the basal and experimental diets (g/kg dry matter, unless stated otherwise)

	Soya Oil Diet	Soya/Fish Oil Diet
Total lipid (g/kg diet)	60.2	72.3
Lipid composition (% weight of total lipid):		
Cholesterol ester	1.2	1.4
Triacylglycerol	78.4	83.1
Free fatty acid	5.7	2.4
Free cholesterol	6.3	4.9
Total phospholipid	8.6	8.4

Table 3.3 - The lipid content and composition of the basal and experimental diets

	Soya Oil Diet		Soya/Fish Oil Diet	
	weight % total fatty acid	**g of fatty acid per kg diet**	**weight % total fatty acid**	**g of fatty acid per kg diet**
16:0	13.8	8.32	14.4	10.4
16:1*n-7*	0.33	0.20	1.45	1.05
18:0	3.30	1.98	3.44	2.49
18:1*n-9*	25.0	15.0	20.5	14.8
18:2*n-6*	51.0	30.7	50.0	36.1
18:3*n-3*	5.00	3.01	6.87	4.97
20:3*n-6*	0.98	0.59	0.32	0.23
20:4*n-6*	-	-	0.08	0.06
20:5*n-3*	0.66	0.40	2.12	1.53
22:5*n-3*	-	-	-	-
22:6*n-3*	-	-	0.87	0.63
Total SAT	17		18	
Total MUFA	25		22	
Total PUFA	58		60	
P:S[1]	3.4		3.4	
U:S[2]	4.8		4.6	
n-6:n-3[3]	9.2		5.1	

[1] polyunsaturated:saturated fatty acid ratio [2] unsaturated:saturated fatty acid ratio
[3] ratio of *n-6* to *n-3* fatty acids

Table 3.4 - The fatty acid composition of the basal and experimental diets (major fatty acids, percentage of total present; shorthand designation as per Table 2.1)

determined as described previously (see Section 2.5.1) was 88.6 μg/g and 94.1 μg/g, respectively. Both diets therefore could be considered to provide sufficient antioxidant protection against oxidative damage arising from the increased presence of polyunsaturated fatty acids.

3.2.2. The pigs and their treatment

Fifteen male and fifteen female Large White x Landrace pigs were randomly allocated to one of three treatment groups with five males and five females assigned per treatment. The fish oil diet was fed *ad libitum* for two, four and six weeks before slaughter following the feeding of the basal (soybean oil) diet for six, four and two weeks, respectively; the treatment groups were thereby designated 2SFOD, 4SFOD and 6SFOD, respectively. At the end of the growth period, the pigs were slaughtered at a commercial slaughter house having achieved a mean liveweight of 78.1 kg (±2.2 standard deviation). Growth performance and carcass characteristics, including backfat firmness, were measured as described previously (see Sections 2.1-2.3). An error at the slaughter house resulted in the loss of one complete carcass.

3.2.2.1. Carcass evaluation

Following slaughter, the pigs were sent to the Carcass Evaluation unit at the Scottish Agricultural College, Edinburgh, for assessment of parameters of carcass quality and for removal of the tissues according to the method described above (see Section 2.2).

3.2.3. Determination of the lipid and fatty acid compositions of the tissues

The lipid and fatty acid compositions of all tissues were determined as described previously in Section 2.6. The outer and inner backfat, *Semitendinosus*, *Longissimus dorsi* and liver were taken from four pigs from each group for determination of the lipid and fatty acid compositions.

3.2.4. Statistical analysis

Data were analysed using the Genstat statistical package (Genstat 5, Release 1.3, Lawes Agricultural Trust, Rothamstead, England) using a one way analysis of variance. Statistical analysis of daily liveweight gain, daily feed intake and feed conversion efficiency was performed using covariates calculated from the first or first two weeks of their measurement. This was done in order to provide an adequate baseline for

comparison of treatment effects. Group and sex were regarded as treatments in the analysis of daily liveweight gain, daily food intake, feed conversion efficiency and killing out proportion. A group effect for ham weight, loin weight, backfat thickness and fatty acid composition was investigated, thus ignoring the effect of sex. This was made necessary due to the loss of a carcass at the abattoir thereby making the residual degrees of freedom unacceptably low. Fat firmness data were analysed by multiple regression using the pre-trial pig liveweights, left-side carcass weights and fat thicknesses as covariates.

3.3. RESULTS

3.3.1. Pig performance and carcass evaluation

The performance of the pigs in terms of growth, food conversion efficiency and other related parameters is presented in Table 3.5. The data show that, with increasing duration of the fish oil diet there was a significant gain in daily liveweight even though the feed intake was not significantly affected. This finding is supported by the marked differences in finishing weight between the groups, in spite of similar starting weights. The results showed a significant group/sex interaction for feed conversion efficiency (FCE). Feed conversion efficiency for the male pigs increased whilst that for females decreased with time of feeding the fish oil diets. The weights of the ham and loin joints increased slightly with increasing time on the fish oil diet, though differences were not significant. With increasing duration on the fish oil diet, there was a significant increase in backfat thickness. Killing-out proportion (i.e. carcass weight/slaughter weight) of the pigs was not significantly affected by duration of the fish oil diet.

3.3.2. Total lipid contents of the pig tissues

Table 3.6 shows the total lipid content of the tissues from the pigs fed the fish oil diet. Outer and inner backfat displayed the highest content of lipid (approximately 70g/100g tissue across treatments); levels of fat displayed by the liver, *Semitendinosus* and *Longissimus dorsi* were considerably lower. In all tissues except outer backfat, there were no significant effects of the duration of the fish oil diet on the total lipid contents. However, in the outer backfat, feeding of the fish oil diet produced a significant increase in the lipid content; there was a similar trend for the inner backfat. The fat content of *L. dorsi* and *Semitendinosus* was not significantly affected by duration of SFOD. However, in both tissues, small increases in lipid content were

Parameter		2SFOD	4SFOD	6SFOD	SED[1]	Signif.[2]
Start weight (kg)		28.3	28.6	31.4	-	-
Finish weight (kg)		71.6	81.6	81.2	-	-
Weight gain (kg/d)		0.77	0.95	0.93	0.06	*
Feed intake (kg/d)		1.82	2.23	1.96	0.18	NS
FCE	male	0.40	0.42	0.47	0.02	**
(kg gain/kg feed)	female	0.49	0.41	0.42	0.02	
Ham joint (kg)		8.28	9.19	9.03	0.60	NS
Loin joint (kg)		5.30	5.77	5.82	0.52	NS
Backfat thickness (mm)		5.7	9.8	8.5	1.1	*
Carcass wt./Slaughter wt.		0.74	0.75	0.75	0.01	NS

[1] SED standard error of the differences between the means

[2] *, ** significant differences, respectively, at P≤0.05 and P≤0.01 NS not significant

Table 3.5 - Growth performance and carcass composition of the pigs fed the soybean oil/fish oil diet for two, four and six weeks; shorthand designations of treatments as per Section 3.2.2

Tissue	2SFOD	4SFOD	6SFOD	SED[1]	Signif.[2]
Outer backfat	59.6	74.6	71.9	3.5	**
Inner backfat	62.4	65.8	76.5	5.8	NS
Longissimus dorsi	1.22	1.23	1.31	0.10	NS
Semitendinosus	1.87	2.19	2.39	0.30	NS
Liver	4.37	4.30	4.22	0.15	NS

[1] SED standard error of the differences between the means
[2] ** significant difference at P≤0.01 NS not significant

Table 3.6 - The total lipid contents of the tissues (g/100g tissue) from the pigs fed the experimental diets; shorthand designations of treatments as per Section 3.2.2

Treatment	Cholesterol ester	Triacylglycerol	Free fatty acid	Free cholesterol	Phospholipid
			Semitendinosus		
2SFOD	0.98	48.5	4.95	5.45	40.1
4SFOD	0.56	54.6	2.87	4.64	37.3
6SFOD	1.80	60.3	4.41	3.90	29.6
SED[1]	0.63	5.7	0.67	0.69	5.0
Signif.[2]	NS	NS	*	NS	NS
			Longissimus dorsi		
2SFOD	1.14	38.2	5.28	7.09	48.3
4SFOD	0.81	38.5	3.36	7.27	50.1
6SFOD	1.29	46.2	3.12	6.24	43.1
SED[1]	0.38	5.1	0.84	0.57	4.7
Signif.[2]	NS	NS	NS	NS	NS
			Liver		
2SFOD	1.15	13.4	10.93	8.33	66.2
4SFOD	1.55	8.6	3.69	8.38	77.7
6SFOD	1.29	9.8	3.40	8.40	76.7
SED[1]	0.56	2.9	1.66	0.49	2.9
Signif.[2]	NS	NS	**	NS	**

[1] SED standard error of the differences between the means
[2] *, ** significant differences, respectively, at P≤0.05 and P≤0.01 NS not significant

Table 3.7 - Relative proportions of the major lipids (g/100g total lipid) within the *Semitendinosus*, *Longissimus dorsi* and liver; shorthand designations of treatments as per Section 3.2.2

associated with increased duration of SFOD. This change may be explained by 'quantity' and 'quality' differences with respect to the lipids between the control and experimental diets.

3.3.3. Composition of the lipids of the tissues

Proportions of the major lipid fractions, i.e. cholesterol ester, triacyglycerol, free fatty acid, free cholesterol and phospholipid within the muscle and liver tissues are shown in Table 3.7 on the previous page. Data for outer and inner backfat are not included as throughout the lipid was comprised almost wholly (i.e. more than 98%) of triacylglycerol. In the muscle tissues, triacylglycerol and phospholipid were consistently the major lipid fractions present, accounting between them for some 85% of the total lipid. Of the other lipid fractions isolated, only free fatty acid and free cholesterol were of major importance. Between the *Semitendinosus* and *Longissimus dorsi*, there was an apparent difference in distribution of lipid between the triacylglycerol and phospholipid; *Semitendinosus* displayed consistently higher proportions of triacylglycerol and lower proportions of phospholipid. With respect to the liver, phospholipid accounted for some two-thirds of the lipid present; proportions of triacylglycerol, free fatty acid and free cholesterol were of a similar order to each other. As in the case of the muscle tissues, cholesterol ester was a minor component.

Although there was a trend for feeding of the fish oil diet to increase the proportion of triacylglycerol within the muscle tissues at the expense of the phospholipid, the effect was not significant. Free cholesterol also tended to decrease in both muscle tissues with time on the SFOD. By contrast to muscle tissues, feeding the fish oil diet resulted in a significant increase in phospholipid levels within the liver with a concomitant reduction in the level of free fatty acid.

3.3.4. Fatty acid composition of the tissue lipid fractions

3.3.4.1. Outer and inner backfat

Tables 3.8 and 3.9 show the fatty acid compositions of the triacylglycerol fractions of the outer and inner backfat from the pigs fed the fish oil diet for varying lengths of time. Other than some small differences, the fatty acid compositions of the outer and inner backfat triacylglycerols were very similar. Thus, in both cases, the two major fatty acids by far were oleic and linoleic acid, together accounting for 60-65% of the total fatty acids present roughly equally divided between them. The levels of linoleic acid in all treatments were over twice those observed by Wood *et al* (1989) at 13% of fatty acids obtained after feeding conventional diets and taking into account differences

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	16.9	18.0	17.3	0.56	NS
16:1*n-7*	2.45	2.53	2.64	0.18	NS
18:0	8.12	10.1	9.17	0.76	NS
18:1*n-9*	29.3	32.8	34.0	1.71	*
18:2*n-6*	36.5	29.8	30.1	1.76	**
18:3*n-3*	4.07	3.82	3.78	0.23	NS
20:3*n-6*	0.17	0.14	0.15	0.02	NS
20:4*n-6*	0.63	0.61	0.59	0.04	NS
20:5*n-3*	0.16	0.28	0.31	0.03	**
22:5*n-3*	0.43	0.46	0.50	0.09	NS
22:6*n-3*	0.31	0.36	0.43	0.04	*

[1] * $P \leq 0.05$ ** $P \leq 0.01$ NS not significant

Table 3.8 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the outer backfat in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	17.2	18.5	17.8	0.68	NS
16:1*n-7*	2.29	2.26	2.27	0.11	NS
18:0	8.92	11.7	10.3	0.94	*
18:1*n-9*	29.2	31.7	32.7	1.23	*
18:2*n-6*	35.7	29.2	30.4	1.61	**
18:3*n-3*	4.11	3.70	3.70	0.23	NS
20:3*n-6*	0.17	0.13	0.13	0.02	NS
20:4*n-6*	0.63	0.57	0.54	0.03	*
20:5*n-3*	0.16	0.26	0.32	0.03	***
22:5*n-3*	0.44	0.44	0.55	0.04	*
22:6*n-3*	0.31	0.36	0.46	0.05	*

[1] * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$ NS not significant

Table 3.9 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the inner backfat in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

in fat thickness (P2) which can affect linoleic acid. Palmitic acid also accounted for a large proportion of the total fatty acids. The level of stearic acid was approximately half that of palmitic. Compared with these acids, proportions of others were very much lower. Collectively, the levels of *n-3* fatty acids were high. Feeding the fish oil diet clearly had an effect on the proportions of the unsaturated fatty acids within the triacylglycerols of both outer and inner backfat. The pattern of change was similar in both cases. Thus, enhanced proportions of C20 and C22 polyunsaturated fatty acids and oleic acids occurred at the expense of a decrease in the levels of linoleic acid. This effect was most marked with increasing time of fish oil feeding.

3.3.4.2. *Semitendinosus*

Tables 3.10, 3.11, 3.12 and 3.13 show the fatty acid compositions of the triacylglycerol, phospholipid, cholesterol ester and free fatty acid fractions, respectively, of the *Semitendinosus*.

The major fatty acid fractions found within the triacylglycerol of the *Semitendinosus* were oleic and linoleic acids, together accounting for some 60% of the total fatty acids present. A substantial proportion also of palmitic acid was accompanied by very much lower levels of stearic acid. Although individually the proportions of *n-3* acids were low, collectively their proportions were high. The effect of feeding the fish oil diets was to significantly increase the proportions of eicosapentaenoic, docosapentaenoic and docosahexaenoic acids. Their levels following six weeks of feeding being double that displayed after two weeks of feeding.

The major fatty acid by far within the phospholipid fraction of the *Semitendinosus* was linoleic acid followed by palmitic and stearic acids. The level of oleic acid was only 9 % of the total fatty acids present. The phospholipids were characterised by high levels of the C20 andC22 polyunsaturated fatty acids. Feeding of the fish oil diet resulted in a highly significant increase in the proportions of eicosapentaenoic and docosahexaenoic fatty acids. There was a significant decrease also in the level of palmitic acid.

In both the cholesterol ester and free fatty acid fractions, linoleic acid was the major component present and was accompanied also by substantial levels of palmitic, stearic and oleic acids. In both lipid fractions, there were also substantial levels of C20 and C22 polyunsaturates. However, within the free fatty acid fraction, not only was the overall level of the C20 and C22 very substantial, but in particular that of docosapentaenoic and docosahexaenoic acids was consistently high. Although feeding the fish oil diet did effect some fatty acid compositional changes within the cholesterol ester and free fatty acid fractions, these were confined to the C16 and C18 components.

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	19.6	19.7	18.7	0.58	NS
16:1*n-7*	3.18	3.01	2.9	0.20	NS
18:0	8.86	10.5	9.63	0.69	NS
18:1*n-9*	35.0	36.1	35.5	1.14	NS
18:2*n-6*	27.1	24.6	26.8	1.50	NS
18:3*n-3*	3.33	3.17	3.48	0.14	NS
20:3*n-6*	0.20	0.18	0.16	0.03	NS
20:4*n-6*	0.82	0.78	0.71	0.05	NS
20:5*n-3*	0.16	0.19	0.25	0.02	**
22:5*n-3*	0.37	0.37	0.50	0.03	**
22:6*n-3*	0.24	0.26	0.38	0.03	**

[1] ** $P \leq 0.01$ NS not significant

Table 3.10 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the *Semitendinosus* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	21.4	22.0	19.9	0.65	*
16:1*n-7*	1.36	1.09	2.06	0.22	NS
18:0	15.0	14.6	14.7	0.49	NS
18:1*n-9*	9.34	9.43	9.68	0.39	NS
18:2*n-6*	35.8	35.6	36.1	0.69	NS
18:3*n-3*	0.71	0.72	0.75	0.05	NS
20:3*n-6*	1.08	0.90	0.96	0.09	NS
20:4*n-6*	9.64	9.39	9.06	0.50	NS
20:5*n-3*	1.62	1.78	2.22	0.10	***
22:5*n-3*	2.30	2.45	2.44	0.19	NS
22:6*n-3*	1.43	1.75	1.87	0.09	**

[1] * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$ NS not significant

Table 3.11 - The fatty acid composition (major fatty acids, percentage of total present) of the phospholipid fraction of the *Semitendinosus* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	26.5	32.6	27.8	3.45	NS
16:1*n-7*	1.47	2.72	2.09	1.28	NS
18:0	14.9	17.6	13.3	2.91	NS
18:1*n-9*	15.0	11.2	15.6	1.17	**
18:2*n-6*	26.7	13.8	24.1	3.29	**
18:3*n-3*	0.74	0.52	0.41	0.18	NS
20:3*n-6*	0.36	0.35	0.19	0.14	NS
20:4*n-6*	4.14	3.33	3.83	1.12	NS
20:5*n-3*	0.70	0.57	0.54	0.10	NS
22:5*n-3*	3.74	4.13	7.46	2.96	NS
22:6*n-3*	0.25	0.52	0.08	0.35	NS

[1] ** $P \leq 0.01$ NS not significant

Table 3.12 - The fatty acid composition (major fatty acids, percentage of total present) of the cholesterol ester fraction of the *Semitendinosus* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	16.3	18.0	16.7	1.47	NS
16:1*n-7*	2.04	2.58	2.27	0.13	**
18:0	9.37	9.82	10.0	0.82	NS
18:1*n-9*	18.4	17.5	17.6	1.05	NS
18:2*n-6*	35.5	32.5	34.2	1.19	NS
18:3*n-3*	2.27	2.13	2.13	0.17	NS
20:3*n-6*	0.83	0.80	0.85	0.13	NS
20:4*n-6*	6.37	5.62	5.31	0.66	NS
20:5*n-3*	2.02	2.19	2.58	0.28	NS
22:5*n-3*	3.79	4.31	4.05	0.82	NS
22:6*n-3*	2.70	3.46	3.52	0.64	NS

[1] ** $P \leq 0.01$ NS not significant

Table 3.13 - The fatty acid composition (major fatty acids, percentage of total present) of the free fatty acid fraction of the *Semitendinosus* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

3.3.4.3. *Longissimus dorsi*

Tables 3.14, 3.15, 3.16 and 3.17 show the fatty acid compositions of the triacylglycerol, phospholipid, cholesterol ester and free fatty acid, respectively, of the *Longissimus dorsi*.

In overall terms, the distribution of fatty acids within the triacylglycerol fraction of the *Longissimus dorsi* was similar to that shown for *Semitendinosus*. Thus, the major fatty acid was oleic acid and was accompanied by high proportions in particular of palmitic acid and linoleic acid. However, in comparison with the *Semitendinosus*, the *Longissimus dorsi* displayed obviously higher proportions of oleic acid accompanied by lower proportions of linoleic acid. As in the case of the *Semitendinosus*, feeding the fish oil diet resulted in significantly enhanced levels of C20 and C22 polyunsaturates.

As for the triacylglycerol fraction, the distribution of fatty acids within the phospholipids of the *Longissimus dorsi* was very similar to that shown for the *Semitendinosus*. Also, the *Longissimus dorsi* displayed a lower level of linoleic acid and a higher level of oleic acid but nowhere near to the extent displayed by the triacylglycerol. The effect of fish oil feeding was similar to that observed for the phospholipids of the *Semitendinosus*, that is, a significant promotion in the levels of eicosapentaenoic and docosahexaenoic acids.

The fatty acid distributions within the cholesterol ester and free fatty acid fractions of the *Longissimus dorsi* were very similar to those for *Semitendinosus*; as in the case of the *Semitendinosus*, fish oil feeding was virtually without effect on the fatty acid compositions.

3.3.4.4. Liver

Tables 3.18, 3.19, 3.20 and 3.21 show the fatty acid compositions of the triacylglycerol, phospholipid, cholesterol ester and free fatty acid, respectively, of the liver.

Although linoleic acid was by far the major fatty acid in the triacylglycerol of the liver, it was accompanied also by substantial levels of other polyunsaturated fatty acids. Thus, collectively, C20 and C22 polyunsaturates together accounted for some 18% of the total fatty acids present. The effect of fish oil feeding was to significantly increase the levels of oleic acid, eicosapentaenoic acid docosapentaenoic acids at the expense of C18 polyunsaturates.

The phospholipids of the liver were characterised by much lower levels of linoleic acid than present within the triacylglycerol but proportions of C20 and C22 polyunsaturated fatty acids were considerably higher. Thus, in total, C20 and C22 polyunsaturates accounted for approximately 26% of total fatty acids present. In contrast to the triacylglcyerol fraction, the effect of feeding the fish oil diet on the fatty acid composition was minimal and confined to a significant increase in the level of

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	21.8	21.7	20.9	0.76	NS
16:1*n-7*	4.23	3.57	3.77	0.23	*
18:0	9.12	10.4	9.54	0.55	NS
18:1*n-9*	41.9	41.8	43.4	1.22	NS
18:2*n-6*	17.9	17.4	17.3	1.36	NS
18:3*n-3*	2.46	2.40	2.43	0.15	NS
20:3*n-6*	0.13	0.15	0.13	0.03	NS
20:4*n-6*	0.64	0.78	0.54	0.09	0.07
20:5*n-3*	0.09	0.15	0.18	0.02	***
22:5*n-3*	0.26	0.28	0.34	0.03	*
22:6*n-3*	0.14	0.18	0.23	0.02	**

[1] * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$ NS not significant

Table 3.14 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerols of the *Longissimus dorsi* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	21.1	21.1	20.4	0.65	NS
16:1*n-7*	1.47	1.27	1.66	0.54	NS
18:0	14.2	13.9	13.8	0.38	NS
18:1*n-9*	11.3	12.0	12.5	0.71	NS
18:2*n-6*	34.6	33.9	33.5	0.63	NS
18:3*n-3*	0.69	0.58	0.69	0.08	NS
20:3*n-6*	1.25	1.11	1.19	0.08	NS
20:4*n-6*	9.89	9.96	9.17	0.34	NS
20:5*n-3*	1.52	1.74	2.12	0.12	**
22:5*n-3*	2.27	2.48	2.64	0.25	NS
22:6*n-3*	1.32	1.65	1.96	0.14	**

[1] ** $P \leq 0.01$ NS not significant

Table 3.15 - The fatty acid composition (major fatty acids, percentage of total present) of the phospholipid fraction of the *Longissimus dorsi* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	23.7	33.0	31.5	3.05	*
16:1*n-7*	1.45	3.15	3.32	1.18	NS
18:0	10.6	14.0	11.4	2.07	NS
18:1*n-9*	13.1	13.1	14.2	1.55	NS
18:2*n-6*	29.2	18.7	25.2	5.77	NS
18:3*n-3*	4.64	0.39	0.49	2.20	NS
20:3*n-6*	0.07	0.36	0.36	0.21	NS
20:4*n-6*	8.38	3.40	3.23	2.05	*
20:5*n-3*	0.37	0.48	0.50	0.16	NS
22:5*n-3*	2.17	4.08	2.45	1.22	NS
22:6*n-3*	-	-	0.13	-	-

[1] * $P \leq 0.05$ NS not significant

Table 3.16 - The fatty acid composition (major fatty acids, percentage of total present) of the cholesterol ester of the *Longissimus dorsi* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	17.8	18.6	19.3	1.22	NS
16:1*n-7*	2.11	2.59	2.34	0.29	NS
18:0	9.17	12.5	11.7	1.65	NS
18:1*n-9*	16.7	17.1	17.8	1.12	NS
18:2*n-6*	34.5	30.2	29.8	2.10	NS
18:3*n-3*	1.93	1.63	1.72	0.14	NS
20:3*n-6*	1.01	0.94	1.05	0.08	NS
20:4*n-6*	7.05	6.79	5.92	0.65	NS
20:5*n-3*	2.22	2.13	2.32	0.29	NS
22:5*n-3*	4.01	3.75	3.88	0.50	NS
22:6*n-3*	2.84	2.99	3.34	0.31	NS

[1] NS not significant

Table 3.17 - The fatty acid composition (major fatty acids, percentage of total present) of the free fatty acid fraction of the *Longissimus dorsi* in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	15.0	15.5	15.5	0.75	NS
16:1*n-7*	2.49	2.48	2.42	0.26	NS
18:0	8.95	10.3	10.2	1.58	NS
18:1*n-9*	15.7	19.0	19.3	1.32	*
18:2*n-6*	35.3	31.5	31.7	1.24	*
18:3*n-3*	3.46	2.25	2.40	0.43	*
20:3*n-6*	0.78	1.02	1.04	0.16	NS
20:4*n-6*	11.02	9.02	8.82	0.91	NS
20:5*n-3*	3.05	3.83	3.40	0.22	*
22:5*n-3*	1.95	2.76	2.92	0.35	*
22:6*n-3*	1.62	1.48	1.49	0.31	NS

[1] * $P \leq 0.05$ NS not significant

Table 3.18 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the liver in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	13.0	12.1	10.9	1.31	NS
16:1*n-7*	1.82	2.02	1.79	0.27	NS
18:0	27.0	28.1	30.8	1.93	NS
18:1*n-9*	9.17	9.51	8.50	0.53	NS
18:2*n-6*	21.8	21.1	20.1	0.99	NS
18:3*n-3*	0.78	0.83	0.63	0.10	NS
20:3*n-6*	0.80	1.00	1.19	0.18	NS
20:4*n-6*	14.1	14.1	14.5	0.78	NS
20:5*n-3*	2.77	4.15	3.55	0.33	**
22:5*n-3*	3.03	2.79	3.19	0.31	NS
22:6*n-3*	5.56	4.23	4.72	0.54	NS

[1] ** $P \leq 0.01$ NS not significant

Table 3.19 - The fatty acid composition (major fatty acids, percentage of total present) of the phospholipid fraction of the liver in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	26.80	18.04	16.10	4.92	NS
16:1*n-7*	1.95	1.59	1.64	0.34	NS
18:0	12.74	8.81	9.24	2.95	NS
18:1*n-9*	16.86	17.48	17.75	2.29	NS
18:2*n-6*	31.87	43.48	45.17	6.14	NS
18:3*n-3*	1.17	1.06	1.20	0.46	NS
20:3*n-6*	0.25	0.18	0.16	0.08	NS
20:4*n-6*	3.52	2.72	3.26	0.49	NS
20:5*n-3*	0.98	1.45	1.63	0.38	NS
22:5*n-3*	0.71	0.12	0.19	0.30	NS
22:6*n-3*	0.25	0.06	0.12	0.14	NS

[1] NS not significant

Table 3.20 - The fatty acid composition (major fatty acids, percentage of total present) of the cholesterol ester fraction of the liver in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

	2SFOD	4SFOD	6SFOD	SED	Signif. level[1]
16:0	15.60	13.34	13.78	1.21	NS
16:1*n-7*	1.99	2.13	2.17	0.35	NS
18:0	21.30	25.84	29.41	2.76	*
18:1*n-9*	15.56	13.51	12.82	0.87	*
18:2*n-6*	27.28	21.48	19.89	1.71	**
18:3*n-3*	2.79	2.11	1.79	0.41	NS
20:3*n-6*	0.67	0.76	0.82	0.13	NS
20:4*n-6*	7.83	9.95	9.51	1.14	NS
20:5*n-3*	1.94	4.56	3.82	0.64	**
22:5*n-3*	1.90	2.67	2.48	0.33	NS
22:6*n-3*	2.18	2.59	2.50	0.26	NS

[1] * $P \leq 0.05$ ** $P \leq 0.01$ NS not significant

Table 3.21 - The fatty acid composition (major fatty acids, percentage of total present) of the free fatty acid fraction of the liver in the pigs fed the soybean oil/fish oil diet (SFOD); shorthand designations as per Table 2.1 (fatty acids) and Section 3.2.2 (treatments)

eicosapentaenoic acid.

Within the cholesterol ester, by far the major fatty acid present was linoleic acid followed by palmitic and oleic acids. Levels of polyunsaturates were very much lower than that displayed by both the triacylglycerol and phospholipid. Duration of fish oil feeding had no effect on fatty acid composition. In common with triacylglycerol and phospholipid fractions, free fatty acid displayed high levels of C20 and C22 polyunsaturates. Fish oil feeding reduced significantly the proportions of oleic and linoleic acids and increased that of stearic and eicosapentaenoic acids.

3.4. DISCUSSION

It is clear from the results that several aspects of pig performance were improved by the fish oil diet. These differences cannot be attributed to the relative energy levels of the diets as the latter were isoenergetic. However, a possible explanation for these differences may be in considering the type of dietary oil which was used in the basal and experimental diets, namely the soybean oil *vs.* fish oil. Several investigations have recorded a benefit in terms of pig growth parameters of feeding a more unsaturated fat. For example, Suomi *et al* (1993) observed that the addition of hydrogenated sunflower oil to pig diets conferred a significantly lower daily gain and FCE compared to the more unsaturated unhydrogenated oil. Improvements in pig performance have also been observed following the consumption of 'long'- as opposed to 'medium'- chain triacylglycerols (Takada *et al*, 1992). In contrast however, Allee *et al* (1972) noted that an isocaloric addition of 10% fat to pig diets, regardless of whether the fat source was lard, tallow, coconut oil or corn oil, resulted in a significant improvement in daily gain and gain/feed ratio. In further contrast to these observations, Viljoen and Ras (1991) reported that the average daily liveweight gain of pigs fed tallow and sunflower oil was significantly lower than for pigs fed a fat-free diet. Based on known principles of nutrition, the latter observation is extremely hard to explain. There has been a whole range of observations in which no significant differences in performance were observed between pigs fed isoenergetic diets but containing different fat levels and differing fatty acid compositions (Leszczynski *et al*, 1992a; Morgan *et al*, 1992; Valaja *et al*, 1992; Prescott and Wood, 1988; Seerley *et al*, 1978; McDonald and Hamilton, 1976). The well-established effect of sex on feed conversion rate observed presently contrasts with previous work (Viljoen and Ras, 1991) in which no differences in FCE between boars and gilts were recorded.

It has been observed that increasing the fat intake of the pig leads to a significant reduction in lean muscle weight (Allee *et al*, 1972). In the present instance, the small differences in total fat intake were presumably insufficient to affect the weights of ham and loin joints. However, backfat thickness increased with increasing duration of the

fish oil diet, a feature which is consistent with the higher total lipid content of the fish oil diet and the improved growth rate observed with increasing length of feeding. This contrasts with the observations of Suomi *et al* (1993) who failed to observe any differences in backfat thickness between pigs fed diets with widely differing fat sources. The lack of any difference in killing out proportion between the pigs fed the fish oil diets for varying periods conforms with previous observations based on pigs which were fed diets that differed considerably in their lipid and fatty acid compositions (Morgan *et al*, 1992; Suomi *et al*, 1993; Allee *et al*, 1972).

The fact that outer and inner backfat contained the highest amounts of lipid, their levels being much higher than those displayed by the *Longissimus dorsi*, *Semitendinosus* and liver, conforms to the expected. Levels of total fat within the backfats and muscle tissues were in general similar to those observed by other workers (Leszczynski *et al*, 1992; Cameron *et al*, 1990; Wood and Lister, 1973). Where differences have been observed, they have been effected through extremes of treatment in terms of total fat intake or as a result of breed differences which affect the rate of fat deposition (Harris *et al*, 1993). Only outer backfat showed any change in lipid content as a result of increasing the duration of feeding the fish oil diet. This effect may be attributed to both changes in the total lipid content of the diet ('quantity effect') and to differences in the level of unsaturation of the dietary fat ('quality effect'). Thus, it has been observed (Leat *et al*, 1964) that the proportion of backfat in pigs was increased by raising the level of unsaturation in the diet. It was proposed that in pigs there is a tendency for a differential deposition of unsaturated dietary fat between subcutaneous and intermuscular regions. The increase in total lipid content presently observed in the outer backfat may also be related to the recorded positive association between lipid content and backfat thickness (Wood *et al*, 1989). With respect to any effect on the fat content of *Semitendinosus* and *Longissimus dorsi* as a result of fish oil feeding, results obtained previously are at considerable variance with each other. For instance, Jurgens *et al* (1970) reported that pigs fed safflower oil contained significantly more intramuscular fat than pigs fed coconut oil whilst by contrast Seerley *et al* (1978) and Allee *et al* (1972) were unable to observe any effect.

The observation that the backfat tissue lipid was almost wholly comprised of triacylglycerol not only conforms with the result which would be expected but is indicative that the backfats were not exposed to any degradation at the time of analysis. In general terms, the distribution of the lipid fractions within the *Semitendinosus* and *Longissimus dorsi* conformed to that expected for muscle tissues in general (Pikul *et al*, 1984). Although observations to date have not reported any differences in the distribution of triacylglycerol and phospholipids between the *Semitendinosus* and *Longissimus dorsi* in pigs, there is evidence that site exerts a significant effect on muscle lipid composition in other domesticated species (Decker and Cantor, 1992; Pikul *et al*, 1984). There exist, however, several references in which dietary treatments

in pigs have affected cholesterol levels in tissues (Harris *et al*, 1993; Leszczynski *et al*, 1992b). It is of interest to note the work of Ranhotra *et al* (1992) in which hypercholesterolaemic rats were fed diets containing either flax seed oil (rich in *n-3* fatty acids) or sunflower oil (rich in *n-6* fatty acids) or mixtures of the two. Serum total cholesterol levels were reduced foremost by flax seed/sunflower oil blends, followed by single flax seed oil- and sunflower oil-containing diets. Kim *et al* (1992) also observed that feeding certain mixtures of *n-3*-rich sardine oil and *n-6*-rich safflower oil had beneficial effects on serum lipid profiles in rats. In all animal species, liver is characterised by high levels of phospholipids. Based on similar feeding trials with pigs and other animal species, there is no obvious explanation for the increase in proportion of phospholipid presently observed within the liver with increasing length of fish oil feeding, especially as there were no major changes in the fatty acid composition of the liver phospholipids.

As would be expected, there were considerable differences in fatty acid composition between the backfat, muscle and liver. Similarly, there were extensive differences in fatty acid compositions between the lipid fractions both in any particular tissue and between tissues. Furthermore, there were several obvious differences in the fatty acid composition between the two muscle tissues sampled. Whereas little difference has been observed in total phospholipid fatty acid composition in muscle from different sites in cattle or sheep (Christie, 1978), in avians bred specifically for meat production obvious differences have been recorded (Pikul and Kummerow, 1989). The normal dietary regime of the pig is based upon a predominance of saturated and monounsaturated fatty acid species which is reflected in the resulting fatty acid composition of the tissues (Morgan *et al*, 1992). The most obvious feature of the present results with respect to the fatty acid compositions of the backfat, muscle and liver tissues was the extremely high contents of polyunsaturated fatty acids attained within each basically comprised of linoleic acid but accompanied also by enhanced levels of C20 and C22 polyunsaturated fatty acids. Thus, in the case of a normal tallow-fed pig, no more than 15% of linoleic acid is found within the backfat (MLC, 1991; Viljoen and Ras, 1991; Madsen *et al*, 1990; Wood, 1973; Christie *et al*, 1972; and see Table 3.22) whereas throughout the present experimentation the level of linoleic acid within the backfat was upwards of 29% of total fatty acids present. This increase in the polyunsaturated fatty acid level consistently occurred at the expense of proportions of oleic acid throughout the tissues (*c.f.* Monahan *et al*, 1992; Brooks, 1971; Leat *et al*, 1964). The ability to achieve such high levels of linoleic acid within the tissues may be attributed to the high digestibility of this fatty acid (Wood, 1984; Brooks, 1971) aided to some extent by the ability of linoleic acid to inhibit the synthetic and desaturase enzymes involved in the formation of other fatty acids (Allee *et al*, 1972; Guarnieri and Johnson, 1970). Within the *L. dorsi*, the levels of linoleic acid were higher than that found in Hertzman *et al* (1988) using diets based on fishmeal,

	Outer backfat	Inner backfat	*Semitendinosus*		*Longissimus dorsi*		Liver	
	TAG[1]	TAG	TAG	PL[2]	TAG	PL	TAG	PL
16:0	21.4	21.8	22.4	26.8	25.3	26.7	26.9	11.5
16:1*n-7*	4.70	4.64	4.86	-	5.27	-	-	2.03
18:0	12.0	12.11	9.74	13.0	9.50	11.4	11.2	31.9
18:1*n-9*	46.2	46.3	51.2	19.7	51.6	21.0	34.6	15.0
18:2*n-6*	12.9	12.3	9.60	26.3	6.48	28.5	16.4	14.5
18:3*n-3*	2.28	2.02	1.23	0.44	0.85	0.41	0.82	0.25
20:3*n-6*	0.02	-	0.29	1.61	0.32	1.02	-	1.94
20:4*n-6*	0.23	0.21	0.21	8.59	0.55	9.05	6.19	17.4
20:5*n-3*	-	-	-	-	-	-	-	-
22:5*n-3*	0.12	0.13	0.10	1.63	-	1.80	1.22	2.45
22:6*n-3*	0.08	0.08	0.07	1.20	-	1.25	0.50	3.13

[1] TAG triacylglycerol fraction [2] PL phospholipid fraction

Table 3.22 - The fatty acid composition (major fatty acids, percentage of total present) of triacylglycerol and phospholipid fractions of tissues from pigs fed a tallow-based diet; based on Morgan, C.A. (unpublished data); shorthand designations of fatty acids as per Table 2.1

rapeseed and rapeseed meal. These workers, after feeding 21.4g linoleic acid/kg diet, found 13.3% linoleic acid in the backfat. In the present investigation, feeding 36.1 g/kg produced 30% linoleic acid in outer and inner backfat, signifying a 30% greater efficiency of deposition of linoleic acid in this study.

Accompanying this feature, there were also changes in the proportions of palmitic and stearic acids within the tissues although the extent of change was less prominent than for oleic. Throughout, levels of palmitic acid were reduced as a result of the fish oil diet compared to that demonstrated when tallow-based diets have been fed (Morgan *et al*, 1992). Changes in the levels of stearic acid were far less consistent than for palmitic acid. Clearly, relative proportions of the saturated fatty acids could most prominently be a reflection of differences in dietary intakes; also, they may reflect underlying mechanisms within the metabolism to accommodate the vast differences in unsaturated fatty acid levels via synthesis of a different range of lipid moieties and molecular species. The levels of palmitic acid presently observed in the backfat were consistent with comparable dietary treatments as a result of which it was concluded that adipose tissue levels of palmitic acid basically reflect those of dietary intake as also recorded by Suomi *et al* (1993); Morgan *et al* (1992); Monahan *et al* (1992); Viljoen and Ras (1991); Madsen *et al* (1990); West and Myer (1987); Whittington *et al* (1986); Wood (1973) and Christie *et al* (1972). In contrast to the present observations, a number of workers has recorded no differences in the levels of palmitic acid within the muscle (*Longissimus*) between pigs fed diets containing very different fatty acid compositions (Leszczynski *et al*, 1992b; Seerley *et al*, 1978; Koch *et al*, 1968; Greer *et al*, 1965). Compared to palmitic acid, it has generally been concluded that tissue levels of stearic acid reflect poorly dietary intakes (Morgan *et al*, 1992; Roberts and Enser, 1988; Whittington *et al*, 1986; Koch *et al*, 1968). The present observations are generally in accord with this. In the case of the liver, it was apparent that any large changes effected with respect to palmitic and stearic acid occurred most prominently in the triacylglycerol fraction.

The deposition of long chain *n-3* polyunsaturated fatty acids has been shown by other workers to be influenced by both their concentration in the diet and the duration of feeding (Taugbøl, 1993; Morgan *et al*, 1992; Monahan *et al*, 1992; Valaja *et al*, 1992). The present investigation demonstrated a similar dose-dependent effect on *n-3* fatty acid levels. The increased levels of the C20 and C22 polyunsaturated fatty acids were brought about primarily at the expense of linoleic acid. This conforms with the observations of Irie and Sakimoto (1992) who attributed an observed decrease in adipose tissue linoleic acid to the preferential deposition of dietary *n-3* polyunsaturated fatty acids. The changes between levels of linoleic acid and C22 polyunsaturated fatty acids may also be related to the observed increase in backfat thickness (see Table 3.5). Whittington *et al* (1986) showed that there was a significant negative correlation between the proportion of linoleic acid in the backfat and its thickness. Clearly, the

most obvious explanation for these differential effects between linoleic acid and the C20/C22 polyunsaturated fatty acids is that of competitive exclusion with respect to esterification within the lipid fraction.

Although it was not significant, there was a trend for tissue levels of arachidonic acid to also accompany the reduction in linoleic acid. A number of workers (Irie and Sakimoto, 1992; Monahan *et al*, 1992; Cameron and Enser, 1991; Leat *et al*, 1964) has noted a clear dependence of adipose tissue and intramuscular fat arachidonic acid levels on dietary arachidonate and/or its metabolic precursor, linoleic acid. Other workers have noted a direct arachidonic acid-lowering effect of dietary fish oil. Thus, Morgan *et al* (1992) detected significant decreases in arachidonic acid levels in pig tissues following fish oil feeding in spite of a similarity of arachidonic and linoleic acid levels in the respective diets. The conversion of linoleic acid to arachidonic acid is subject to inhibition by an increased presence of *n-3* acids (Hwang *et al*, 1988). It is therefore not surprising that the markedly higher levels of these fatty acids in the fish oil diet of the present experiment would not be without effect on arachidonic acid levels.

With respect to contemporary interest in the enhancement of *n-3* fatty acid levels within animal tissues for human consumption, it is clear from the present and other observations (Morgan *et al*, 1992; Irie and Sakimoto, 1992) that their levels can be manipulated to a high degree. With regard to the value or otherwise of the long chain polyunsaturates in human dietary components, it has been the recent policy to express them in terms of ratios of particular fatty acids or groups of fatty acids. Thus, it has been common practice for several years (COMA, 1991) to use the ratio of total polyunsaturates to total saturates (P:S ratio). More recently, with the increasing recognition of the *n-3* polyunsaturates in particular, it has been found useful to quote the ratio of the total *n-6* acids to the total of the *n-3* acids. With respect to this ratio the British Nutrition Foundation (1992) have stated that this ratio be restricted to that between linoleic acid (the major *n-6* acid) to α-linolenic acid (the major *n-3* acid) whilst other work has chosen to express this in terms of the total *n-6* to total *n-3* ratio (Galli and Simopoulos, 1989; see Section 1.6).

Tables 3.23 and 3.24 show the P:S and *n-6*:*n-3* fatty acid ratios, respectively, obtained for the backfat, muscle and liver tissues in the present work. With respect to the *n-6*:*n-3* ratios, feeding the fish oil diets effected highly significant changes in the backfat and muscle tissues but no change in the liver. As can be seen, increasing the duration of feeding produced further reduction of the ratio. Greater changes in the ratio were observed in the phospholipid fraction compared to the triacylglycerol. This accords with the observation that, consistent with its structural role, phospholipid contains more polyunsaturated fatty acids than triacylglycerol. In contrast to the present results, the phospholipid fractions from tallow-fed pigs demonstrated very high *n-6*:*n-3* ratios within the muscle tissues (see Table 3.22), e.g. *Semitendinosus* and *Longissimus dorsi* 11.2 and 11.1, respectively. It has been suggested by the British

Tissue	TRIACYLGLYCEROL					PHOSPHOLIPID				
	2SFOD	4SFOD	6SFOD	SED	Signif.[1]	2SFOD	4SFOD	6SFOD	SED	Signif.[1]
Outer backfat	1.7	1.2	1.3	0.1	*	-	-	-	-	-
Inner backfat	1.6	1.1	1.2	0.1	*	-	-	-	-	-
Semitendinosus	1.1	1.0	1.1	0.1	NS	1.4	1.4	1.5	0.03	**
Longissimus dorsi	0.7	0.6	0.7	0.1	NS	1.5	1.5	1.5	0.04	NS
Liver	2.4	2.0	2.0	0.2	NS	1.2	1.2	1.1	0.03	NS

[1] *, ** significant differences, respectively, at P≤0.05 and P≤0.01 NS not significant

Table 3.23 - The ratios of polyunsaturated to saturated fatty acids (P:S ratio) in the tissues of the pigs fed the soybean oil/fish oil diet; shorthand designations of treatments as per Section 3.2.2

Tissue	TRIACYLGLYCEROL					PHOSPHOLIPID				
	2SFOD	4SFOD	6SFOD	SED	Signif.[1]	2SFOD	4SFOD	6SFOD	SED	Signif.[1]
Outer backfat	7.5	6.2	6.2	0.3	**	-	-	-	-	-
Inner backfat	7.3	6.3	6.2	0.3	**	-	-	-	-	-
Semitendinosus	6.9	6.4	6.0	0.3	*	7.7	6.9	6.3	0.3	**
Longissimus dorsi	6.3	6.1	5.7	0.2	*	7.9	7.0	5.9	0.4	**
Liver	4.7	4.1	4.1	0.3	*	3.0	3.0	3.0	0.2	NS

[1] *, ** significant differences, respectively, at P≤0.05 and P≤0.01 NS not significant

Table 3.24 - The ratios of *n-6* to *n-3* fatty acids in the tissues of the pigs fed the soybean oil/fish oil diet; shorthand designations of treatments as per Section 3.2.2

Nutrition Foundation that an optimum *n-6:n-3* ratio is of the order of 6:1. As can be seen from Table 3.24, the ratio obtained presently from feeding the fish oil diets were near to the optimal ratio. In contrast to both the backfat and muscle tissues, the *n-6:n-3* ratio of the liver was considerably lower. Nevertheless, such a ratio is offset to a large extent by the fact that the liver is by far the richest source in qualitative terms for the C20 and C22 *n-3* acids.

With respect to the polyunsaturated:saturated fatty acid (P:S) ratio, the current recommended value for dietary fat intake lies at 1.00 (NACNE, 1983). The present feeding of the fish oil diets resulted in significant reductions in the P:S ratio within the backfat and muscle. In general, all the tissues displayed a P:S ratio near to the optimum recommended taking into account relative proportions of the major lipid components. Table 3.25 shows the summary characteristics with respect to total proportions of saturates (SAT), monounsaturates (MUFA), polyunsaturates (PUFA) and the *n-6:n-3* and P:S ratios of outer backfat/subcutaneous adipose tissue in pigs as observed presently and by other workers. There exists widespread agreement between the levels of SAT, MUFA and PUFA in fat from pigs fed 'control' or 'tallow' diets. Thus, levels of SAT, MUFA and PUFA consistently approximated to 35, 50 and 15, respectively. In all cases where modified fat diets were fed there was a considerable alteration in this distribution, the degree and direction of change differing as a result of both the level and type of dietary fat addition. The P:S ratios in Irie and Sakimoto (1992) and Rhee *et al* (1988) are more similar to tallow-fed pigs than to the present work or that of Monahan *et al* (1992). Circumstances in which there has been an 'overkill' with respect to improving the *n-6:n-3* ratio exist; for instance, Irie and Sakimoto (1992) produced backfats with ratios of 3.1 and 1.8, respectively, in pigs fed diets containing 2% and 6% sardine oil. Thus, amongst contemporary work involving specific feeding of polyunsaturated fatty acids to pigs, the greatest perceived health benefit would accrue from the consumption of the fish oil diet which was fed in the present investigation for six weeks.

Table 3.26 shows the overall fat composition of fat separable from the *Semitendinosus* muscle and the inner backfat. Within the *Semitendinosus*, the absolute amount of total polyunsaturated fatty acids (0.9 g/100 g) is three times that recorded by Holland *et al* (1991) for raw cod (0.3 g/100 g), and one-third of that for raw mackerel (3.3 g/100 g). The relatively high levels of both the *n-6* and *n-3* polyunsaturates recorded within the fat tissue highlights the important contribution which could be made to the levels of these fatty acids in trimmings for meat product preparation. Such products (e.g. sausages, meat pies) ordinarily contain upwards of 24% total fat, a level of fat which is much higher than that occurring within fresh pork as consumed. With respect to the levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), it is estimated that a *Longissimus dorsi* chop from the 6SFOD treatment and comprising 100 g lean + 10 g fat could supply 0.08 g EPA+DHA; similarly, a 50 g

Pigs fed control/tallow diets				
	Morgan *et al* (1992)	**Rhee *et al* (1988)**	**McDonald and Hamilton (1976)**	**Koch *et al* (1968)**
Total SAT	35	35	36	35
Total MUFA	50	56	50	51
Total PUFA	15	9	13	13
P:S[1]	0.46	0.25	0.36	0.37
Pigs fed modified fat diets				
	6SFOD	**Monahan *et al* (1992)**	**Irie and Sakimoto (1992)**	**Rhee *et al* (1988)**
Fat level and source	5% soya oil/1% fish oil	3% soya oil	4% fish oil	12% high 18:1 sunflower oil
Total SAT	28	34	41	19
Total MUFA	37	36	41	72
Total PUFA	36	30	17	9
P:S	1.3	0.88	0.43	0.47
n-6:n-3[2]	6.2	4.9	2.3	nd[3]

[1] polyunsaturated to saturated fatty acid ratio [2] ratio of total *n-6* to total *n-3* fatty acids
[3] not determinable from the results

Table 3.25 - Comparison of fatty acid characteristics of pig outer backfat: published data *versus* present findings

	Semitendinosus	**Inner backfat**
Per 100 g Fat, of which:	2.4 g	76.5 g
Saturated	0.7	22
Monounsaturated	0.7	27
Polyunsaturated	0.9	28
of which:		
n-6	0.8	24
n-3	0.1	4

Table 3.26 - Composition of the separable fat from the *Semitendinosus* and inner backfat from the pigs on the 6SFOD treatment

sausage prepared from the meat and fat tissues and comprising 26% total fat could supply 0.10 g EPA+DHA. Consumption of these products could therefore make a valuable contribution to the intake of 0.3 to 1.0 g EPA+DHA/day which has been recommended (Barlow *et al*, 1990; Galli and Simopoulos, 1989). However, the actual amounts consumed are likely to be influenced by a range of factors including differences in total fat contents, fat losses in cooking as well as the amounts of fat which are trimmed away 'on the plate'.

As previously outlined, a major drawback for the inclusion of polyunsaturates in the diet of the pig is that arising from a deterioration in backfat firmness. Firmness of the backfat as measured by standard penetrometry was corrected to values at 1 °C. After adjusting for the effects of the pre-trial pig liveweights, left-side carcass weights and fat thickness (P2), the results obtained were, respectively, 687.7, 748.2 and 728.4 for the two, four and six week dietary treatments. It is interesting to note that increasing the time of fish oil feeding significantly enhanced backfat firmness ($P \leq 0.02$; standard error of the differences between the means: 11.5), even allowing for factors which could influence the results. All of the values were above the critical level of 629 units below which the fat has been described as unacceptably 'soft' (Dransfield and Kempster, 1988). Furthermore, there were no observations of soft or oily fat after subjective inspection by professional visual observation. Such results were highly unexpected in view of the extremely high polyunsaturated fatty acid content of the backfat, in particular linoleic acid levels, and run contrary to the established dogma of unsaturation and physical properties of backfat (Maynard and Loosli, 1956). Up to the present date, a correlation between fluidity and backfat polyunsaturated fatty acid content has been observed. Thus, unacceptably soft backfat has been observed at linoleic acid levels of 19% and 43% (Suomi *et al*, 1993), 25% (West and Myer, 1987) and 39% of total fatty acids (Christensen, 1964). Prescott and Wood (1988) identified a 133 mg linoleic acid/g fat (i.e. approximately 13%) critical point beyond which undesirable fat-softening could occur. Similarly, Whittington *et al* (1986) stated a maximum advisable linoleic acid level of 15% in bacon fat in response to concerns over fat softening. The fact that in the present investigation there were no adverse physical characteristics of fat containing linoleic acid concentrations which were well above such 'critical' levels is surprising. This may be attributed to the enforced rearrangement of fatty acids in the triacylglycerols of the fat which is able to offset any physical changes expected from a straightforward consideration of the increased levels of polyunsaturates. An explanation may therefore be found in considering the relationship between the physical properties and the triacylglycerol structural characteristics of the fat. Furthermore, there exists the need to determine the effects which the increased presence of polyunsaturated fatty acids, as well as the presence of pro- and antioxidant substances, may have on the resulting fatty acid composition and sensory quality of meat from pigs fed the modified diet.

4. The effect of alterations in dietary vitamin E and copper on the fatty acid composition and olfactory characteristics of tissues of pigs fed a soybean oil/fish oil diet

4.1. INTRODUCTION

In the previous experiment, the fatty acid content of pig tissues was modified in accordance with human dietary recommendations. In brief, the levels of saturated, monounsaturated and polyunsaturated fatty acids, particularly the long chain *n-3* polyunsaturated fatty acids, and ratios of specific fatty acids in porcine tissues were significantly altered by the inclusion in the diet of soybean oil and fish oil. These changes were wrought in the absence of deleterious effects on the firmness of the backfat.

The presence in the tissues of increased levels of polyunsaturated fatty acids raises the possibility of increased lipid peroxidation (Rhee *et al*, 1988b; Dahle *et al*, 1962). Consequently, the British Nutrition Foundation (1992) recommended that any increase in the consumption of polyunsaturated fatty acids should be accompanied by increases in the intake of antioxidant nutrients. Vitamin E is the principal antioxidant nutrient currently used in animal feeds. Supplementation of diets with vitamin E has resulted in significant increases in the oxidative stability of poultry (Sheehy *et al*, 1993) and pig (Monahan *et al*, 1990) meats. As indicated previously, fish oil is characterised by high levels of eicosapentaenoic acid and docosahexaenoic acid which, due to the presence of high unsaturation, are extremely susceptible to peroxidative deterioration. The enhancement of long chain polyunsaturated fatty acid levels in pig tissues therefore incurs a greater threat of the development of oxidative rancidity. Other factors in the diet may contribute to the development of oxidative rancidity. One important feed additive used commonly in commercial pig feeds as a growth promoter is inorganic copper as Cu^{2+} (Bowland, 1990). The addition of copper to pig diets has been associated with increases in the proportions of unsaturated fatty acids in the depot fat (Amer and Elliot, 1973a; Moore *et al*, 1969; Christie and Moore, 1969). It may be inferred therefore that there is likely to be a consequential decrease in the oxidative stability of the fat with a potential for the development of unacceptable odours (Klaus *et al*, 1995).

It was observed in the previous experiment that the fatty acid composition of porcine tissues could be brought nearer to the optimum with respect to human dietary requirements after feeding a soybean oil/fish oil diet for six weeks preceding slaughter. This diet contained a low level of copper (approximately 20 mg/kg dry matter; see Table 3.2) but a relatively high level of vitamin E (94 μg/g; see Section 3.2.1.2) which, according to the rationale proposed, would be conditions for a low level of lipid peroxidation. In the converse situation, i.e. feeding a high level of copper and low level of vitamin E, deleterious effects on the quality of the resulting meat could be envisaged. The investigation now being reported was an attempt to evaluate the effects of alterations of dietary vitamin E and copper on the fatty acid composition and physical and sensory properties of the tissues of pigs fed the soybean oil/fish oil diet which was

fed in the previous experiment.

4.2. MATERIALS AND METHODS

4.2.1. Composition of diets

The ingredient compositions of the diets used in this experiment are presented in Table 4.1 and the proximate and mineral compositions of the diets are shown in Table 4.2. The copper and vitamin E contents of the diets were determined according to the methods described in Sections 2.4 and 2.5.1, respectively. Diets were based on barley and soybean meal and contained similar levels of soybean oil (35 g/kg) and fish oil (9.5 g/kg) but differed in their levels of copper (Cu^{2+}, cupric) sulphate and vitamin E (α-tocopheryl acetate). The levels of copper designated as 'low' and 'high' were nominally 20 and 100 mg/kg, respectively. Although in general terms the levels of copper within the diet reflected to a large degree the amount of copper added as a supplement, the overall difference was not as great as was intended. Thus, the mean levels of copper in the low and high level diets were 28 and 78 mg/kg, respectively. No obvious reason for this discrepancy could be found. Unfortunately, a similar discrepancy occurred between the intended and actual levels of vitamin E in the diets. Thus, the actual levels of vitamin E in the low and high level diets were 75 and 375 mg/kg. In the case of vitamin E however the reason for the difference was found to arise from the mistaken over-supplementation at compounding. The contents of total fat and linoleic acid were determined according to the methods described previously (see Section 2.6). The contents of total fat, linoleic acid, crude protein and mineral and trace elements for all diets were broadly similar and approximated to those recorded for the soybean oil/fish oil diet in Experiment 1 (see Tables 3.2 to 3.4).

4.2.1.1. Fatty acid composition

The fatty acid compositions of the diets were determined according to the procedure described in Section 2.6 and are shown in Table 4.3. The diets contained a relatively high proportion of linoleic and α-linolenic acids due to the inclusion of a significant amount of soybean oil (see Table 3.1 for fatty acid composition of soybean oil). Consequently, the overall content of polyunsaturated fatty acids approached 60% of total fatty acids. The levels of eicosapentaenoic acid and docosahexaenoic acid were high in all of the diets due to the presence of the fish oil.

	LL	HL	LH	HH
Description	Low Cu-Low Vit. E	High Cu-Low Vit. E	Low Cu-High Vit. E	High Cu-High Vit. E
Barley	624.6	624.3	617.1	616.8
Soybean meal	271	271	271	271
Dicalcium phosphate	8.9	8.9	8.9	8.9
Fish meal	25	25	25	25
Min/vit suppl.	20	20	20	20
Wafolin	6	6	6	6
Soybean oil	35	35	35	35
Boost oil	9.5	9.5	9.5	9.5
Copper added (mg/kg)	20	100	20	100
Vitamin E suppl. (IU/kg)	75	75	375	375
Total fat	63.0	63.2	63.2	66.6
Linoleic acid	28.7	29.9	29.8	29.5

Table 4.1 - Ingredient composition of the soybean oil/fish oil diets differing with respect to the contents of copper and vitamin E (g/kg, unless stated otherwise)

Component	LL	HL	LH	HH
Dry matter	859	861	861	866
Digestible energy (MJ/kg DM)	15.5	15.1	15.0	14.8
Crude protein	238	240	238	240
Ash	74	74	80	81
Acid hydrolysed ether extract	71.1	55.8	72.6	59.1
Crude fibre	54	48	49	51
Neutral detergent fibre	182	196	203	202
Calcium	14.8	14.1	16.4	16.7
Phosphorus	8.7	8.6	8.3	8.1
Sodium	1.5	1.5	1.4	1.5
Copper (mg/kg DM)	28.1	63.5	28.9	92.5
Zinc (mg/kg DM)	148	140	136	137
Iron (mg/kg DM)	302	253	267	288
Selenium (mg/kg DM)	0.35	0.34	0.34	0.32

Table 4.2 - The proximate and mineral compositions of the experimental diets (g/kg dry matter, DM, unless stated otherwise)

	LL	HL	LH	HH
14:0	1.23	1.19	1.17	1.21
16:0	15.7	14.6	14.5	15.0
16:1*n-7*	1.95	1.83	1.85	1.88
18:0	3.79	3.76	3.71	3.64
18:1*n-9*	21.0	20.5	20.4	23.9
18:2*n-6*	45.5	47.4	47.1	44.3
18:3*n-3*	6.67	6.61	7.08	5.53
20:3*n-6*	0.10	0.11	0.10	0.12
20:4*n-6*	0.19	0.17	0.17	0.18
20:5*n-3*	2.27	2.33	2.35	2.67
22:5*n-3*	0.24	0.25	0.26	0.29
22:6*n-3*	1.30	1.30	1.35	1.32
Total SAT	21	19	19	20
Total MUFA	23	22	22	26
Total PUFA	56	58	58	54
P:S[1]	2.7	3.0	3.0	2.7
U:S[2]	3.8	4.1	4.2	4.0
n-6:n-3[3]	4.4	4.5	4.3	4.5

[1] polyunsaturated to saturated fatty acid ratio; [2] unsaturated to saturated fatty acid ratio
[3] ratio of total *n-6* to *n-3* fatty acids

Table 4.3 - The fatty acid composition of the experimental diets (major fatty acids, percentage of total present); shorthand designations as per Table 2.1 (fatty acids) and Table 4.1 (treatments)

4.2.2. The pigs and their treatment

Twenty-four Large-White x Landrace pigs of mean liveweight 39 kg (±3.2 standard deviation) were randomly assigned to four groups, each comprising three entire males and three females and which were fed one of the diets described above for six weeks pre-slaughter. During the growth phase of the trial, pigs were weighed weekly for calculation of the rate of liveweight gain. A record was kept of the feed consumption of each pen. The four groups of pigs were started on trial at two different times, 'staggered' by one week, to allow sufficient time for full carcass evaluation of each pig after slaughter. Pigs were slaughtered at an overall mean liveweight of 76 kg (±7.8 standard deviation).

4.2.2.1. Carcass evaluation

Following slaughter, pigs were sent to the Carcass Evaluation Unit at the Scottish Agricultural College, Edinburgh, for removal of the outer and inner backfat, *Semitendinosus*, *Longissimus dorsi* and liver. Tissues were excised from the carcasses according to the method described previously (see Section 2.2). From each pig, a loin chop with the backfat layers left intact over the loin was sent to the Division of Food Animal Science (University of Bristol) for measurement of fat firmness by penetrometry, as described in Section 2.3.

4.2.3. Determination of the lipid and fatty acid compositions of the tissues

The lipid and fatty acid compositions of the tissues were determined as described in Section 2.6. For the outer and inner backfats, only the triacylglycerol fraction was of interest due to the fact that it alone accounts for over 98% of the total amount of lipid. In the remaining tissues, i.e. *Semitendinosus*, *Longissimus dorsi* and liver, only the phospholipid fraction was analysed for fatty acid composition as from previous observations this fraction has been shown to be associated with the highest level of unsaturation (see Section 3.3.3).

4.2.4. Determination of the levels of copper and vitamin E in the diets and tissues

The levels of vitamin E in the *Semitendinosus*, *Longissimus dorsi* and liver were determined by high performance liquid chromatography as described in Section 2.5. The level of inorganic copper within the same tissues was determined as described in section 2.4.

4.2.5. Olfactory sensory analysis

Samples of cooked outer backfat underwent sensory evaluation by trained panellists under the supervision of Dr. Sue Marie at the Sensory Laboratory of the Meat and Livestock Commission (MLC), Winterhill House, Milton Keynes, England. The method employed for the sensory evaluation of the fat was as follows:

4.2.5.1. Samples

Fat samples weighing 50 g each were placed in individual 11 cm diameter coded screw-cap jars and cooked from the frozen state in a 650 Watt microwave oven at 70% of full power for 9.5 minutes. Caps were loosely fitted during microwaving. The jars were then placed on hot plates, one per booth, in the sensory testing room.

4.2.5.2. Panellists

The panellists included six women and three men of varying ages who were trained by the MLC and experienced in the sensory assessment of beef, pork and lamb. The panellists generated their own vocabulary to describe the odours of these samples and agreed upon 17 attributes that, for them, would fully describe and discriminate between the samples.

4.2.5.3. Procedure

Both the effect of diet and the effect of frozen storage time on the olfactory characteristics of the outer backfat were investigated. Samples of backfat were taken from two of the experimental groups, namely the high copper/low vitamin E (HL) group and the low copper/high vitamin E (LH) group, and were stored at -18 °C for two and six months. Of the twelve samples at each storage time, the six from male animals were panelled on one occasion and the six from female animals on another, separated in time by 48 and 24 hours, respectively. In this way, the variation between samples, except those of experimental interest, i.e. diet and storage time, would be minimised. A practice session preceded each set of two testing sessions at each time period. At each session, panellists removed the lid of each jar, nosed the contents and rated each of the attributes on an intensity scale of 1 (low) to 8 (high). As a means of counterbalancing order effects, the order of sample presentation to each panellist was varied according to a partial Latin-square design.

4.2.6. Statistical Analysis

Data were analysed using the Genstat statistical package (Genstat 5, Release 1.3 Lawes Agricultural Trust, Rothamstead, England) using analysis of variance (one way).

In the analysis of the sensory data, a statistical package employing the restricted maximum likelihood (REML) model was used. This took into account differences between panellists and animals (random effects) and tests for differences between diets and storage times (fixed effects) as well as any interactions between these fixed effects.

4.3. RESULTS

4.3.1. Pig performance and carcass evaluation

The growth performance and carcass composition of the pigs fed the experimental diets are shown in Table 4.4. The feed intake was a reflection of the intake of the pen as opposed to that of each pig as it was not possible to make an individual record of intake. The daily feed intake was slightly higher for the two high vitamin E diets than for the low vitamin E diets. The rate of liveweight gain of the pigs approximated to 0.90 kg/day and was not significantly different between the dietary treatments. Males had a significantly higher daily liveweight gain than females (0.95 *vs.* 0.83 kg/day, data not shown). There was no effect of alterations in either dietary copper or vitamin E on the growth performance or weights of major cuts of the pigs.

4.3.2. Copper and vitamin E contents of the tissues

The levels of copper, zinc and iron in the *Semitendinosus*, *Longissimus dorsi* and liver of the pigs fed the low or high level of copper are presented in Table 4.5. The highest levels of copper, zinc and iron were displayed by the liver, followed by the *Semitendinosus* and *Longissimus dorsi* muscles. There were no significant effects of dietary copper level on the levels of copper, zinc or iron in these tissues.

The contents of vitamin E in the *Semitendinosus*, *Longissimus dorsi* and liver in the pigs fed the experimental diets are illustrated in Figure 4.1. The liver was characterised by a higher concentration of vitamin E than the muscle tissues which displayed similar contents of the vitamin. The levels of vitamin E in the *Semitendinosus* and *Longissimus dorsi* muscles were significantly higher in the pigs fed the high level of vitamin E than in those fed the lower level. In the liver, the HH treatment produced a significantly higher level of vitamin E than any of the other treatments.

4.3.3. Total lipid contents of the pig tissues

The total lipid contents of the tissues are shown in Table 4.6. The outer and inner

Parameter	LL	HL	LH	HH	SED[1]	Signif.[2]
Start weight (kg)	37.8	37.5	39.3	40.8	-	-
Finish weight (kg)	74.6	74.1	78.4	78.6	-	-
Weight gain (kg/d)	0.88	0.87	0.93	0.90	0.08	sex*
Feed intake (kg/d)	2.39	2.40	2.91	2.78	nd	nd
Whole carcass (kg)	57.3	61.8	60.4	61.9	3.05	NS
Carcass wt./Slaughter wt.	0.74	0.74	0.74	0.76	0.01	NS
Ham joint (kg)	9.1	9.7	9.5	9.7	0.65	NS
Loin joint (kg)	5.1	5.1	5.2	5.2	0.40	NS
Shoulder joint (kg)	7.9	7.9	8.6	8.6	0.58	NS
Flank (kg)	3.6	3.5	3.8	3.8	0.30	NS
Residual (kg)	20.2	22.6	21.3	21.7	1.17	NS
Waste (kg)	4.3	4.2	4.6	4.7	0.36	NS
Backfat thickness at P2 (mm)	6.8	9.0	8.2	8.5	1.01	NS
Fat firmness (penetrometer units)	789	728	669	715	64.0	NS

[1] SED standard error of the differences between the means

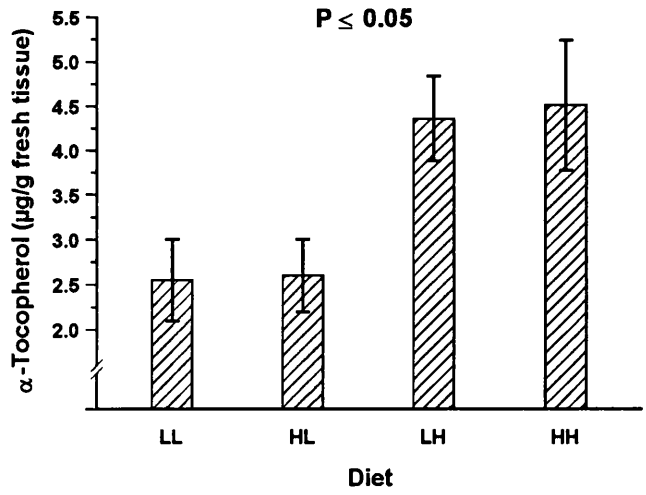
[2] * significant difference at P≤0.05 NS not significant nd not determinable

Table 4.4 - The growth performance and carcass composition of the pigs fed the experimental diets; shorthand designations of treatments as per Table 4.1

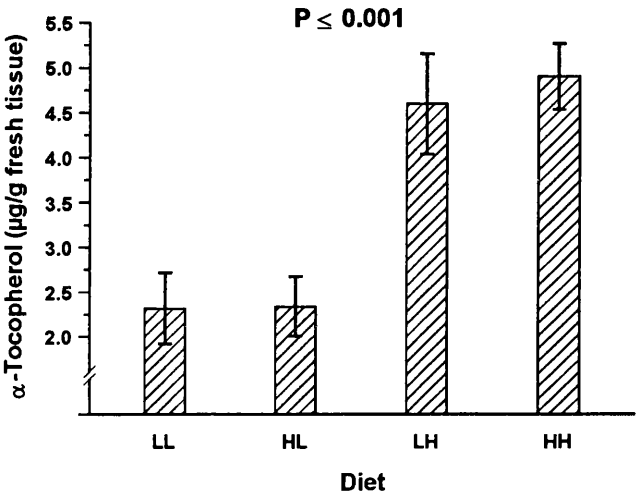
	Low Cu	High Cu	SED[1]	Significance[2]
Semitendinosus				
Cu	2.92	3.21	0.265	NS
Zn	85.9	88.1	5.21	NS
Fe	33.4	35.4	2.82	NS
Longissimus dorsi				
Cu	2.10	2.24	0.178	NS
Zn	71.0	78.8	4.28	NS
Fe	23.6	29.1	3.45	NS
Liver				
Cu	20.3	22.2	2.53	NS
Zn	204	198	9.9	NS
Fe	575	507	99.0	NS

[1] SED standard error of the differences between the means
[2] NS not significant

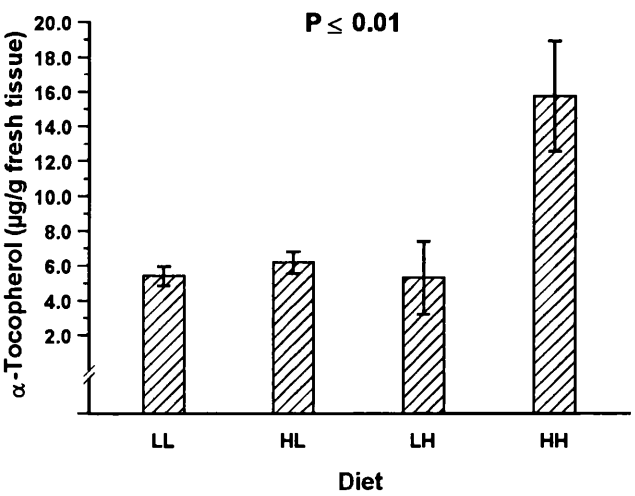
Table 4.5 - The content of the elements copper (Cu), zinc (Zn) and iron (Fe) in the *Semitendinosus*, *Longissimus dorsi* and liver in the pigs fed the low and high levels of copper (mg/kg tissue dry matter)



A.



B.



C.

Figure 4.1 - The content of α-tocopherol in the *Semitendinosus* (A), the *Longissimus dorsi* (B) and the liver (C) in the pigs fed the experimental diets; significance of diet effect as shown; shorthand designations of diets as per Table 4.1

Tissue	LL	HL	LH	HH	SED[1]	Signif.[2]
Outer backfat	68.2	71.8	67.3	70.4	2.92	sex** sex×Cu**
Inner backfat	62.8	65.8	54.6	61.8	4.99	NS
Semitendinosus	2.65	2.42	2.67	2.06	0.33	NS
Longissimus dorsi	2.71	3.05	3.03	1.90	0.50	NS
Liver	3.86	3.77	3.83	3.98	0.26	sex*

[1] SED standard error of the differences between the means

[2] * ** significant differences, respectively, at P≤0.05 and P≤0.01 NS not significant

Table 4.6 - The total lipid contents (g/100g tissue) of the porcine tissues as affected by the level of copper (Cu), vitamin E (Vit. E) and the sex of the animal; shorthand designations of diets as per Table 4.1

backfats were characterised by lipid contents in the region of 70 g/100 g with the outer backfat containing slightly more total lipid than the inner backfat. The *Semitendinosus* and *Longissimus dorsi* muscles contained appreciably less lipid, approximately 2.5 g/100 g, than the backfats. These figures are in accordance with those observed in Experiment 1 (see Section 3.3.2). The total lipid contents of the outer backfat and liver were significantly affected by the sex of the animal. In both tissues, females contained a greater amount of lipid compared with males. There was a significant interaction between sex and the level of copper. Males on the high copper diets had a significantly higher total lipid content than those on the low copper diets.

4.3.5. Fatty acid composition of the tissue lipid fractions

4.3.5.1. Outer backfat triacylglycerol

The fatty acid composition of the triacylglycerol from the outer backfat is shown in Table 4.7. Differences in the levels of fatty acids attributable to sex, the level of copper and/or the level of vitamin E are shown. Summations of the various classes of fatty acids and their ratios are also presented. The results showed a similarity in fatty acid content between the treatments and are similar to results obtained in Experiment 1 for the comparable (6SFOD) diet. In all treatments, the levels of oleic acid and linoleic acid were 33-35% and 26-27% of total fatty acids, respectively. Palmitic acid was the next most prevalent fatty acid followed in quantity by stearic acid and α-linolenic acid. Together, the C20 and C22 *n-3* polyunsaturated fatty acids accounted for approximately 1.5% of total fatty acids. The distribution of saturated, monounsaturated and polyunsaturated fatty acids was approximately 30, 38 and 32% of total fatty acids, respectively. The ratio of polyunsaturated to saturated fatty acids (P:S ratio) approximated to 1 whilst the ratio of linoleic to α-linolenic acid was approximately 7 in all of the treatments. The ratio of total *n-6* to total *n-3* fatty acids was roughly 5 between the treatments.

Several significant differences in the fatty acid composition of the outer backfat triacylglycerol were observed. The level of oleic acid was affected by the sex of the animal with females having a slightly higher level of this acid than males in all treatments (35.3 *vs* 33.2% of total fatty acids, data not shown). This difference was also reflected in the level of total monounsaturated fatty acids. The feeding of the high copper diets caused a lower level of α-linolenic acid to be present than in the low copper diet (3.90 *vs.* 3.60%, data not shown). The ratios of *n-6* to *n-3* fatty acids were markedly affected by the type of diet. For both ratios, the LL and HH diets gave rise to values which were significantly higher than the HL and LH diets.

	LL	HL	LH	HH	SED[1]	Significance[2]
14:0	1.31	1.36	1.34	1.32	0.067	NS
16:0	19.2	19.5	19.4	19.6	0.38	NS
16:1*n-7*	3.34	3.36	3.31	3.19	0.197	NS
18:0	8.86	9.13	9.17	9.61	0.758	NS
18:1*n-9*	34.0	35.2	33.2	34.8	1.35	sex*
18:2*n-6*	27.1	25.1	27.1	25.9	1.60	NS
18:3*n-3*	3.78	3.80	4.03	3.39	0.198	Cu* Cu×Vit. E*
20:3*n-6*	0.14	0.14	0.13	0.13	0.011	NS
20:4*n-6*	0.57	0.56	0.56	0.50	0.026	NS
20:5*n-3*	0.36	0.40	0.42	0.42	0.044	NS
22:5*n-3*	0.72	0.72	0.75	0.61	0.086	NS
22:6*n-3*	0.67	0.69	0.69	0.62	0.056	NS
Total SAT[3]	29	30	30	30	1.0	NS
Total MUFA[4]	37	39	36	38	1.4	sex*
Total PUFA[5]	33	31	34	32	1.9	NS
P:S[6]	1.1	1.1	1.1	1.0	0.09	NS
18:2*n-6*/ 18:3*n-3*	7.2	6.6	6.7	7.6	0.12	Cu* Vit. E** Cu×Vit. E***
n-6:*n-3*[7]	5.0	4.6	4.7	5.3	0.15	Cu×Vit. E***

[1] SED standard error of the differences between the means
[2] * ** *** significant effects, respectively, at P≤0.05, P≤0.01 and P≤0.001 NS not significant
[3] the weight percentage of total saturated fatty acids
[4] the weight percentage of total monounsaturated fatty acids
[5] the weight percentage of total polyunsaturated fatty acids
[6] polyunsaturated to saturated fatty acid ratio
[7] the ratio of total *n-6* to total *n-3* fatty acids

Table 4.7 - The fatty acid composition (major fatty acids, percentage of total present) of the outer backfat triacylglycerol as affected by the level of copper (Cu) and/or vitamin E (Vit. E) in the diet and the sex of the animal; shorthand designations of fatty acids and of treatments as per Table 2.1 and Table 4.1, respectively

4.3.5.2. Inner backfat triacylglycerol

The fatty acid composition of the triacylglycerol from the inner backfat is shown in Table 4.8. Overall, the fatty acid composition was similar to that in the 6SFOD treatment observed in the previous experiment (see Section 3.3.4.1) with the major fatty acids being oleic and linoleic acids followed by palmitic and stearic acids. Compared to the fatty acid composition of the outer backfat, the inner backfat contained a higher level of saturated fatty acids and a lower level of monounsaturated and polyunsaturated fatty acids. There was a number of effects of diet and sex on the inner backfat fatty acid composition. A number of effects was marginally outside of statistical significance but have been shown in the Table. As observed in the outer backfat, females had a higher content of oleic acid than males and consequently a higher level of total monounsaturated fatty acids. Males had a significantly higher ratio of total *n-6* to total *n-3* fatty acids (5.0 *vs.* 4.8, data not shown). The level of vitamin E in the diet significantly affected the level of a number of fatty acids. Thus, the presence of additional vitamin E resulted in a slight reduction in the level of arachidonic acid. The *n-6* to *n-3* fatty acid ratios were significantly increased due to the presence of higher vitamin E. The presence of additional vitamin E increased the linoleic acid to α-linolenic acid ratio from 6.9 to 7.1 and the total *n-6* to total *n-3* fatty acid ratio from 4.8 to 5.0. As observed in the outer backfat, there were significant copper×vitamin E interactions for the *n-6* to *n-3* fatty acid ratios. For both ratios, the LL and HH diets gave rise to values which were significantly higher than the HL and LH diets.

The fatty acid content of the inner backfat from the pigs fed low and high levels of copper is shown in Table 4.9. No similar data is shown for the effect of vitamin E as in all cases no significant effects were observed. In general, increasing the level of dietary copper caused small increases in the levels of saturated fatty acids although only the values for palmitic acid approached statistical significance. The level of palmitoleic acid was unaffected by the addition of copper whilst although the level of oleic acid was higher in the high copper category, the difference failed to reach significance. The levels of polyunsaturated fatty acids generally decreased with diets supplying the higher level of copper, an observation which is reflected in the summations of all polyunsaturates. However, differences were only significant for linoleic acid, α-linolenic acid and arachidonic acid. The P:S ratio was significantly reduced in accordance with the alterations in individual saturated and polyunsaturated fatty acids. Unlike in the outer backfat, the ratios of *n-6* to *n-3* fatty acids were not affected by the level of copper in the diet.

4.3.5.4. *Semitendinosus* phospholipid

The fatty acid composition of the phospholipid from the *Semitendinosus* is shown in Table 4.10. Overall, the fatty acid composition was similar to that in the 6SFOD

	LL	HL	LH	HH	SED	Significance
14:0	1.43	1.46	1.37	1.36	0.073	NS
16:0	20.9	21.5	20.8	21.4	0.45	Cu 0.07 sex×Cu 0.08
16:1*n-7*	3.17	3.17	3.16	3.08	0.217	NS
18:0	10.3	10.9	10.1	11.0	0.80	NS
18:1*n-9*	30.5	32.4	31.2	33.0	1.54	sex 0.08 sex×Vit. E*
18:2*n-6*	26.8	24.5	27.0	24.6	1.66	Cu 0.06 sex×Vit. E 0.07
18:3*n-3*	3.77	3.66	3.96	3.31	0.193	Cu** Cu×Vit. E 0.07
20:3*n-6*	0.13	0.12	0.12	0.11	0.009	sex 0.06
20:4*n-6*	0.54	0.51	0.52	0.46	0.023	Cu* Vit. E 0.06
20:5*n-3*	0.40	0.39	0.38	0.35	0.036	NS
22:5*n-3*	0.66	0.62	0.70	0.63	0.050	NS
22:6*n-3*	0.72	0.69	0.70	0.65	0.051	NS
Total SAT	33	34	32	34	1.1	Cu 0.09
Total MUFA	34	36	34	36	1.7	sex 0.09 sex×Vit. E*
Total PUFA	33	30	33	30	2.0	Cu 0.06
P:S	1.0	0.9	1.0	0.9	0.09	Cu*
18:2*n-6*/ 18:3*n-3*	7.1	6.7	6.8	7.4	0.13	Vit. E* Cu×Vit. E*** sex×Vit. E*
n-6:n-3	4.9	4.7	4.8	5.1	0.09	sex** Vit. E* sex×Cu* sex×Vit. E* Cu×Vit. E***

Table 4.8 - The fatty acid composition (major fatty acids, percentage of total present) of the inner backfat triacylglycerol as affected by the level of copper (Cu) and/or vitamin E (Vit. E) in the diet and the sex of the animal; effects lying marginally outside of statistical significance quoted as probability values; all shorthand designations as per Table 4.7

	Low Cu	High Cu	SED	Signif.
14:0	1.40	1.41	0.052	NS
16:0	20.8	21.5	0.32	0.07
16:1*n-7*	3.17	3.12	0.153	NS
18:0	10.2	11.0	0.56	NS
18:1*n-9*	30.9	32.7	1.09	NS
18:2*n-6*	26.9	24.6	1.18	*
18:3*n-3*	3.87	3.49	0.137	**
20:3*n-6*	0.12	0.12	0.006	NS
20:4*n-6*	0.53	0.48	0.016	*
20:5*n-3*	0.39	0.37	0.026	NS
22:5*n-3*	0.68	0.63	0.036	NS
22:6*n-3*	0.71	0.67	0.036	NS
Total SAT	32	34	0.8	0.09
Total MUFA	34	36	1.2	NS
Total PUFA	33	30	1.4	0.06
P:S	1.0	0.9	0.06	*
18:2*n-6*/ 18:3*n-3*	6.9	7.0	0.13	NS
n-6:n-3	4.9	4.9	0.06	NS

Table 4.9 - The fatty acid composition (major fatty acids, percentage of total present) of the inner backfat triacylglycerol as affected by the level of copper in the diet; effects lying marginally outside of statistical significance quoted as probability values; all shorthand designations as per Table 4.7

	LL	HL	LH	HH	SED	Significance
14:0	0.14	0.14	0.13	0.12	0.015	sex** Vit. E 0.07
16:0	23.1	22.9	22.4	22.1	0.79	NS
16:1*n-7*	1.21	1.48	1.42	1.34	0.299	NS
18:0	15.4	14.5	14.7	14.7	0.30	Cu* CuxVit. E*
18:1*n-9*	10.5	10.9	11.2	11.2	0.81	NS
18:2*n-6*	34.1	33.8	33.8	34.0	0.81	NS
18:3*n-3*	0.52	0.65	0.65	0.60	0.056	CuxVit. E*
20:3*n-6*	0.90	1.06	0.98	1.11	0.090	Cu*
20:4*n-6*	7.76	7.95	7.69	7.83	0.541	NS
20:5*n-3*	2.28	2.34	2.50	2.61	0.211	Vit. E 0.09
22:5*n-3*	2.31	2.41	2.56	2.46	0.119	Vit. E 0.08
22:6*n-3*	1.96	1.91	2.02	2.02	0.199	NS
Total SAT	39	38	37	37	1.2	Vit. E 0.06
Total MUFA	12	12	13	12	0.7	NS
Total PUFA	50	50	50	51	1.1	NS
P:S	1.3	1.3	1.3	1.4	0.08	NS
18:2*n-6*/ 18:3*n-3*	65	52	52	57	2.4	CuxVit. E*
n-6:n-3	6.0	5.9	5.5	5.6	0.26	NS

Table 4.10 - The fatty acid composition (major fatty acids, percentage of total present) of the *Semitendinosus* phospholipid as affected by the level of copper (Cu) and/or vitamin E (Vit. E) in the diet and the sex of the animal; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

treatment observed in the previous experiment (see Section 3.3.4.2) with the major fatty acid being linoleic acid followed by palmitic and stearic acids. The level of myristic acid was significantly affected by sex with males exhibiting a higher level of this acid than females (0.15 *vs.* 0.11%, data not shown). The presence of the higher level of dietary copper significantly reduced the level of stearic acid (15.1 *vs.* 14.6%, data not shown). The level of α-linolenic acid was significantly lower after feeding of the LL and HH diets than after feeding the HL or LH diets. This copper×vitamin E interaction was also manifested in the ratio of linoleic acid to α-linolenic acid. The fatty acid composition of the *Semitendinosus* phospholipid in the pigs fed the low and high levels of vitamin E is presented in Table 4.11. Although there were no statistically significant effects, differences in fatty acid levels depending on the level of dietary vitamin E were apparent. The levels of saturated fatty acids were lower and monounsaturated fatty acids higher in the pigs fed the high level of vitamin E. There was virtually no difference in the levels of linoleic and arachidonic acids. However, the long chain polyunsaturated fatty acids eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid were noticeably higher in the pigs fed the additional vitamin E. The *n-6* to *n-3* ratios were consequently reduced in these pigs.

4.3.5.5. *Longissimus dorsi* phospholipid

The fatty acid composition of the phospholipid from the *Longissimus dorsi* is shown in Table 4.12. Overall, the fatty acid composition was similar to that in the 6SFOD treatment observed in the previous experiment (see Section 3.3.4.3). There was a number of effects of diet on the fatty acid composition with the principal effects arising from differences in the level of dietary vitamin E. Thus, unlike in the *Semitendinosus* phospholipid, copper on its own had no effect on the fatty acid composition. The fatty acid composition of the *Longissimus dorsi* phospholipid in the pigs fed the low and high levels of vitamin E is presented in Table 4.13. The level of palmitic acid was significantly lower in the pigs fed the high level of vitamin E. Although not significant, the level of stearic acid showed a similar trend. In contrast, the levels of the monounsaturated and polyunsaturated fatty acids were elevated in the high vitamin E groups. This effect was significant for docosahexaenoic acid but marginally outside of significance for eicosapentaenoic acid and docosapentaenoic acid. These alterations in individual fatty acids were mirrored in the summations of the classes of fatty acids and their ratios.

4.3.5.3. Liver phospholipid

The fatty acid composition of the phospholipid from the liver is shown in Table 4.14. Overall, the fatty acid composition was similar to that in the 6SFOD treatment observed in the previous experiment (see Section 3.3.4.4). There was a number of

	75 mg/kg Vitamin E	**375 mg/kg Vitamin E**	SED	Signif.
14:0	0.14	0.12	0.011	0.07
16:0	23.0	22.3	0.56	NS
16:1*n-7*	1.36	1.38	0.210	NS
18:0	15.0	14.6	0.21	NS
18:1*n-9*	10.7	11.2	0.57	NS
18:2*n-6*	33.9	33.8	0.57	NS
18:3*n-3*	0.59	0.63	0.039	NS
20:3*n-6*	0.98	1.05	0.064	NS
20:4*n-6*	7.83	7.79	0.380	NS
20:5*n-3*	2.30	2.57	0.148	0.09
22:5*n-3*	2.36	2.52	0.084	0.08
22:6*n-3*	1.92	2.03	0.140	NS
Total SAT	38	37	0.5	0.06
Total MUFA	12	13	0.6	NS
Total PUFA	50	50	0.6	NS
P:S	1.3	1.4	0.03	NS
18:2*n-6*/ 18:3*n-3*	59	55	3.9	NS
n-6:n-3	6.0	5.6	0.27	NS

Table 4.11 - The fatty acid composition (major fatty acids, percentage of total present) of the *Semitendinosus* phospholipid as affected by the level of vitamin E in the diet; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

	LL	HL	LH	HH	SED	Significance
14:0	0.16	0.22	0.22	0.13	0.060	NS
16:0	23.5	23.0	21.9	21.5	0.87	Vit. E*
16:1*n-7*	1.56	1.68	1.92	1.77	0.523	NS
18:0	15.3	14.7	13.9	14.4	0.62	Vit. E 0.09
18:1*n-9*	11.5	12.0	13.6	12.4	2.0	NS
18:2*n-6*	34.2	33.5	32.5	33.2	0.91	NS
18:3*n-3*	0.68	0.84	1.03	0.71	0.15	Cu×Vit. E*
20:3*n-6*	0.96	0.98	0.99	1.19	0.095	Vit. E 0.07
20:4*n-6*	6.58	7.25	7.25	7.84	0.920	NS
20:5*n-3*	2.22	2.24	2.49	2.65	0.242	Vit. E 0.06
22:5*n-3*	2.07	2.21	2.42	2.47	0.215	Vit. E 0.07
22:6*n-3*	1.22	1.46	1.74	1.78	0.270	Vit. E*
Total SAT	39	38	36	36	1.1	Vit. E**
Total MUFA	13	14	16	14	2.1	NS
Total PUFA	48	48	48	50	2.0	NS
P:S	1.2	1.3	1.3	1.4	0.07	Vit. E*
18:2*n-6*/ 18:3*n-3*	51	41	36	47	5.6	Cu×Vit. E*
n-6:*n-3*	6.9	6.2	5.3	5.6	0.42	Vit. E**

Table 4.12 - The fatty acid composition (major fatty acids, percentage of total present) of the *Longissimus dorsi* phospholipid as affected by the level of copper (Cu) and/or vitamin E (Vit. E) in the diet and the sex of the animal; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

	75 mg/kg Vitamin E	375 mg/kg Vitamin E	SED	Signif.
14:0	0.19	0.17	0.040	NS
16:0	23.2	21.7	0.61	*
16:1*n-7*	1.63	1.84	0.364	NS
18:0	15.0	14.2	0.43	0.09
18:1*n-9*	11.8	13.0	1.40	NS
18:2*n-6*	33.8	32.9	0.64	NS
18:3*n-3*	0.77	0.85	0.104	NS
20:3*n-6*	0.97	1.10	0.066	0.07
20:4*n-6*	6.95	7.57	0.641	NS
20:5*n-3*	2.23	2.58	0.169	0.06
22:5*n-3*	2.15	2.44	0.150	0.07
22:6*n-3*	1.35	1.76	0.188	*
Total SAT	38	36	0.8	**
Total MUFA	13	15	1.5	NS
Total PUFA	48	49	1.4	NS
P:S	1.3	1.4	0.05	*
18:2*n-6*/ 18:3*n-3*	45	42	3.9	NS
n-6:n-3	6.5	5.5	0.29	**

Table 4.13 - The fatty acid composition (major fatty acids, percentage of total present) of the *Longissimus dorsi* phospholipid as affected by the level of vitamin E in the diet; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

	LL	HL	LH	HH	SED	Significance
14:0	0.07	0.08	0.10	0.08	0.016	NS
16:0	12.4	12.0	16.3	12.4	1.40	Cu* Vit. E* Cu×Vit. E 0.07
16:1*n-7*	1.95	1.85	2.31	1.66	0.218	Cu* Cu×Vit. E 0.08
18:0	30.1	30.5	27.2	30.6	1.26	Cu*
18:1*n-9*	8.29	8.46	11.03	9.24	0.917	Vit. E**
18:2*n-6*	17.8	18.0	13.2	16.9	1.30	Cu* Vit. E** Cu×Vit. E*
18:3*n-3*	0.63	0.77	0.47	0.57	0.077	Cu* Vit. E**
20:3*n-6*	1.11	1.16	1.62	1.30	0.154	Vit. E** Cu×Vit. E 0.09
20:4*n-6*	14.6	13.9	11.5	13.0	0.740	Vit. E*** Cu×Vit. E*
20:5*n-3*	3.86	4.31	3.37	3.65	0.619	NS
22:5*n-3*	3.49	3.51	4.07	3.70	0.270	Vit. E 0.06
22:6*n-3*	5.74	5.46	8.93	6.84	0.681	Cu* Vit. E*** Cu×Vit. E 0.06 sex×Cu×Vit. E*
Total SAT	42	43	44	43	1.1	sex* Vit.E*
Total MUFA	10	10	13	11	1.3	Vit. E*
Total PUFA	47	47	43	46	1.5	Vit. E**
P:S	1.1	1.1	1.0	1.1	0.06	Vit. E**
18:2*n-6*/ 18:3*n-3*	28	23	28	30	2.6	NS
n-6:*n-3*	2.4	2.4	1.6	2.1	0.32	Cu×Vit. E*

Table 4.14 - The fatty acid composition (major fatty acids, percentage of total present) of the liver phospholipid as affected by the level of copper (Cu) and vitamin E (Vit. E) in the diet and the sex of the animal; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

effects of differences in copper and vitamin E levels on the fatty acid composition. The single effect of sex was only observed in the level of total monounsaturates; in overall terms, females showed a higher level than the males, an observation similar to that for other tissues. The fatty acid composition of the liver phospholipid in the pigs fed the low and high levels of copper is presented in Table 4.15. The levels of palmitic and palmitoleic acids were significantly lower in the pigs fed the high copper diet. The levels of stearic, linoleic and α-linolenic acids were significantly higher in the pigs fed the high copper diet. The level of docosahexaenoic acid was significantly lower in the pigs fed the high copper diet. There was no apparent effect of copper on the summations of fatty acids and their ratios.

The fatty acid composition of the liver phospholipid in the pigs fed the low and high levels of vitamin E is presented in Table 4.16. The effect of vitamin E was more pronounced than that of copper. In the presence of the high level of vitamin E, palmitic acid and oleic acid significantly increased. In contrast, the levels of linoleic, α-linolenic and arachidonic acids significantly decreased. The levels of docosapentaenoic acid and docosahexaenoic acid increased significantly in the presence of additional vitamin E. As a consequence, the levels of total saturates and monounsaturates increased and total polyunsaturates decreased significantly in the pigs fed the high level of vitamin E. Both the P:S ratio and the total *n-6* to total *n-3* fatty acid ratio were significantly reduced in the presence of added vitamin E.

4.3.6. Olfactory sensory analysis

Table 4.17 shows the results for the olfactory sensory analysis of the outer backfat from the pigs fed the high copper/low vitamin E (HL) diet and the low copper/high vitamin E (LH) diet. These diets could potentially be seen as giving rise to the greatest difference in terms of susceptibility to the development of oxidative rancidity. The least significant difference (LSD) has been presented at the 5% level of probability. Among the 17 odour descriptors, the highest intensities were generally exhibited by the 'fatty', 'piggy' and 'sweaty' odours. Of the 17 flavour descriptors, only the intensity of 'ammonia' odour gave rise to a significant difference where the LH diet generated a higher score.

Table 4.18 shows the results for the olfactory sensory analysis of the outer backfat from the pigs fed the HL and LH diets after two and six months of frozen storage. A number of odours were perceived as significantly more intense after 6 months storage than after two months storage, irrespective of diet. These were 'skatole', 'androstenone', 'rancid', 'fatty', 'sweaty', 'fruity', 'nutty', 'chemical' and 'cabbage'.

	Low Cu	High Cu	SED	Signif.
14:0	0.09	0.08	0.011	NS
16:0	14.3	12.2	0.98	*
16:1*n-7*	2.13	1.76	0.153	*
18:0	28.6	30.6	0.88	*
18:1*n-9*	9.66	8.85	0.642	NS
18:2*n-6*	15.5	17.5	0.91	*
18:3*n-3*	0.55	0.67	0.054	*
20:3*n-6*	1.36	1.23	0.108	NS
20:4*n-6*	13.1	13.4	0.52	NS
20:5*n-3*	3.61	3.98	0.433	NS
22:5*n-3*	3.78	3.61	0.189	NS
22:6*n-3*	7.33	6.15	0.477	*
Total SAT	43	43	0.3	NS
Total MUFA	12	11	0.7	NS
Total PUFA	45	47	0.9	NS
P:S	1.1	1.1	0.02	NS
18:2*n-6*/ 18:3*n-3*	29	27	2.4	NS
n-6:n-3	2.0	2.3	0.19	NS

Table 4.15 - The fatty acid composition (major fatty acids, percentage of total present) of the liver phospholipid as affected by the level of copper in the diet; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

	75 mg/kg Vitamin E	375 mg/kg Vitamin E	SED	Signif.
14:0	0.08	0.09	0.011	NS
16:0	12.2	14.5	0.98	*
16:1*n-7*	1.90	2.02	0.153	NS
18:0	30.3	28.7	0.88	NS
18:1*n-9*	8.36	10.22	0.642	**
18:2*n-6*	17.8	14.9	0.91	**
18:3*n-3*	0.69	0.51	0.054	**
20:3*n-6*	1.14	1.47	0.108	**
20:4*n-6*	14.3	12.2	0.52	***
20:5*n-3*	4.07	3.49	0.433	NS
22:5*n-3*	3.51	3.89	0.189	*
22:6*n-3*	5.62	7.96	0.477	***
Total SAT	42	43	0.3	*
Total MUFA	10	12	0.7	*
Total PUFA	47	44	0.9	**
P:S	1.1	1.0	0.02	**
18:2*n-6*/ 18:3*n-3*	27	29	2.4	NS
n-6:n-3	2.4	1.9	0.18	*

Table 4.16 - The fatty acid composition (major fatty acids, percentage of total present) of the liver phospholipid as affected by the level of vitamin E in the diet; effects lying marginally outside of statistical significance quoted as probability levels; all shorthand designations as per Table 4.7

Parameter	Diet HL	Diet LH	LSD[1]	Significance[2]
Musty	2.20	2.57	0.381	NS
Skatole	1.82	2.16	0.359	NS
Androstenone	1.62	1.83	0.281	NS
Urine	2.20	2.46	0.420	NS
Rancid	2.11	2.13	0.333	NS
Fishy	2.35	2.19	0.534	NS
Sweet	2.49	2.36	0.337	NS
Fatty	2.88	2.55	0.340	NS
Piggy	2.85	2.78	0.512	NS
Porky	2.53	2.27	0.371	NS
Sweaty	2.63	2.54	0.364	NS
Ammonia	2.00	2.72	0.547	***
Fruity	1.86	1.77	0.280	NS
Burnt	1.58	1.72	0.389	NS
Nutty	2.08	1.95	0.371	NS
Chemical	2.07	2.38	0.396	NS
Cabbage	1.57	1.56	0.256	NS

[1] LSD least significant difference

[2] *** significant difference at P≤0.001 NS not significant

Table 4.17 - The olfactory sensory analysis of outer backfat from the pigs fed the high copper/low vitamin E (HL) and the low copper/high vitamin E (LH) diets; scores 1 (low) to 8 (high) for each parameter; shorthand designations of treatments as per Table 4.1

Parameter	Storage 1	Storage 2	LSD[1]	Significance[2]
Musty	2.25	2.52	0.384	NS
Skatole	1.72	2.26	0.363	**
Androstenone	1.54	1.90	0.284	**
Urine	2.23	2.44	0.434	NS
Rancid	1.86	2.38	0.335	**
Fishy	2.30	2.24	0.537	NS
Sweet	2.47	2.38	0.340	NS
Fatty	2.41	3.02	0.343	***
Piggy	2.61	3.02	0.516	NS
Porky	2.33	2.47	0.375	NS
Sweaty	2.24	2.93	0.368	***
Ammonia	2.28	2.43	0.551	NS
Fruity	1.64	1.99	0.283	*
Burnt	1.50	1.80	0.391	NS
Nutty	1.77	2.27	0.375	**
Chemical	1.88	2.53	0.399	**
Cabbage	1.40	1.73	0.259	*

[1] LSD least significant difference

[2] * ** *** significant differences, respectively, at P≤0.05, P≤0.01 and P≤0.001 NS not significant

Table 4.18 - The olfactory sensory analysis of outer backfat from the pigs fed the HL and LH diets after frozen storage for 2 and 6 months, labelled as Storage 1 and Storage 2, respectively; scores 1 (low) to 8 (high) for each parameter

4.4. DISCUSSION

The present experiment was designed to investigate the effects of alterations in dietary copper and vitamin E on the fatty acid composition and sensory properties of tissues of pigs which had received the soybean oil/fish oil diet similar to that used in the first experiment (see Section 3.2.1). In addition to its role as an enhancer of pig growth performance (Moore *et al*, 1969), copper has been associated with a reduction in the oxidative stability of pig tissues due to the increase in the levels of unsaturated fatty acids (Amer and Elliot, 1973b; Christie and Moore, 1969). On the other hand, the presence of vitamin E in the diet has been associated with a significant increase in the oxidative stability of porcine tissues on account of its antioxidant effects (Monahan *et al*, 1992). In the previous experiment, the feeding of a soybean oil/fish oil diet resulted in an improvement in the fatty acid composition of muscle, fat and liver tissues with respect to human health requirements. The present study has been an attempt to determine the effects of two elements of the pig diet, namely copper and vitamin E, which can be seen to have contrasting effects, on the optimum fatty acid distribution which was obtained previously.

The performance of the pigs was not affected by the inclusion of either copper or vitamin E. An effect of dietary vitamin E on growth performance would not have been expected. The inclusion of supplemental vitamin E in both the pig and poultry diets has not been associated with any increase in growth performance (Amer and Elliot, 1973b; Sheehy *et al*, 1993). However, the inclusion of copper in the pig diet has generally been associated with a significant improvement in pig performance (Dove and Haydon, 1992; Braude, 1967). Braude (1967) observed that the average improvement arising from the addition of 250 mg/kg feed was approximately 8% in average daily liveweight gain and 3% in feed conversion efficiency. Myres and Bowland (1973) also observed that carcass quality was improved by copper supplementation. An effect of copper on performance and/or carcass characteristics would therefore have been expected to occur in the present study; however, no such effect was found. This could partly be accounted for by an insufficient difference in the copper contents between the 'low' and 'high' copper diets (means of 29 and 78 mg copper/kg diet, respectively) to manifest the previously reported effects. Most trials involving the feeding of copper to pigs for the enhancement of growth performance have used much higher levels of copper in the range 125 to 250 mg/kg (see Bowland, 1990). However, even after feeding copper at the rate of 250 mg/kg, a number of workers has observed that pig performance was not improved or that it was adversely affected (Ho *et al*, 1975; Amer and Elliot, 1973b; Elliot and Amer, 1973). Elliot and Amer (1973) furthermore observed that young pigs tended to respond better to supplemental copper as a result of an apparent decreasing response to dietary copper as they got older.

The contents of vitamin E in the muscle and liver tissues were markedly affected by the level of vitamin E supplementation. The levels of vitamin E were approximately doubled in the muscles of the pigs fed the high vitamin E diets compared to those of the pigs fed the lower level of the vitamin. The level of vitamin E observed in the muscle tissues was similar to that recorded by Monahan *et al* (1992) for muscle (unspecified) from pigs fed a diet containing 3% soybean oil and vitamin E at the level of 200 mg/kg diet. In the muscle tissues there was no effect on the level of vitamin E as a result of feeding the different levels of copper. However, in the liver, copper levels did have an effect on vitamin E contents. The reason for this tissue-specific difference is not apparent.

There was no significant effect of copper intake on the levels of copper in either the muscle tissues or the liver. However, the copper levels within all of the tissues tended to be higher at the higher dietary intake of the element. Insofar as the liver is concerned, increases in this element would have been expected as various workers have observed highly significant increases in liver copper concentrations under conditions of high intakes of the element (Elliot and Amer, 1973; Myres and Bowland, 1973). Elliot and Amer (1973) also observed that male pigs tended to accumulate more copper in the liver than females. Again, the absence of significant between-treatment differences in tissue copper levels observed in the present experiment could be due to the insufficient difference in the dietary levels between the experimental diets. The levels of zinc and iron in the muscle tissues tended to increase with the higher intake of copper. This was in contrast to the liver where the iron level was slightly lower in the pigs receiving the high copper diets. Copper supplementation of diets has been shown to affect the absorption and utilisation of zinc by various enzymes (Cunnane, 1984). Thus, in the presence of a high level of dietary copper, zinc deficiency may occur unless the level of zinc in the diet is also increased. Similarly, the presence of excess copper has been shown to cause iron-deficient anaemia and increased lipid peroxidation (Jain *et al*, 1983).

Although the feeding of copper at 250 mg/kg has been associated with significant reductions in the firmness of pig backfat (Astrup and Matre, 1987), the firmness of the backfat in the present investigation was not significantly affected. The similarity of fat firmness was probably also the result of the lack of extreme difference in the copper contents of the diets. The melting points of adipose tissues have also been shown to be lowered in pigs fed supplemental copper (Amer and Elliot, 1973a; Moore *et al*, 1969). These copper-induced alterations in physical properties have been shown to be the result of increases in the levels of unsaturated fatty acids (Amer and Elliot, 1973a) and to specific alterations in the levels of triacylglycerol molecular species within the fat (Christie and Moore, 1969). The fact that females were found to have a significantly higher content of total lipid in the outer backfat than males (72.3 *vs.* 66.5 g/100g, data not shown) accords with previous observations (Barton-Gade, 1987). The lack of significant differences due to sex in the total lipid contents of the muscle tissues also

accords with the findings of Barton-Gade (1987). In overall terms, the fatty acid compositions of the tissues were similar to those observed in the previous experiment (see Section 3.3.4). Most notably, all tissues were characterised by significant increases in the levels of long chain *n-3* polyunsaturated fatty acids with consequential alterations in the ratios of *n-6* to *n-3* fatty acids, expressed either as the ratio of linoleic acid to α-linolenic acid or as the ratio of total *n-6* to total *n-3* fatty acids. In the backfats, the ratio of linoleic acid to α-linolenic acid approximated to the stated optimum of 6:1 (British Nutrition Foundation, 1992). However, in the muscle and liver phospholipid fractions, the ratio was far higher than this optimum although if the contributions of the other lipid fractions in the provision of fatty acids is taken into account the ratio would be brought closer to that required. The ratios of total *n-6* to total *n-3* fatty acids were in the region of the suggested human dietary optimum of 4:1 (Galli and Simopoulos, 1989); in the case of the liver, the presence of substantial levels of *n-3* fatty acids reduced the ratio to approximately 2:1. It was clear that, as shown previously by Barton-Gade (1987), gender influenced tissue fatty acid composition.

The inner backfat was generally more responsive to changes in dietary copper and vitamin E than the outer backfat which may be a result of the view held that the inner backfat is the more metabolically active layer of backfat (Dziubajlo, 1991). Overall, there was only a small effect of copper level on the fatty acid composition of the outer and inner backfats. Furthermore, some differences were not in the direction which would have been expected as saturated fatty acids actually increased on the high copper diets. Significant increases in the levels of monounsaturated fatty acids in pig depot fat as a result of feeding 250 ppm copper as copper sulphate have been observed previously (Dziubajlo, 1991; Amer and Elliot, 1973a; Christie and Moore, 1969). These effects have been attributed to copper-induced increases in the activity of stearoyl coenzyme A desaturase (a Δ^9 desaturase, EC 1.14.99.5), the enzyme responsible for inserting a double bond at carbon number 9 on a molecule of stearic acid (Dziubajlo, 1991; Ho *et al*, 1975). Ho *et al* (1975) also observed that the conversions of oleoyl coenzyme A and palmitoyl coenzyme A to their respective products increased with copper supplementation on account of the stimulation of the desaturase enzyme systems. Thus, as a result of supplemental copper, increases in the levels of myristic, palmitoleic, oleic and octadecadienoic acids have been observed along with decreases in palmitic, stearic and linoleic acids (Dziubajlo, 1991; Ho *et al*, 1975). The small effect of copper supplementation on fatty acid composition presently observed may not only be related to the copper status of the diets but also to the ability of high tissue levels of linoleic acid to inhibit stearoyl coenzyme A desaturase activity (Allee *et al*, 1972). The design of the present experiment was to produce particularly high levels of linoleic acid within the tissues. Alterations in the zinc to copper ratio have also been implicated in the modification of tissue levels of a number of fatty acids (Cunnane, 1984). Whereas copper promotes Δ^9 desaturase activity, zinc stimulates the conversion of linoleic acid to

gamma-linolenic acid by enhancing the activity of Δ^6 desaturase (Clejan *et al*, 1982) and by affecting a range of other factors related to linoleic acid metabolism (Cunnane, 1984). Thus, in these respects, the activities of zinc and copper may be viewed as being physiologically competitive, with copper giving rise to fatty acids of the *n-9* family and zinc giving rise to fatty acids of the *n-6* family.

It is clear from the present investigations that both copper and vitamin E are implemental in the *n-6* to *n-3* fatty acid ratios within the muscle and fat tissues. The ratios may also be affected by the differing oxidative susceptibilities of the tissues as the result of alterations in copper and vitamin E levels. An increase in the copper to iron ratio in erythrocytes has been associated with increased peroxidation of long chain polyunsaturated fatty acids within tissues (Jain *et al*, 1983). In the present investigation, the fatty acid compositions of the muscle tissues were affected to a far greater extent by vitamin E level than by copper. This is in line with the antioxidant role of vitamin E and the protection it provides against oxidative degradation of polyunsaturated fatty acids (Burton, 1994). As a consequence, associated changes in *n-6* to *n-3* fatty acid ratios were observed. Such effects of vitamin E on the tissue levels of polyunsaturated fatty acids have been described by various authors (Klaus *et al*, 1995; Buttriss and Diplock, 1988) although Monahan *et al* (1992) observed no such effect in the tissues of pigs fed supplemental vitamin E. The inclusion of vitamin E in the pig diet has also been shown to significantly reduce the formation of secondary lipid oxidation products within the raw and cooked muscle (Monahan *et al*, 1990). Although liver tissue changes were similar in character to those displayed by the muscle and adipose tissues, the extent of the changes was significantly greater.

The olfactory characteristics of the outer backfats were not significantly different. These results are in agreement with previous observations (Morgan *et al*, 1992) in which no difference in organoleptic acceptability was observed for meats from pigs fed diets containing a wide range of fatty acid compositions. However, a number of characteristics were significantly affected by the time of frozen storage. The higher ratings with storage time for skatole and androstenone were unexpected as it would not be envisaged that the levels of these compounds would increase in the carcasses post-mortem. A range of possible explanations exist for these observations including interactive features of odour development and panellist inconsistencies (Meat and Livestock Commission, 1992, personal communication). In spite of this possibility, there was no evidence of a session effect at each period of testing. There was no indication of the acceptability or otherwise of the fat to the panellist. Further in-depth investigations of more of the characteristics related to eating quality are therefore warranted.

5. Evaluation of the methodology for separation of the triacylglycerol molecular species in pig backfat by silver ion high performance liquid chromatography

5.1. Introduction

As a result of experimentation to date, two major features have become apparent. These were: i) extensive differences in polyunsaturated fatty acid levels in the tissues achieved by appropriate dietary manipulation. The end result was the ability to manipulate the composition to conform to that of modern theory of human dietary requirements (*c.f.* British Nutrition Foundation, 1992); ii) whereas increasing polyunsaturation was correlatable with increasing fluidity of pig backfat, the extent to which this occurred was considerably less than expected from previous work (Whittington *et al*, 1986) and in spite of considerable polyunsaturation acceptable backfats were obtained. The question therefore arises as to how very fluid backfats were not observed under conditions in which extreme unsaturation was present. The possibility arose therefore that the fundamental changes in fatty acid composition were associated with changes at the molecular species level that to a large degree offset appropriate and conventionally accepted changes in physical properties. A range of methodologies exist to investigate molecular species of individual major lipid components. Initially, as an adjunct to overall fatty acid analyses, such data were obtained using analytical systems based on enzymic hydrolysis. Thus, in the case of triacylglycerols, meaningful results could be obtained based on pancreatic lipase hydrolysis (Luddy *et al*, 1964) and in a more sophisticated manner through stereospecific analysis based on Grignard degradation followed by lipase hydrolysis (Yurkowski and Brockerhoff, 1966). The advent of silver ion (argentation) thin layer chromatography provided further molecular discrimination to be undertaken based on the relative degree of unsaturation of the fatty acid components via inorganic adduct formation (Christie, 1982). Although effective in separating triacylglycerols with major differences in unsaturation, there were severe limitations based on the inability of the medium to effect separation of the more highly unsaturated moieties. More recently, with the routine introduction of high performance liquid chromatography, a methodology for molecular species differentiation based on silver ion chromatography has been evolved (Christie, 1988). Thus, in the case of sheep adipose tissue, some ten individual triacylglycerol molecular species were able to be separated ranging from species comprised entirely of saturated fatty acid moieties to species containing a saturated, a monounsaturated and a diunsaturated fatty acid. In the light of the present tissues to be investigated, that is, pig outer backfat, this methodology presented the most appropriate means for triacylglycerol species analysis. For the application to the pig samples, particular modification and evaluation had to be undertaken. This is now described.

5.2. Sample preparation

Following extraction of the total backfat lipid by chloroform:methanol as described previously (Section 2.6.1), triacylglycerol sample was prepared by elution on short columns of 'Florisil', 100-120 mesh (BDH, Lutterworth, England) according to the method of Christie (1988). The Florisil was added to a depth of 5 cm to a glass pasteur pipette containing a glass wool plug at the narrow end. Following 'washing' with several column volumes of hexane-diethyl ether (4:1 v/v), 20 mg of outer backfat total lipid in chloroform were discreetly added to the column. The column was then eluted with further additions (minimum 5 ml) of hexane:diethyl ether to elute the triacylglycerols which were then collected in a small glass tube with PTFE seal. The eluant was then taken to dryness at 60 °C under nitrogen gas and the triacylglycerols redissolved in an appropriate volume of 1,2-dichloroethane such that the final concentration of triacylglycerol was of the order of 100 $mgml^{-1}$.

5.3. Separation conditions

For triacylglycerol molecular species separations, a selection of solvent systems and timed elution regimes were available depending upon likely molecular species to be encountered based on sample source (Christie, 1988). Inspection of these methodologies indicated that the basic solvents and their time gradients as shown in the table below would be most appropriate for the pig backfats to be investigated. Preliminary investigations were undertaken and showed this to be the case and enabled complete elution of the triacylglycerol components. The recovery was verified by comparison of the fatty acid composition of the whole with that derived from the relative proportions in each fraction as outlined by Christie (1988). The agreement between the two fell within acceptable limits. In all cases, the solvents which were of an HPLC grade were de-gassed with helium prior to and during use to remove dissolved oxygen. The mobile phase flow rate was 1.0 $mlmin^{-1}$. Prior to the application of the sample for analysis, the system was purged with the most polar solvent (Solvent C) and ending with the least polar solvent (Solvent A).

	A	B	C
Time (min)	1,2-Dichloroethane-1,1-Dichloromethane 1:1 v/v	Acetone	Acetone-Acetonitrile 9:1 v/v
0	100 %	0 %	0 %
15	50	50	0
40	0	0	100
48	0	0	100

The pump was a SP8800 Ternary HPLC Pump (Thermo Separation Products, Stone, England). The column was a Nucleosil 100 5μ SA length 25 cm, o.d. 6.4 mm, i.d. 4.6 mm (Hichrom Ltd., Reading, England) fitted with a 2μ column prefilter (Alltech Associates Applied Science Ltd., Carnforth, England) for removal of particulate matter. In order to effect separation of the triacylglycerols on the basis of their unsaturation, the sulphonic acid moieties of the column packing had to be loaded with silver ions. This was achieved as follows: firstly, the column was flushed with 1% (w/v) ammonium nitrate in water at a rate of 0.5 $mlmin^{-1}$ for one hour followed by flushing with deionised water at a rate of 1 $mlmin^{-1}$ again for one hour. 1 ml of a 0.2% (w/v) solution of silver nitrate in water was injected onto the column via the Rheodyne valve in 50 μl aliquots at one minute intervals. Twenty minutes after the last silver nitrate injection, the column was washed with 60 ml methanol over a period of one hour followed by 60 ml of a mixture of 1,2-dichloroethane:1,1-dichloromethane (1:1 v/v) for a further hour.

Detection of the solutes was via an Evaporative Light Scattering Detector Model IIA (Varex Inc., Burtonsville, Maryland, USA). For detection and measurement of the separated solutes, the detector requires the vapourisation of solvent in a heated stream of gas followed by measurement of the degree of light scattering caused by the non-volatile solute. The nebulizer gas was air supplied via a Jun-Air Oil-less Compressor (Nørresundby, Denmark) and was passed through a desiccant prior to entry into the detector. By inspection, an optimal detector tube temperature and nebulizer gas flow were found; an optimum signal:noise ratio and highest peak area occurred at a tube temperature of 93 °C and a gas flow of 1.9 $lmin^{-1}$.

5.4. Sample injection and fraction collection

Triacylglycerol samples contained in 70 μl aliquots of 1,2-dichloroethane were introduced into the HPLC system via a Rheodyne valve with a 10 μl sample loop. Prior to injection, the valve was washed thoroughly with a suitable quantity of 1,2-dichloroethane. Consistent separation of triacylglycerol molecular species was obtained for the backfats tested. A typical trace obtained from a computer scanned image of a HPLC chromatogram from an outer backfat sample is shown in Figure 5.1, . A total of 14 separated triacylglycerol species was able to be collected. Their separation, which was based on the degree of unsaturation, was performed with initial elution of wholly saturated triacylglycerols. In order to determine the relative amounts of each fraction, as well as the fatty acid composition of the triacylglycerols, the fractions were collected manually for methylation and gas-liquid chromatographic analysis. To achieve this, a Nupro® stream-splitter (P.S. Instruments Ltd., Sevenoaks, England) was positioned between the column and the light-scattering detector. By altering the resistance to flow

through the action of a needle-valve, the stream-splitter was adjusted to give a satisfactory split ratio between the light-scattering detector and the eluant collection point. The action of a micrometer screw gauge allowed adjustment of the needle valve to give consistent and accurate split ratios. A split ratio was chosen of 1:8 between the light-scattering detector and the collection point, respectively; it was found that this was obtained when the rate of solvent flow from the collection point was 1.00 ±0.05 g solvent A/min.

Triacylglycerol fractions were collected as designated from the moment the integrator plot showed the start of a peak until the start of the next significant peak. Verification of synchronisation between the time at which the detector responded and the time at which the fraction was collected was undertaken by inspection using a tristearin standard dissolved in 1,2-dichloroethane and collecting the eluant before, during and after the appearance of a peak on the integrator.

5.5. Gas-liquid chromatographic analysis of eluted triacylglycerols

In all cases the triacylglycerol fractions were methylated according to methodology described previously (see Section 2.6.4.1) in the presence of 0.0322 mg of pentadecanoic acid internal standard. To take into account the discrepancies of the absolute levels of triacylglycerol present, appropriate reductions in volumes of methylating agent and solvent extraction media were made.

Gas-liquid chromatographic analysis of each fraction was undertaken as described in Section 2.6.4.2.

5.6. Processing of data

Following gas-liquid chromatographic analysis, the peak area data was manually transferred to a spreadsheet program (Microsoft® Excel 4.0). In fractions containing saturated, monoenoic and dienoic fatty acids, identification of the corresponding TAG molecular species was straightforward. In such fractions, the presence of small amounts of more unsaturated fatty acids (i.e. those containing three or more double bonds) were omitted in the re-processing of chromatograms. In triacylglycerol species containing more highly unsaturated fatty acids, identification of any triacylglycerol peak was not as straightforward as for those cited above. Considerable care therefore had to be taken to identify those fatty acids which were important but which occurred in relatively small quantities.

5.7. Evaluation of triacylglycerol separations

This was performed by undertaking replicate elutions of two series of triacylglycerols: i.) standard triacylglycerols of known fatty acid composition and combination. These were obtained commercially (Sigma Chemical, Poole, England); ii.) a combination of triacylglycerols obtained from biological tissues with a known overall fatty acid composition. For example, pig backfat triacylglycerols. Clearly, the triacylglycerol molecular species able to be obtained commercially were confined to relatively simple combinations. Only by reference to biological samples could the more complex triacylglycerol species, in particular those containing the longer chain polyunsaturates, be obtained. As can be seen from Figure 5.1 and Table 5.1, the backfat obtained from pigs that had received fish oil produced a chromatogram with some 14 separable fractions, all of which could be delineated with respect to their fatty acid compositions. Based on the work of Christie and Moore (1970b), fatty acids in the first six triacylglycerol species in Table 5.1 have been described in the order that they are positioned on the glycerol moiety, i.e. positions 1, 2 and 3, respectively. Saturated and monounsaturated triacylglycerol species contained only three principal fatty acids whereas fractions containing a mixture of triacylglycerol species necessarily contained more fatty acids. Separation was achieved on the basis of overall unsaturation with the more saturated fatty acid fractions eluting first. In general, each peak differed from the next by a difference of one double bond only. However, in some cases, two adjacent and clearly separated peaks contained the same number of double bonds, e.g. SMM and SSD. This would appear to be due to the greater retention by the column of triacylglycerols containing a single highly unsaturated fatty acid. This phenomenon was also observed in more highly unsaturated fractions such as the MDD/SMT fraction in which SMT of four double bonds coeluted with MDD of five double bonds. In certain instances, adjacent fractions eluted closely together and had to be collected as a single fraction, e.g. triacylglycerols with configurations MMD and SDD.

The detector response was unable to be checked other than for a few standard triacylglycerols of known fatty acid composition. In the circumstances therefore, the chromatograms derived from the light-scattering detector were not used for quantification. In all cases, quantification of the separated triacylglycerols was undertaken from data produced by the subsequent gas-liquid chromatographic analyses and internal standardisation. Reproducibility of the analyses was verified by replicate runs and was found to be acceptable.

5.8. Evaluation of the method

Although a total of 14 triacylglycerol fractions was able to be separated, some 19 species were recorded. Complete separation and measurement of all species was not

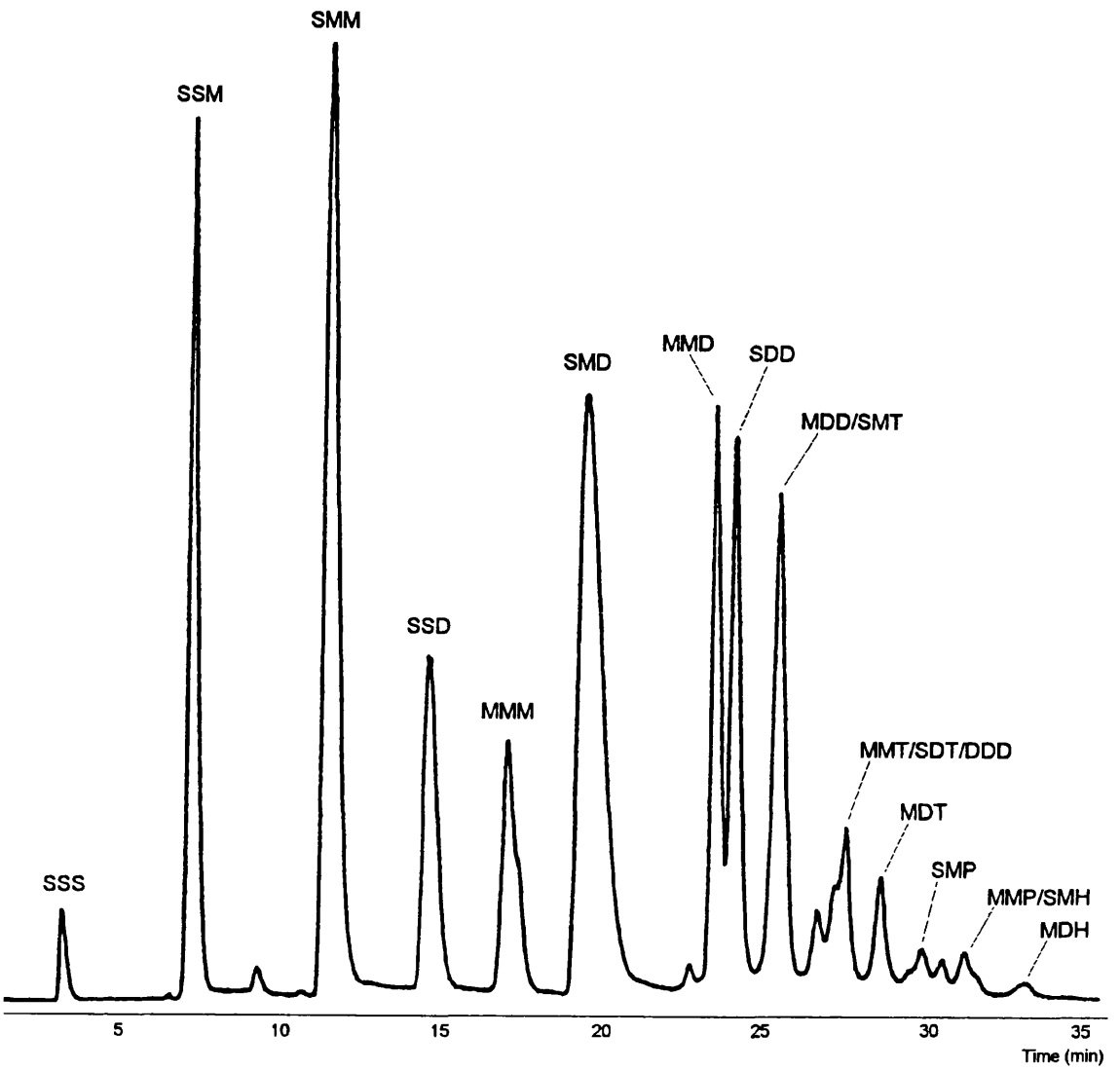


Figure 5.1 - Chromatogram showing the separation of molecular species of triacylglycerol from pig outer backfat by silver ion high performance liquid chromatography; fatty acids designated as S (saturated), M (monoenoic), D (dienoic), T (trienoic), P (pentaenoic) and H (hexaenoic)

Fraction	Principal fatty acids
SSS	18:0 - 16:0 - 18:0
SSM	18:0 - 16:0 - 18:1*n-9*
SMM	18:1*n-9* - 16:0 - 18:1*n-9*
SSD	18:0 - 16:0 - 18:2*n-6*
MMM	18:1*n-9* - 18:1*n-9* - 18:1*n-9*
SMD	18:1*n-9* - 16:0 - 18:2*n-6*
MMD/SDD	18:1*n-9* - 16:0 - 18:2*n-6*
MDD/SMT	18:2*n-6* - 18:1*n-9* - 18:3*n-3*
MMT/SDT/DDD	18:2*n-6* - 18:1*n-9* - 18:3*n-3*
MDT	18:2*n-6* - 18:1*n-9* - 18:3*n-3*
SMP	16:0 - 18:1*n-9* - 18:2*n-6* - 18:3*n-3* - 22:5*n-3*
MMP/SMH	18:1*n-9* - 16:0 - 22:5*n-3* - 22:6*n-3*
MDH	18:1*n-9* - 18:2*n-6* - 22:6*n-3*
DDH	18:2*n-6* - 18:2*n-6* - 22:6*n-3*

Table 5.1 - The content of major fatty acids in the triacylglycerol fractions; fatty acids designated as S (saturated), M (monoenoic), D (dienoic), T (trienoic), P (pentaenoic) and H (hexaenoic) and according to Table 2.1

possible due to the proximity of peaks and coelution of some of the triacylglycerol species. Undoubtedly however, the separation and determination of the fractions obtained were markedly superior to those achievable by other methodologies. The major methodology which had been previously used for triacylglycerol species analysis of pig backfat was silver ion thin-layer chromatography. Using this technique, Anderson *et al* (1970) were only able to separate triacylglycerols of SSS, SSM, SMM and MMM configuration in pig backfat whilst Christie and Moore (1970) by suitable modification of the methodology were additionally able to separate species with SSD and SMD configuration. Dziubajlo (1991) purported to have separated other triacylglycerol species in pig backfat using silver ion thin-layer chromatography but with noticeable inconsistencies. Thus, in conclusion, the availability of silver ion high performance liquid chromatography allowed markedly improved and quite consistent separation of triacylglycerols to that able to be achieved previously.

6. The effect of dietary inclusion of long chain polyunsaturated fatty acids on the fatty acid composition and physical properties of pig backfat

6.1. INTRODUCTION

The experiments described previously (see Section 3) which involved pigs receiving diets containing high levels of soybean oil in conjunction with a relatively low level of refined fish oil resulted in backfats in which linoleic acid constituted between 25 and 35% of total fatty acids. As has been indicated previously (Section 3.4), the presence of very high levels of linoleic acid in the backfat of pigs, although readily achievable, has always been associated with unacceptable physical properties. Indeed, a linear correlation between the linoleic acid content and consistency has been shown to occur (Whittington *et al*, 1986) and is routinely used to demonstrate the deleterious effects arising from feeding linoleic acid. These authors suggested that a maximum level of linoleic acid in the backfat commensurate with acceptable physical properties should not exceed 15% of total fatty acids. It was therefore surprising to observe that in the present series of experiments (see Section 3.3) the incorporation of linoleic acid into the backfat at levels of 25-35% of total fatty acids failed to have any extreme effect on the physical property. Indeed, 'fluidity' of such backfats was only marginally above the cut-off point between acceptability and non-acceptability whilst the presence of the fish oils in the diet appeared to have a reversal effect on fluidity and promoted the production of a firmer fat.

It would thus appear that there existed some factor which was able to compensate for the presence of high linoleic acid levels and consequential effects on fluidity. The investigations of Dziubajlo (1991) indicated the importance of triacylglycerol structure as a factor influencing the melting point of pig backfat. Significantly, these investigations demonstrated that pigs of a wide variety of breeds but having similar backfat fatty acid compositions could give rise to a wide range of melting points. In the reverse, different fatty acid compositions were associated with similar melting points. Furthermore, randomisation of the fatty acids of the fats from different breeds through interesterification produced a unification of melting points. These investigations in conjunction with previous work (Moore *et al*, 1969; Christie and Moore, 1969) showed that the physical properties of the backfat, or to be more specific, the triacylgycerols, are not explained solely by a consideration of the fatty acid composition. It is thus possible that the observations presently described can be accounted for by specific changes in triacylglycerol structure. In addition, the presence in the diet of the fish oil may not promote the level of fat softness which might be expected to arise from the highly unsaturated nature of the fatty acids present (Irie and Sakimoto, 1992; Hertzman *et al*, 1992). The likelihood that this effect may be brought about by a specific interaction of the long chain *n-3* polyunsaturated fatty acids with alterations in triacylglycerol structure has therefore to be considered.

The present experiment was designed to examine the changes in physical properties

of pig backfat and triacylglycerol structural characteristics occurring as a result of changes in the proportions of soybean oil and fish oil - and thus the polyunsaturated fatty acids - within the diets.

6.2. MATERIALS AND METHODS

6.2.1. Composition of diets

Six diets were prepared according to the ingredient composition shown in Table 6.1 and were fed *ad libitum* from feed hoppers. The main components were barley and soybean meal. Each diet contained tallow or soybean oil or a mixture of soybean oil and fish oil fed at different levels. The level of barley was adjusted in order to maintain similar levels of energy content between the diets. The 50 g/kg tallow diet served as a control whilst diets containing soybean oil or soybean oil/fish oil comprised the treatments for investigations of the interrelationships between backfat fluidity and chemical composition. The proximate and mineral composition of the diets are shown in Table 6.2. The differing oil contents of the diets were reflected in the values for the respective ether extract value. The levels of the macro- and micro-minerals were similar between the diets. The diets differed with respect to total fat content in the range 4.4 to 8.2% by weight of diet; the linoleic acid content of the diets differed widely as appropriate to the levels of inclusion of the soybean oil over a wide range with good agreement between soybean oil diets and their paired soybean oil/fish oil diets.

6.2.1.1. Fatty acid composition

The major fatty acids present within the soybean oil and the fish oil ('Boost' oil; Seven Seas Ltd., Hull, England) were identical to those described previously (Section 3.2.1). The fatty acid composition of the diets is shown in Table 6.3. The most notable differences were commensurate with the various oil additions. Levels of saturated and monounsaturated fatty acids were higher in the tallow diet than in any of the other diets. The presence of fish oil in the soybean oil/fish oil diets resulted in higher levels of saturated and monounsaturated fatty acids than in corresponding soybean oil diets. This is accounted for by the presence of approximately 50% saturated plus monounsaturated fatty acid components in the original fish oil. The level of linoleic acid in all of the experimental diets was over twice that observed in the control. The level of linoleic acid was similar between diets SO1, SO2 and SO3 and

	Tallow	SO1	SOFO1	SO2	SOFO2	SO3
Description*	5% Tallow	2.5% SO	2.5% SO + 1.0% FO	5.0% SO	5.0% SO + 1.0% FO	7.5% SO
Barley	627.2	652.7	642.5	627.2	617.0	601.7
Soybean meal	265	265	265	265	265	265
Dicalcium phosphate	7.8	7.3	7.5	7.8	8.0	8.3
Fish meal	25	25	25	25	25	25
Pig Breeder 20E	20	20	20	20	20	20
Wafolin	5	5	5	5	5	5
Tallow	50	0	0	0	0	0
Soybean oil	0	25	25	50	50	75
Fish oil	0	0	10	0	10	0
Total fat	65.5	44.3	54.2	63.0	70.6	82.2
Linoleic acid	13.3	24.0	25.8	34.9	33.9	45.9

*SO soybean oil FO fish oil

Table 6.1 - Ingredient composition of the control and experimental diets (g/kg diet)

Component	Tallow	SO1	SOFO1	SO2	SOFO2	SO3
Dry matter	879	876	874	881	876	878
Digestible energy (MJ/kg DM)	16.3	15.3	15.5	15.3	15.8	15.4
Crude protein	237	242	226	236	238	227
Ash	62	63	60	65	57	59
Acid hydrolysed ether extract	62.0	57.8	73.8	89.6	94.6	102.8
Crude fibre	38	43	40	36	36	37
Neutral detergent fibre	141	206	210	238	224	250
Calcium	11.1	11.9	11.2	13.2	10.8	11.4
Phosphorus	8.5	8.3	8.3	8.9	8.3	8.2
Sodium	1.54	1.51	1.58	1.83	1.77	1.53
Copper (mg/kg DM)	25.9	25.7	24.5	25.4	26.3	22.5
Zinc (mg/kg DM)	145	159	153	187	145	156
Iron (mg/kg DM)	291	303	319	314	268	281
Selenium (mg/kg DM)	0.33	0.34	0.38	0.36	0.33	0.29

Table 6.2 - The proximate and mineral compositions of the control and experimental diets (g/kg dry matter, DM, unless stated otherwise); shorthand designations of treatments as per Table 6.1

	Tallow	SO1	SOFO1	SO2	SOFO2	SO3
14:0	2.06	0.30	1.41	0.21	0.99	0.17
16:0	22.4	14.8	15.7	13.5	14.1	12.6
16:1*n-7*	3.77	0.60	2.17	0.46	1.55	0.36
18:0	15.24	3.07	3.22	3.29	3.21	3.37
18:1*n-9*	31.6	17.6	17.5	18.8	21.4	18.9
18:2*n-6*	20.4	54.2	47.5	55.4	48.1	55.8
18:3*n-3*	3.57	7.32	6.68	7.35	6.26	7.59
20:3*n-6*	0.08	0.30	0.29	0.31	0.66	0.35
20:4*n-6*	0.18	0.16	0.34	0.04	0.34	0.11
20:5*n-3*	0.35	0.79	2.95	0.41	2.14	0.36
22:5*n-3*	0.09	0.09	0.31	-	0.12	0.04
22:6*n-3*	0.27	0.70	1.96	0.27	1.21	0.32
Total SAT	38	18	19	17	17	16
Total MUFA	35	18	20	19	23	19
Total PUFA	25	64	60	64	59	65
P:S[1]	0.7	3.6	3.2	3.8	3.5	4.1
U:S[2]	1.6	4.6	4.2	4.9	4.7	5.3
n-6:n-3[3]	4.8	6.1	4.0	6.9	5.0	6.8

[1] polyunsaturated to saturated fatty acid ratio [2] unsaturated to saturated fatty acid ratio
[3] the ratio of total *n-6* to *n-3* fatty acids

Table 6.3 - The fatty acid composition of the control and experimental diets (major fatty acids, percentage of total present); shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

accounted for some 55% of the total fatty acids; the percentage was reduced accordingly in diets SOFO1 and SOFO2. The levels of long chain polyunsaturated fatty acids, in particular eicosapentaenoic acid and docosahexaenoic acid were markedly higher in diets containing the fish oil. The significant presence of eicosapentaenoic acid and docosahexaenoic acid within the tallow and soybean oil diets were accountable by inclusion of the fish meal as a component of all the diets. The ratios of polyunsaturated:saturated and unsaturated:saturated fatty acids were markedly lower for the tallow diet than for the other diets. The presence of eicosapentaenoic acid and docosahexaenoic acid was reflected in the *n-6:n-3* fatty acid ratios of diets containing fish oil.

6.2.2. The pigs and their treatment

Twenty-four Large-White x Landrace entire male pigs were randomly assigned to six treatment groups and were fed one of the six diets described above for five weeks before slaughter. Although it was planned that pigs would be fed for six weeks, the liveweight gain of the pigs was such that slaughter weight was attained earlier than anticipated. During the growth phase the pigs were weighed weekly for determining their liveweight gain and a detailed record was kept of group feed intakes. The absence of individual recording precluded collection of data for each pig. Commencement of the trial was 'staggered' to allow sufficient time for full carcass evaluation of each pig. The pigs were slaughtered at an overall mean liveweight of 81.8 kg (±5.0 standard deviation).

6.2.2.1. Carcass evaluation

Following slaughter, pigs were sent to the Carcass Evaluation unit at the Scottish Agricultural College, Edinburgh, for removal of the *Semitendinosus* and outer and inner backfat. Tissues were excised from the pigs according to the method described previously (Section 2.2). From each pig, samples of intact backfat from the shoulder and the loin were sent to the Division of Food Animal Science (University of Bristol) for measurement of fat firmness by penetrometry as described in Section 2.3.

6.2.3. Determination of the lipid and fatty acid compositions of the tissues

The lipid and fatty acid compositions of all tissues were determined as described previously in Section 2.6.

6.2.4. Determination of the distribution of triacylglycerol molecular species

Qualitative and quantitative estimation of triacylglycerol molecular species was based on the method of Christie (1988) as described previously (Section 5). As outlined, separation of intact triacylglycerols was achieved on the basis of differences in the degree of unsaturation using silver ion high performance liquid chromatography and evaporative light-scattering detection.

6.2.5. Statistical analysis

Data were analysed using the Genstat statistical package (Genstat 5 Release 1.3 Lawes Agricultural Trust, Rothamstead Experimental Station) using one-way analysis of variance and multiple regression. Potential differences between the treatments in the firmness of backfats over the shoulder and loin were investigated by multiple regression using ultimate fat thickness (P2) and whole carcass weight measurements as covariates. Where statistically significant effects occurred, differences between treatments were identified by the method of orthogonal contrasts. Five separate contrasts were devised, as shown in the table below. The fatty acid and triacylglycerol species data were also analysed by one-way analysis of variance; differences between treatments were identified by the method of contrasts. Potential correlations between physical properties of pig fat and fatty acids or triacylglycerol molecular species were examined and expressed as Pearson (r) coefficients.

Contrast	Tallow	SO1	SOFO1	SO2	SOFO2	SO3
Tallow *vs.* rest	5	-1	-1	-1	-1	-1
SO1,SOFO1,SO2, SOFO2 *vs.* SO3	0	-1	-1	-1	-1	4
SO1,SOFO1 *vs.* SO2,SOFO2	0	1	1	-1	-1	0
SO1 *vs.* SOFO1	0	1	-1	0	0	0
SO2 *vs.* SOFO2	0	0	0	1	-1	0

6.3. RESULTS

6.3.1. Pig performance and carcass evaluation

The results obtained from the various measurements of growth are shown in Table 6.4. The results indicated no significant effects of diet on these parameters of pig performance. All groups displayed daily liveweight gain with average growth in excess of expected figures (SAC, 1994). Feed intake was highest in the SOFO2 and SO3 groups. No statistical comparison of feed intake between groups was possible as pigs were not fed individually. Killing out proportion approximated to the national average and was similar between treatments. Backfat thickness measurements showed no difference between treatments. Although not shown, measurements of fat thickness were made at slaughter using a standard optical probe; there was also no difference between treatments.

6.3.1.1. Backfat firmness

Results obtained from the penetrometer measurements of the backfats are shown in Figure 6.1. Measurements indicated relatively wide variation within treatments, as shown by the length of error bars which in a number of cases exceeded 100 penetrometer units. A figure of 575 penetrometer units has been deemed to provide a division between acceptable and unacceptable firmness (Dransfield and Kempster, 1988). As expected, the pigs fed the exclusively tallow-based diet gave the firmest fat for both the loin and shoulder; penetrometer values of over 700 units were obtained in both cases. Compared to the values obtained for the tallow group, all treatments resulted in a significant decrease in fat firmness (see Table 6.5). For the loin, fat firmness resulting from all the soybean oil and soybean oil/fish oil treatments was lower than the recommended minimum of 575 units; there were no significant differences between the treatments. For the shoulder, there were significant effects of diet on fat firmness. Thus, the SOFO1 diet resulted in a 23% increase in firmness compared to the SO1 diet. In overall terms, the firmness of the shoulder fat was higher than the loin by some 50 penetrometer units (604 *vs.* 552, respectively).

6.3.2. Composition of the lipids of the tissues

The total lipid contents of the outer backfat, inner backfat and *Semitendinosus* are shown in Table 6.6. The outer and inner backfats were characterised by lipid contents in the region of 70 g/100 g whilst the values for the *Semitendinosus* muscle tissue were approximately 2.5 g/100 g. These figures accord with those obtained previously in the

Parameter	Tallow	SO1	SOFO1	SO2	SOFO2	SO3	SED[1]	Signif.[2]
Start weight (kg)	44.3	44.9	43.4	44.3	43.1	44.5	-	-
Finish weight (kg)	85.0	82.2	77.8	83.9	81.2	81.4	-	-
Weight gain (kg/d)	0.93	0.85	0.82	0.90	0.87	0.84	0.06	NS
Feed intake (kg/d)	2.59	2.47	2.39	2.40	2.91	2.78	nd	nd
Whole carcass (kg)	63.3	61.3	57.3	61.8	60.4	61.9	3.05	NS
Carcass wt./Slaughter wt.	0.74	0.75	0.74	0.74	0.74	0.76	0.01	NS
Ham joint (kg)	10.1	9.5	9.1	9.7	9.5	9.7	0.65	NS
Loin joint (kg)	5.6	5.6	5.4	5.3	5.7	5.9	0.41	NS
Shoulder joint (kg)	10.2	9.7	8.4	10.0	9.0	9.3	0.49	*
Flank (kg)	3.9	3.7	3.4	3.8	3.5	3.4	0.27	NS
Residual (kg)	23.3	22.2	20.2	22.6	21.3	21.7	1.17	NS
Waste (kg)	4.5	4.5	4.3	4.2	4.6	4.7	0.36	NS
Backfat thickness (mm)	6.0	7.5	7.5	7.7	7.0	6.3	1.79	NS

[1] SED standard error of the differences between the means [2] * significant difference at P≤0.05 NS not significant nd not determinable

Table 6.4 - The growth performance and carcass composition of the pigs fed the control and experimental diets; shorthand designations of treatments as per Table 6.1

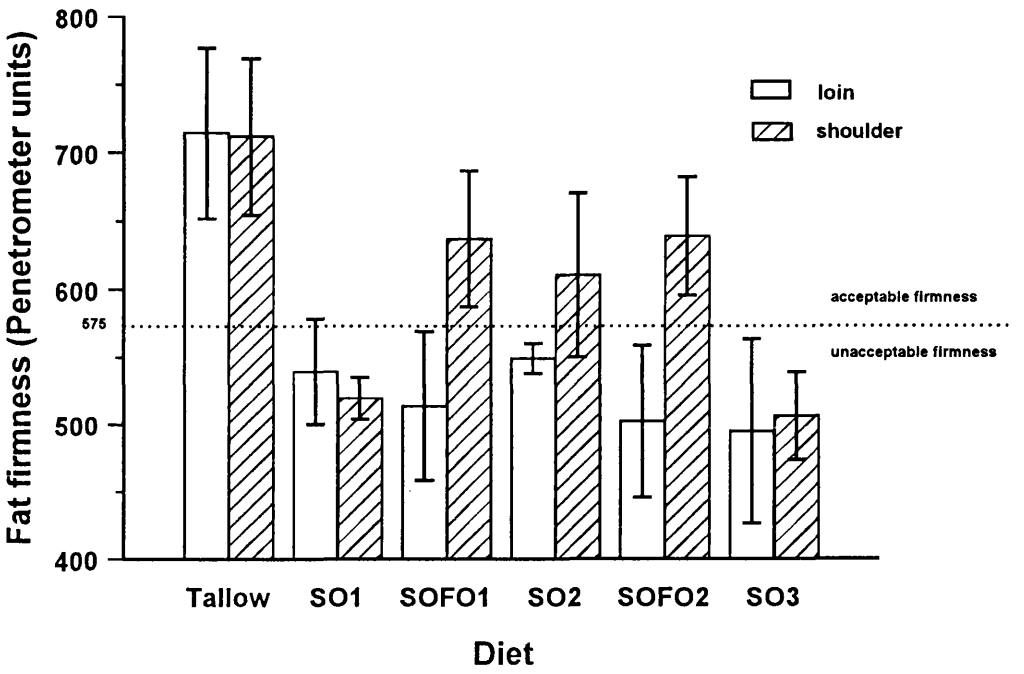


Figure 6.1 - Effect of dietary fat treatments on firmness of the backfat measured over the loin and shoulder; all measurements corrected to 4 °C; each result is the mean ±standard error.

first experiment (Section 3.3.2).

The lipid composition of the *Semitendinosus* muscle tissue is shown in Table 6.7. As shown previously, triacylglycerol comprised some 70% of the total fat within the tissue and as accompanied by substantial levels also of phospholipid. The lipid composition was similar in all respects to that observed previously (see Section 3.3.3). There were no significant differences in lipid composition as a result of dietary treatment.

6.3.3. Fatty acid composition of the tissue lipid fractions

The fatty acid composition of the triacylglycerols of the outer and inner backfats and all the major lipid moieties of the *Semitendinosus* muscle were determined and accorded with the tissue compositions described previously. In the present circumstances, only the fatty acid compositions of the triacylglycerol fractions of the outer and inner backfat and the triacylglycerol and phospholipid fractions of the *Semitendinosus* muscle are of relevance here.

6.3.3.1. Outer backfat

Table 6.8 shows the effect of the diets on the fatty acid composition of the outer backfat triacylglycerol including the relevant summaries with respect to total saturated-, total monounsaturated- and polyunsaturated fatty acids (i.e. total SAT, total MUFA, total PUFA, respectively), total polyunsaturated:saturated (P:S) fatty acid ratio and total *n-6:n-3* fatty acid ratio. Table 6.9 shows the associated individual orthogonal contrasts applied to the results. As observed previously in Section 3, the fatty acid compositions of the tissues were significantly influenced by the fatty acid compositions of the diets. Marked differences in fatty acid composition were shown between outer backfats from pigs receiving the basic tallow diet and the diets supplemented with vegetable/fish oils. The fatty acid composition of the triacylglycerols from the pigs fed the basic tallow diet were similar to those obtained previously (Morgan *et al*, 1992) with the major acid being oleic acid followed by palmitic and linoleic acids; apart from linoleic and α-linolenic acids, long chain polyunsaturated fatty acids were at low levels. In general, the oil-based diets significantly reduced the levels of saturated and monounsaturated fatty acids, the greatest effect resulting from the SO3 treatment. Apart from stearic acid, the levels of saturates and monounsaturates were lower in SO3 than in any of the other treatments. Levels of myristic, palmitic and stearic acids were also significantly reduced in pigs receiving increasing amounts of oil. The inclusion of fish oil was without further effect on the levels of these fatty acids. The level of stearic acid was less affected by treatment than the other fatty acids.

The levels of polyunsaturated fatty acids were markedly affected by diet. Figure

	Comparison 1	Comparison 2	Comparison 3	Comparison 4	Comparison 5	Overall level of significance
	Tallow *vs.* rest	SO1, SOFO1, SO2, SOFO2 *vs.* SO3	SO1, SOFO1 *vs.* SO2, SOFO2	SO1 *vs.* SOFO1	SO2 *vs.* SOFO2	
Loin[1]	0.001	NS	NS	NS	NS	0.014
Shoulder	0.001	0.07	NS	0.05	NS	0.004

[1] NS not significant

Table 6.5 - Orthogonal contrasts for the firmness of fat over the loin and shoulder; values quoted as probabilities; shorthand designations of treatments as per Table 6.1

Tissue	**Tallow**	**SO1**	**SOFO1**	**SO2**	**SOFO2**	**SO3**	SED[1]	Signif.[2]
Outer backfat	68.3	70.5	71.2	72.0	70.7	71.2	4.21	NS
Inner backfat	72.5	75.0	74.3	77.4	78.1	75.2	4.35	NS
Semitendinosus	2.15	2.23	2.40	3.24	2.29	2.86	0.54	NS

[1] SED standard error of the differences between the means [2] NS not significant

Table 6.6 - The total lipid contents of the tissues (g/100g tissue) from the pigs fed the control and experimental diets; shorthand designations of treatments as per Table 6.1

Treatment	**Cholesterol ester**	**Triacylglycerol**	**Free fatty acid**	**Free cholesterol**	**Phospholipid**
Tallow	0.41	69.0	4.34	4.03	22.3
SO1	0.27	69.3	3.87	3.19	23.4
SOFO1	0.30	70.5	2.99	3.33	22.9
SO2	0.38	78.9	3.28	2.05	15.4
SOFO2	0.36	67.5	4.38	3.56	24.2
SO3	0.22	75.0	3.76	2.72	18.3
SED[1]	0.074	6.00	0.751	0.821	4.92
Signif.[2]	NS	NS	NS	NS	NS

[1] SED standard error of the differences between the means [2] NS not significant

Table 6.7 - Relative proportions of the major lipids (g/100g total lipid) within the *Semitendinosus*; shorthand designations of treatments as per Table 6.1

	Tallow	SO1	SOFO1	SO2	SOFO2	SO3	SED[1]	Signif.[2]
14:0	1.52	1.25	1.38	1.07	1.25	1.10	0.080	***
16:0	21.6	20.6	20.7	19.4	19.6	18.3	0.55	***
16:1*n-7*	4.09	2.81	2.98	2.60	2.90	2.25	0.200	***
18:0	10.7	10.0	10.5	8.93	8.89	8.82	0.721	*
18:1*n-9*	44.0	36.7	35.8	35.9	35.8	32.7	2.20	***
18:2*n-6*	14.1	24.0	23.0	27.4	26.1	31.5	2.44	***
18:3*n-3*	2.97	3.41	3.54	3.81	3.52	4.24	0.240	***
20:3*n-6*	0.07	0.10	0.10	0.11	0.15	0.10	0.038	NS
20:4*n-6*	0.37	0.54	0.53	0.42	0.41	0.51	0.103	NS
20:5*n-3*	0.09	0.10	0.42	0.11	0.34	0.09	0.034	***
22:5*n-3*	0.22	0.27	0.51	0.12	0.47	0.17	0.075	***
22:6*n-3*	0.25	0.26	0.57	0.18	0.49	0.25	0.073	***
Total SAT	34	32	33	29	30	28	1.1	***
Total MUFA	48	40	39	38	39	35	2.2	***
Total PUFA	18	29	29	32	31	37	2.8	***
P:S[3]	0.5	0.9	0.9	1.1	1.1	1.3	0.13	***
n-6:n-3[4]	4.1	6.1	4.7	6.7	5.6	6.8	0.27	***

[1] SED standard error of the differences between the means [2] * *** significant differences, respectively, at P≤0.05 and P≤0.001 NS not significant
[3] polyunsaturated to saturated fatty acid ratio [4] ratio of total *n-6* to *n-3* fatty acids

Table 6.8 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the outer backfat in the pigs fed the control and experimental diets; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

	Comparison 1	Comparison 2	Comparison 3	Comparison 4	Comparison 5
	Tallow *vs.* rest[1]	SO1, SOFO1, SO2, SOFO2 *vs.* SO3	SO1, SOFO1 *vs.* SO2, SOFO2	SO1 *vs.* SOFO1	SO2 *vs.* SOFO2
14:0	0.001	0.02	0.02	0.10	0.04
16:0	0.001	0.001	0.011	NS	NS
16:1*n-7*	0.001	0.001	NS	NS	NS
18:0	0.03	NS	0.014	NS	NS
18:1*n-9*	0.001	0.05	NS	NS	NS
18:2*n-6*	0.001	0.002	0.07	NS	NS
18:3*n-3*	0.001	0.001	NS	NS	NS
20:3*n-6*	NS	NS	NS	NS	NS
20:4*n-6*	NS	NS	NS	NS	NS
20:5*n-6*	<0.001	<0.001	NS	<0.001	<0.001
22:5*n-3*	0.09	0.004	NS	0.003	<0.001
22:6*n-3*	0.06	0.02	NS	<0.001	<0.001
Total SAT	<0.001	0.003	0.003	NS	NS
Total MUFA	<0.001	0.024	NS	NS	NS
Total PUFA	<0.001	0.005	NS	NS	NS
P:S[2]	<0.001	0.002	0.046	NS	NS
n-6:n-3[3]	<0.001	<0.001	0.003	<0.001	<0.001

[1] NS not significant [2] polyunsaturated to saturated fatty acid ratio
[3] ratio of total *n-6* to *n-3* fatty acids

Table 6.9 - Orthogonal contrasts for the fatty acid composition of the outer backfat triacylglycerol; values quoted as probabilities; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

6.2 shows a strictly linear increase in the levels of linoleic acid observed in the outer backfat as a result of increasing dietary intake of linoleic acid. There were significant differences in the levels of linoleic acid within the backfat depending upon the degree of inclusion of the soybean oil in the diet. The level of linoleic acid in those pigs receiving the highest levels of soybean oil was some two-fold greater than for pigs fed the tallow-based diet. The presence of fish oil was without further effect on linoleic acid levels. The levels of C20 and C22 long chain polyunsaturated fatty acids were significantly affected by diet. As can be seen in Tables 6.8 and 6.9, the levels of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid were significantly enhanced as a result of the inclusion of fish oil in the diet. The presence of the soybean oil/fish oils brought about highly significant increases in the content of both *n-6* and *n-3* polyunsaturated fatty acids with consequential effects on polyunsaturated fatty acid ratios.

6.3.3.2. Inner backfat

The fatty acid composition of inner backfat is shown in Table 6.10 and respective orthogonal contrasts in Table 6.11. The distribution of fatty acids between the different dietary treatments was similar to that observed in the outer backfat triacylglycerol but with several exceptions. As a general observation, the effects of the diets were less marked than those observed in the outer backfat. The greatest difference in fatty acid composition was observed between the tallow control and the experimental groups. In particular, the dietary effects on eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid levels were less than for the outer backfat with concomitant effects on *n-6*:*n-3* ratios.

6.3.3.3. *Semitendinosus*

The fatty acid composition of the triacylglycerols of the *Semitendinosus* muscle tissue is shown in Table 6.12 and associated orthogonal contrasts in Table 6.13. In overall terms, the fatty acid composition was similar to that observed previously (Section 3.3.4.2). Inclusion of the soybean oil/fish oil had significant effects on the saturated and monounsaturated fatty acid levels with stearic acid being less affected than palmitic. The levels of linoleic acid and α-linolenic acid increased significantly with increasing level of soybean oil in the diet. The inclusion of fish oil resulted in marked increases in the levels of long chain *n-3* polyunsaturated fatty acids although these differences were not reflected in the *n-6*:*n-3* ratios.

The fatty acid composition of the phospholipids of the *Semitendinosus* muscle is shown in Table 6.14 with associated orthogonal contrasts shown in Table 6.15. As reported above for the triacylglycerol fraction, in the phospholipid fraction there were marked changes in fatty acid composition depending on the diet. There were

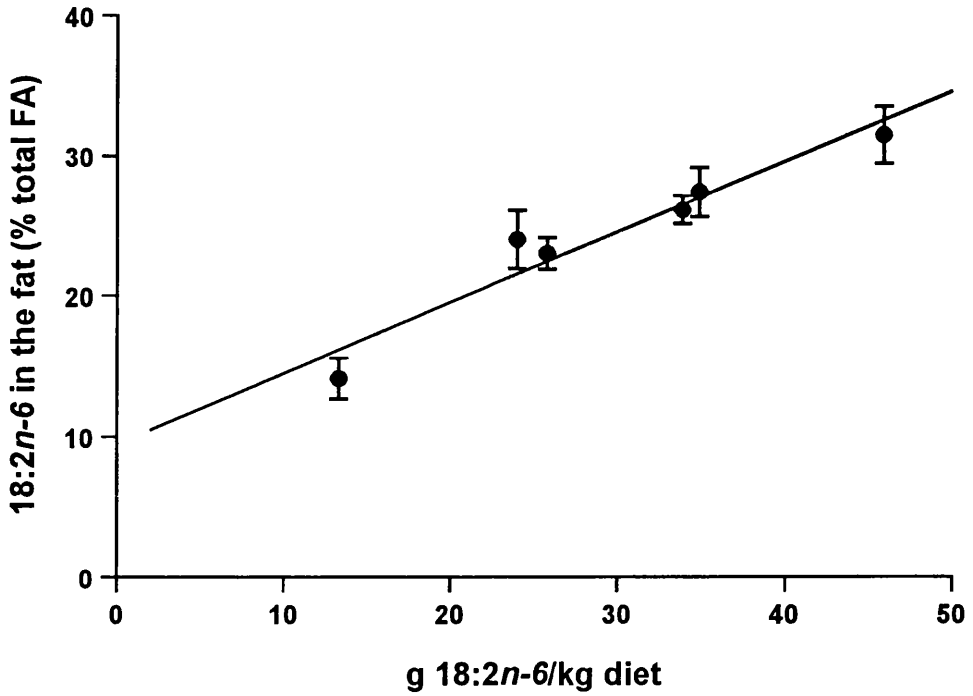


Figure 6.2 - Deposition of linoleic acid in the outer backfat following tallow, soybean oil and soybean oil/fish oil based diets; each result is the mean ±standard error; regression coefficient (r^2) = 0.93; equation of the line: y = 0.5x + 9.45; 'FA' - fatty acid

	Tallow	SO1	SOFO1	SO2	SOFO2	SO3	SED[1]	Signif.[2]
14:0	1.48	1.17	1.20	0.97	1.17	0.97	0.100	***
16:0	22.6	21.4	20.9	19.7	20.4	18.3	0.57	***
16:1*n-7*	3.89	2.51	2.68	2.27	2.62	1.90	0.179	***
18:0	12.0	11.6	11.7	10.3	10.5	9.88	0.76	*
18:1*n-9*	42.6	35.6	35.2	34.1	35.5	31.2	2.36	**
18:2*n-6*	13.8	23.3	23.6	27.7	24.9	32.5	2.53	***
18:3*n-3*	2.87	3.30	3.44	3.83	3.38	4.19	0.214	***
20:3*n-6*	0.06	0.09	0.09	0.08	0.07	0.10	0.020	NS
20:4*n-6*	0.30	0.42	0.32	0.54	0.35	0.57	0.123	NS
20:5*n-3*	0.09	0.09	0.19	0.09	0.28	0.08	0.041	***
22:5*n-3*	0.19	0.30	0.31	0.26	0.39	0.17	0.089	NS
22:6*n-3*	0.19	0.34	0.39	0.28	0.46	0.22	0.088	*
Total SAT	36	34	34	31	32	29	1.1	***
Total MUFA	46	38	38	36	38	33	2.3	***
Total PUFA	18	28	28	33	30	38	2.7	***
P:S[3]	0.5	0.8	0.8	1.1	0.9	1.3	0.11	***
n-6:n-3[4]	4.2	5.9	5.6	6.3	5.6	7.1	0.50	***

[1] SED standard error of the differences between the means [2] * ** *** significant difference, respectively, at P≤0.05, P≤0.01 and P≤0.001 NS not significant
[3] polyunsaturated:saturated fatty acid ratio [4] ratio of total *n-6* to *n-3* fatty acids

Table 6.10 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the inner backfat in the pigs fed the control and experimental diets; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments).

	Comparison 1	Comparison 2	Comparison 3	Comparison 4	Comparison 5
	Tallow *vs.* rest[1]	SO1, SOFO1, SO2, SOFO2 *vs.* SO3	SO1, SOFO1 *vs.* SO2, SOFO2	SO1 *vs.* SOFO1	SO2 *vs.* SOFO2
14:0	<0.001	0.04	NS	NS	0.06
16:0	<0.001	<0.001	0.02	NS	NS
16:1*n-7*	<0.001	<0.001	NS	NS	0.07
18:0	0.05	0.05	0.03	NS	NS
18:1*n-9*	<0.001	0.04	NS	NS	NS
18:2*n-6*	<0.001	<0.001	NS	NS	NS
18:3*n-3*	<0.001	<0.001	NS	NS	0.05
20:3*n-6*	NS	NS	NS	NS	NS
20:4*n-6*	NS	NS	NS	NS	NS
20:5*n-6*	0.06	0.01	0.04	0.01	<0.001
22:5*n-3*	NS	0.04	NS	NS	NS
22:6*n-3*	0.03	0.03	NS	NS	0.06
SAT	<0.001	<0.001	0.006	NS	NS
MUFA	<0.001	0.02	NS	NS	NS
PUFA	<0.001	<0.001	NS	NS	NS
P:S[2]	<0.001	<0.001	0.05	NS	NS
n-6:n-3[3]	<0.001	0.003	NS	NS	NS

[1] NS not significant [2] polyunsaturated to saturated fatty acid ratio
[3] ratio of total *n-6* to *n-3* fatty acids

Table 6.11 - Orthogonal contrasts for the fatty acid composition of the inner backfat triacylglycerol; values quoted as probabilities; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

	Tallow	SO1	SOFO1	SO2	SOFO2	SO3	SED[1]	Signif.[2]
14:0	1.38	1.27	1.31	1.05	1.12	1.08	0.062	***
16:0	21.5	21.4	21.0	19.8	19.2	18.8	0.55	***
16:1*n-7*	4.30	3.31	3.22	2.95	3.25	2.43	0.172	***
18:0	10.3	10.9	10.8	9.55	8.93	9.23	0.697	*
18:1*n-9*	47.3	43.7	41.5	40.8	42.3	35.9	2.20	***
18:2*n-6*	11.7	15.7	17.7	21.4	20.5	27.8	2.33	***
18:3*n-3*	2.53	2.67	2.79	3.24	2.98	3.78	0.224	***
20:3*n-6*	0.07	0.10	0.10	0.10	0.11	0.13	0.025	NS
20:4*n-6*	0.39	0.53	0.51	0.58	0.52	0.62	0.071	*
20:5*n-3*	0.09	0.09	0.28	0.08	0.25	0.06	0.032	***
22:5*n-3*	0.24	0.20	0.43	0.24	0.38	0.12	0.071	**
22:6*n-3*	0.24	0.22	0.44	0.24	0.39	0.15	0.074	**
Total SAT	33	34	33	30	29	29	1.2	***
Total MUFA	52	47	45	44	46	38	2.3	***
Total PUFA	15	19	22	26	25	33	2.7	***
P:S[3]	0.5	0.6	0.7	0.9	0.9	1.1	0.11	***
n-6:n-3[4]	3.9	5.1	4.6	5.8	5.3	6.9	0.343	***

[1] SED standard error of the differences between the means [2] * ** *** significant difference, respectively, at P≤0.05, P≤0.01 and P≤0.001 NS not significant
[3] polyunsaturated to saturated fatty acid ratio [4] ratio of total *n-6* to *n-3* fatty acids

Table 6.12 - The fatty acid composition (major fatty acids, percentage of total present) of the triacylglycerol fraction of the *Semitendinosus* in the pigs fed the control and experimental diets; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

	Comparison 1	Comparison 2	Comparison 3	Comparison 4	Comparison 5
	Tallow *vs.* rest[1]	SO1, SOFO1, SO2, SOFO2 *vs.* SO3	SO1, SOFO1 *vs.* SO2, SOFO2	SO1 *vs.* SOFO1	SO2 *vs.* SOFO2
14:0	<0.001	0.021	<0.001	NS	NS
16:0	0.003	0.001	<0.001	NS	NS
16:1*n-7*	<0.001	<0.001	NS	NS	0.10
18:0	NS	NS	0.003	NS	NS
18:1*n-9*	<0.001	0.001	NS	NS	NS
18:2*n-6*	<0.001	<0.001	0.02	NS	NS
18:3*n-3*	0.003	<0.001	0.03	NS	NS
20:3*n-6*	0.03	NS	NS	NS	NS
20:4*n-6*	0.006	NS	NS	NS	NS
20:5*n-6*	0.014	<0.001	NS	<0.001	<0.001
22:5*n-3*	NS	0.001	NS	0.002	0.06
22:6*n-3*	NS	0.004	NS	0.004	0.06
Total SAT	0.031	0.01	<0.001	NS	NS
Total MUFA	<0.001	<0.001	NS	NS	NS
Total PUFA	<0.001	<0.001	0.02	NS	NS
P:S[2]	<0.001	<0.001	0.01	NS	NS
n-6:n-3[3]	<0.001	<0.001	0.02	NS	NS

[1] NS not significant [2] polyunsaturated to saturated fatty acid ratio
[3] ratio of total *n-6* to *n-3* fatty acids

Table 6.13 - Orthogonal contrasts for the fatty acid composition of the *Semitendinosus* triacylglycerol; values quoted as probabilities; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

	Tallow	SO1	SOFO1	SO2	SOFO2	SO3	SED[1]	Signif.[2]
14:0	0.17	0.13	0.16	0.14	0.16	0.15	0.023	NS
16:0	20.0	22.3	21.8	20.2	21.3	20.6	0.85	*
16:1*n-7*	3.48	1.92	2.48	2.45	2.70	2.42	0.486	NS
18:0	13.7	13.9	13.6	14.7	13.1	13.9	0.55	NS
18:1*n-9*	15.4	10.9	9.59	9.48	9.88	8.92	0.67	***
18:2*n-6*	29.5	35.6	35.2	38.3	36.3	39.5	1.11	***
18:3*n-3*	0.67	0.61	0.61	0.64	0.53	0.69	0.078	NS
20:3*n-6*	1.41	1.29	1.10	1.20	1.06	1.03	0.122	*
20:4*n-6*	8.43	8.05	7.19	8.70	7.60	8.48	0.711	NS
20:5*n-3*	2.26	1.43	2.87	1.12	2.36	1.09	0.141	***
22:5*n-3*	2.25	2.02	2.54	1.74	2.30	1.67	0.153	***
22:6*n-3*	2.73	1.92	2.74	1.39	2.73	1.59	0.237	***
Total SAT	34	36	36	35	35	35	1.1	NS
Total MUFA	19	13	12	12	13	11	0.5	***
Total PUFA	47	51	52	53	53	54	1.1	***
P:S[3]	1.4	1.4	1.5	1.5	1.5	1.6	0.1	NS
n-6:n-3[4]	5.0	7.6	5.0	9.9	5.7	9.8	0.55	***

[1] SED standard error of the differences between the means [2] * *** significant difference, respectively, at P≤0.05 and P≤0.001 NS not significant
[3] polyunsaturated to saturated fatty acid ratio [4] ratio of total *n-6* to *n-3* fatty acids

Table 6.14 - The fatty acid composition (major fatty acids, percentage of total present) of the phospholipid fraction of the *Semitendinosus* in the pigs fed the control and experimental diets; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

	Comparison 1	Comparison 2	Comparison 3	Comparison 4	Comparison 5
	Tallow *vs.* rest[1]	SO1, SOFO1, SO2, SOFO2 *vs.* SO3	SO1, SOFO1 *vs.* SO2, SOFO2	SO1 *vs.* SOFO1	SO2 *vs.* SOFO2
14:0	NS	NS	NS	NS	NS
16:0	0.06	NS	0.04	NS	NS
16:1*n-7*	0.01	NS	NS	NS	NS
18:0	NS	NS	NS	NS	0.01
18:1*n-9*	<0.001	0.05	NS	0.06	NS
18:2*n-6*	<0.001	<0.001	0.03	NS	0.08
18:3*n-3*	NS	NS	NS	NS	NS
20:3*n-6*	0.01	NS	NS	NS	NS
20:4*n-6*	NS	NS	NS	NS	NS
20:5*n-6*	<0.001	<0.001	0.004	<0.001	<0.001
22:5*n-3*	NS	<0.001	0.05	0.002	0.002
22:6*n-3*	0.002	0.001	NS	0.002	<0.001
Total SAT	NS	NS	NS	NS	NS
Total MUFA	<0.001	0.01	NS	NS	NS
Total PUFA	<0.001	0.04	0.07	NS	NS
P:S[2]	0.07	NS	0.09	NS	NS
n-6:n-3[3]	<0.001	<0.001	0.01	<0.001	<0.001

[1] NS not significant [2] polyunsaturated to saturated fatty acid ratio
[3] ratio of total *n-6* to *n-3* fatty acids

Table 6.15 - Orthogonal contrasts for the fatty acid composition of the *Semitendinosus* phospholipid; values quoted as probabilities; shorthand designations as per Table 2.1 (fatty acids) and Table 6.1 (treatments)

significant increases in linoleic acid with consequential reductions in oleic acid. The levels of the long chain polyunsaturated fatty acids increased markedly and in some cases the levels were nearly doubled between fish oil- and non-fish oil containing treatments. The changes in *n-3* polyunsaturated fatty acids were reflected in changes in *n-6*:*n-3* ratios but not in P:S ratios.

6.3.4. Distribution of triacylglycerol molecular species in outer backfat

When the total triacylglycerols of the backfats were subjected to molecular species analysis as described in Section 5, distinctive differences were noted between the standard tallow diet and the oil supplemented diets and also between the various oil supplemented diets. The most extreme differences in molecular configuration were exhibited between the backfats of pigs that received the tallow diet and those which received the SOFO1 diet. These chromatograms are shown in Figure 6.3. Based on identification of the various separated triacylglycerols (see Section 5.7), it can be seen that, in particular, the high levels of the saturated and monounsaturated fatty acid-containing triacylglycerols associated with the tallow feeding were considerably reduced in the fat of the SOFO1-fed pigs (see Tables 6.16 and 6.17). It was also clear that in the SOFO1-fed pigs both the level and variety of polyunsaturated triacylglycerol species were considerably enhanced. Thus, between the two dietary groups there was a considerable shift in the profile of the triacylglycerol species giving rise to a significant prominence of species containing polyenoic fatty acids. The major triacylglycerol species in the tallow-fed pigs were those containing a saturated and two monounsaturated fatty acids (SMM), followed quantitatively by SSM, SMD and MMD+SDD species. There were marked changes in triacylglycerol species occurring with increasing inclusion of the vegetable oil. Throughout, significant differences were observed between the backfat obtained from the tallow-fed pigs and pigs receiving all of the other diets. The level of SSM species was halved, and that of MDD+SMT species was doubled in the SO3 diet compared to the tallow control.

There was a number of differences apparent in triacylglycerol species which were clearly commensurate with the differing dietary levels of soybean oil and fish oil and levels of polyunsaturated fatty acids being received. Thus, under conditions of increasing linoleic acid, SSM and MMM triacylglycerol species significantly decreased whilst the proportions of MMD+SDD, MDD+SMT and MMT+SDT+DDD progressively increased. Although the presence of fish oil in the diet diminished the levels of SMM, SMD and MMD+SDD species and enhanced those species containing long chain *n-3* polyunsaturated fatty acids, the comparisons indicated significant differences for only a few of the triacylglycerol species. In general, saturated and monounsaturated species (apart from SMM) increased slightly as a result of feeding fish oil. The most marked differences were confined to triacylglycerol species

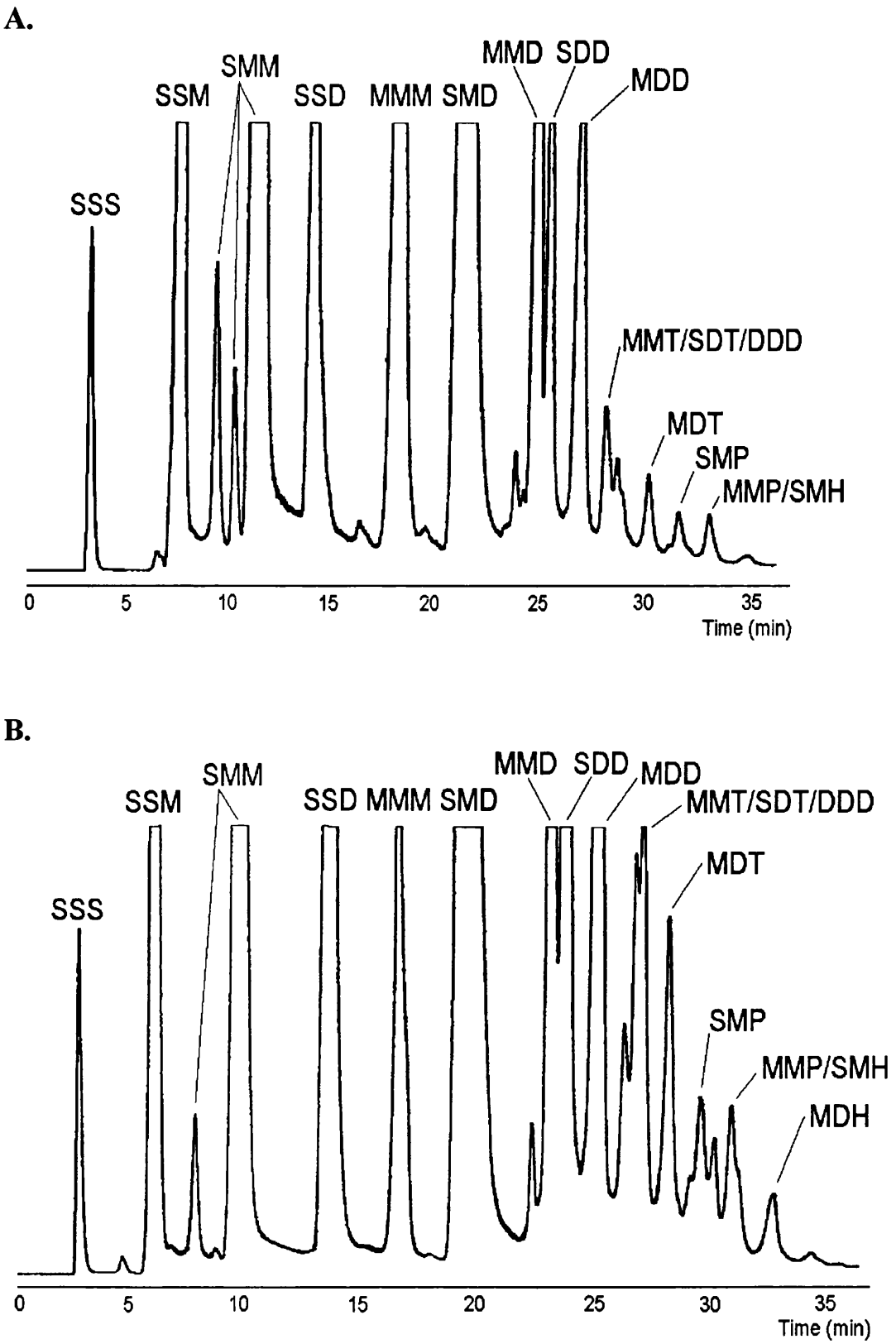


Figure 6.3 - Chromatograms showing the distribution of triacylglycerol molecular species in outer backfat from a tallow-fed pig (A) and from a pig fed the SOFO1 diet (B); fatty acids designated as S (saturated), M (monoenoic), D (dienoic), T (trienoic), P (pentaenoic) and H (hexaenoic)

Fraction	Tallow	SO1	SOFO1	SO2	SOFO2	SO3	SED[1]	Signif.[2]
SSS	2.10	1.55	1.70	1.25	1.25	1.35	0.224	**
SSM	17.2	11.9	12.5	9.78	9.96	8.77	1.28	***
SMM	27.7	19.3	16.3	17.0	15.3	14.2	2.40	***
SSD	5.87	7.71	8.51	7.61	7.40	7.48	1.236	NS
MMM	8.70	5.16	5.47	5.50	5.24	4.00	0.897	***
SMD	16.0	19.6	17.7	18.2	17.5	18.9	1.37	NS
MMD/SDD	11.5	15.8	13.7	16.9	15.5	17.7	1.23	***
MDD/SMT	4.97	8.16	7.58	10.2	11.4	11.5	1.11	***
MMT/SDT/DDD	2.66	5.57	5.77	7.06	6.82	8.27	0.861	***
MDT	0.95	2.08	2.10	2.74	3.47	3.21	1.156	***
SMP	1.03	1.66	2.75	2.03	2.33	2.74	0.417	**
MMP/SMH	1.04	1.00	2.53	1.11	2.04	1.15	0.453	**
MDH	0.29	0.46	1.20	0.60	1.27	0.81	0.261	**
DDH	-	-	0.47	-	0.52	-	0.050	NS

[1] SED standard error of the differences between the means [2] ** *** significant difference, respectively, at P≤0.01 and P≤0.001 NS not significant

Table 6.16 - The distribution of the triacylglycerol molecular species (major species, percentage of total present) in the outer backfat of the pigs fed the control and experimental diets; shorthand designations as per Table 5.1 (triacylglycerol species) and Table 6.1 (treatments)

	Comparison 1	Comparison 2	Comparison 3	Comparison 4	Comparison 5
Triacylglycerol fraction	Tallow *vs.* rest[1]	SO1, SOFO1, SO2, SOFO2 *vs.* SO3	SO1, SOFO1 *vs.* SO2, SOFO2	SO1 *vs.* SOFO1	SO2 *vs.* SOFO2
SSS	<0.001	NS	0.02	NS	NS
SSM	<0.001	0.02	0.02	NS	NS
SMM	<0.001	NS	NS	NS	NS
SSD	0.05	NS	NS	NS	NS
MMM	<0.001	0.06	NS	NS	NS
SMD	0.025	NS	NS	NS	NS
MMD/SDD	<0.001	0.02	NS	0.08	NS
MDD/SMT	<0.001	0.02	<0.001	NS	NS
MMT/SDT/DDD	<0.001	0.005	0.05	NS	NS
MDT	0.02	NS	NS	NS	NS
SMP	<0.001	0.10	NS	0.01	NS
MMP/SMH	NS	NS	NS	0.002	0.06
MDH	0.006	NS	NS	0.007	0.02

[1] NS not significant

Table 6.17 - Orthogonal contrasts for the molecular species composition of the outer backfat triacylglycerol; values quoted as probabilities; shorthand designations as per Table 5.1 (triacylglycerol species) and Table 6.1 (treatments)

containing the long chain polyunsaturated fatty acids; such differences were two- to three-fold. With the exception of SMP species, these differences were mirrored between the SO2 and SOFO2 diets. The presence of fish oil in the diet resulted in the appearance of a triacylglycerol with the configuration DDH which was not observed in any of the other groups.

6.4. DISCUSSION

Previous data in which increasing levels of unsaturated fatty acids have been achieved in the backfat of pigs have routinely demonstrated a concomitant increase in fat softness (Whittington *et al*, 1986). Therefore, it has become an established part of pig husbandry to avoid enhancement of unsaturated fatty acid levels to maintain both marketability and consumer acceptance. Indeed, fat in the diet of the pig has generally been restricted to a role as an energy provider rather than as a means of manipulating the lipid component of the carcass. From the present results it was clear that in both the shoulder and loin the fluidity of the fat was markedly increased by the presence of substantial levels of unsaturated fatty acid components, the effect being less for the shoulder than for the loin. Using a penetrometer value of 575 units as the dividing line between acceptably and unacceptably soft backfat (Dransfield and Kempster, 1988), the difference between shoulder and loin backfat softness becomes apparent. In the case of the loin, all dietary treatments can be designated as unacceptable which is not the case for the shoulder. This difference between firmness of shoulder and loin fats may have been the result of the observed thinness of the fat over the loin, making accurate measurements more difficult to obtain. Furthermore, it has been established that the firmness of pig backfat decreases with decreasing fat thickness (Prescott, 1988). The effect of diet may therefore have been masked by the thinness of the fat over the loin. The difficulty of adequately assessing the firmness of fat at this site has been noted by Wood *et al* (1989). However, these workers also observed that the firmness of fat over the shoulder could be taken as a good predictor of fat firmness over the loin. Consequently, firmness could be assessed in the shoulder and the results applied to the loin. Their study also showed that thinner backfat contains more water and collagen and less total lipid than thicker fat. Firmness measurements made on loin fat may therefore not be as reliable as those made on the shoulder for studying influences of dietary fatty acid on firmness.

In overall terms, the feeding of the unsaturated oil diets effected backfat fatty acid changes similar to those observed and described previously (see Section 3.3). With particular reference to the present experiment, the extent of deposition of linoleic acid in the backfat was even greater than that previously observed by Hertzman *et al*

(1988). It has also been observed (Prescott, 1988) that some 58% of total linoleic acid consumed by the pig was deposited in body fat depots regardless of the sex of the animal or the concentration of linoleic acid in the diet. Furthermore, it was observed that 60% of the amount incorporated into the tissue was associated with the depot fat. As in the case of linoleic acid, both docosapentaenoic acid and docosahexaenoic acid levels in backfat were highly correlatable with levels in the feed (Hertzman *et al*, 1988). However, it is noteworthy that eicosapentaenoic acid incorporation was markedly less efficient, presumably due to the channelling of eicosapentaenoic acid to various metabolic interpolations, e.g. synthesis of prostaglandins. This observation is supported by the finding of Lin and Connor (1990) who showed that the ratio of docosahexaenoic acid to eicosapentaenoic acid in rabbit adipose tissue was higher than in the diet.

Whereas it would be expected that increased levels of linoleic acid via soybean oil inclusion and increased polyunsaturation per unit weight of fatty acid by fish oil inclusion would create the conditions for pronounced increases in fat fluidity, this was not so. Considerably enhanced tissue levels of linoleic acid between soybean oil treatments (from 24% of total fatty acids up to 31%) failed to have a truly marked effect on softness as would have been expected (Whittington *et al*, 1986). The presence of even higher levels of unsaturation via fish oil treatment seemingly attenuated the softening such that the penetrometer values for the backfat were above the 'critical' 575 units. These effects were observed even after allowing for innate differences in P2 fat thickness and whole carcass weight.

The firmness of fat has been shown to be influenced by a number of individual fatty acids. Thus, the firmness of pig fat can be correlated with levels of linoleic acid (Whittington *et al*, 1986; Enser *et al*, 1984), stearic acid (Enser, 1984), palmitic acid (Whittington *et al*, 1986; Enser *et al*, 1984) and the ratio of stearic to linoleic acid (Dziubajlo, 1991). Among these parameters, the level of linoleic acid and the stearic:linoleic acid ratio have shown the strongest correlation with backfat firmness. Dziubajlo (1991) noted that small changes in the stearic:linoleic acid ratio were associated with large effects on melting point. Enser *et al* (1984) proposed that unsatisfactory fat will arise when two criteria are met: firstly, when the level of linoleic acid is higher than 9.2% total fatty acids and secondly, when the ratio of stearic acid to linoleic acid is less than 1.5. In considering the present results for backfat firmness, it is notable that fat from the SOFO1 treatment was significantly firmer than that from the SO1 treatment despite the absence of any differences in the respective levels of palmitic, palmitoleic, stearic or linoleic acid which, as indicated above, have been shown to be associated, to varying extents, with fat consistency. Figures 6.4 and 6.5 show the correlations obtained from the present backfat data between fat firmness and levels of oleic acid and linoleic acid, respectively. In the case of oleic acid, there were similar correlations for the shoulder and loin. The strongest positive correlation

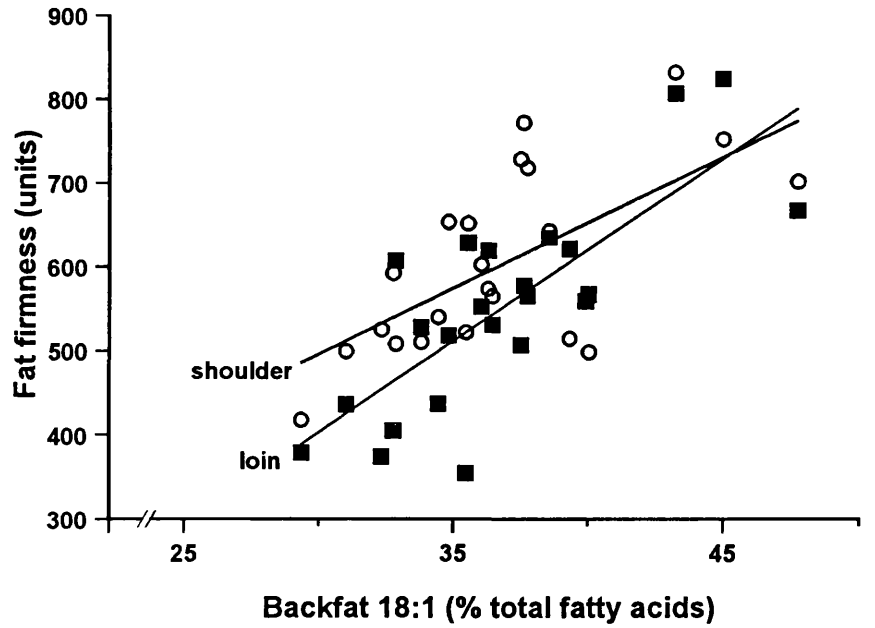


Figure 6.4 - Correlation between the backfat firmness and oleic acid concentration (■ - loin; O - shoulder)

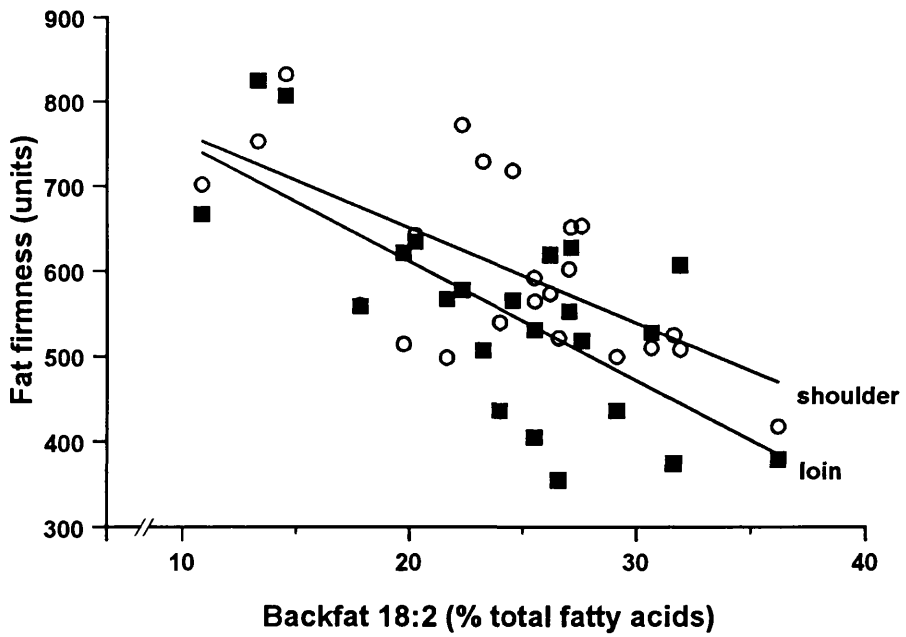


Figure 6.5 - Correlation between the backfat firmness and linoleic acid concentration (■ - loin; O - shoulder)

involved oleic acid. This is in contrast with the findings of Enser *et al* (1984) who observed that oleic acid appeared to make little contribution to the consistency of pig fats. However, Dziubajlo (1991) had observed that there was a significant correlation between oleic acid and adipose tissue triacylglycerol slip point. As can be seen from Table 6.18, the ratios of stearic acid to linoleic acid revealed strong positive correlations, in agreement with the work of Enser *et al* (1984). Also, the concentrations of stearic, palmitic and palmitoleic acids were not related to fat firmness as would be expected from the work of Enser *et al* (1984) who observed that instrumental puncture force values were best correlated with the proportion of stearate, followed by palmitic acid. The strong negative correlation presently observed between linoleic acid and fat firmness accords with the work of Enser *et al* (1984) who described it as the best discriminator of fat firmness. Under conditions of α-linolenic acid enhancement, a similar negative correlation to that for linoleic acid has also been observed (Prescott, 1988). The present correlation coefficients recorded for α-linolenic acid accord with this observation.

Conventionally, increased polyunsaturation of pig fat has been viewed as affecting physical properties such as melting point and membrane fluidity in a linear fashion (Enser *et al*, 1984, and see above). However, based on a number of studies (Applegate and Glomset, 1986; Stubbs and Smith, 1990), a more enlightened view is that in some circumstances there is not a linear relation between degree of unsaturation and physical properties such as fluidity. Membrane fluidity studies have shown that, after introduction of 2 or 3 double bonds, fluidity begins to level off and even decrease again (Neuringer *et al*, 1988). Furthermore, it is believed that highly polyunsaturated fatty acids such as docosahexaenoic acid adopt a helical configuration which allows close packing of the molecules (Stubbs and Smith, 1990). Computer modelling studies have confirmed that the minimal energy conformation of glyceride-linked docosahexaenoic acid can be an 'angle iron' shape or, more probably, a helix (Applegate and Glomset, 1986). With respect to molecular size, the consequences of these findings are that DHA is significantly shorter than both arachidonic acid and stearic acid. It was interesting to note that when the ratio between stearic acid plus total C20+C22 polyunsaturated fatty acids and linoleic acid is compiled, the correlation coefficients obtained for both loin and shoulder fats were similar to those obtained for stearic acid relative to linoleic acid. The possibility is therefore that the long chain C20 and C22 polyunsaturated fatty acids have a tendency to behave more like stearic acid in their effects on fat firmness. Unexpected findings of other studies where fish oil was fed to pigs may be explained by the aforementioned observations. Irie and Sakimoto (1992) observed that the melting point of fat was not different between pigs fed diets containing 0, 2, 4 and 6% fish oil. Although hardness of fat measured with a texturometer decreased with increasing fish oil, even the fat from pigs fed the 6% fish oil diet was not classified as very soft. Hertzman *et al* (1988) observed that

A. OUTER BACKFAT

	Loin	Shoulder
14:0	0.284	0.364
16:0	0.444	0.506
16:1	0.503	0.515
18:0	0.464	0.422
18:1	0.777	0.644
18:2	-0.706	-0.647
18:3	-0.639	-0.636
20:3	-0.350	0.053
20:4	-0.288	-0.525
20:5	-0.367	0.099
22:5	-0.256	0.105
22:6	-0.394	-0.036
18:0/18:2	0.668	0.565
(18:0+20:5+22:5+ 22:6)/18:2	0.647	0.580

B. INNER BACKFAT

	Loin	Shoulder
14:0	0.238	0.233
16:0	0.384	0.396
16:1	0.476	0.473
18:0	0.526	0.476
18:1	0.766	0.688
18:2	-0.709	-0.658
18:3	-0.619	-0.611
20:3	-0.342	-0.478
20:4	-0.307	-0.543
20:5	-0.353	0.058
22:5	0.129	0.364
22:6	-0.098	0.132
18:0/18:2	0.711	0.606
(18:0+20:5+22:5+ 22:6)/18:2	0.710	0.623

Table 6.18 - Pearson (r) correlation coefficients showing degree of relationship between loin/shoulder firmness and individual fatty acids in the outer (A) and inner (B) backfat

consistency of fat from pigs on fishmeal-containing diets was firmer than that from pigs fed rapeseed meal-containing diets, despite the fact that the fishmeal diets contained more total polyunsaturated fatty acid. However, no differences were statistically significant.

The physical properties of fat are also affected by the positional distribution of fatty acids on the glycerol (Christie and Moore, 1970). By submitting triacylglycerols to pancreatic lipase hydrolysis, it is possible to determine the composition of fatty acids esterified to the *sn*-2-position but only the collective composition of fatty acids at the *sn*-1- and *sn*-3-positions. Pig adipose tissue triacylglycerols are characterised by a relatively unique positional distribution of fatty acids: position *sn*-1 contains mostly stearic acid; position *sn*-2 is almost solely occupied by palmitic acid and position *sn*-3 contains unsaturated fatty acids - mostly oleic and linoleic acids; the fatty acid composition of position *sn*-3 is least open to change (Christie and Moore, 1970a).

Several workers have examined possible relationships between the positional distribution of fatty acids and physical properties of the resulting backfat. However, the investigations were constrained to measurements of the fatty acids in the *sn*-(1+3) and *sn*-2 positions. Nevertheless, Dziubajlo (1991) observed that only the linoleic acid concentration at position *sn*-2 correlated significantly with melting point. At positions *sn*-(1+3), stearic acid correlated positively and linoleic acid negatively with melting point. Enser *et al* (1984) examined the positional distribution of fatty acids in triacylglycerols from pigs with 'satisfactory' and 'unsatisfactory' fat. The proportions of fatty acids esterified to the *sn*-2-position were similar in satisfactory and unsatisfactory samples with palmitic acid as the main acid. The only significant difference was that linoleic acid formed 3.3% of the fatty acids at position *sn*-2 in satisfactory fat and 5.4% in unsatisfactory fat. In positions *sn*-(1+3), it was observed that triacylglycerols in fat from bacon with unsatisfactory physical properties contained 44% less palmitic acid, 15% less stearic acid and 220% more linoleic acid than those present in satisfactory bacon. These authors therefore proposed that, as the linoleic acid content of the fat increases, there is an enhancement of triacylglycerols in which the stearic acid on the *sn*-1 or *sn*-3 positions is displaced, by inference, with linoleic acid. Christie and Moore (1970a) observed that position *sn*-3 showed the least change in fatty acid composition. At levels up to 10% of linoleic acid within the triacylglycerols, the acid was found mainly in position *sn*-3 where it accounted for 10-20% of the fatty acids within that position. As linoleic acid increased, it was deposited in position *sn*-1, displacing stearic acid and, as levels further increased, it was placed in position *sn*-2 where it displaced palmitic acid.

With respect to the long chain *n-3* fatty acids, Leray *et al* (1993), using a stereospecific procedure, observed that at the onset of fish oil feeding in rats, eicosapentaenoic acid and docosahexaenoic acid became concentrated in position *sn*-3 of adipose tissue triacylglycerols. After four weeks of fish oil feeding,

eicosapentaenoic acid had been progressively incorporated into position *sn*-1 and docosahexaenoic acid into position *sn*-2. It was suggested that this was a result of the intermolecular rearrangement of triacylglycerol fatty acids.

It is interesting to note that in-depth investigations of the structure of adipose tissue triacylglycerols of modern breeds of pigs compared with more primitive traditional breeds have shown that, in spite of similar overall fatty acid compositions, there were significant differences in physical properties. These differences were correlatable with differences in the intramolecular rearrangements of the major fatty acids - both saturated and unsaturated (Dziubajlo, 1991). In the present and previous investigations, it can be suggested that the situations that exist between overall fatty acid composition, molecular species and intramolecular fatty acid arrangements collectively exert considerable influence on the physical properties of the triacylglycerol and that predicting any particular outcome with regard to physical properties has to take cognisance of all these features.

It is apparent from the present results that the feeding of the unsaturated oils resulted in a significant shift in the type and range of triacylglycerol molecular species within the backfats of the pigs. A similar, though less well defined, shift in unsaturated triacylglycerol species in pig fats in which the only addition to the diet was 4% maize oil has been recorded by Dziubajlo (1991). In the present experiment, the comparisons of particular interest were those between triacylglycerol species from pigs fed only soybean oil and those fed fish oil in addition to the soybean oil. These comparisons would potentially reveal differences in triacylglycerol structure which could account for the absence of an effect of a high level of linoleic acid on backfat firmness in the present experiment, as demonstrated when the pigs were fed the SO1 and SOFO1 diets. The fact that differences between soybean oil and soybean oil/fish oil diets were restricted to the triacylglycerol species containing the long chain *n-3* polyunsaturated fatty acids would lend support to the view that these fatty acids had a 'hardening' effect on the backfat. Table 6.19 shows the correlation coefficients for the relationships between triacylglycerol species and fat firmness in the present experiment. Strong positive relationships are indicated for SMM and MMM species. The firmness of fat was strongly negatively correlated with the MMT/SDT/DDD fraction.

It is clear from the present investigations that further in-depth studies on the relationships between dietary oil content, fatty acid composition, positional distribution of the fatty acids in the triacylglycerols, pig genotype and the ensuing physical properties of the fat in pigs are required for any predictions of fat firmness. Such in-depth data can obviously be obtained. However, the combinations of such parameters are potentially infinite and therefore meaningful data in terms of simple rules may not be possible. At the very least, it is apparent that simplistic rules that have determined thinking on the relationship between fat composition and physical properties are not tenable.

	Loin	Shoulder
SSS	0.547	0.511
SSM	0.682	0.580
SMM	0.699	0.489
SSD	-0.423	-0.342
MMM	0.738	0.681
SMD	-0.387	-0.519
MMD/SDD	-0.458	-0.587
MDD/SMT	-0.540	-0.489
MMT/SDT/DDD	-0.737	-0.566
MDT	-0.559	-0.200
SMP/DDT	-0.567	-0.201
MMP/SMH	-0.346	0.119
MDH	-0.422	-0.105

Table 6.19 - Pearson (r) correlation coefficients showing the relationships between the triacylglycerol molecular species and the firmness of fat over the loin and shoulder

7. Overall conclusions

1. A review of the literature was conducted with regard to past manipulations of the fatty acid composition of porcine depot fat. Whilst previous work has indicated the susceptibility of porcine tissues to manipulation of the fatty acid composition by alterations in the fatty acid content of the pig diet, such changes have been limited by deleterious effects on the quality of the resulting tissues, exemplified by an unacceptable firmness of the backfat and an increased incidence of undesirable flavours. Recommendations for limiting the content of polyunsaturated fatty acids, the primary sources of excessively soft fat and malodorous compounds, have been made.

   A further review of the literature comprised an examination of current theory regarding the principal dietary factors implicated in the onset and development of cardiovascular disease. The findings that the consumption of long chain *n-3* polyunsaturated fatty acids have exhibited ameliorating effects on this disease have found expression in current dietary recommendations. The desirability therefore of enhancing the content of long chain *n-3* polyunsaturated fatty acids within non-fish foods was considered. With respect to achieving this in meat and meat products, the pig presents a well-established means of modifying the fatty acid composition by altering the pig diet and therefore in providing a means of contributing to human dietary requirements.

2. An attempt was made to provide further information with respect to enhancing the nutritional quality of pig meat and fat for human consumption in accordance with contemporary human dietary guidelines. The first experiment was conducted to determine the effects on pig performance and carcass fatty acid composition of feeding a diet containing soybean oil and fish oil. The diet was fed over different pre-slaughter periods in order to determine the optimum length of time required to produce the most desirable changes in fatty acid composition. There was no adverse effect of the modified fat diet on pig growth but rather improvements in performance were observed with increasing time of feeding the modified diet. The fatty acid composition was significantly altered in the outer and inner backfats, *Semitendinosus*, *Longissimus dorsi* and liver with increasing duration of feeding the soybean oil/fish oil diet. Whilst the levels of saturated and monounsaturated fatty acids were depressed by feeding the modified diet, the levels of the long chain polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic acid were significantly enhanced. The ratios of *n-6* to *n-3* fatty acids and polyunsaturated to saturated fatty acids in the tissues increasingly conformed to the stated optima with increasing length of time that the pigs were fed the modified diet. The changes in fatty acid composition were the same in the tissues of pigs fed identical/similar diets in the second and third experiments. The human dietary optima were approximated

most closely in the pigs fed the soybean oil/fish oil diet for six weeks before slaughter. The results were found to be an improvement upon the findings of other work where the aim was to enhance the value of pig meat with respect to human health. It was observed that a useful contribution to the human dietary intake of *n-3* polyunsaturated fatty acids could arise from the consumption of both pork meat and meat products derived from the pigs fed the modified diet and particularly so for meat products which are composed of a relatively high percentage of total fat.

3. In seeking to address the contradictory effects of dietary components commonly utilised in commercial practice, the effect of alterations in inorganic copper and vitamin E on the fatty acid composition and sensory properties of porcine tissues was investigated. Whereas there was no effect of diet on the performance or carcass characteristics of the pigs fed different levels of dietary copper and vitamin E, the levels of vitamin E in the tissues were markedly affected by those in the diet. Differences in copper and vitamin E significantly affected the levels of a number of fatty acids. An effect of dietary copper on fatty acid composition was most apparent in the liver followed by the inner backfat although the changes exhibited were not as would have been expected. This may be the result of a lack of a marked difference in the dietary copper levels. The effect of dietary vitamin E was more apparent than the effect of copper in modifying the fatty acid composition of the tissues. Contingent upon these effects were alterations in the salient ratios of fatty acids.

4. As a result of feeding fats containing high levels of polyunsaturated fatty acids concerns have been expressed over the development of unacceptable flavours and odours due to the increased susceptibility of such fatty acids to peroxidative decay. The olfactory characteristics of cooked fat were assessed from two soybean oil/fish oil diets which differed in the contents of inorganic copper and vitamin E. Altering the inorganic copper to vitamin E ratio of the diet had little effect on the olfactory characteristics of the fat. However, there were significant differences in flavour parameters between 2 and 6 months of frozen storage of the fat which was indicative of oxidative deterioration of the fat.

5. In spite of the presence of high levels of polyunsaturation within the backfats of the pigs fed the modified soybean oil/fish oil diet, the firmness of the fat assessed both objectively and subjectively was not inordinately soft. By employing methodology based on silver ion high performance liquid chromatography, the hypothesis that the observations made on the physical properties of the fat could be related to specific alterations in the distribution of triacylglycerol molecular species was tested. Alterations in the fatty acid composition of the diet were associated with significant shifts in the distribution of triacylglycerol molecular species in the backfat. The fat

from pigs fed a tallow-based diet contained a predominance of triacylglycerol species containing saturated and monounsaturated fatty acids whereas the presence of soybean oil in the diet generated a reduction in the levels of these species and a concomitant increase in the content of species containing polyunsaturated fatty acids. The presence of fish oil in the diet in addition to soybean oil caused an increased presence in the backfat of triacylglycerol species containing one or more long chain *n-3* polyunsaturated fatty acids. The shoulder fat from pigs fed the soybean oil/fish oil diet was significantly firmer than that from pigs fed the soybean oil diet. These results would imply that, in the present context, the presence of the long chain *n-3* polyunsaturated fatty acids exerted a stabilising effect on backfat firmness.

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