

FACTORS AFFECTING THE COMPOSITION AND PHYSICAL
PROPERTIES OF PIG ADIPOSE TISSUE TRIACYLGLYCEROLS

by

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ABSTRACT

In four separate experiments, groups of pigs, some of different breeds, were given diets that varied in the source of protein and in the contents of maize oil and copper sulphate. The animals were slaughtered at about 95 kg liveweight and samples of adipose tissue were taken for enzyme assay and determination of the composition, structure and physical properties of the constituent triacylglycerols; blood samples were taken for lipid analysis.

Dietary copper increased the activity of microsomal stearyl-CoA desaturase in the inner layer of subcutaneous adipose tissue, and it decreased the stearic/oleic acid ratio and the melting point of the constituent triacylglycerols. Dietary maize oil decreased the activities of microsomal stearyl-CoA desaturase in both layers of subcutaneous adipose tissue, and although it resulted in a threefold increase in the linoleic acid content of the constituent triacylglycerols, the melting points of these were depressed to a relatively small extent.

The nature of the dietary protein had little effect on the fatty acid composition of the adipose triacylglycerols but it exerted a marked influence on the positional distribution of fatty acids within the triacylglycerol molecules.

In an experiment in which pigs from eleven different breeds were given the same diet, the results for the adipose triacylglycerols from each breed fell into one of three distinct categories, each exhibiting a characteristic pattern of positional distribution of the constituent fatty acids and a separate correlation between melting point and saturated/unsaturated fatty acid ratio. These three correlations were significantly different from one another and indicated substantial breed differences in the extent to which a given change in fatty acid composition affected the physical properties of the adipose triacylglycerols. Examination of the results obtained after the elimination of structural effects by random interesterification of the adipose triacylglycerols from the eleven breeds of pig revealed only a single correlation between melting point and saturated/unsaturated fatty acid ratio.

In general, adipose triacylglycerols with a more asymmetric distribution of constituent fatty acids exhibited greater changes in physical properties with changes in fatty acid composition than did those triacylglycerols with a more random pattern of fatty acid distribution.

*This work is dedicated
to my parents*

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Abbreviations

BHT	2,6-di- <u>tert</u> -butyl-p-cresol (butylated hydroxy toluene)
FA	Fatty acid
FAS	Fatty acid synthetase
G3P	Glycerol-3-phosphate
GPAT	Glycerol phosphate acyl transferase
HDL	High density lipoprotein
IDL	Intermediate density lipoprotein
LDL	Low density lipoproteins
MG	Monoacylglycerol
TG	Triacylglycerol
UFA	Unesterified fatty acid
VLDL	Very low density lipoprotein

Shorthand nomenclature of fatty acids

The shorthand system of naming fatty acids is as follows. The number preceding the colon indicates the number of carbon atoms in the chain; the number succeeding it denotes the number of *cis* double bonds. The superscript Δ refers to the position of these double bonds. Thus 16:0 denotes palmitic acid and 18:1 Δ^9 denotes oleic acid. However 18:1 would indicate any of the monounsaturated 18 carbon fatty acids when the position of the double bond is not specified. Fatty acids with trans double bonds have the prefix *tr* e.g. *tr* 18:1 Δ^9 is the abbreviated notation for elaidic acid.

Nomenclature of triacylglycerol species

The abbreviated notation for the molecular species of triacylglycerols is as follows: S and U denote saturated and unsaturated fatty acids respectively. Thus SSU describes a triacylglycerol with two saturated and one unsaturated fatty acid. When abbreviated in this form no positional distribution of the fatty acids is indicated.

Section 1
LITERATURE REVIEW

1.1 Introduction

The consistency of the subcutaneous adipose tissue plays a major role in the quality assessment of pig carcasses. Fat which is too soft is not only difficult to handle during processing but also is usually more prone to oxidation. As such it is less attractive both to the producer and the consumer alike.

With this in mind, much research has been devoted to the study of adipose tissue metabolism in the pig. The major cause of soft fat appears to be a change in the fatty acid composition of the triacylglycerols which are stored in the adipocytes. These changes can be effected by alterations to the diet and in particular by the addition of dietary fats and copper. These factors have been well studied but a knowledge of the physical properties of the triacylglycerols is also required if the causes of fat softness are to be fully understood.

1.2 Physical properties of triacylglycerols

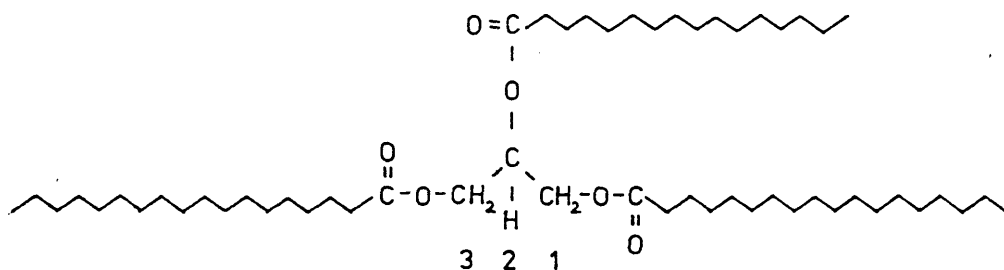
1.2.1 Structure of fatty acids and triacylglycerols

Triacylglycerols are molecules of glycerol with a fatty acid esterified to each of the three hydroxyl groups (*Fig. 1.2.1*). Their physical characteristics are affected by both the composition and distribution of their constituent fatty acids.

/Fig. 1.2.1

Fig. 1.2.1

Triacylglycerol structure

1-octadecanoyl-2-hexadecanoyl-3-octadecanoyl-*sn*-glycerol

(1,3-Distearoyl-palmitin)

One of the most obvious effects is on the melting point which, in general, increases with fatty acid chain length. This is due to the London-Van der Waals dispersion forces. Although weak these forces are proportional to the number of overlapping $-\text{CH}_2-$ groups and the distances between them. With long acyl chains the bonding strength is sufficient to affect the melting point (Cook, 1985). However this applies only for homologous series of fatty acids as there are differences caused by unsaturation, chain branching or other alterations to the acyl chains.

The introduction of a *cis* double bond causes a bend in the chain of about 30° so that the shape is distinctly different from that of the parent saturated acid. This weakens the London forces as the hydrocarbon chains cannot pack as tightly together. The crystalline structure is therefore disrupted and so the melting point is lowered. The presence of more double bonds decreases the melting point even further and their position is also of importance. *Trans* isomers have a configuration more like that of the saturated chain than the corresponding *cis* isomer and this is reflected in the physical data. Table 1.2.1 illustrates these effects using the 18 carbon

fatty acids as an example (Small, 1986).

Table 1.2.1

Melting points (β crystalline form) of various 18 carbon fatty acids.

Fatty acid	Shorthand notation	Melting point, $^{\circ}\text{C}$
Stearic acid	18:0	69.6
Oleic acid	18:1 Δ^9	16.3
<i>Cis</i> -Vaccenic acid	18:1 Δ^{11}	14.5
Elaidic acid	18:1 Δ^9	46.5
Linoleic acid	18,2 $\Delta^{9,12}$	-5.0
Linolenic acid	18,3 $\Delta^{9,12,15}$	-11.3

(Data from Small, 1986)

The same trend is seen with the triacylglycerols as with the fatty acids. Thus the melting point increases with the number and chain length of saturated fatty acids. Tristearin, triolein and trilinolein have melting points of 73.1, 5.5 and -13.1°C respectively (Small, 1986).

1.2.2 Polymorphism of fatty acids and triacylglycerols

Fatty acids and triacylglycerols both exhibit multiple melting points. This polymorphism, the ability to crystallize in several different forms, is a common feature of long chain compounds. It arises either from different types of chain packing or from variations in the angle of tilt of the chains to the base of the molecule. These different packing arrangements all have similar lattice energies but display their own physical characteristics such as X-ray diffraction and infra-red absorption spectra (Fahey *et al.*, 1985;

Small, 1986). The multiple melting points are associated with the transition from one crystalline form to another and this can also be seen in differential thermal analysis curves (Chapman, 1965). Of the three main crystal structures adopted by simple triacylglycerols i.e. those containing a single fatty acid species, the β form is the most stable and exhibits the highest melting point. It is the melting point of this particular form which is most commonly measured (Chapman, 1969). The properties of the various crystalline states are fully discussed by Small (1986).

Triacylglycerols which have similar fatty acid compositions may have melting points which are very close. For example, 1,2-dipalmito-olein and 1-palmito-2-oleo-stearin differ in melting point by only 0.4°C. Added together such closely-related triacylglycerols can form mixed crystals and in the past this caused problems when melting points were used as a criterion of purity (Litchfield, 1972). When a more heterogeneous mixture of triacylglycerols is heated, those with the higher melting points may dissolve in the ones which have already melted. This would account for the wide range of temperature over which melting was seen to occur in a triacylglycerol sample isolated from pig adipose tissue (Moore *et al.*, 1969).

1.2.3 Positional distribution of fatty acids in triacylglycerols

Positional isomerism of the fatty acids also affects the melting points. Random interesterification of pig adipose triacylglycerols resulted in marked differences in melting point before and after the esterification procedure (Moore *et al.*, 1969). The presence of these specific triacylglycerol structures may be important for the physical properties of the molecules and hence their subsequent metabolism (Fahey *et al.*, 1985).

Differences in the positional distribution of fatty acids have been noted both between species and between the tissues of individual animals (Brockerhoff *et al.*, 1966; Christie and Moore, 1970a). In most mammalian triacylglycerols position 2 is mainly occupied by the shorter and the more unsaturated fatty acids. Thus linoleic and often oleic acids accumulate in the 2-position whereas the saturated acids are found in the outer 1- and 3-positions. Triacylglycerols from pig liver follow this trend but those isolated from the adipose tissue do not. Here the 2-position is preferentially occupied by palmitic acid (Christie and Moore, 1970a). Marine mammals are similarly exceptional (Brockerhoff and Hoyle, 1963).

There are several theories which attempt to explain the pattern of fatty acid distribution within the triacylglycerols and these are discussed by Litchfield (1972). Foremost among them is the 1-random, 2-random, 3-random hypothesis which assumes that there are three pools of fatty acids each of which is separately distributed to the 1-, 2- and 3- positions of all the glycerol molecules in a fat.

Christie and Moore (1970a) isolated triacylglycerols from five different sites in the pig and compared the fatty acid data with the proportions predicted by this theory. They found that the agreement was very good for inner and outer backfats, acceptable with liver and milk fats, but much less satisfactory with the blood lipids. This last result reflects the heterogenous origin of the blood triacylglycerols which are derived from several different sources.

1.3 Synthesis of triacylglycerols and fatty acids

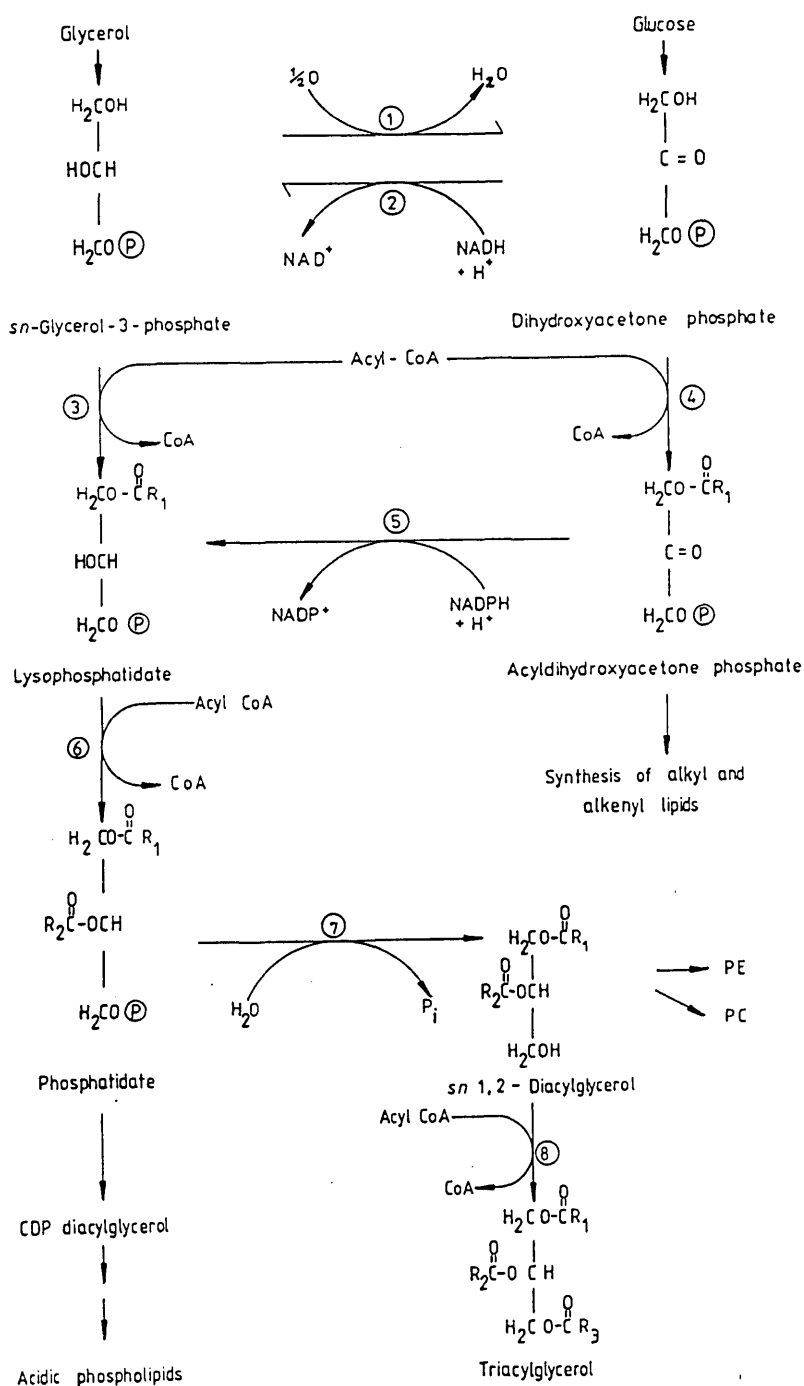
1.3.1 Synthesis of triacylglycerols

In pig adipocytes the pathway of esterification of fatty acids to give triacylglycerols involves glycerol-3-phosphate (G3P). This can be formed either from glucose via the glycolytic pathway or by the phosphorylation of glycerol. The glycerol-3-phosphate is acylated to 1-monoacyl glycerol-3-phosphate (lysophosphatidate) by the enzyme glycerol phosphate acyltransferase. A second acyltransferase esterifies another fatty acid to the 2 position to give phosphatidate. This is dephosphorylated to a 1, 2-diacylglycerol which is then acylated by a third acyltransferase to yield triacylglycerol (see figure 1.3.1).

Experiments on rats have shown that the non-random positional distribution of fatty acids seen in the triacylglycerols arises during the synthesis of phosphatidic acid (Elovson *et al.*, 1969; Yamashita *et al.*, 1981). Studies have therefore been concentrated on the substrate specificity of the two acyltransferases involved in the synthesis of this intermediate.

The first of these enzymes, glycerol phosphate acyltransferase (GPAT), is located in equal quantities in both the mitochondrial and the microsomal fractions of rat liver. However, in adipose tissue the mitochondria are only responsible for about 10% of the total GPAT activity. Although these two acyltransferases catalyse the same reaction they are different enzymes and exhibit different substrate specificities. The mitochondrial enzyme prefers saturated CoA esters whereas the microsomal acyltransferase can utilize both saturated and unsaturated fatty acyl CoAs (Brindley, 1985b). However, Yamashita *et al.* (1972) found that partially purified GPAT from rat liver microsomes used saturated fatty acyl CoA as the acyl donor and that the unsaturated analogues were poor substrates for the reaction.

Figure 1.3.1 Biosynthesis of triacylglycerols



- 1 Glycerol-3-phosphate dehydrogenase
- 2 Glycerol-3-phosphate dehydrogenase (NAD⁺)
- 3 Glycerol phosphate acyltransferase
- 4 Dihydroxyacetone phosphate acyltransferase
- 5 Acyl dihydroxyacetone phosphate reductase
- 6 Monoacylglycerol phosphate (lysophosphatidate) acyl transferase
- 7 Phosphatidate phosphohydrolase
- 8 Diacylglycerol acyltransferase

Monoacylglycerol phosphate (lysophosphatidate) acyltransferase activity is also found both in the endoplasmic reticulum and the mitochondria. Enzymes from each of these sites select for unsaturated fatty acyl CoA with the microsomal enzyme being the more important (Brindley, 1985b; Yamashita *et al.*, 1972, 1973).

Thus mitochondria tend to produce lysophosphatidate whereas the microsomal system produces phosphatidic acid. In both cases an unsaturated fatty acid will be esterified to the 2-position and a saturated one to the 1-position. Position 3, acylated by the enzyme diacylglycerol acyltransferase shows preferential esterification of stearic acid, followed by palmitic acid (Yamashita *et al.*, 1973).

Although this illustrates how the positional distribution of fatty acids can arise, it must be remembered that these experiments were carried out on rats. Direct comparisons with the pig are therefore not possible, especially as the fatty acid distribution patterns between the two species are so markedly different. However a similar situation is likely to exist in the pig, although the substrate specificity of the acyltransferases will be different to account for the preferential esterification of palmitic acid to the 2-position of the adipose tissue triacylglycerols. Christie and Moore (1970a) have suggested that the differences between pig liver and adipose tissue triacylglycerols could be accounted for by inter-organ variations in the fatty acid specificities of the acyltransferases. Further work needs to be carried out on the pig lipid metabolism to clarify this point.

1.3.2.1 Biosynthesis of saturated fatty acids.

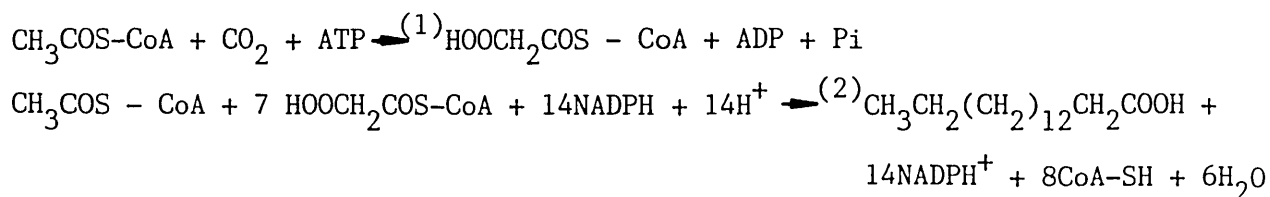
The fatty acids present in the adipose tissue triacylglycerols are

obtained either by *de novo* synthesis in the tissue itself or from dietary sources. In a pig receiving a diet containing 2-4% fat, it is estimated that 73-82% of the fat deposited is newly synthesized from carbohydrate (Leat, 1976). The adipose tissue is the principal site of fatty acid synthesis in the pig (O'Hea and Leveille, 1969).

The biosynthesis of fatty acids occurs in two distinct steps. The first is the carboxylation of acetyl CoA to malonyl CoA catalysed by the enzyme acetyl CoA carboxylase. This is the rate limiting step in the pathway. The second step is a cyclical series of reactions involving the condensation of acetyl CoA and malonyl CoA catalysed by fatty acid synthetase (FAS) in the presence of NADPH. This gives firstly butyric acid and then the higher fatty acids, as their CoA esters. The sequential reactions involved in the synthesis of palmitate are reviewed by Wakil *et al.* (1983). However the overall reactions catalysed by acetyl CoA carboxylase and fatty acid synthetase are summarised in Figure 1.3.2 (Wakil *et al.*, 1983; Gurr, 1985).

The control of long chain fatty acid biosynthesis is complex and will not be discussed here. There are several articles which can be consulted for a detailed account (Goodridge, 1985; Gurr, 1985; Wakil *et al.*, 1983). Briefly, fatty acid synthesis is increased when high carbohydrate or low fat diets are fed. High fat diets, particularly those rich in polyunsaturated fatty acids, tend to decrease fatty acid synthesis.

Fig. 1.3.2 Biosynthesis of palmitate



(1) Acetyl CoA carboxylase

(2) Fatty acid synthetase

1.3.2.2 Elongation and desaturation of fatty acids.

In pig adipose tissue the major end product of fatty acid synthesis is palmitic acid. This can then be either elongated or desaturated or both to give a wider spectrum of fatty acids.

Two-carbon chain elongation systems exist in both the endoplasmic reticulum and the mitochondria of several tissues with the former being the more important. In both cases the fatty acyl acceptors are only active as their CoA derivatives. The two-carbon condensing unit is malonyl CoA in the microsomal system and acetyl CoA in the mitochondrial system. In common with other enzyme systems the end product fatty acid inhibits further elongation (Cook, 1985).

In keeping with other mammals, the pig possesses several desaturase enzymes but it is the Δ^9 desaturase which is involved in the formation of oleic acid from stearic acid. Stearoyl CoA desaturase introduces a double bond between carbon atoms 9 and 10 of the stearic acid molecule. The enzyme acts on the CoA ester of the fatty acid and the reaction requires both molecular oxygen and a reduced pyridine nucleotide, usually NADH. The enzymology of the desaturases is more fully discussed by Jeffcoat (1979) but the Δ^9 desaturase reaction is given here in Figure 1.3.3.

In general the Δ^9 desaturase responds to dietary changes in a similar manner to other lipogenic enzymes such as fatty acid synthetase. When the rate of fatty acid synthesis is high, so is the activity of the desaturase thus ensuring that there are sufficient unsaturated fatty acids to maintain the fluidity of the tissue lipids. This is important as triacylglycerols containing predominantly palmitic and stearic acids have high melting points

and so could crystallize under physiological conditions (Cook, 1985; Jeffcoat, 1979).

An interesting feature of the Δ^9 desaturase is the effect of copper (as Cu^{2+}) on its activity. A 250 ppm dietary supplement of this metal has been found to increase enzyme activity in both liver and adipose microsomes from the pig. However it is unlikely that copper is an active component of the desaturase. The enzyme activity is unaffected by *in vitro* addition of CuSO_4 to rat microsomal preparations. Copper chelators were also found to be ineffective (Jeffcoat, 1979). This point will be dealt with again in Section 1.7.

Despite the presence of several desaturase systems, animals cannot introduce double bonds beyond the Δ^9 position on the acyl chain. Thus fatty acids, such as linoleic acid ($18:2^{\Delta^9,12}$) which are required by the animal have to be obtained from the diet.

1.3.3. Dietary fat - uptake and metabolism

Dietary triacylglycerols are emulsified in the gut and then hydrolysed by pancreatic lipase to yield free fatty acids and 2-monoacylglycerols. These more polar products are solubilized into mixed bile acid micelles from which they are absorbed by passive diffusion into the intestinal epithelial cells. Once inside the cells the triacylglycerols are resynthesized, mainly from the 2-monoacylglycerols and fatty acids. However shorter chain fatty acids escape esterification and enter the portal blood unchanged (Brindley, 1985a).

In order to be transported, the triacylglycerols have to be physically

stable in an aqueous environment. To achieve this they aggregate with amphiphilic compounds, namely phosphatidyl choline, cholesterol and various apoproteins, to form chylomicrons. These contain 85-95% triacylglycerol but the intestine can also secrete VLDL which only contain about 60% triacylglycerol.

The nascent lipoproteins enter the bloodstream via the thoracic duct of the lymphatic system. Once in the blood they interact with other classes of lipoproteins, resulting in a loss of surface film phospholipid and cholesterol and a concomitant gain of apoproteins C and E.

Adipose, muscle and various other tissues can utilize chylomicron and VLDL triacylglycerols. The lipoproteins bind, through their surface apo C-II, to the enzyme lipoprotein lipase (clearing factor lipase) which is located on the endothelial walls of the tissue capillary network. This enzyme hydrolyses the triacylglycerols extracellularly, resulting in a high local concentration of fatty acids. These then diffuse through the endothelial cells into the adipocytes where they are esterified back to triacylglycerols (see Section 1.3.2). There is evidence that lipoprotein lipase hydrolyses the more fluid unsaturated triacylglycerols faster than the more solid, saturated analogues (Morrisset *et al.*, 1977). Thus, unsaturated fatty acids would be preferentially released from the VLDL and chylomicrons. This is probably a result of a more efficient interaction between the enzyme and the more fluid lipid and also between the lipid and the apo C-II moiety of the lipoprotein.

The chylomicrons and VLDL thus lose the major part of their triacylglycerol core and so become smaller. With this decrease in size, less surface material

is required and the excess is transferred to other lipoproteins. Apo-C and apo-A, for example, are transferred to high density lipoproteins (HDL). When the degraded lipoproteins can no longer compete with their nascent equivalents and lose their affinity for the enzyme, they leave the capillary bed. The chylomicron remnants are taken up by the liver and the components are degraded in the lysosomes. In peripheral tissues the VLDL are converted in an analogous fashion into IDL which are then metabolized by the liver into LDL. The fate of the chylomicrons and VLDL is shown in Fig. 1.3.4.

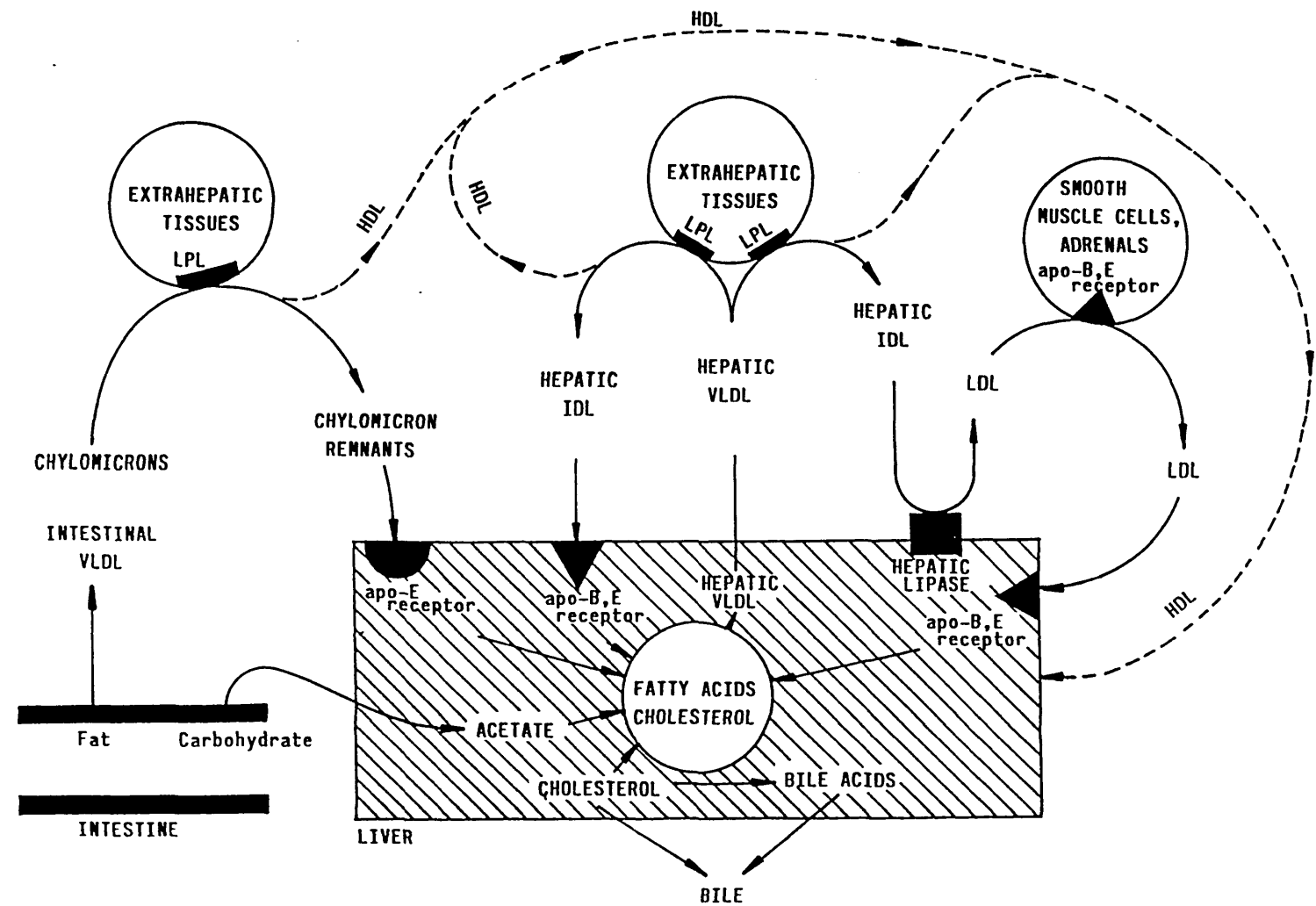
Approximately 90-95% of the ingested triacylglycerol is absorbed by the intestine. The fate of the constituent fatty acids depends to a large extent on the site of uptake. Those taken up by muscle are mainly oxidised whereas those in the adipose tissue are esterified and stored as triacylglycerols. Uptake by the various tissues is regulated by differential control of lipoprotein lipase. In adipose tissue, fatty acid uptake is enhanced by insulin and glucocorticoids and is decreased by several factors including catecholamines, corticotropin and glucagon (Brindley, 1985, a,b).

1.4 Quality of porcine backfat

1.4.1 Structure and function of backfat

Pig adipose tissue consists of clusters of fat cells within a matrix of connective tissue. These adipocytes hypertrophy during fat deposition so that even though they represent only a third of the cell number they actually form about 80% of the tissue mass. The stored lipid is comprised almost entirely of triacylglycerols (Enser, 1984; Leat, 1976).

Figure 1.3.4: Metabolism of plasma lipoproteins.



The largest site of fat deposition in the pig is the subcutaneous backfat region. The adipose tissue here is divided into two anatomically distinct layers separated by a thin band of connective tissue. These two layers differ in composition; the outer layer, just under the skin, is more unsaturated and contains more connective tissue than the inner. There are also differences in total lipid and water content (Santoro, 1984; Sturm *et al.*, 1982).

During normal growth the thickness of the inner layer increases more than the outer layer. Thus in the mature tissue the inner layer is often the thicker one. There is also a variation along the length of the pig. Backfat is thickest at the shoulder and thinnest in the midback region (Mersmann & Leymaster, 1984).

Several studies have shown that the inner layer is more metabolically active as measured by the activities of the lipogenic enzymes (De Wilde, 1984; Koch *et al.*, 1968a; Sturm *et al.*, 1982). This higher turnover is thought to account for the differences in fatty acid composition observed between the two layers. The greater unsaturation of the outer layer can be explained by the fact that the fat in this layer is deposited while the pig is young, when the rate of deposition is slow. The inner layer is mainly deposited during rapid growth; more *de novo* synthesis of fatty acids occurs and this results in a more saturated fat (Hart & Van der Veen, 1957). Hence both the saturated to unsaturated fatty acid ratio and the melting point of the inner layer are higher than those of the outer (Moore *et al.*, 1969).

Differences in the degree of backfat unsaturation have also been noted

between sexes. Ho and Elliot (1974) and Koch *et al.* (1968b) both found that the backfat of gilts was more unsaturated than that of barrows (castrated males). The unsaturation of backfat decreases in the order: boars, gilts, barrows. Leat (1976) attributed this to hormonal differences which affected the desaturase enzymes. However females exhibit lower rates of lipogenesis than barrows and so might be expected to have a more unsaturated backfat (Allee *et al.*, 1972).

Mersmann and Leymaster (1984) have suggested that whereas the primary function of the inner backfat is a metabolic one, the outer layer may act in a more thermo-insulatory manner. This supports earlier work by MacGrath *et al.* (1968) who found that pigs exposed to the cold (0-5°C) exhibited greater backfat unsaturation than those exposed to a 25-30°C environment. The higher proportion of unsaturated acids lowers the melting point of the stored triacylglycerols and so may help to maintain tissue fluidity. These experimenters concluded that the relationship between depot fat unsaturation and environmental temperature is influenced by the intake of polyunsaturated fatty acids which are not synthesized by the pig.

1.4.2 Consistency of backfat

The fatty acid composition is the main factor affecting the quality of the fat. The lipid melting point has therefore been used as an indication of the fat softness. However, net fatty acid composition and melting point are not always directly related (Section 1.2.3). More specific correlations have since been suggested such as that of the melting point with the sum of the unsaturated fatty acids. Elliot and Bowland (1969) found that for each decrease of 0.87°C in melting point there was a 1% increase in the percentage

of unsaturated fatty acids in the depot fat lipid samples. The monounsaturated to saturated ratio of the 16 and 18 carbon fatty acids has also been used as an index of softness (Lea *et al.*, 1970; Walker *et al.*, 1971). Similarly employed are the oleic to stearic acid ratio, the percentage of stearic acid and the percentage of linoleic acid (Enser *et al.*, 1983).

The last of these indexes, the amount of linoleic acid present is particularly relevant in commercial bacon production. High levels of this fatty acid (>15%) are frequently associated with the down-grading of pig carcasses on the basis of soft fat (Enser, 1984). The more unsaturated fat results in a softer adipose tissue when measured either by thumb pressure or commercial testing devices (Dransfield & Jones, 1984).

Although a slightly softer adipose tissue is generally tolerated by the bacon manufacturing industry, carcasses displaying excessively soft backfat are not acceptable. This is because of problems arising during processes such as slicing and vacuum packing. Soft fat is rejected as it loses definition of the individual slices and appears discoloured and pliable (Enser *et al.*, 1984).

In view of this, a considerable effort has been made to investigate the causes of soft fat and so prevent its occurrence. In particular the effect of various dietary constituents has been studied.

1.5 The effect of diet on fat quality

1.5.1 Energy level

The energy level provided by a particular diet is known to affect the

consistency of the backfat. Diets providing less than 80% of the full feed requirement were found to have the greatest effect on the fatty acid composition of porcine backfat (Koch *et al.*, 1968a). The lower energy levels used by these authors resulted in a significantly more ^{un-}saturated fat than that from the corresponding control pigs. Stearic acid accounted for most of the decrease in the amount of saturated fatty acids while there was a concomitant rise in the concentration of linoleic acid.

Conversely an increased level of fatness reduces the proportion of linoleic acid in the stored triacylglycerols. This is because high levels of caloric intake leading to greater fat deposition are associated with high levels of *de novo* fatty acid synthesis whose balance is predominantly saturated. Thus if diets rich in linoleic acid are also underfed so that fat deposition is reduced then the consequences for fat firmness are greater than for other diets (Wood, 1984).

A firmer fat is obtained when both the growth rate and fat thickness are high as these conditions lead to greater *de novo* fatty acid synthesis and desaturase activity. The fat is thus relatively enriched in saturated and mono-unsaturated fatty acids but contains less of the polyunsaturated fatty acids which are of dietary origin. Although a low level of linoleic acid is an important factor governing fat firmness, there is also a higher concentration of lipid in bigger, more tightly packed cells which contain less associated water (Wood, 1984).

1.5.2 Dietary fat

1.5.2.1 Level of dietary fat

When a low fat diet is given to pigs then most of the fat deposited is

necessarily from *de novo* synthesis. Analysis of the fatty acids from adipose tissue triacylglycerols shows that the lipid is comprised mainly of palmitic, stearic and oleic acids. Negligible amounts of linoleic and linolenic acids are present (Brooks, 1971).

When high fat diets (10% fat) are fed to pigs there is a marked decrease in fatty acid synthesis from glucose. The activities of the lipogenic enzymes are also decreased in the adipose tissue. Allee and co-workers (1971; 1972) measured fatty acid synthesis, citrate cleavage enzyme and malic enzyme activities in outer backfat homogenates from pigs fed diets supplemented with various types and levels of fat. They noted that although the activities decreased linearly as the level of dietary fat increased, there was a concomitant rise in the concentration of plasma free fatty acids. The effect was observed with all types of dietary fat used irrespective of their degree of unsaturation. This increase in the level of plasma free fatty acids is inconsistent with the anticipated increase in fat deposition generally associated with high fat diets. However, Romsos and Leveille (1974) found that although high fat diets suppressed the activities of lipogenic enzymes such as glucose-6-phosphate dehydrogenase, the effect was greater with polyunsaturated fats. Similarly Abraham *et al.* (1983) found that fat synthesis in the mouse was decreased by linoleate but not by the more saturated fatty acids.

Increasing the level of dietary fat from 1 to 13% also increased the daily weight gain. This improved feed utilization is probably due to the higher calorific value of the fat-supplemented diets (Allee *et al.*, 1971; 1972).

1.5.2.2 Quality of dietary fat

The feeding of high fat diets decreases *de novo* synthesis of fatty acids in adipose tissue. However the animals tend to have thicker backfat than those on lower fat regimes. This implies that more of the storage triacylglycerols are of dietary origin. Under these conditions the fatty acid composition of the tissue lipid can to some extent reflect that of the dietary fat source. This is particularly evident when *de novo* fat synthesis is low and the fatty acid composition of the dietary lipid differs largely from that which is synthesized by the pig. The fat synthesized from carbohydrate follows a composition pattern which is genetically determined (De Wilde, 1984; Enser & Buller, 1985, Freeman, 1983; Lea *et al.*, 1970).

Koch *et al.* (1968b) reported that pigs fed a 10% safflower oil diet had a more unsaturated^{er} backfat than control animals receiving a basal corn-soyabean meal diet. However, feeding the safflower oil diet for five weeks and then replacing it with a 10% tallow diet for a similar period tended to restore the fatty acid profile to that of the control animals. This is consistent with an earlier study by Blumer *et al.* (1957) where feeding a diet containing 10% coconut oil for the last month prior to slaughter was sufficient to harden satisfactorily the fat of pigs which had previously been fed a more unsaturated fat.

Similarly, Brooks (1967) found that a 10% soyabean oil diet resulted in a fat depot level of linoleic acid which was three times that of the control animals. The adipose tissue concentration of linoleic acid was found to increase linearly with the amount eaten. This elevated tissue level leads to a concomitant decrease of the other fatty acids, particularly oleic acid. This is in accordance with Koch *et al.* (1968b) who noted that dietary linoleic

acid was preferentially deposited over oleic acid. Thus in animals receiving the safflower oil supplement the adipose tissue concentration of oleic acid was significantly decreased. To a lesser extent this was also observed with palmitoleic acid. As a result of these variations the level of saturated acids remains fairly constant. This supports earlier work by Dahl and Persson (1965). Christensen (1973) suggested that these changes in fatty acid profile are an attempt to maintain lipids with the physico-chemical properties required for biological function.

The presence of linoleic acid has a marked effect on tissue firmness and lipid melting point. There is a negative correlation between the proportion of linoleic acid and the slip point (open capillary melting point) of the lipid (Enser, 1984). This is to be expected considering the physical properties of the unsaturated fatty acids in triacylglycerols (Section 1.2.1).

Although dietary provision of this fatty acid makes fat soft, stearic acid is considered to be more important in regulating backfat consistency. Oleic acid does not play a significant role (Blumer *et al.*, 1957). Hence there must be some relationship between dietary linoleic acid and adipose tissue stearic acid. With diets high in linoleic acid the synthetic pathway might be expected to produce increased levels of stearic acid at the expense of oleic acid. In some species, dietary linoleic acid inhibits the desaturation of stearate to oleate, but there is no direct evidence for this in the pig as desaturase activity is neither repressed by dietary linoleate nor induced by saturated fatty acids in suet*. Also the changes in stearic acid concentration during growth are not related to changes in the activity of the Δ^9 desaturase (Enser, 1984; Enser & Buller, 1985).

* beef perirenal fat

In summary it can be said that diets supplemented with fats rich in linoleic acid are a major cause of soft fat. Although this can be tolerated to some extent, the situation can be aggravated if other factors contributing to the softening of tissue lipid are also present. Copper, as Cu^{2+} ions, in particular is known to affect fat consistency.

1.5.3 Dietary copper

1.5.3.1 Growth performance

The effect of dietary copper in promoting the growth of pigs has been well established (Braude, 1967). Although the trace requirement of this element is in the region of 5-10 mg Cu/kg feed, dietary inclusion of copper (as Cu^{2+}) at much higher concentrations (250mg Cu/kg feed) results in improved feed conversion efficiency and hence an improved growth rate (Barber *et al.*, 1957; Braude, 1967; Braude, 1981; Lucas *et al.*, 1961). However there have also been several reports where this level of dietary copper had no significant effect on growth performance (Castell & Bowland, 1968a; Elliot & Amer, 1973; Bradley *et al.*, 1983). Cases of toxicity have also been noted (Mills, 1968).

Despite these detrimental reports, supplementation of copper in pig diets is now widespread. The dietary concentration of copper has been reduced by the EEC to 125 mg Cu/kg feedstuff. At this level the increases in weight gain and feed conversion ratio are still appreciable but environmental pollution is reduced. One of the main problems arising from the use of copper as a growth promoter is the subsequent spreading of pig slurry on grazing land. Sheep are particularly susceptible to copper poisoning and also, crops grown on copper-rich soil may also suffer (CEC, 1984; Meyer & Kroger, 1973).

1.5.3.2 Liver copper

Even at a reduced level of dietary copper supplementation there are noticeable differences between copper-loaded and control pigs. The liver acts as the main body store of this metal and the liver copper concentration can rise dramatically as a result of dietary copper supplementation (Elliot & Amer, 1973). However the distribution of the copper between the liver lobules is uneven, with the higher concentrations located in the cells adjacent to the portal tracts or at the periphery of the lobule. As the copper accumulates, so more and more cells become involved in its storage (Bloomer & Lee, 1978). Serum, kidney, spleen and hair copper levels are also raised under conditions of high copper intake (Castell & Bowland, 1968b).

1.5.3.3 Fat quality

Another major effect of copper supplementation is on the depot fat quality, particularly the backfat. There are reports of backfat from copper-fed pigs being softer than that from the controls, sometimes unacceptably so. Braude and Ryder (1973) found that feeding diets containing 150–250 mg Cu/kg diet resulted in slightly lower backfat melting points than in the controls. With all treatments they found that the inner layer had a melting point ($\sim 47^{\circ}\text{C}$) about 2°C higher than the corresponding outer backfat layers ($\sim 45^{\circ}\text{C}$). There was a similar difference between the 0 and 250 ppm Cu treatments for the same layer of backfat. Walker *et al.* (1971) reported similar depressions in both slip point and melting point, although the overall melting points were lower than those of Braude and Ryder.

In contrast, Moore *et al.* (1969) observed differences of a much greater magnitude. They found that the inner layer of backfat from animals receiving

the basal diet had a melting point of 43-45°C, compared with 30-33°C for the outer layer. The corresponding values for the copper-supplemented pigs are 33-34°C and 32-36°C respectively. Thus dietary copper had the effect of lowering the melting point of the inner layer by about 10°C, so that in melting point it resembled the outer layer of the basal-fed pigs. The outer layer of the copper-fed pigs had a melting point roughly similar to that of the control animals. Similarly, Amer and Elliot (1973a) found that the dietary inclusion of 250 ppm Cu reduced the melting point of the backfat by 8-10°C compared to the control pigs. However the backfat was not separated and so the values are for the combined fat layer.

These changes in melting point are related to the differences in depot fat triacylglycerols between basal-fed and copper-fed pigs. The total backfat i.e. both layers analysed together, is significantly more unsaturated when the pigs are fed 250 ppm of copper in the diet. This is attributed to the presence of more monounsaturated fatty acids (16:1 and 18:1) and less saturated fatty acids (16:0 and 18:0) (Elliot & Bowland, 1968). Amer and Elliot (1973a) found similar changes in composition but also noted a rise in the level of linoleic acid when the pigs were fed the high copper ration. However, Moore *et al.* (1969) only found differences in the concentrations of oleic and stearic acids.

Contrary to these findings, Walker *et al.* (1971) reported that the unsaturation of backfat samples, measured as iodine values, was not significantly increased by dietary copper. Looking at specific fatty acids they did find changes in the level of palmitoleic and stearic acids similar to those previously mentioned. They also found that the concentration of palmitic acid increased and that of oleic acid decreased with the addition of copper

to the diet. This pattern of distribution is in disagreement with the other work. However, Elliot and Bowland (1968) reported that the differences in backfat between treatments noted up to a liveweight of 70 kg were not significant at 90 kg even though the more unsaturated pattern of distribution was still apparent. Despite these differing observations there was still a decrease in lipid melting point consistent with the addition of copper to the diet.

As noted before, Moore *et al.* (1969) found differences of 10–15°C in melting point resulting from the presence of 250 ppm dietary copper. Analysis of the separated layers of backfat showed marked differences in composition between the inner backfat of control pigs and the corresponding outer layer. The copper-fed pigs had backfat layers similar in composition to each other and to the outer backfat of the control animals. The inner layer of the controls had 3–5% more stearic acid and 3–5% less oleic acid than samples taken from the other three sites. This parallels the melting point data.

Such changes in fatty acid composition might be expected to result in differences in melting point of a similar magnitude; Amer and Elliot (1973a) found a significant negative correlation between the melting point and the total weight per cent of unsaturated fatty acids. However this does not explain the findings of Walker *et al.* (1971) who reported a depression of backfat melting point owing to the presence of dietary copper, even though they found no significant differences in the fatty acid composition.

This suggests that the positional distribution of the fatty acids within the triacylglycerols may be partly responsible for the observed changes in melting point. Work by Moore *et al.* (1969) showed that random intersterification

of backfat lipids resulted in melting points differing by only a few degrees, compared to the larger differences seen before the esterification procedure.

This is supported by Amer and Elliot (1973b) who found that high levels of dietary copper altered the positional distribution of fatty acids in the backfat triacylglycerols. They used pancreatic lipase hydrolysis techniques to determine the composition at the 2- position of the triacylglycerols. The 1- and 3- positions are not distinguished from each other by this method. Dietary supplementation with 250 ppm Cu was found to increase the amount of 16:1, 18:1 and 18:2 at the 2- position. As a result, analysis of the triacylglycerol species showed that there was significantly less SSU present, although the data for SUS and SUU were not significant. The decreases in melting point caused by dietary copper were paralleled by the variation in concentration of triacylglycerol types. It is the more saturated triacylglycerols i.e. S_3 and S_2U which are thought to be responsible for determining fat consistency. Thus the increase in unsaturation at position 2 would reduce the concentration of these two triacylglycerol species and so would effectively lower the lipid melting point (Amer & Elliot, 1973b).

In a more detailed study, Christie and Moore (1970b) used stereospecific analysis to determine the fatty acid distribution in all three positions of pig adipose triacylglycerols. The samples were chosen so as to cover a wide spectrum of oleic acid content. This was effected by the addition of varying levels of copper (0-250 ppm) to the pig diets. In general they found that as the overall oleic acid concentration rose so did the levels of this acid and palmitoleic acid at positions 1 and 2 on the triacylglycerol molecule. A small rise in linoleic acid content at position 2 was also noted. Accompanying these rises were corresponding decreases in stearic acid (and myristic acid,

14:0) at position 1, and palmitic acid (and 14:0 and 18:0 to a lesser extent) at position 2. Position 3 was the least affected by changes in the overall fatty acid composition. However as the oleic acid content of the triacylglycerols increased so did the levels of oleic acid, palmitoleic acid and linoleic acid, with a concomitant decrease of stearic acid (and palmitic acid) concentration.

Thus the concentration of unsaturated acids at position 2 on the triacylglycerol molecule increases with increasing tissue levels of oleic acid. This is in accordance with Amer and Elliot (1973b) who found that the increase in unsaturation caused by dietary copper resulted in less SSU triacylglycerols. In both cases, a decrease in melting point was observed, indicating that the positional distribution of the fatty acids affects the physical properties of the triacylglycerols.

1.6 Factors affecting the action of dietary copper

1.6.1 Protein

The level of protein in the diet is known to affect the uptake of copper. The main site of storage is the liver and so the liver copper concentration is indicative of the copper status of the animal. Liver copper concentrations of pigs fed 250 ppm Cu together with a 17% protein diet were lower than those of pigs fed a similar diet containing only 14% protein (Lucas *et al.*, 1962). The reason for this is unclear.

The source of protein is thought to be more important than the quantity fed. Animal protein is more effective than vegetable protein for increasing growth parameters. This is partly due to the differing amino acid profiles but copper uptake is also involved (Evans, 1973).

Combs *et al.* (1966) reported that pigs accumulated significantly more copper in the liver when fed casein than when fed soyabean meal as a protein source. Similarly fishmeal diets induced higher retention of copper in the liver than diets containing soyabean meal (Drouliscos *et al.*, 1970; Wallace *et al.*, 1966). Barber *et al.* (1981) found that although liver copper was reduced in animals fed soyabean meal compared to white fishmeal there was no difference in performance except between the controls and the copper-fed animals.

The decreased copper absorption observed in animals fed soyabean diets has been attributed to the high level of phytic acid (hexa phospho-myo-inositol) present in the soyabean meal. The phytate is thought to form insoluble metal complexes with the trace elements (Van Campen, 1971). This idea is disputed by the findings of Lepen and Adrian (1983) that sodium phytate increased the digestibility of copper and by Suttle and Mills (1966) who found that the soyabean meal diet did not exert its expected effect of reducing the availability and hence the toxicity of copper. In the latter case diets had either soyabean meal, white fishmeal or dried skimmed milk as the protein source. Of the three treatments only that containing white fishmeal resulted in toxicosis, perhaps because of the higher calcium level in this diet. High calcium levels in the diet can induce zinc deficiency in the animal and this can lead to increased copper toxicity (see Section 1.6.3).

In summary animal protein in the diet leads to greater copper absorption than with dietary vegetable protein. The amount of copper absorbed decreases with increasing protein level. As a result the effects of copper supplementation are more marked in animals receiving protein from animal sources. However the implications for fat quality have not been widely investigated.

1.6.2 Feeding scale

The differing responses to copper feeding noted by various authors (Section 1.5.3.1) may be, in part, due to the feeding scale employed. Elliot and Bowland (1968) reported that *ad libitum* diets resulted in less cases of soft fat than if a more restricted feeding scale was imposed. Under restricted feeding conditions less synthesis of fatty acids occurs and so more of the fat deposited is of dietary origin and as such may be more unsaturated. Also Cooke (1981) stated that in general faster growing pigs respond less to growth promoters than do the more slowly growing animals.

Thus the restricted feeding scales employed by Braude and Ryder (1973) and Walker *et al.* (1971) may have resulted in a greater response to copper-feeding than the *ad-lib* diets of Bradley *et al.* (1983) and Elliot and Amer (1973). Although this would help to account for the differences observed between these authors' results it must be noted that the feeding scale is only a single factor affecting the response to dietary copper.

1.6.3 Other metals

High intakes of copper raise the requirement for zinc and iron in the diet as there is competition amongst these metals at the site of absorption. Thus in animals receiving a high level of copper, symptoms of zinc deficiency may occur unless the dietary concentration of this element is also raised. Likewise increased levels of dietary zinc can help to prevent the adverse effects seen at dietary copper levels of 250 ppm or more (Underwood, 1971; Van Campen, 1971).

1.6.4 Other factors

Both the sex and breed of pig may have a role in determining the animal's response to dietary copper. However there appears to be no comprehensive information concerning this point (Cooke, 1981). Studies on fat metabolism have shown differences between Pietrain and Large White pigs. This may be important as copper does interfere with adipose tissue metabolism (see Section 1.5.3.3) (Muller, 1984). Also, in a study on soft fat in cross-bred pigs, Lea *et al.* (1970) found that most of the very low melting point fats came from half-Hampshire crosses.

The addition of other growth promoting substances has also been studied in relation to the effect of dietary copper. Barber *et al.* (1971) reported that dietary arsanilic acid and sodium salicylate gave similar growth responses to those obtained when 250 ppm copper was fed. However no additive effects were noted when either of these compounds was added to the diet together with the copper. In similar experiments, Barber *et al.* (1955) reported no synergistic action between dietary copper and the antibiotic aureomycin.

1.7 Mode of action of copper

The various effects of dietary copper in the pig have been the subject of much research. In particular the changes in growth performance and fat quality have been studied.

Increases in growth performance have been attributed to the effect of copper on the gut microflora (Hawbaker *et al.*, 1961). It has been suggested by Lenis (1981) that the improved protein digestibility observed in the presence

of dietary copper is due to a reduction in the microbial degradation of essential nutrients such as amino acids. However other work has revealed no apparent differences in the bacterial flora of the faeces or throughout the alimentary canal between copper-fed and basal-fed animals (Braude, 1967; Underwood, 1971).

Another possible site of action in the gut is on the digestive enzymes. Dietary CuSO_4 has been found to increase pepsin activity and so improve protein digestibility and hence the growth rate (Kirchgessner, 1976). However, this does not account for the changes in fat quality.

It is generally agreed that the presence of copper in pig diets leads to an increase in the unsaturated fatty acids in adipose tissue (see Section 1.5.3.3). The feeding of fishmeal-based diets has been implicated as a cause of the increase in lipid unsaturation. Fishmeal may contain polyunsaturated fatty acids which could ultimately be deposited in the storage triacylglycerols (Banks & Hilditch, 1932). However, in view of the small quantities involved it is unlikely that this would have a significant effect on tissue unsaturation.

Ho and Elliot (1973) investigated that the dietary copper-induced increase in oleic acid was due to an enhanced activity of the stearoyl CoA desaturase. They found a significant increase in the desaturase activity of both hepatic and adipose (outer backfat) microsomes from pigs fed 250 ppm Cu-supplemented diets. In later work they reported that the desaturation of palmitoyl CoA and oleoyl CoA was also increased by the presence of dietary copper (Ho & Elliot, 1974).

Contrary to the original findings of these authors, Thompson *et al.* (1973)

found that the desaturase activity in pig adipose tissue (outer backfat and perirenal fat) was not affected by copper supplementation. This was attributed to the much lower copper levels found in adipose tissue compared to the liver. As confirmation for this idea they studied the effect of the addition of CuSO_4 to microsomal preparations *in vitro*. The desaturase system of the livers from pigs receiving no dietary copper was activated by CuSO_4 additions *in vitro*. Ho *et al.* (1975) in a similar study reported that the enhancement of activity was much lower than expected from the *in vivo* data. In contrast, experiments on rat liver microsomes showed no effect of CuSO_4 additions *in vitro* on desaturase activity (Wahle & Davies, 1975).

Investigations by Wahle and Davies (1975) have shown that copper-deficient rats have lower Δ^9 desaturase activity than animals of normal copper status. Repletion of copper either by dietary supplement or intraperitoneal injection of CuSO_4 restored the enzyme activity.

The effect of copper chelators such as L-histidine and diethyl dithiocarbamate has also been studied (Ho *et al.*, 1975; Jeffcoat, 1979). Use of these compounds resulted in no significant change in activity relative to the controls. This suggested that the copper acts as an integral part of the system, rather than as an activating agent, probably at the terminal component of the electron transport chain. However this idea has been invalidated as the rat liver enzyme is an iron-containing protein and is inhibited by iron-chelators (Jeffcoat, 1979). These discrepancies may be due to differences between species but further work is required to fully elucidate these changes in desaturase activity.

The role played by the circulating free fatty acids (FFA) has also been investigated. Work by Myres and Bowland (1973) on fasted and fed pigs

receiving either basal or copper-supplemented diets has shown changes in free fatty acid metabolism. In the fed state dietary copper increased the level of plasma FFA but changes in their composition were also noted. These authors suggested that copper might decrease the rate of re-esterification of the free fatty acids in adipose tissue. As the unsaturated fatty acids appeared to be affected more than their saturated analogues, this could account for the well-documented changes in adipose tissue fatty acid composition due to dietary copper. However this work did not include any other factors controlling the relative rates of lipolysis and re-esterification.

Another possible mode of action of copper has been suggested by Saggerson *et al.* (1976). These workers reported that, in the rat, transition metal ions such as nickel (Ni^{2+}) and copper (Cu^{2+}) appeared to mimic the effects of insulin at the adipocyte plasma membrane, but not those on intracellular processes. Thus these metal ions could decrease lipolysis and stimulate glucose transport and hence fatty acid synthesis. This increase in fatty acid synthesis could in turn lead to the enhancement of desaturase activity discussed earlier in this section. Also, Freeman (1983) has shown that high levels of insulin stimulate the Δ^9 desaturase and it is possible that the Cu^{2+} ions may imitate this particular effect of the hormone. For dietary copper to produce this result the adipose tissue copper levels would have to be higher than those of the control animals. Castell and Bowland (1968b) found no such increase but they did report elevated serum copper levels as a result of copper-feeding. Thus circulating copper could be responsible for this effect if Cu^{2+} ions have a similar hormone-like action in the pig.

In the work by Moore *et al.* (1969) it was noted that the effect of dietary copper on melting point was more marked on the inner backfat layer

than the outer. It may be said that this is a growth effect as the two layers are deposited during different stages of development (see Section 1.4.1). The higher metabolic activity of the inner layer may also be a contributing factor and there may be differences in the blood supply. Little work appears to have been done on these differential effects except from the analytical point of view.

From the research undertaken it can be seen that there is no singular mode of action of copper on the pig. The changes in growth performance and fat quality have been attributed to several metabolic effects of dietary copper but the relative importance of these remains unclear.

1.8 Summary

In conclusion, the quality of pig backfat can be affected by several factors. Quantitatively the most important of these are the level and type of dietary fat and the presence of high levels of dietary copper. Other factors may also play a significant role but it is the dietary inclusion of these two which affects the nature of the adipose tissue triacylglycerols. The resulting increase in unsaturation of the triacylglycerol species lead to lower melting points and hence an increase in fat softness.

Although much research has been directed at the effects of dietary copper and dietary fat, less has been devoted to the interactive effects of the two. It was the aim of this project to investigate this interaction in particular reference to the changes in triacylglycerol structure and composition and their subsequent effect on carcass quality. The experimental approach involved studying the effects of 4% dietary maize oil and 250 mg Cu/kg feed on fat quality. The effects of several variables including protein source, breed and another growth promoter were also investigated.

Section 2
MATERIALS AND METHODS

Section 2

MATERIALS AND METHODS

2.1 Materials

Chemicals were obtained from the following suppliers:

Aldrich (Dorset):

Sodium methoxide

Amersham International plc (Amersham):

[1 - ^{14}C] Stearoyl - Coenzyme A; specific activity 55 mCi/mmol
(2.04 GBq/mmol); radioactive concentration 0.93 M Bq/ml

BDH (Poole, Dorset):

2-amino-2- (hydroxymethyl)-propane-1,3-diol (tris)

2', 7' - dichlorofluorescein

2:6 - di - tert - butyl - p - cresol (butylated hydroxytoluene, BHT)

Scintran [2 - (4' - tert - Butylphenyl) -5- (4'' - biphenyl) -
1, 3, 4-oxadiazole]

Merck (supplied by BDH, Dorset):

Kieselgel 60 H (TLC, 70-230 mesh, particle size 0.063 - 0.200 mm)

Silica gel 60 (column, 70-230 mesh, particle size 0.063 - 0.200 mm)

Sigma (Poole, Dorset):

Bile salts (sodium cholate and sodium deoxycholate)

Bovine serum albumin

Folin reagent (Folin - Ciocalteu phenol reagent)

Heptadecanoic acid methyl ester (C17:0 methyl ester)

Lipid standards

NADH

Pancreatic lipase, porcine (EC 3.1.1.3, triacylglycerol acyl-hydrolase)

20,000-50,000 units/mg

Stearoyl Coenzyme A (n-octadecanoyl Coenzyme A)

Sulpelco (Sawbridgeworth, Herts):

10% Silar 9 CP on Chromosorb WHP (100-120 mesh)

rac - Glyceryl - 1,3- palmitate - 2 - oleate

All other reagents were supplied by Sigma Chemical Company Ltd., (Poole, Dorset).

2.2 Methods

2.2.1 Lipid extraction from tissues

2.2.1.1 Folch wash method

This method, used for the extraction of lipids from adipose tissue, is a modification of the original procedure of Folch *et al.* (1957) (Ways & Hanahan, 1972).

Approximately 1 g of tissue was homogenised in 10 ml of methanol for one minute using a Virtis homogeniser. 20 ml of chloroform was added and the mixture was then homogenised for a further two minutes. This was filtered and the solid residue was resuspended in 30 ml of chloroform:methanol (2:1 v/v and containing 100 mg l⁻¹ 2:6 -di - tert - butyl - p - cresol (BHT) as antioxidant) and homogenised for three minutes. The mixture was filtered a second time and the remaining solid was washed with 20 ml of chloroform and 10 ml of methanol. The filtrates were combined and transferred to a separating funnel. A volume of aqueous 0.88% potassium chloride solution, equivalent to one quarter of the total volume of the filtrate was then added, the mixture was shaken thoroughly and allowed to separate. The upper aqueous layer was then removed and the washing procedure was repeated using water:methanol (1:1, v/v) instead of the potassium chloride solution. The lower layer contains the purified lipid extract and was filtered before the solvent was removed on a Buchi rotary evaporator. Samples were stored in a small volume of chloroform at -20°C until further analysis.

2.2.1.2 Hajra extraction

This method, essentially an acidic version of the Bligh and Dyer extraction method, was used for the extraction of blood lipids (Bligh & Dyer, 1959; Hajra *et al.*, 1968).

To each 0.5 ml of plasma the following were added:

1.88 ml chloroform:methanol (1:2 v/v + 100 mg l^{-1} BHT)

0.625 ml 2M KCl/0.2 M H_3PO_4

0.625 ml $CHCl_3$

20 μ l 6M HCl

The mixture was shaken vigorously and then separated by centrifugation. The upper layer was discarded and the lower phase was washed with 2 ml of synthetic top phase (see below). The lipid-containing lower phase was dried down in a stream of N_2 and stored at $-20^\circ C$ until further analysis. Synthetic top phase was made by mixing together 500 ml chloroform, 500 ml methanol, 200 ml water, 250 ml 2M KCl/0.2 M H_3PO_4 and 16 ml 6M HCl in a separating funnel. The layers were allowed to separate and the lower layer was discarded.

2.2.2 Purification of triacylglycerols

Triacylglycerols in the lipid extract prepared by the 'Folch wash' method (Section 2.2.1.1) were purified by thin layer chromatography. The lipid sample was redissolved in chloroform and a small amount, equivalent to about 5 - 10 mg of lipid, was applied as a band to the silica gel plate. A lipid standard containing mono-, di- and tri-olein was also applied. The plate was developed in a solvent system containing:

Hexane	:	Diethyl ether	:	Acetic acid (glacial)	
60	:	40	:	1	v/v/v .

The triacylglycerol band, identified by means of the lipid standard, was scraped from the plate and eluted with 2 x 2 ml chloroform:methanol (2:1). The silica gel was removed by centrifugation and the triacylglycerol sample was dried in a stream of nitrogen.

2.2.3 Thin layer chromatography

Preparation of plates

a) Silica gel plates

An aqueous slurry of 25g kieselgel 60 H and 55ml water was spread onto five 20 x 20cm glass plates to a thickness of 0.25 mm. The plates were allowed to air dry at room temperature before heating at 110°C for one hour. After this activation procedure the plates were either used immediately after they had cooled or else were stored in a desiccator.

b) 10% silver nitrate plates

The plates used for argentation chromatography were spread to a thickness of 0.25 mm. The silica gel slurry was made using 25g kieselgel 60 H and 55ml of water containing 2.5g silver nitrate. The method of preparation was the same as for the ordinary silica gel plates. After activation the plates were stored desiccated and in the dark.

c) Preparative 10% silver nitrate plates

These were prepared in the same manner as the 0.25mm AgNO₃ plates. However the preparative plates were spread to a thickness of 0.5 mm using a slurry made from 60g kieselgel 60H and 140 ml water containing 6g AgNO₃. Activation and storage of the plates was as before.

Development of plates

The thin layer plates were developed in lined glass tanks containing the solvent system quoted in the relevant text. The silver nitrate plates were developed in the dark to prevent blackening of the plate. Following development of the plate the solvents were allowed to evaporate. The component bands were then visualized by spraying with a 0.1% solution of

2', 7'-dichlorofluorescein in methanol. The lipids appear as yellow spots or bands on a dark background when viewed under ultra-violet light (254 nm). This spray reagent does not affect subsequent fatty acid analysis (Christie, 1982).

2.2.4 Determination of the fatty acid composition of lipids

The fatty acid composition of the lipid samples was determined by gas chromatography of their methyl esters. These were prepared from purified triacylglycerols (or other lipid) by the method of Gurr and Robinson (1972).

3ml of transmethylation reagent (see below) and one anti-bumping granule were added to a small sample of the purified lipid and refluxed for 30 to 40 minutes at 90°C. The tubes were allowed to cool and 3ml of water was then added. Light petroleum ether (bpt 40-60°C) was added to give two phases of approximately equal volume. The mixture was shaken and separated by centrifugation. The lower aqueous layer was removed and the upper layer, containing the fatty acid methyl esters, was dried over anhydrous sodium sulphate. This solution was used for the gas chromatographic analysis of fatty acid composition (see Section 2.2.5) and, if necessary, was concentrated in a stream of nitrogen.

Transmethylation reagent:

Methanol	:	Toluene	:	conc. H_2SO_4	
20	:	10	:	1	by volume + 100 mg l ⁻¹ BHT

2.2.5 Gas liquid chromatography

Gas chromatography was used for the analysis of the methyl esters of fatty

acids derived from lipid samples. All analyses were performed on a Packard 430 or 429 gas chromatograph fitted with a flame ionisation detector.

The 2m glass column (internal diameter = 2mm; external diameter = $\frac{1}{4}$ inch) was silanised before packing with 10% Silar 9CP on Chromosorb WHP (100 - 120 mesh). Newly-packed columns were conditioned overnight at 240°C before analytical use to remove excess liquid phase. Drying tubes were fitted to both the hydrogen and air lines and an Oxisorb tube was fitted to the nitrogen line to prevent oxidation of the column packing.

The operating conditions were an isothermal column temperature of 180°C and both the injection port and detector were at 200°C. Gas flows were set at 25 ml min⁻¹ for both the carrier gas (oxygen-free nitrogen) and hydrogen, and at 250 ml min⁻¹ for air.

Peaks were recorded on a Packard 611 chart recorder and peak areas were calculated either by the (peak height x peak width at half peak height) method or using the Packard 430 integrator. Work by Ettre and Kabot (1963) has shown that with a flame ionisation detector the relative peak area values can be taken as concentration by weight i.e. the response factors for the individual fatty acids are the same.

Methyl esters of fatty acids were identified by comparison of their retention times with those of known standards. Columns were replaced when peak trailing became evident.

2.2.6 Determination of the positional distribution of fatty acids in triacylglycerols

Complete stereospecific analysis of triacylglycerols is possible but is a complex procedure. Thus a method was used to determine the composition of the fatty acids at the 2- position of the triacylglycerols. The composition at the 1- and 3- positions can be obtained by difference but these two are not distinguishable from each other by this technique.

The method, pioneered by Luddy *et al.* (1964) involves the use of porcine pancreatic lipase which specifically hydrolyses the fatty acids esterified to the outer 1- and 3- positions of the triacylglycerols. This yields 2- monoacylglycerols, the fatty acid composition of which reflects that of the 2- position in the original triacylglycerols.

The incubation mixture consisted of:

1ml	1 M tris buffer, pH8
100 μ l	2.2% CaCl_2 in water
250 μ l	0.05% bile salts in water
5-10 mg	triacylglycerol (extracted by Folch wash and purified by tlc)

This mixture, in screw-cap tubes, was equilibrated at 40°C for five minutes. The reaction was started by the addition of 15 μ l porcine pancreatic lipase ($\sim$ 20,000 units per ml in 0.1 M tris buffer, pH 8). The tubes were shaken at 2200 rpm using a Janke and Kunkel Ika-Vibrax-VXR shaker for one minute at 40°C.

The reaction was stopped by the addition of 10 ml diethyl ether, and the mixture was shaken and allowed to separate. The ether layer was removed and

the extraction procedure was repeated with a further 2 x 10 ml diethyl ether. The combined ether extracts were washed with 2 x 5ml of 0.88% KCl in water and then dried with anhydrous Na_2SO_4 .

The ether extract contains 2-monoacylglycerols, free fatty acids and unreacted triacylglycerols. These are separated from each other by thin layer chromatography on silica gel plates as follows. The extract was dried down with nitrogen and then redissolved in 50 μ l $\text{CHCl}_3:\text{CH}_3\text{OH}$ (2:1 v/v). 25 μ l of this was applied to the thin layer plate as a band. 2-monopalmitin was used as a standard. The plate was developed in:

Chloroform	:	Methanol	:	Acetic acid (glacial)
98	:	2	:	1 (v/v/v)

After visualization of the plate with 0.1% 2', 7' - dichloro-fluorescein in methanol, the 2-monoacylglycerol band was scraped into centrifuge tubes and eluted with 2x2 ml $\text{CHCl}_3 : \text{CH}_3\text{OH}$ (2:1 v/v). The lipid was then transmethylated and analysed by gas chromatography as described earlier in Sections 2.2.4 and 2.2.5.

The fatty acid composition of the 2-position of the triacylglycerols is obtained by this method. The percentage composition of the outer 1- and 3-positions is calculated by the following relationship between the concentrations of the fatty acid in the complete triacylglycerol and at position 2 :

$$(\text{Positions 1 and 3}) = \frac{3 \times [\text{triacylglycerol}] - [\text{position 2}]}{2}$$

Analysis of the free fatty acid band would also give the fatty acid composition of the 1- and 3- positions. However acyl migration can occur leading to the formation of 1-monoacylglycerols from 2-monoacylglycerols. These would then be hydrolysed so giving a less accurate result. The reaction conditions permit $\sim 40\%$ hydrolysis of the triacylglycerols. This is sufficient to measure the composition of the 2-monoacylglycerols whilst minimising acyl migration.

The accuracy of the method was checked by hydrolysing a specific triacylglycerol i.e. rac -glyceryl - 1, 3- palmitate -2-oleate. Analysis of the monoacylglycerol band showed a fatty acid composition of 90% oleic acid and 10% palmitic acid. Thus the method is at least 90% efficient as it assumes that no acyl migration has occurred in the original sample, and that hydrolysis is completely random.

2.2.7 Determination of lipid classes in plasma samples

The method of Christie *et al.* (1970) was used as it allows the simultaneous determination of the lipid classes and their fatty acid compositions. Individual lipid classes are separated by their layer chromatography and a known amount of internal standard is then added to each. Following gas chromatographic analysis the weight of fatty acids can be calculated by reference to the internal standard. Triacylglycerols, cholesteryl esters and free fatty acids were determined by this technique. Free cholesterol concentration was determined as described in Section 2.2.8.

Lipids were extracted from plasma samples by the Hajra method (Section 2.2.1.2); 5-10 mg of lipid extract was then chromatographed on silica gel

plates (Section 2.2.1) and developed in the solvent system:

Hexane	:	Diethyl ether	:	Acetic acid (glacial)
60	:	40	:	1 v/v/v

Lipid standards including triolein, cholesteryl oleate, oleic acid and cholesterol were routinely applied to the thin layer plate in order to identify the individual lipid species. These were visualized by spraying the plate with a 0.1% solution of 2', 7' - dichlorofluorescein in methanol. The bands of lipids were scraped and eluted with 2 x 2ml CHCl_3 : CH_3OH (2 : 1 v/v).

1ml of the internal standard, methyl heptadecanoate (0.285 g l^{-1} in cyclohexane) was then added to each lipid fraction. Transmethylation of the samples and subsequent gas chromatographic analysis was carried out as described in Section 2.2.4. The total weight of fatty acids present in each lipid class is directly related to the amount of internal standard added. Thus the weight per cent (or mole per cent) of the fatty acids can be calculated. However, multiplication of these values by a calculated factor gives a good approximation to the actual weight of lipid present (Christie *et al.*, 1970).

2.2.8 Determination of cholesterol concentration in plasma

5ml of isopropanol was rapidly added to 200 μ l of plasma (or cholesterol standard), mixed well and then centrifuged. To 1ml of the supernatant, 3ml of glacial acetic acid was added. This was then mixed and 300 μ l of ferric chloride reagent was added (see below). After mixing 3ml of conc. H_2SO_4 was carefully added. This was then mixed again and left to stand for 10 minutes to allow the full colour to develop.

The optical density was read at 560 nm against an isopropanol-water blank in a Pye-Unicam SP8 - 100 uv/vis spectrophotometer.

A standard curve was constructed by assaying a series of cholesterol standards. The relationship was linear up to at least 1.5 mg/ml of cholesterol in isopropanol (Holme & Peck, 1983).

Ferric chloride reagent consists of 2.5 g l^{-1} $\text{FeCl}_3 \cdot 6 \text{ H}_2\text{O}$ in 85% orthophosphoric acid.

2.2.9 Determination of the slip point of lipids

Samples of triacylglycerols, purified by tlc (Section 2.2.2) and thoroughly dried by vacuum desiccation were melted. Four capillary tubes were then inserted so that the fat rose into them to a length of $10 \pm 2 \text{ mm}$. The fat was then solidified by briefly placing the tubes on ice, taking care to avoid contact with water. They were then left at a temperature of $15 - 17^\circ\text{C}$ for at least 16 hours to allow the fat to stabilize in the capillary tubes. It is important to treat all samples the same as triacylglycerols can exhibit polymorphic behaviour.

Slip points were then determined, in triplicate, by the British Standard Institute method (BS 684 : General Introduction, 1982 and BS 684 : Section 1.3, 1976). The slip point (open capillary melting point) is the temperature at which the column of fat rises in the capillary tube, under the conditions specified by the British Standard test. If repeated determinations differed by more than 0.3°C then the determination was repeated.

2.2.10 Separation and determination of triacylglycerol species

Pooled samples of triacylglycerols were applied to preparative (0.5mm thick) 10% silver nitrate tlc plates. A standard mixture, containing saturated, mono-, di- and tri-unsaturated simple triacylglycerols, was also applied. The plates were developed in the solvent system:

Hexane	:	Diethyl ether	:	Toluene	:	Methanol	
60	:	20	:	20	:	1	by volume

The triacylglycerol bands were visualized under u.v light after spraying with 2', 7' - dichlorofluorescein (0.1% w/v in methanol). The bands were scraped and the triacylglycerols were eluted with 3 x 3ml of chloroform : methanol (2 : 1 v/v + 100mg l⁻¹ BHT). The combined eluants were then washed with 2ml of 100mM NH₄OH to remove the fluorescent dye. The chloroform layer was filtered and then dried down in a stream of nitrogen.

The triacylglycerols were redissolved in 1ml of the CHCl₃ : CH₃OH mixture. 200 µl was then taken for fatty acid determination (Section 2.2.4). Prior to the transmethylation step 50 µl of methyl heptadecanoate (0.285 g l⁻¹ in cyclohexane) was added as an internal standard. Following transmethylation the fatty acid composition was determined by gas chromatography (Section 2.2.5). The addition of the internal standard enables the relative concentrations of the triacylglycerol groups to be calculated. The 0.05 µmoles of standard added is equivalent to 0.01425 mg of the methyl heptadecanoate.

2.2.11 Random interesterification of triacylglycerols

Pooled samples of triacylglycerols were randomly interesterified according to the method of Parodi (1979).

Approximately 500 mg of triacylglycerols, previously dried by vacuum desiccation, was heated at 100°C for 1 hour with 0.2% sodium methoxide as a catalyst. The tubes were allowed to cool before 3 ml of 10% citric acid solution (w/v) was added to destroy the catalyst. The lipid was recovered by extraction with 3 x 5 ml of light petroleum ether (bpt. 40 - 60°C).

The randomly interesterified triacylglycerols were purified on a silicic acid column (30 mg lipid per g absorbent). The triacylglycerols were eluted with twenty column volumes of 5% diethyl ether in hexane (v/v). The eluant was collected in 10 ml batches and a small sample of each was applied to a silica gel thin layer plate in order to check the purity of each fraction. A triacylglycerol standard was also applied. The plates were developed in :

Hexane	:	Diethyl ether	:	Acetic acid (glacial)
60	:	40	:	1 v/v/v

The developed plates were sprayed with 2', 7' - dichlorofluorescein (0.1% w/v in methanol) and the bands were then viewed under uv light. The first 10 ml fraction was found to contain another lipid component and so was discarded. The remaining fractions, containing purified triacylglycerol, were pooled and the solvent was removed by rotary evaporation.

A small sample of the lipid was hydrolysed by pancreatic lipase (Section 2.2.6) to check that the interesterification was random. The remaining triacylglycerol was thoroughly dried by vacuum desiccation for subsequent slip point determination (Section 2.2.9).

2.2.12 Preparation of microsomes

Samples of adipose tissue were obtained immediately after slaughter and were placed on ice during transport back to the laboratory.

Approximately 50g of tissue was weighed out and then roughly chopped into a chilled Waring blender. Three volumes (150 ml) of cold 50mM potassium phosphate buffer, pH 7.4 and containing 250mM NaCl and 5mM EDTA, was added and homogenised for 2 x 1 minute periods. The mixture was poured into chilled centrifuge bottles and centrifuged at 1,000g at 4°C for 10 minutes in a MSE Hi Spin 21 centrifuge.

The fat cake was then removed and the mixture centrifuged at 10,000g at 4°C for 30 minutes. This step removed mitochondria, cell debris and more dense cell organelles from the suspension. Microsomes were sedimented from the resulting postmitochondrial supernatant fraction by centrifugation at 100,000 g for 1 hour at 4°C. A Kontron Centrikon T-2070 ultracentrifuge was used for this step.

The microsomes were resuspended in fresh 0.1 M potassium phosphate buffer, pH 7.4, and protein concentration was determined by the Lowry method (Section 2.2.13) and adjusted to 7 mg protein/ml. The microsomal suspension was stored at -20°C until further analysis.

2.2.13 Determination of protein concentration

Protein concentration was determined by the method of Lowry *et al.* (1957). A series of standard bovine serum albumin solutions were assayed in order to construct a standard curve. This was found to be linear up to a protein

concentration of at least 1 mg protein/ml.

2.2.14 Assay for stearoyl Coenzyme A desaturase activity

This enzyme is responsible for the desaturation of stearic acid to oleic acid. It acts on the CoA ester of stearic acid and requires both oxygen and NADH. The assay procedure, based on that of Jones and Gaylor (1981), involves the use of a radiolabelled substrate, $[1 - ^{14}\text{C}]$ - stearoyl Coenzyme A.

The incubation mixture contained the following :

1.2mM NADH (in 10mM potassium phosphate buffer, pH 7.4, prepared daily).

60 μ M $[1 - ^{14}\text{C}]$ - stearoyl CoA (5.5 μ Ci/ μ mol and stored in 10mM potassium phosphate buffer, pH6).

0.5 mg microsomal protein (prepared as in Section 2.2.12)

in 1 ml total volume of 0.1 M potassium phosphate buffer, pH 7.4.

The reaction was started by the addition of the microsomal protein and the mixture was incubated for 10 minutes at 37°C in a shaking water bath. The reaction was stopped with 1 ml of a 10% solution of methanolic KOH and the mixture was then refluxed at 90°C for 30 minutes. After cooling, 1 ml of 3M HCl was added and the organic layer was extracted with 2 x 5 ml of light petroleum ether (bpt. 40 - 60°C). The solvent was evaporated in a stream of nitrogen, 2 ml of transmethylation reagent was added (Section 2.2.4) and the mixture was refluxed at 90°C for 40 minutes. After cooling an equal volume of water was added, followed by sufficient light petrol ether to give two phases of equal volume. This mixture was shaken and the layers separated by centrifugation. The organic phase was then recovered and dried down in a stream of nitrogen.

The dried sample, containing the methyl esters of stearic and oleic acids, was then redissolved in 125 μ l of light petroleum ether. 50 μ l of this was then applied to 10% silver nitrate thin layer plates (Section 2.2.3b). Methyl stearate and methyl oleate standards were also applied. The plate was developed, in the dark, in :

Hexane : Diethyl ether

9 : 1 v/v

and the bands were visualized as described in Section 2.2.3.

The methyl oleate and methyl stearate bands were identified by means of the standards and were scraped into scintillation vials to which 10 mls. of Scintran (a toluene-based scintillant) was then added. The origin from each assay spot and a small sample of silica gel from an unused part of the plate were also scraped. The amount of activity in the vials was then counted using an LKB 1211 ambient temperature scintillation counter.

A series of ^{14}C standards were used to set up a quench curve for the scintillation counter and the samples were corrected for quench using the external standard ratio method. The count from the scraped blank part of the thin layer plate was used as a measure of the background radioactivity, a high count indicating possible contamination of the plate.

The percentage desaturation from each incubation was calculated by the ratio of radioactivity in the methyl oleate band to the total radioactivity of the assay spot. The band scraped from the origin normally has a very low count and was used to check that both the saponification and methylation reactions had gone to completion.

Incubations were normally carried out in triplicate and blanks were performed using boiled microsomal protein.

Determination of optimum assay conditions.

In order to determine the optimum conditions for the assay the concentrations of stearyl CoA, NADH and protein were varied independently of each other. The length of incubation period was also varied. The concentrations of NADH and stearyl CoA chosen were close to the plateaux of the relevant requirement curves (see Figures 2.2.14 a,b). The incubation was carried out for the time and at the protein concentration corresponding to the linear portion of the relevant curves (Figures 2.2.14 c,d).

Differences between means were compared using Students t test.

Figure 2.2.14 a,b

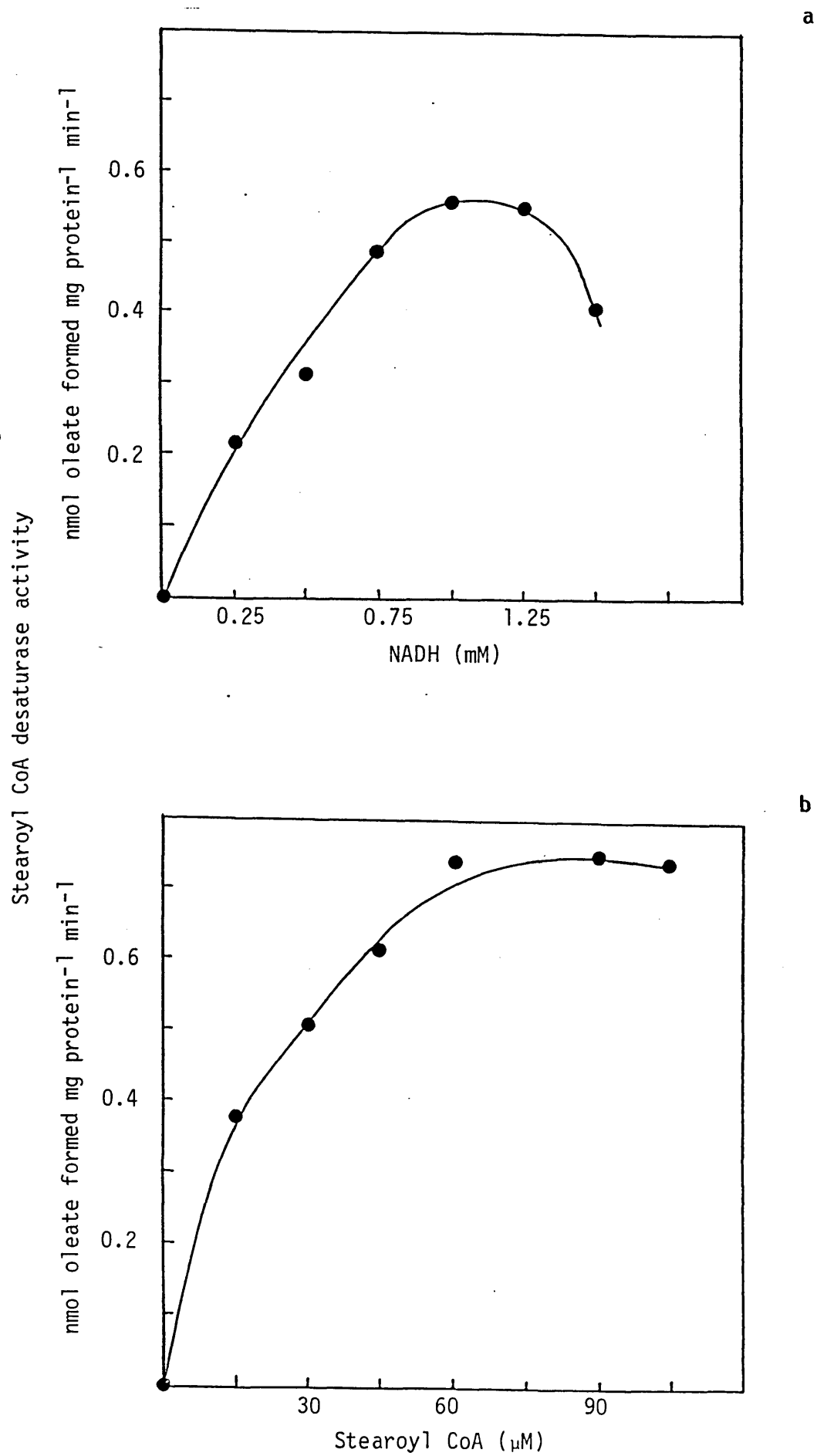
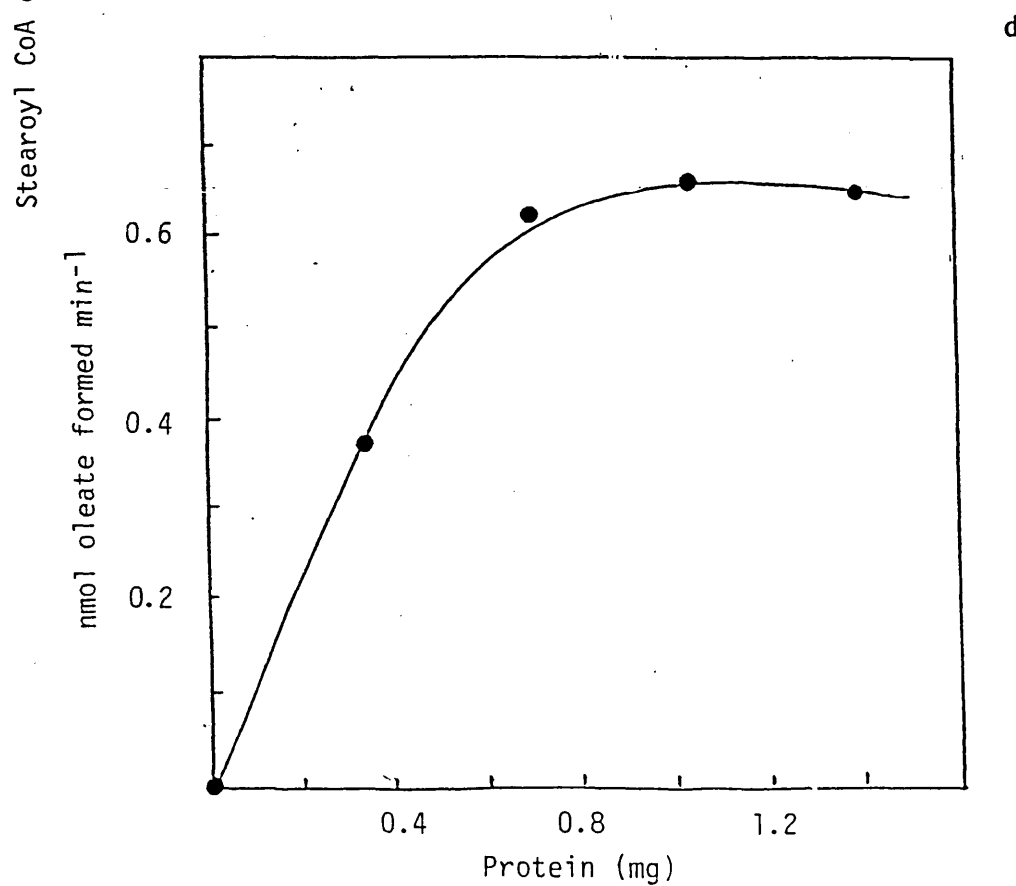
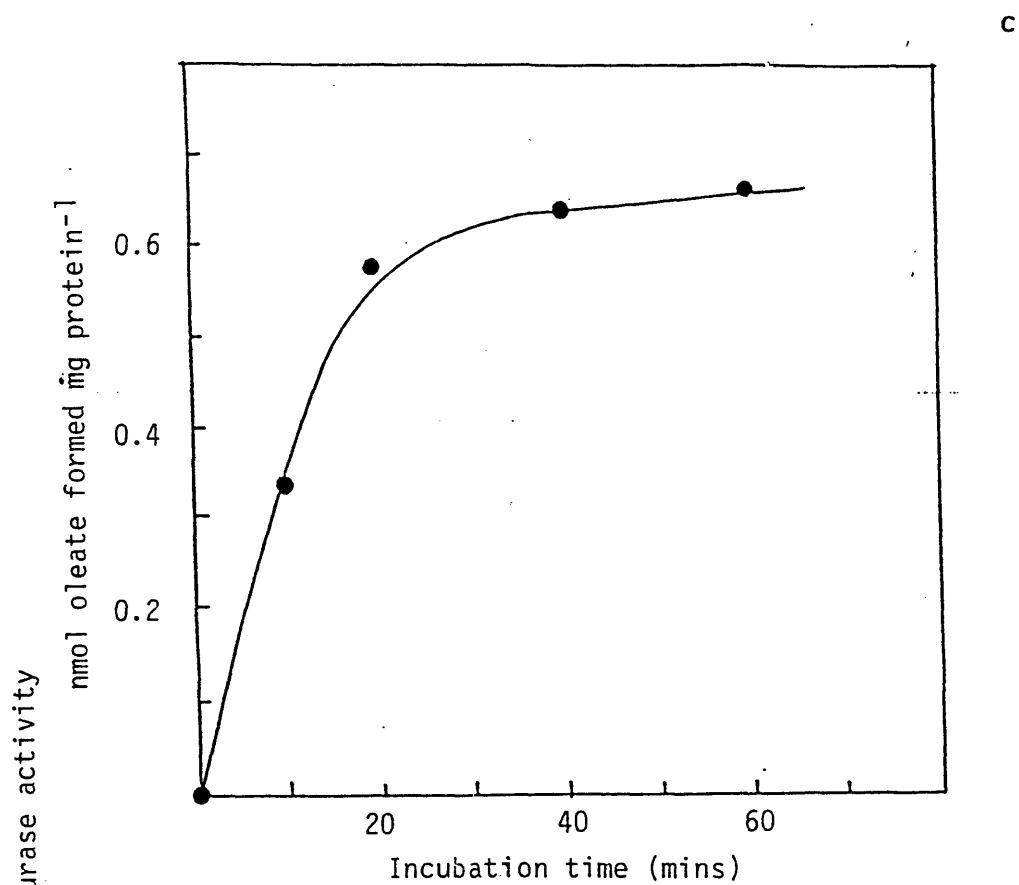


Figure 2.2.14 c.d



Section 3
EXPERIMENT 1

SECTION 3

Experiment 1

The effect of dietary copper, maize oil and protein source
on backfat quality

3.1.1 Introduction

Dietary copper was originally included in pig diets as a growth promoter since improved feed conversion efficiencies were reported when copper (as Cu^{2+}) was added at concentrations of up to 250 mg Cu/kg feed (Section 1.5.3.1). This feed supplement was also found to affect the quality of the depot fat by increasing the concentration of monounsaturated fatty acids, particularly oleic acid, in the adipose tissue triacylglycerols (Elliot & Bowland, 1968). As a result the melting point of the backfat lipids was decreased and in some cases this led to depot fat which was unacceptably soft.

The inclusion of polyunsaturated oils in the diet can also increase lipid unsaturation by increasing the concentration of linoleic acid in the stored lipid (Brooks, 1967). One of the aims of this experiment was to investigate whether or not copper increased the softness and unsaturation of backfat when included in a diet already containing a high proportion of unsaturated fatty acids. Thus barley-based diets were supplemented with either 200 mg Cu/kg feed, 4% maize oil or both of these.

In addition the effect of copper supplementation can, to some extent, be altered by the type of protein present in the diet. Drouliscos *et al.* (1970) noted that the retention of copper by the liver was lower in pigs fed soyabean

meal diets compared with animals receiving similar diets based on fishmeal protein. Soyabean meal contains high levels of phytic acid which are thought to chelate with Cu^{2+} ions and so reduce copper uptake. This effect, together with that of dietary maize oil was studied by supplementing maize oil and copper to diets containing either fishmeal or soyabean meal as the source of protein.

3.1.2 Dietary treatments

There were eight dietary treatments in this experiment. Four contained white fish meal (13g/100g diet) as the protein supplement whereas soyabean meal (24g/100g diet) was used in the other four treatments. The effects of dietary copper (200 mg Cu/kg diet) and maize oil (4g/100g diet) were examined separately and together with each of the two protein supplements. A summary of the treatments is given below:

Fishmeal treatments	F	Fishmeal
	FCu	Fishmeal + copper
	FM	Fishmeal + maize oil
	FCu	Fishmeal + maize oil + copper
Soyabean treatments	S	Soyabean
	SCu	Soyabean + copper
	SM	Soyabean + maize oil
	SMCu	Soyabean + maize oil + copper

Table 3.1.1 gives the composition of the eight experimental diets. Samples of the fishmeal and soyabean meal supplements were retained for the determination of fat content and fatty acid composition. The overall fat contents and fatty acid compositions of the eight diets are given in Table 3.1.2.

Table 3.1.1: Experiment 1. Composition (kg/1000kg) of the experimental diets

Diets	F	FCu	FM	FMCu	S	SCu	SM	SMCu
Barley meal	855.5	854.8	815.5	814.8	736.2	735.6	696.2	695.6
White fish meal	132.0	131.9	132.0	131.9	-	-	-	-
Soyabean meal	-	-	-	-	240.0	239.8	240.0	239.8
Limestone flour	-	-	-	-	2.6	2.6	2.6	2.6
Dicalcium phosphate	-	-	-	-	8.7	8.7	8.7	8.7
Mineral/vitamin supplement*	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Maize oil	-	-	40.0	40.0	-	-	40.0	40.0
CuSO ₄ .5H ₂ O	-	0.8	-	0.8	-	0.8	-	0.8
Calculated composition (g/100g)								
Crude protein	17.5	17.5	17.1	17.1	18.0	18.0	17.6	17.6
Lysine	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Calcium	1.0	1.0	1.0	1.0	0.8	0.8	0.8	0.8
Phosphorus	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6
MJDE/kg	12.8	12.8	13.7	13.7	12.9	12.9	13.9	13.9

* See Appendix 1 for composition of this supplement.

Table 3.1.2: Experiment 1.

Fat contents (%) and fatty acid compositions (weight %) of the experimental diets.

Wt % of fatty acid	Dietary Treatment			
	F, FCu	FM, FMCu	S, SCu	SM, SMCu
16:0	14.2	13.4	11.1	12.5
16:1	3.1	1.0	0.2	0.1
18:0	4.8	3.2	5.4	3.3
18:1	19.1	27.0	20.8	28.0
18:2	43.6	49.2	59.0	53.8
18:3	4.5	2.1	2.6	1.4
a	0.8	0.3	-	-
20:4	4.3	1.4	-	-
b	2.3	0.7	-	-
c	2.3	0.7	-	-
% fat in diet	1.8	5.8	1.4	5.4

a,b,c - unidentified fatty acids with retention times of 10.8, 19.6 and 35.6 minutes respectively.

Fatty acid compositions were calculated from data obtained by analysis of fishmeal and soyabean meal and from literature (Kuksis, 1978; Hilditch, 1956). (barley,maize oil)

3.1.3 Animals

At six weeks of age thirty-two Large White x (LW x Landrace) pigs were selected in eight littermate groups of four. Each littermate was then randomly assigned to one of the four dietary treatments with either fishmeal or soyabean meal as the protein supplement. There was an overall balance of sex so that two intact males and two females were allocated to each of the experimental diets.

3.1.4 Experimental procedure

All of the experimental animals received a copper-supplemented diet from three to six weeks of age. This was replaced in the following three weeks *i.e.* from six to nine weeks of age by one of the two diets not supplemented with copper or maize oil. Thus for six to nine weeks of age all sixteen pigs allocated to the fishmeal treatments were given the experimental diet containing fishmeal but no copper or maize oil (Diet F). Similarly, the sixteen pigs allocated to the four soyabean meal treatments were given the basal soyabean meal diet (Diet S) for the same period. The pigs were given the eight experimental diets when they reached nine weeks of age and about 18 kg liveweight.

Housing was in individual pens so that the food intake for each animal could be measured. The experimental diets were given wet and twice daily according to a semi-*ad lib.* scale up to a maximum daily intake of 2.95 kg diet/pig. Details of the feeding scale are given in Appendix 2. The animals were weighed on the same day and at the same time each week and ultrasonic P_2 measurements were taken every two weeks.

Samples of faeces were collected from each of the pigs on diets FCu and

SCu *i.e.* those receiving copper but no maize oil, at four and eight weeks after the start of the experiment. Sufficient faeces were collected to provide two composite samples each of about 1 kg of freeze-dried and ground faeces. The samples were then stored at -20°C for analysis of copper content.

Each pig was slaughtered on the day following the weekly weighing when it reached a minimum of 87.5 kg. No food was given on the day of slaughter. A strip of backfat, 3 cm in width, was taken along the whole length of the right side of each carcass *i.e.* from shoulder to loin. Liver samples were taken immediately after slaughter. Both liver and backfat samples were rapidly transferred to a deep freeze where they were stored at -20°C until analysis. On the day following slaughter maximum thickness of the shoulder, loin and P₂ fat layers were measured on the left side of the carcass.

3.1.5 Methods of analysis

The strips of backfat taken from the carcass were separated into the component inner and outer layers. Three sections were then taken from each layer, corresponding to the shoulder, middle and loin regions, and these together with the perirenal adipose tissue were used for lipid analysis. The lipids were extracted from each of these samples (Section 2.2.1.1) and the triacylglycerol fraction purified (Section 2.2.2). The fatty acid compositions and melting points of the triacylglycerol samples were determined as described in Sections 2.2.4 and 2.2.9 respectively. The pancreatic lipase hydrolysis technique was used to determine the positional distribution of the fatty acids in the triacylglycerols (Section 2.2.6). In addition, pooled triacylglycerol extracts from each layer of pigs on each treatment were randomly interesterified (Section 2.2.11) and their subsequent melting point ascertained.

In general the differences between treatment means were compared by analysis of variance. Although the experiment was randomized for littermates within treatments containing the same protein source the details of this were not recorded and so had to be excluded from the analysis. Thus the experiment was regarded as a factorial design, and the main effects of protein source(1df), maize oil(1df), copper(1df), sex(1df) and interactions(10df) ^e_k were looked for. The protein source*copper*maize oil*sex interaction was not tested, so giving 17 df for error rather than 16.

Where statistically significant effects of protein source, copper or maize oil ^e_k were noted, further analysis of treatment means was carried out ie. for protein source the F and S treatment means were compared, for copper the F and FCu and S and SCu treatments were compared, and for maize oil the F and FM and the S and SM treatments were compared by t test using the error degrees of freedom(17). Differences in triacylglycerol composition and slip point between sample sites within each backfat layer (within treatments) were compared by t test(6df). These differences were all found to be not significant at the 5% level and so the results within each backfat layer were pooled. Differences between adipose tissue sites within treatments were also compared by t test(22df between backfat layers and 14df between backfat layers and perirenal fat). Differences in faecal copper content were compared by t test with 4 df. Where linear regressions were calculated significance was tested by reference to the correlation coefficient(n-2df). Differences between regression were compared by t test of the gradients and intercepts.

3.2 Results of Experiment 1

3.2.1 Growth rates, food intakes and feed conversion ratios

The mean weekly weights of the animals are given in Table 3.2.1. There were no significant differences at the 5% level in mean growth rates between pigs on any of the dietary treatments despite the slightly higher calorific value of the oil-supplemented diets. In keeping with this, the daily feed consumptions, daily liveweight gains and feed conversion ratios were also similar between pigs on different dietary treatments. Table 3.2.2 gives details of these and other feed related results. There were statistically significant interactions between maize oil and copper for total liveweight gain ($p < 0.05$) and between fishmeal and soyabean meal for feed conversion ratio ($p < 0.05$). However these findings are probably of little importance as they are not evident with the other feed related results and, in addition, no significant differences were noted between individual treatments for total liveweight gain ($p > 0.05$). No significant difference was noted between the mean feed conversion ratios of the pigs on the F and S diets respectively ($p > 0.05$). There was a significant effect of sex on the total amount of feed consumed ($p < 0.001$) but this is probably of little importance as it was not noted with the other feed related data.

3.2.2 Thickness of subcutaneous backfat

The P_2 fat depth is defined as the fat thickness measured over the M. longissimus 4.5cm from centre at the level of the head of the last rib (Kempster et al, 1982). In pigs from all treatments there was a rapid increase in P_2 fat depth of approximately 7mm during weeks 2 to 4 of the experimental period

Table 3.2.1: Experiment 1. Mean weekly weight (kg) and P_2 backfat thickness measured (mm) for all four pigs on each dietary treatment during the experimental trial.

a) Mean weekly weights

Dietary treatment

Week of experiment	F	FCu	FM	FMCu	S	SCu	SM	SMCu	LSD (17df)
1	20.9	21.6	21.3	22.0	20.9	21.3	22.5	21.3	3.3
2	26.4	26.3	26.3	28.0	25.4	25.6	27.3	26.1	4.4
3	31.5	32.3	31.5	34.0	29.5	30.0	31.8	31.3	5.0
4	34.5	39.0	31.9	40.6	35.5	36.6	37.8	37.5	8.8
5	38.3	42.5	35.0	44.8	40.8	42.3	43.5	43.3	9.9
6	44.9	48.6	41.6	50.9	48.3	49.1	50.4	50.0	9.4
7	51.0	54.8	48.8	57.0	54.0	55.8	56.8	56.8	8.6
8	58.3	61.8	55.5	64.0	57.8	61.0	62.8	63.0	8.6
9	65.8	67.3	63.5	70.8	66.0	69.3	71.8	71.8	8.5
10	73.1	74.8	71.0	78.6	73.8	76.3	79.0	79.0	8.5

b) P_2 backfat thickness

Dietary treatment

Week of experiment	F	FCu	FM	FMCu	S	SCu	SM	SMCu	LSD (17df)
2	2.8	2.8	3.5	3.3	2.8	4.0	4.3	3.3	1.7
4	7.5	9.0	9.3	10.3	9.5	10.3	10.8	9.8	3.4
6	8.3	9.3	9.8	11.3	8.5	9.0	9.8	10.3	2.3
8	11.0	11.3	10.8	13.3	10.0	10.3	10.5	10.3	3.5
10	12.3	12.3	13.5	13.5	11.8	11.0	11.5	12.8	2.8

Table 3.2.2: Experiment 1. Mean liveweight gains, weights of food consumed and food conversion ratios for all four pigs on each of the eight dietary treatments.

Dietary Treatments	F	FCu	FM	FMCu	S	SCu	SM	SMCu	LSD
Total liveweight gain (kg)	74.5	70.6	71.1	72.3	72.3	73.0	72.3	74.4	4.6
Total food consumed (kg)	180	176	170	185	179	175	160	166	9.5
Food consumed per day (kg)	2.04	2.06	1.91	2.24	2.05	2.04	1.95	2.01	0.4
Liveweight gain per day (g)	826	833	800	870	834	852	889	907	170.6
Food conversion ratio	2.4	2.5	2.4	2.6	2.5	2.4	2.2	2.2	0.5

21df

Table 3.2.3: Experiment 1. Mean backfat thickness (mm) at the shoulder, P₂ and loin sites and mean carcass lengths (mm) of all pigs on each dietary treatment.

Dietary Treatments	F	FCu	FM	FMCu	S	SCu	SM	SMCu	LSD
Carcass measurement									
Shoulder - maximum fat thickness (mm)	31.8	35.8	35.8	35.0	27.3	29.3	33.0	31.8	8.3
Loin - maximum fat thickness (mm)	14.5	16.3	17.5	16.3	11.0	12.8	13.8	14.8	8.3
P ₂ fat thickness (mm)	17.0	16.5	19.3	16.8	18.5	14.3	16.6	14.0	5.8
Carcass length (mm)	798	784	769	797	812	809	812	817	52.0

21df

Measurements were taken on the day after slaughter.

(Table 3.2.1). This was followed by a steady rate of increase in the depth of the fat layer which was similar for pigs on all eight dietary treatments.

There were statistically significant effects of protein source on the loin and shoulder backfat measurements ($p < 0.05$ and $p < 0.01$ respectively). However a comparison between pigs on the F and S treatments respectively was not significant ($p > 0.05$). The P_2 backfat thickness appeared to be decreased by the presence of copper in the diet ($p < 0.05$ overall) although this difference was not evident with individual treatments comparisons.

Mean carcass length was longer in the pigs receiving the soyabean based treatments ($p < 0.001$). However the mean carcass lengths of the pigs given the F and S diets respectively were not significantly different from each other ($p > 0.05$). There were no significant effects of sex on any of the carcass measurements ($p > 0.05$).

3.2.3 Copper analyses

The faecal copper concentrations are shown in Table 3.2.4. It was anticipated that the phytic acid present in the soyabean meal would chelate with the dietary copper in the gastrointestinal lumen, effectively reducing the intake of copper compared with the pigs receiving fishmeal, as the source of protein (Section 1.6.1). The results here show no differences at the 5% level between the faecal copper concentrations and so the pigs on the FCu and SCu treatments would appear to be comparable with respect to the level of dietary copper absorbed. This would be consistent with the lack of differences in growth rates between pigs on analogous fishmeal and soyabean based treatments.

Although there seemed to be no difference in the faecal

Table 3.2.4: Experiment 1.

Effect of dietary protein source on the faecal copper concentrations (mg/g dry weight) of pigs receiving diets containing 200 mg Cu/kg feed.

Faecal copper concentration (mg/g dry weight)	FCu	SCu	SED
Week 4	1.022	0.999	0.04
Week 8	1.091	1.129	0.03

4df

Results given are the means of three determinations from pooled samples of faeces from pigs on each of the two treatments.

Table 3.2.5: Experiment 1. Mean liver weights (g) and liver copper concentrations (ppm in dry matter) for all pigs on each dietary treatment.

	Dietary Treatment							
	F	FCu	FM	FMCu	S	SCu	SM	SMCu
Liverweight (g)	1672	1623	1630	1703	1583	1597	1625	1750
Liver copper concentration (ppm in dry matter)	24	199	29	125	27	103	25	116

21df

excretion of copper, the effect of dietary copper on the liver was more pronounced. The mean liver weights and liver copper concentrations are shown in Table 3.2.5. There were no treatment or sex differences in liver weight ($p>0.05$) but the copper concentration varied markedly between the livers from pigs fed the low- and high-copper diets. The liver copper concentrations from the pigs on the copper containing diets were significantly higher than those on the treatments lacking copper ($p<0.001$ overall and $p<0.01$ for the F vs FCu comparison only).

3.2.4 Composition, structure and physical properties of the adipose tissue triacylglycerols

3.2.4.1 Comparison between different adipose tissue sites

The various lipid analyses were performed on adipose tissue from the perirenal fat and from both the inner and outer layers of backfat. Within each layer of backfat, samples were taken from the shoulder, middle and loin regions. This was done as preliminary experiments, not reported here, indicated differences in Δ^9 desaturase activity at various sites within each layer of backfat. However the fatty acid compositions at the shoulder, middle and loin regions within each layer were found not to be significantly different for each pig ($p>0.05$). These results were pooled but the mean values for these sites within each layer are listed in Appendix 3. In addition, there was no significant effect of sex on adipose tissue triacylglycerol composition ($p>0.05$).

Table 3.2.6: Experiment 1. Mean fatty acid compositions (weight per cent) of triacylglycerols isolated from the inner and outer backfat and perirenal fat of all pigs on each dietary treatment.

Dietary Treatment		F	FCu	FM	FMCu	S	SCu	SM	SMCu	LSD
Fatty acid (%)										21df
16:0	Inner	27.4	24.9	22.0	20.9	25.7	25.2	20.0	19.2	4.2
	Outer	25.5	23.3	22.0	19.6	24.4	26.2	19.5	18.0	3.4
	Perirenal	28.1	26.3	24.1	21.9	28.7	27.9	23.7	22.9	4.0
	LSD(a,b)	1.8, 2.8	1.4, 3.2	1.2, 2.5	1.4, 2.4	1.5, 4.5	1.2, 2.9	1.2, 4.7	1.5, 5.2	
16:1	Inner	3.1	3.5	1.8	2.0	1.9	2.5	1.4	1.0	1.4
	Outer	3.3	4.0	1.8	2.1	2.0	2.7	1.6	1.2	1.3
	Perirenal	2.5	2.2	1.0	1.4	2.0	2.4	1.1	1.1	0.9
	LSD(a,b)	0.5, 0.5	0.7, 1.1	0.6, 0.6	0.4, 0.7	0.4, 0.5	0.6, 0.4	0.4, 0.6	0.5, 0.5	
18:0	Inner	17.0	15.8	15.7	12.5	18.6	16.7	13.4	14.7	3.2
	Outer	15.5	13.4	13.1	10.5	16.1	13.5	12.0	12.2	2.4
	Perirenal	21.2	20.0	18.7	16.4	21.7	20.9	16.9	17.3	2.5
	LSD(a,b)	1.2, 4.8	2.3, 4.7	2.7, 3.9	2.3, 4.1	2.4, 3.5	3.0, 5.3	1.7, 5.2	2.6, 4.3	
18:1	Inner	43.7	47.7	36.2	39.8	43.0	44.5	37.0	37.5	1.4
	Outer	45.8	49.8	37.7	42.3	44.4	43.6	37.7	38.0	4.0
	Perirenal	41.0	43.4	31.5	36.2	38.5	38.8	30.5	32.3	2.5
	LSD(a,b)	2.2, 2.9	1.4, 5.2	1.7, 6.3	2.8, 6.3	1.6, 5.0	1.4, 5.7	1.2, 7.3	1.1, 6.2	
18:2	Inner	7.2	6.6	21.0	21.6	9.3	9.5	24.6	24.5	4.2
	Outer	8.4	7.9	21.9	22.1	11.3	12.3	25.9	27.2	2.7
	Perirenal	5.7	6.7	20.6	20.4	7.6	8.3	24.2	22.7	1.7
	LSD(a,b)	1.3, 3.1	1.4, 1.4	1.2, 1.8	0.8, 2.2	2.3, 3.8	3.1, 4.2	1.4, 1.7	2.8, 4.8	
18:3	Inner	0.5	0.5	2.4	2.3	0.5	0.7	2.7	2.2	0.8
	Outer	0.4	0.6	2.3	2.4	0.7	0.7	2.3	2.8	1.0
	Perirenal	0.5	0.4	3.0	2.6	0.5	0.8	3.1	2.7	1.2
	LSD(a,b)	0.3, 0.3	0.4, 0.4	0.4, 1.0	0.5, 0.5	0.4, 0.5	0.3, 0.4	0.6, 0.9	0.4, 0.6	

Results for the inner and outer backfat layers of backfat are the means of three sample sites. Appendix 3 lists the mean compositions of triacylglycerols from each of these three sites.

LSD values between sites are with 22df between backfat layers (a) and 14df between backfat layers and perirenal fat. (b)

The fatty acid compositions of the extracted triacylglycerols are given in Table 3.2.6. Differences in composition between inner and outer backfat layers and perirenal fat for pigs on individual treatments were significant in a number of cases.

With pigs on each treatment the differences between sites followed the same general trends. Saturated fatty acid content was highest in the perirenal fat and lowest in the outer backfat; the concentration of palmitic acid was significantly higher in the inner layer compared with the outer layer in the pigs on both the F and FCu treatments ($p < 0.05$). Stearic acid concentration was significantly greater in the inner backfat layer relative to the outer in pigs on all treatments which did not contain maize oil ($p < 0.001$). Also the perirenal fat had significantly greater concentrations of stearic acid than did the outer backfat of pigs on all treatments except SM ($p < 0.001$).

This variation between sites in the concentration of saturated fatty acids corresponds with the results of the melting point determinations (Table 3.2.7). Thus, within pigs on each treatment the perirenal fat had the highest slip point and the outer backfat had the lowest. These differences were, on the whole, very highly significant ($p < 0.001$). The complete melting point results for the three sites along the length of the pig back are given in Appendix 4.

The concentration of monounsaturated acids followed an opposite trend to that of the saturated fatty acids. The

Table 3.2.7: Experiment 1. Mean slip point (°C) of triacylglycerols isolated from the inner and outer backfat and perirenal fat of all pigs on each of the dietary treatments.

Dietary Treatment	F	FCu	FM	FCu	S	SCu	SM	SMCu	LSD
<u>Sample Site</u>									21df
Inner backfat	42.3	39.1	38.7	35.4	44.5	42.6	38.7	37.8	1.9
Outer backfat	40.1	37.7	35.6	31.7	42.5	40.1	35.3	33.7	2.2
Perirenal fat	45.6	44.8	43.2	40.6	46.8	46.8	42.7	42.3	1.1
LSD a	1.8	1.7	2.0	2.3	1.8	1.9	1.7	3.2	
b	3.2	5.6	4.4	3.8	2.3	3.5	3.9	4.4	

Results given are the means of the slip point determination taken from all four pigs on each treatment. The backfat results represent the means of all three sites sampled from each backfat layer. Appendix 4 lists the mean slip points at these three sites for each backfat layer, for all eight dietary treatments.

LSD values between sites are with 22df between backfat layers (a) and 14df between backfat layers and perirenal fat. (b)

perirenal fat contained lower concentrations of palmitoleic acid than did the backfat from pigs on the F, FCu and FM treatments ($p < 0.05$). Similarly, oleic acid concentration in the perirenal fat was significantly lower than in the outer backfat of pigs given the four treatments lacking maize oil ($p < 0.001$).

There were no statistically significant inter-site differences in linoleic acid content between pigs on any of the eight experimental treatments ($p > 0.05$). However the concentration of linoleic acid in the outer backfat triacylglycerols was slightly higher than that in the perirenal triacylglycerols.

The differences in linolenic acid content between the three sites were small and no general trend was evident. However, with pigs on the SMCu treatment the outer layer of backfat had a significantly greater concentration of linolenic acid than did the inner layer ($p < 0.05$).

Thus, in general, with pigs on the same dietary treatment, triacylglycerols from each adipose tissue site displayed distinct fatty acid profiles. The perirenal fat had lower concentrations of the unsaturated fatty acids compared with the two backfat layers which also differed from each other; the inner layer tending to be more saturated than the outer. These differences in fatty acid composition were reflected in the melting points of the samples which corresponded with the degree of saturation.

Complete stereospecific analysis was not carried out, but as an alternative the pancreatic lipase hydrolysis technique was

Table 3.2.8: Experiment 1.

Mean fatty acid compositions (weight percent) at the 2 and 1 + 3 positions of triacylglycerols isolated from the inner and outer backfat of all pigs on each of the dietary treatments.

Position of distribution of fatty acids (wt.%)			Dietary Treatment								LSD 21df
			F	FCu	FM	FMCu	S	SCu	SM	SMCu	
16:0	Inner	2	48.7	46.8	43.6	47.8	66.4	72.1	60.8	60.8	30.0
		1 + 3	16.5	14.4	10.9	8.4	6.1	1.6	-0.7	-1.9	
	Outer	2	47.4	44.4	51.3	51.4	69.2	73.0	66.1	61.9	29.5
		1 + 3	16.5	12.9	6.0	4.8	2.0	3.7	-3.2	-4.3	
16:1	Inner	2	3.0	2.8	2.6	2.9	4.4	6.3	3.6	4.9	2.8
		1 + 3	3.3	4.0	1.5	1.6	0.8	0.6	0.8	-1.1	
	Outer	2	3.6	3.4	2.6	2.7	6.5	5.5	4.3	4.9	2.5
		1 + 3	3.2	4.4	1.3	2.1	0.1	2.0	0.4	-0.6	
18:0	Inner	2	11.0	15.3	16.2	13.1	7.4	5.1	5.3	5.6	3.4
		1 + 3	20.3	16.8	15.2	13.9	24.8	22.7	16.4	19.7	
	Outer	2	16.5	16.6	11.5	10.8	5.3	5.2	5.5	6.2	7.3
		1 + 3	14.3	12.0	12.7	9.8	19.7	15.5	13.8	15.0	
18:1	Inner	2	29.4	25.8	25.9	24.4	16.1	13.3	16.8	15.1	12.7
		1 + 3	50.1	55.7	39.7	46.9	53.7	56.8	45.4	47.5	
	Outer	2	24.5	29.1	21.1	23.5	13.6	11.5	13.5	15.7	7.8
		1 + 3	55.3	58.8	46.6	51.4	60.5	55.9	50.7	51.4	
18:2	Inner	2	7.6	9.5	11.2	12.8	5.8	3.2	13.4	13.6	3.4
		1 + 3	6.9	4.7	25.9	26.1	11.5	13.1	31.4	31.0	
	Outer	2	9.2	7.4	14.5	12.3	5.3	4.8	10.7	11.4	3.5
		1 + 3	8.1	8.8	25.1	26.3	15.5	18.2	31.7	32.9	

The composition of fatty acids at position 2 of the triacylglycerols was determined by the pancreatic lipase hydrolysis technique and the composition at positions 1 + 3 was determined by calculation.

used to determine the distribution of fatty acids between position 2 and positions 1+3 of the adipose tissue triacylglycerols. The method was performed on the inner and outer backfat triacylglycerols only, and the results are shown in Table 3.2.8. In general, the results indicate similar patterns of fatty acid distribution between the three sites with palmitic acid mainly esterified to position 2 and oleic acid the major component at the 1+3 positions. A few statistical differences ($p < 0.05$) were noted at the 2-position; between the stearic and oleic acid concentrations of the triacylglycerols from the two backfat layers of pigs on the F treatment and in the linoleic acid content of the triacylglycerols from the backfat of pigs on the SCu and SM treatments.

3.2.4.2 Effect of the source of dietary protein

The source of dietary protein had a statistically significant effect on the concentration of linoleic acid at all three adipose tissue sites ($p < 0.01$, $p < 0.001$ and $p < 0.01$ for inner and outer backfat and perirenal fat respectively). At each of the three sites the presence of soyabean meal in the diet was associated with a higher concentration of linoleic acid in the adipose tissue triacylglycerols. However, comparisons of the treatment means between pigs receiving the F and S diets respectively were only significant for the outer backfat and perirenal fat ($p < 0.05$).

In contrast to linoleic acid, triacylglycerol oleic acid concentrations were lower overall in pigs fed the soyabean meal

compared with the fishmeal diets ($p < 0.001$ for outer backfat and perirenal sites). Differences between the F and S treatment means were only statistically significant for the perirenal adipose tissue ($p < 0.05$).

There was a statistically significant difference between the palmitoleic acid concentrations of the outer backfat triacylglycerols of pigs receiving diets based on fishmeal and soyabean meal respectively ($p < 0.001$ overall, $p < 0.05$ for the F vs S comparison), with the pigs fed on soyabean meal tending to have the lower values.

The concentrations of the other fatty acids measured in the triacylglycerol samples were not statistically significantly affected by the source of dietary protein.

Although the nature of the protein supplement had a small effect on the overall composition of the backfat triacylglycerols, the effect on the positional distribution of the fatty acids was much more pronounced (Table 3.2.8). Triacylglycerols from the pigs receiving the soyabean treatments had much higher levels of palmitic acid at position 2 than did those from pigs receiving the analogous fishmeal treatments ($p < 0.001$ overall and $p < 0.05$ for F vs S comparisons for both backfat layers), but much lower concentrations of this fatty acid at the 1+3 positions.

Palmitoleic acid concentration was higher at position 2 of the triacylglycerols in the inner and outer backfat of pigs receiving the soyabean diets ($p < 0.05$ overall and for the F vs S comparisons) when compared with the pigs given the fishmeal

diets. In addition, the concentration of palmitic acid at position 2 was much greater than at position 1+3 in the triacylglycerols of the pigs receiving the soyabean based treatments. This difference in distribution pattern was very much smaller in the adipose tissue triacylglycerols of the pigs given the fishmeal diets.

Stearic acid concentration was similar between position 2 and positions 1+3 of the triacylglycerols from the pigs on the fishmeal treatments. However, with the pigs on the soyabean treatments the concentration at the 1+3 positions was much greater than at position 2. In addition, the triacylglycerols from the pigs given the fishmeal based treatments had much higher concentrations of stearic acid at position 2 than did those given the soyabean treatments (inner backfat $p < 0.001$ overall and for comparison of F vs S treatment means) while the opposite was true for positions 1+3.

With all of the experimental animals the concentration of oleic acid in positions 1+3 was greater than in position 2 but this differential distribution was more pronounced in the pigs receiving the soyabean-containing diets. In addition, the triacylglycerol oleic acid concentration was higher in both backfat layers for the pigs receiving fishmeal compared with soyabean meal based diets ($p < 0.001$ overall and $p < 0.05$ for F vs S comparison in both backfat layers).

The positional distribution of linoleic acid in the backfat triacylglycerols depended on the nature of the diet. When maize oil was present in the diet the concentration of linoleic acid

was two or three times greater at positions 1+3 than at position 2. With the pigs on the F and FCu treatments there were no consistent differences in the distribution of this fatty acid between position 2 and positions 1+3 of the adipose tissue triacylglycerols. However with the pigs given the S and SCu treatments the distribution was similar to that of pigs given the treatments supplemented with maize oil i.e. the linoleic acid concentration was approximately three times greater at positions 1+3 than at position 2.

In addition, the concentration of linoleic acid in the triacylglycerols was significantly lower in the outer backfat of pigs fed soyabean based diets compared with those fed fishmeal diets ($p < 0.01$ overall and $p < 0.05$ for comparison F and S treatment means).

The source of dietary protein also had a statistically significant effect on the slip point of the adipose tissue triacylglycerols (Table 3.2.7). At all three adipose tissue sites the slip point was greater with the triacylglycerols from the pigs fed the diets based on soyabean meal ($p < 0.05$ overall and for the F versus S treatment mean comparison at all three adipose tissue sites).

3.2.4.3 Effect of supplementing the diets with maize oil

There were marked differences in the fatty acid compositions of the adipose tissue triacylglycerols owing to supplementation of the diets with maize oil (Table 3.2.6). For all six fatty

acids assayed at each of the three adipose tissue sites there was a very highly statistically significant effect of maize oil in the diet ($p < 0.001$ overall at all three sites). Further analysis showed that mean palmitic, palmitoleic, stearic and oleic acid concentrations in the adipose tissue triacylglycerols were, in general, statistically significantly different between pigs given the F and the FM and between the S and the SM treatments ($p < 0.05$). Similarly mean triacylglycerol linoleic acid and linolenic acid concentrations were significantly different at all three adipose tissue sites between pigs given the F and FM and the S and SM diets ($p < 0.001$ for linoleic acid and $p < 0.01$ for the linolenic acid comparisons).

At each of the three sites the effects of maize oil supplementation of the diet were consistent. The levels of linoleic acid in the triacylglycerols from the pigs on the maize oil supplemented treatments were approximately three times those of the pigs given the basal treatments and this was offset largely by a decrease in oleic acid concentration and smaller decreases in the saturated fatty acid content. Linolenic acid concentration was also increased in the adipose tissue triacylglycerols of pigs receiving the maize oil-supplemented treatments.

The effect of dietary maize oil on the positional distribution of fatty acids in the adipose tissue triacylglycerols was less marked. The only statistically significant differences were noted in the concentration of linoleic acid at position 2 in the triacylglycerols from both backfat layers ($p < 0.01$ and $p < 0.001$ for inner and outer backfat layers respectively both overall and

with the F vs FM and S vs SM comparisons).

The backfat triacylglycerols of the pigs on the maize oil supplemented treatments had higher levels of linoleic acid at both position 2 and positions 1+3 of the triacylglycerols owing to the increased amount of this acid in the overall fatty acid composition. The increase in concentration at the 1+3 positions is greater than twice that at the 2-position, suggesting that the linoleic acid is preferentially esterified to one or both of the 1 and 3 sites.

Dietary maize oil decreased the slip point of the triacylglycerols compared with those from the pigs given the basal treatments (Table 3.2.7) ($p < 0.001$ overall for all three adipose tissue sites and $p < 0.05$ for the F vs FM and S vs SM comparisons at each site). These findings are in keeping with the marked effects of dietary maize oil supplementation on the fatty acid composition of the adipose tissue triacylglycerols.

3.2.4.4 Effect of supplementing the diets with copper

The mean fatty acid compositions of the extracted adipose tissue triacylglycerols (Table 3.2.6) show no major differences attributable to dietary copper. The concentration of stearic acid in the triacylglycerols at all three adipose tissue sites was lowered when copper was present in the diet but the differences were not always statistically significant ($p < 0.01$ and $p < 0.05$ overall for inner and outer backfat layers respectively). A comparison of treatment means between pigs

receiving the basal and copper supplemented treatments found only the S versus SCu comparison for outer backfat to be significant ($p < 0.05$).

In contrast, oleic acid concentrations in the triacylglycerols were greater in the adipose tissue of pigs receiving copper supplemented diets ($p < 0.01$ and $p < 0.05$ overall for inner and outer backfat layers respectively). When treatment means were compared between pigs receiving the basal and copper supplemented treatments, significant differences were noted between the F and FCu treatments in both backfat layers ($p < 0.05$) and between the S and SCu treatments for the inner backfat triacylglycerols only ($p < 0.05$).

There was a statistically significant interaction between copper and protein source for the outer backfat triacylglycerol concentration of linoleic acid ($p < 0.01$ overall and $p < 0.05$ for the FCu versus SCU comparison). However as the concentrations of the various fatty acids in the triacylglycerols are not independent of each other and this interaction was only statistically significant in this instance then this finding is probably of limited importance.

There were no other significant effects of copper for any of the other fatty acids measured ($p > 0.05$). In addition the concentrations of the various fatty acids at position 2 of the triacylglycerols were not significantly affected by the presence of copper in the diet ($p > 0.05$).

The melting point temperatures of the adipose tissue triacylglycerols are given in Table 3.2.7. The presence of

copper in the diet was associated with a statistically significant decrease in triacylglycerol melting point in both inner and outer layers of backfat ($p < 0.01$ overall and $p < 0.05$ for F vs FCu and S vs SCu comparisons in each backfat layer). There was an overall effect of copper on slip points from the perirenal triacylglycerols ($p < 0.05$) but individual treatment comparisons were not statistically significant.

3.2.5 Random interesterification of triacylglycerol samples

Samples of triacylglycerols from the inner backfat layer of pigs from each of the eight experimental treatments were randomly interesterified as described in Section 2.2.11. A comparison of the slip points before and after the esterification procedure is shown in Table 3.2.9. With pigs from all treatments the slip point was lower in the interesterified triacylglycerols compared with those from the original sample; this is consistent with the observation that the fatty acids present in the triacylglycerols deposited in pig adipose tissues are esterified in a non-random manner.

There were overall significant effects of copper, protein source and maize oil on the randomly interesterified triacylglycerol melting points ($p < 0.05$, 0.05 and 0.01 respectively). Comparisons between treatments for the effects of copper were not significant ($p > 0.05$). The F versus S treatment comparison was statistically significant ($p < 0.05$) and so were the comparisons for the effects of maize oil ($p < 0.01$ for F vs FM and

Table 3.2.9: Experiment 1.

Mean slip point (°C) of pooled samples of inner backfat triacylglycerols from all pigs on each of the dietary treatments both before and after random in interesterification.

	F	FCu	FM	FMCu	S	SCu	SM	SMCu	LSD
Before	42.3	39.1	38.7	35.4	44.5	42.6	38.7	37.8	3.6
After	37.6	36.6	32.5	29.4	39.6	40.1	34.4	34.0	4.1

4df

Values given are the mean of three determinations using pooled samples of triacylglycerols from all pigs on each treatment.

S vs SM comparisons).

The results to some extent reflect the fatty acid composition of the triacylglycerols (Table 3.2.6). Lipids from pigs given the soyabean treatments were more saturated than those from their counterparts given the fishmeal treatments and so even after interesterification, the triacylglycerols from the adipose tissue of pigs given the soyabean treatments have the higher melting points.

3.2.6 Separation and analysis of triacylglycerol species

The separation of the triacylglycerol samples into classes with the same number of double bonds in each molecule, was carried out as described in Section 2.2.10. These bands were then analysed for their fatty acid compositions; the results of these analyses are discussed in Appendix 5.

3.2.7 Summary

The results of the growth and carcass measurements indicate no major differences between the experimental treatments. The results of the copper analyses suggest that there is no difference in either copper uptake or excretion between animals receiving the same level of copper in diets based on different protein sources. However differences were noted between pigs on the FCu and SCu treatments with regard to the fatty acid compositions of the adipose tissue triacylglycerols.

Comparison of triacylglycerols from the three adipose tissue

sites showed that the perirenal fat was the most saturated of the three sites and the outer backfat the least saturated. These differences in saturated fatty acid content were paralleled by the slip points. The positional distribution of fatty acids in the triacylglycerols was similar at all three adipose tissues sites.

The source of dietary protein had little effect on gross fatty acid composition. However the monounsaturated acid content was higher in the triacylglycerols from the pigs given the fishmeal-based treatments and linoleic acid concentration was increased in adipose tissue triacylglycerols from pigs receiving the soyabean treatments. The effect of dietary protein source on the positional distribution of the fatty acids in the triacylglycerols was much more pronounced.

Dietary maize oil greatly increased the concentration of linoleic and linolenic acid in the adipose tissue triacylglycerols at the expense of all the other fatty acids, particularly oleic acid and the saturated fatty acids. There was a concomitant decrease in triacylglycerol melting point. The maize oil supplement also affected the positional distribution of fatty acids in the adipose tissue triacylglycerols, particularly linoleic acid.

The presence of supplementary dietary copper increased the oleic acid content of the adipose tissue triacylglycerols, at the expense of stearic acid. In accordance with the changes in fatty acid composition, dietary copper lowered the slip point of the triacylglycerols.

3.3 Discussion of results of Experiment 1

3.3.1 Growth rates, food intake, feed conversion ratios and copper analyses

The use of two different types of protein caused no apparent changes in either the rates of growth or feed-related results between analogous treatments based on different sources of protein (Tables 3.2.1 and 3.2.2). Both the source and level of dietary protein are known to affect growth rates, with vegetable protein considered less effective than animal protein for increasing growth performance (Evans, 1973). In this present experiment, the diets were supplemented with either fishmeal or soyabean meal protein, but the amounts of each were adjusted to provide similar levels of amino acids, as measured by available lysine (Table 3.1.1). The quality of protein in each treatment was therefore comparable and so not likely to influence the growth rate. In addition, Kennington *et al.* (1958) reported that when pigs were given diets containing between 14 and 20% protein, the level of protein did not appear to influence the growth rates, feed consumptions or feed conversion ratios of the animals. This observation would suggest that any small differences in protein content between the fishmeal and soyabean treatments of this present experiment would not be apparent in the growth performance of the animals.

The treatments based on fishmeal contained slightly higher levels of fat than did the corresponding diets based on soyabean meal (Table 3.1.2). However this small difference in fat content had no apparent effect on the growth rates of the pigs from Experiment 1. This is in keeping with the work of Low (1985) who reported that supplementing diets with lipid while maintaining a constant amino acid intake had no effect on the rate or efficiency of amino acid use (as measured by growth rate and area of eye muscle).

The addition of 4% maize oil to the diet had no apparent effect on the

rates of growth of the animals (Table 3.2.1). This is contrary to the work of Allee *et al.* (1971) who reported that increasing the level of dietary fat from 1 to 13% increased the daily liveweight gains of the animals, with the greatest gains occurring with the pigs receiving the 4% fat diet. However, with this present experiment, a beneficial effect (but not significant at the 5% level) of maize oil on the feed conversion ratios was only noted with the pigs on the soya-based treatments. This last observation is consistent with the work of Baird *et al.* (1958) who reported that increasing the caloric density of the diets improved the feed conversion efficiency but had no significant effect on the rates of liveweight gain of the animals.

In addition, the total feed consumptions of the pigs on the maize oil treatments of this present experiment were slightly lower than those on the oil-free treatments (Table 3.2.2). This is in agreement with Abernathy *et al.* (1958) and Pond *et al.* (1960) who reported that an increase in the caloric density of the ration was accompanied by a decrease in the total amount of food consumed by the pigs. The maize oil diets of the experiment reported here were only slightly more calorific than those lacking this supplement (Table 3.1.1) and this relatively small difference in energy values may account for the lack of significant improvements in the growth rates and feed conversion efficiencies of the pigs receiving the maize oil supplemented diets. However, Noland and Scott (1960) observed that small differences in energy intake, comparable with those noted between the pigs on the maize oil supplemented and unsupplemented treatments of the experiment reported here, had no significant effects on liveweight gains.

The differences in growth rates and feed-related results between the pigs on the copper-supplemented and basal treatments did not reach the 5% level of

significance (Tables 3.2.1 and 3.2.2 respectively) although the animals receiving the copper-containing diets appeared to have slightly higher daily liveweight gains. This comparatively small response to supplemental copper was not entirely unexpected. There are many accounts of the response by pigs to supplemental dietary copper which range from the large increases in pig growth rates demonstrated by Braude (1967) to reports of no apparent response (Elliot & Amer, 1973; Bradley *et al.*, 1983) and even cases of toxicity (Mills, 1968) (Section 1.5.3.1). The various factors such as the source of protein and the feeding scale which affect the action of copper on growth parameters are discussed in Section 1.6..

Although the pigs from this present experiment received isonitrogenous diets, others have reported that the level of protein may affect the response to dietary copper. Lucas *et al.* (1962) noted that pigs fed a copper-supplemented diet retained less of the copper (as measured by liver copper levels) and grew more rapidly if they were fed a 17% protein diet compared with a 14% protein diet. The animals in this present experiment received diets containing approximately 17-18% protein and so this may, at least in part, account for the similarity of growth rates between the control and copper-supplemented treatments. In addition, the feeding scale was of a semi-*ad lib.* nature; Cooke (1981) reported that, in general, faster growing pigs respond less to growth promoters than do more slowly growing animals. Elliot and Bowland (1968) also noted that the action of dietary copper was to some extent influenced by the feeding scale, with the more restricted diets producing a greater response.

The uptake of dietary copper was investigated by determining the levels of this metal in both the livers and faeces of the animals from Experiment 1. The faecal copper levels of the pigs receiving the FCu and SCu treatments were

not significantly different from each other at the 5% level (Table 3.2.4). Differences in liver copper concentrations were only significant between pigs on the copper-supplemented and -unsupplemented treatments with the former having liver copper levels approximately four times those of the animals receiving the basal treatments (Table 3.2.5). These liver copper results are in agreement with studies by Bradley *et al.* (1983) and Castell and Bowland (1968). Also, in this present experiment there was no interaction between liver copper and protein source and this accords with the work of Suttle and Mills (1966) and Lepen and Adrian (1983) who also found no effect of soyabean meal on growth rates.

Soyabean meal contains about 1.7% phytic acid and this high level of phytate, compared with fishmeal, was expected to chelate with the dietary copper in the gastro-intestinal lumen (O'Dell *et al.*, 1972; Van Campen, 1971). This would effectively reduce its uptake by the animal and so reduce any growth promoting action associated with the dietary copper. However no differences were observed in this present experiment between the daily liveweight gains or feed conversion ratios of the animals on the FCu and SCu treatments. Barber *et al.* (1981) also reported that the feeding of pigs with copper-supplemented diets (250 mgCu/kg diet) based on either white fish meal or soyabean meal protein resulted in similar carcass and overall performance results.

The faecal copper measurements of pooled samples of freeze-dried faeces were not significantly different at the 5% level between the pigs on the fish-meal and soyabean-based copper treatments of this present experiment. However the liver copper levels from the individual pigs exhibited a considerable disparity within each of the copper-supplemented treatments. Although the excretion of copper may be similar between animals on the FCu and SCu treatments, it must be noted that the faecal copper levels within treatments may be as

variable as the liver copper concentrations but that these differences were concealed by the pooling of the samples.

The copper levels were consistently higher in the livers of the animals receiving the copper-supplemented diets compared with the basal diets but were not significantly different, at the 5% level, between pigs receiving the same level of dietary copper. Thus the phytic acid present in the soyabean meal did not appear to bind with the copper in the gut contents and so diminish its uptake. However, there is evidence that the pig can digest phytic acid, the intestinal phytase responsible being stimulated by vitamin D. Phytases present in the plant material itself may also break down the phytic acid, but this occurs in the stomach where the pH is more suited to the plant enzyme (Taylor, 1980). Experiments on rats have shown that phytate digestibility is increased by feeding diets low in phosphorus (Nahopetian & Young, 1980). In addition, the binding of trace elements by phytic acid *in vitro* exhibits a synergistic effect so that if two or more cations are present then increasing amounts of phytate-metal complexes are precipitated (Davies & Olpin, 1979). Thus there appears to be a complex relationship between the dietary provision of minerals and their actual absorption. The mineral content of the diet and the presence of enzymes within the soyabean meal may therefore be key factors affecting the degree of copper uptake by the animals of this present experiment.

There is some evidence that the growth promoting effects of supplemental dietary copper are only apparent up to about 46 kg liveweight (Elliot & Amer, 1973). In order to investigate this, the daily liveweight gains of the pigs from the present experiment were calculated up to week 6 of the trial by which time most of the animals had reached this weight. The results of the calculations are given in Table 3.3.1.

Table 3.3.1: Experiment 1. Mean daily live weight gains up to 46 kg live weight of all four pigs on each dietary treatment

	Treatment							
	F	FCu	FM	FMCu	S	SCu	SM	SMCu
Daily LWG (kg)	0.664	0.735	0.589	0.777	0.714	0.735	0.765	0.768

21 df

With the fishmeal treatments, dietary copper was associated with improved growth rates of the pigs compared with those on the analogous treatments which lacked added copper ($p \leq 0.05$). Similar differences were noted between the growth rates of the pigs on the S and SCu treatments, although the improvement did not reach the 5% level of significance. There was no apparent difference between the growth rates of the animals on the SM and SMCu treatments. Thus the presence of the soyabean supplement appeared to reduce the improvement in growth rate associated with the presence of copper in the diet. However the pigs receiving the soya-based treatments did have slightly higher daily live-weight gains than their counterparts given the analogous fishmeal-based treatments. The apparently improved growth rate owing to the addition of 200 mgCu/kg feed to the diet in this present experiment is much less than that reported by Braude (1967) and others. However these present findings are similar to those of Elliot and Amer (1973) who reported that whereas up to 69 kg liveweight there was a trend towards improved growth rates when dietary copper (250 mg Cu/kg feed) was supplied, this effect was not apparent if the copper-supplemented diet was fed to a liveweight of 92 kg.

3.3.2 Thickness of subcutaneous backfat.

The carcass fat thicknesses show that the backfat is thickest at the shoulder, thinnest at the P_2 site and of intermediate thickness at the loin region (Table 3.2.3). This is in agreement with Mersmann and Leymaster (1984) who reported similar variations in backfat thickness along the length of the pig.

In the experiment now reported, there were no significant ($p > 0.05$) between-treatment differences in the thicknesses of backfat of the animals at

the shoulder, P₂ and loin sites. This is consistent with the growth rates which were also similar between treatments (Table 3.2.1). Although the pigs on the copper-supplemented treatments exhibited slightly greater growth rates up to 46 kg liveweight, compared with those on the basal treatments, these differences were not reflected in any increases in P₂ fat thickness up to this weight (Table 3.3.1).

It has been widely reported that increased levels of dietary fat are associated with an increased thickness of the subcutaneous backfat (De Wilde, 1984; Allee *et al.*, 1971). However in the present experiment, the carcass backfat thicknesses of the animals which received the maize oil treatments were not significantly thicker than those of the pigs given the diets lacking this supplement ($p > 0.05$). The supplemented diets used in the present study contained 4% maize oil whereas those of De Wilde (1984) were supplemented with up to 10% of tallow or coconut oil and as such were much more calorific. In addition, tallow contains a high proportion of saturated fatty acids and coconut oil is rich in medium chain saturated fatty acids (Gunstone, 1986); the saturated nature of these dietary fats, together with their high rate of inclusion in the diets may account for the occurrence of significantly thicker backfat in the animals fed these particular treatments.

In contrast, the diets used by Allee *et al.* (1971) were formulated to contain between 1 and 13% maize oil and with these treatments the pigs given the 4% maize oil diet had backfat significantly thicker than that of the animals receiving the 1% maize oil treatment. However, increasing the level of dietary fat above 4% did not result in a corresponding increase in the thickness of subcutaneous backfat, but instead tended to be associated with slightly thinner backfat.

With the results of Experiment 1, reported here, there were no significant ($p>0.05$) treatment differences in carcass backfat thickness. This appears to be inconsistent with the work of Allee *et al.* (1971) but the basal diets used in the present study did not contain any maize oil whereas those of Allee *et al.* (1971) were supplemented with 1% maize oil. In addition, with the animals, diets and feeding regime employed in this present experiment, the dietary fat supplied might suppress *de novo* synthesis of fatty acids and yet also be deposited in the adipose tissue such that there is no increase in backfat thickness associated with the presence of dietary maize oil. This is in partial agreement with Allee *et al.* (1971) as the aim of their experiment was to find a level of dietary fat which would decrease the conversion of dietary carbohydrate to fat to such an extent that, despite the deposition of the dietary fat, the total body fat would be reduced. In addition, the pigs used in this present experiment may have had a lower propensity to fat deposition than those used by both Allee *et al.* (1971) and De Wilde (1984).

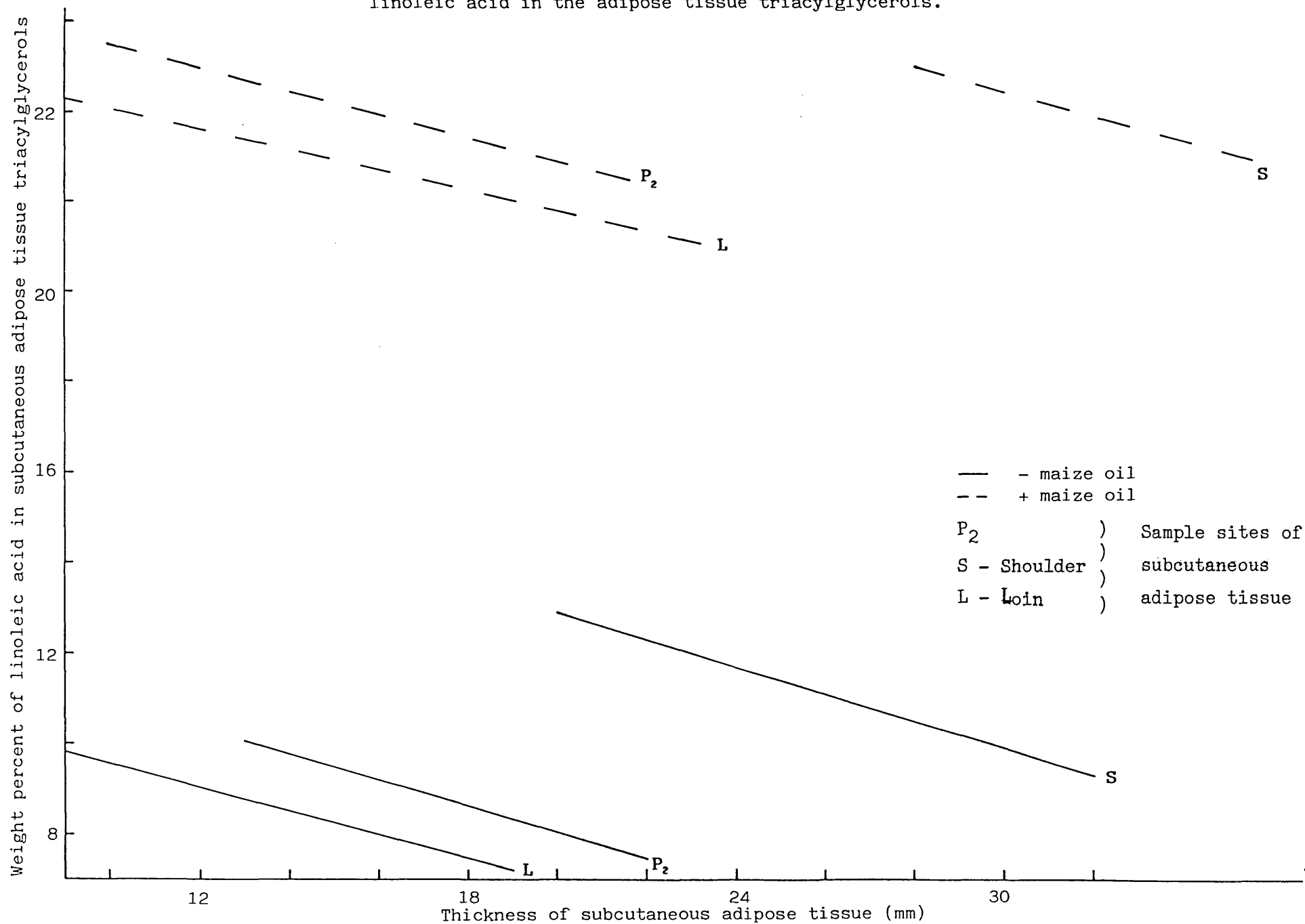
As suggested earlier, the nature of the dietary fat may also affect the thickness of the subcutaneous backfat. Wood and Enser (1982) reported that the thickness of pig backfat is inversely correlated with the concentration of linoleic acid in the adipose tissue. This relationship may arise from the effects of dietary fat on adipose tissue lipogenesis. Increased levels of dietary fat, particularly those which are rich in polyunsaturated fatty acids, decrease *de novo* synthesis of fat in the adipose tissue (Allee *et al.*, 1972; De Wilde, 1984; Romsos & Leveille, 1974). Thus supplemental dietary maize oil would be expected to decrease endogenous synthesis of fatty acids in the backfat tissue. Although the dietary fat would be deposited at the expense of newly synthesized fatty acids, the level of fat fed may inhibit *de novo* synthesis sufficiently to result in an overall reduction in backfat thickness (Allee *et al.*, 1971).

The carcass backfat thicknesses at slaughter were plotted against the mean linoleic acid concentrations in both layers of backfat for all eight dietary treatments of Experiment 1. Significant correlations ($p \leq 0.01$) were found when, for each site, the results from the maize oil-supplemented and -un-supplemented treatments were plotted separately, and these are illustrated in Figure 3.3.1. The linear regressions had gradients which were not significantly different at the 5% level either between sites or between plus and minus maize oil treatments. However the intercepts were much higher for the results obtained from the maize oil treatments owing to the higher adipose tissue concentrations of linoleic acid.

It is interesting to note that at each of the three backfat sites measured, similar relationships were obtained between the backfat thickness and the linoleic acid concentration. This suggests that the response to dietary changes is similar at all three sites, despite the differing thicknesses of backfat along the length of the pig. To some extent this also justifies the use of the P_2 fat thickness as a measure of fatness; an increase in thickness at the P_2 site is indicative of similarly increased fat thickness at the other points of fat measurement. However it must be noted that, despite its widespread use, the P_2 site is the thinnest part of the pig backfat and so measurement of the fat thickness at this site is subject to greater inaccuracy.

From Figure 3.3.1 it appears that for the maize oil-supplemented and -un-supplemented treatments respectively, higher levels of linoleic acid are associated with thinner backfat layers. This is consistent with the observed slightly thinner carcass fat thicknesses of the pigs from the soyabean compared with the fishmeal treatments (Table 3.2.3); with analogous treatments based on different protein sources the pigs fed the soya-based diets had slightly higher levels of linoleic acid in the adipose tissue triacylglycerols (Table 3.2.1).

Figure 3.3.1: Experiment 1. Relationship between the thickness of subcutaneous adipose tissue and the weight percent of linoleic acid in the adipose tissue triacylglycerols.



Wood and Enser (1982) found a negative correlation between the P₂ backfat thickness and linoleic acid concentrations when these varied between 10 and 16% of the total fatty acids. For a range of linoleic acid concentrations from 7 to 10% of the total fatty acids a negative relationship was also observed with the results from the experiment reported here although the gradient was not as steep as that observed by Wood and Enser (1982), possibly as a result of differences in the breed, age and nutrition of the animals. However when the linoleic acid content of the adipose tissue was greatly increased *i.e.* when 4% maize oil diets were fed to the pigs, then the backfat was not significantly thinner than that of the animals given the basal diets.

This lack of between-treatment differences for the fat thicknesses at the shoulder, P₂ and loin sites may be a result of variations in lipogenic activity between individual pigs. Food intakes and backfat thicknesses were similar between treatments (Tables 3.2.2. and 3.2.3) and so the presence of significant correlations between linoleic acid and backfat thickness must have arisen from variations between the pigs. With the treatments lacking maize oil the intake of linoleic acid was similar for all pigs, as the food consumptions were the same for all treatments. The negative correlation suggests that some pigs have a greater propensity to fat deposition than others, with the deposited fat being derived mainly from *de novo* fatty acid synthesis. Linoleic acid cannot be synthesized by the pig and so a higher rate of endogenous fat synthesis would result in both thicker backfat and a lower concentration of linoleic acid.

A similar situation exists with the pigs on the maize oil-supplemented treatments except that with these the overall linoleic acid content is higher, owing to the greater concentration of this fatty acid in the diet. There is some

evidence which suggests that dietary polyunsaturated fatty acids can influence backfat thickness by inhibiting both *de novo* fatty acid synthesis and Δ^9 desaturase activity (Allee *et al.*, 1971; Jeffcoat, 1979). However the two regression lines obtained from the results at each site are parallel to each other, suggesting that this inhibition does not occur to any great extent in the pigs on the maize oil-supplemented treatments of this experiment.

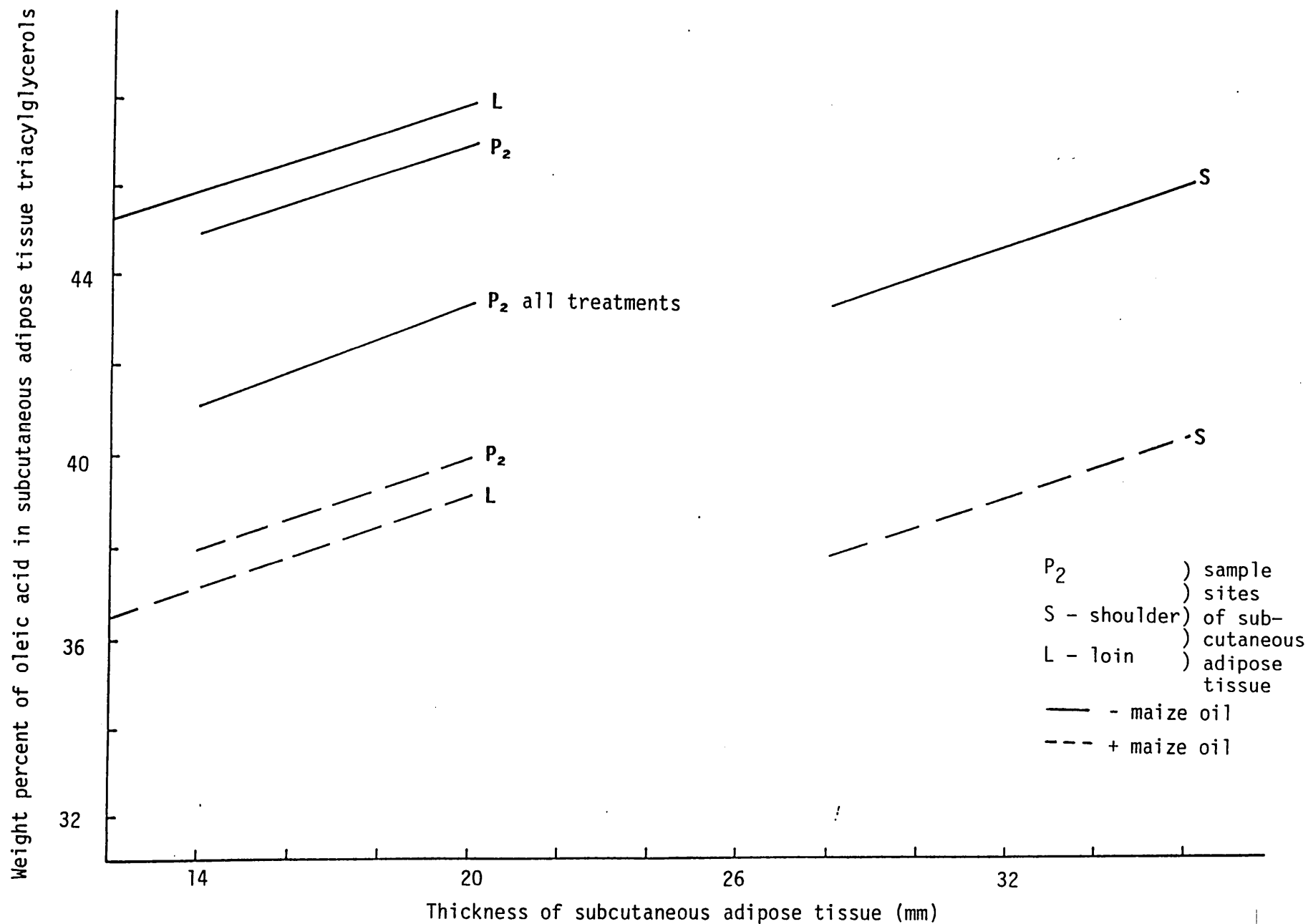
The relationships between the concentrations of the other fatty acids and the backfat thickness were also investigated. However with the exception of oleic acid, the correlations between the carcass fat thickness at the shoulder, P₂ and loin sites and fatty acid concentrations were not significant at the 5% level. With oleic acid, significant correlations were obtained at all three sites, when the results from the maize oil-supplemented and -unsupplemented treatments were plotted separately. In addition, at the P₂ site a significant correlation ($p < 0.05$) was observed with the results from all eight dietary treatments. These relationships are illustrated in Figure 3.3.2. The regression lines had positive gradients which were not significantly different from each other at the 5% level. Thus increasing amounts of oleic acid in the adipose tissue were associated with thicker layers of backfat.

Food intakes were similar between treatments and so the dietary intake of oleic acid was also similar for all animals. However some pigs have a greater propensity to fat deposition than others and with these animals the rates of *de novo* fatty acid synthesis are greater. Oleic acid is a major product of fatty acid biosynthesis and so pigs with thicker backfat have higher levels of oleic acid in the adipose tissue. This particular fatty acid may be derived from both dietary and endogenous sources and so the concentration of oleic acid is representative of the deposition of fat from both these sources.

Figure 3.3.2:

Experiment 1.

Relationships between the thickness of subcutaneous adipose tissue and the weight percent of oleic acid in the adipose tissue triacylglycerols.



Wood and Enser (1982) did not report any correlations between the concentrations of the various fatty acids, other than linoleic acid, and the P₂ backfat thickness. However, Mersmann and MacNeil (1985) reported that the thickness of pig backfat is significantly correlated with the plasma concentrations of free fatty acids and triacylglycerols as these values are representative of both fat mobilization and deposition.

3.3.3 Composition, structure and physical properties of the adipose tissue triacylglycerols

3.3.3.1 Comparison between different adipose tissue sites

Analysis of the fatty acid composition of triacylglycerols from the inner and outer layers of backfat and perirenal fat of pigs from each treatment showed differences between these three sites (Table 3.2.6). In agreement with Moore *et al.* (1969) and Santoro (1984) the triacylglycerols from the outer layer of backfat contained the highest proportion of unsaturated fatty acids whereas the perirenal fat was the most saturated.

These overall differences in fatty acid composition between sites may have arisen from the differences in lipogenic activity between the three adipose tissue sites. It has been widely reported that the inner backfat layer is more metabolically active than the outer layer as it has higher activities of the lipogenic enzymes (De Wilde, 1984; Sturm *et al.*, 1982). However, lipoprotein lipase activity is higher in the outer compared with the inner backfat tissue (Enser, 1973). This suggests that the outer layer of backfat contains a higher proportion of dietary fatty acids than the inner layer which has a greater rate of endogenous fatty acid synthesis. Thus the contribution of dietary and newly synthesized fatty acids to the composition

of the triacylglycerols is different in the two backfat layers and this may contribute to the differences in overall composition between triacylglycerols from these two sites. Similarly the perirenal fat is more metabolically active than the subcutaneous backfat and so contains greater concentrations of endogenously synthesized fatty acids (Hood & Allen, 1973). The differences in lipoprotein lipase activity between the perirenal and subcutaneous adipose tissue sites were not measured by Enser (1973), but the fatty acid compositions of the triacylglycerols would suggest that the activity of this enzyme is lower in the perirenal adipose tissue.

Another factor which may contribute to the differences in fatty acid unsaturation between the three tissue sites is the relative activities of the Δ^9 desaturase enzymes. A higher activity of this enzyme in the outer compared with the inner backfat layer would contribute to the greater degree of unsaturation of the triacylglycerols from the outer layer.

Also to be considered is the effect of temperature on the composition of the adipose tissue triacylglycerols. Lower environmental temperatures are associated with increased backfat unsaturation as this maintains the tissue lipids in a fluid state (MacGrath *et al.*, 1968). Similar differences may arise between the three adipose tissue sites sampled from pigs in this present experiment. The outer backfat layer, just under the skin, is subjected to the ambient temperature whereas the perirenal fat is maintained at the body temperature of the pig; thus the outer layer of backfat would be expected to contain higher concentrations of unsaturated fatty acids in order to maintain the tissue lipids at their optimum state of fluidity (Cook, 1985). This suggestion is in agreement with an early study of the effect of temperature on fat composition in the pig. Henriques and Hanson (1901) reported that the

backfat from a pig kept at 0°C but covered with a sheepskin coat had a lower iodine value *i.e.* was more saturated, than that from a littermate kept unprotected at the same temperature. This effect of temperature on triacylglycerol composition may be related to its effect on the Δ^9 desaturase. Lower ambient temperatures may stimulate the activity of this enzyme so that there are sufficient unsaturated fatty acids to maintain tissue fluidity. Work on *Tetrahymena* microsomes has shown a rapid increase in fatty acyl CoA desaturase activities accompanying a decrease in environmental temperature from 39.5 to 15°C (Umeki *et al.*, 1982).

The lipogenic activity at the various sites may also be influenced by the supply of blood to the adipose tissue. Mersmann and Leymaster (1984) have suggested that differences in the rates of blood flow might influence the differing rates of deposition (and depletion) of the individual backfat layers of the pig. In addition, work on dogs and rabbits has shown that the blood supply is related to the number rather than to the size of the adipocytes so that the mean blood flow is greater in tissue containing smaller adipocytes (Di Girolamo *et al.*, 1971; Di Girolamo & Esposito, 1975).

Despite the differences in composition, the positional distribution of the fatty acids in the triacylglycerols remained much the same between the inner and outer layers of backfat. This agrees with the work of Christie and Moore (1970a) who also found a consistent pattern of fatty acid distribution between adipose tissue triacylglycerols from different sites. They attributed this to similar specificities at each site of the acyltransferases involved in triacylglycerol synthesis.

In a different study, Christie and Moore (1970b) investigated the relationships between the component fatty acids from these same three adipose

tissue sites. By plotting the concentrations of each fatty acid against the oleic acid content of the triacylglycerols they found highly significant correlations for each fatty acid studied. Similar plots using the results from the experiment reported here also revealed significant relationships at all three sites for each fatty acid. The gradients and correlation coefficients of these are given in Table 3.3.2.

Although for each fatty acid the gradients at the three sites were not significantly different from each other at the 5% level, there is a slight tendency towards less steep slopes with the regressions from the perirenal sites. This suggests that changes in the oleic acid concentrations of the perirenal adipose tissue are associated with smaller changes in the concentrations of the other fatty acids when compared with the two backfat layers. In contrast, the similarity between the gradients at the inner and outer backfat sites suggests that changes in oleic acid concentration are accompanied by similar responses at each of these sites, possibly as a result of changes in the activities of the lipogenic enzymes.

Interestingly, the gradients of the palmitic and stearic acid regressions are similar to each other, suggesting a similar response to increases in the concentrations of the saturated fatty acids. The steepest gradient is that of the linoleic acid regression and so changes in the concentrations of this fatty acid are accompanied by marked changes in the concentrations of oleic acid.

The changes in triacylglycerol fatty acid composition brought about by the different dietary treatments, and which are discussed later, appear to vary with the site of the fat sample. Table 3.2.6 shows that most of the

Table 3.3.2: Experiment 1.

Gradients and correlation coefficients for the comparisons at each adipose tissue site between the concentrations (weight per cent) of various fatty acids (x) and the concentration (weight per cent) of oleic acid (y) in the adipose tissue triacylglycerols.

<u>Fatty acid</u>	<u>Site</u>	<u>Gradient</u>	<u>Correlation Coefficient</u>	
16:0	Inner backfat	0.35	0.47	
	Outer backfat	0.24	0.31	
	Perirenal fat	0.20	0.32	
16:1	Inner backfat	0.12	0.55	
	Outer backfat	0.12	0.53	
	Perirenal fat	0.08	0.51	
18:0	Inner backfat	0.35	0.17	ns
	Outer backfat	0.16	0.32	
	Perirenal fat	0.24	0.44	
18:2	Inner backfat	-1.40	-0.84	
	Outer backfat	-1.39	-0.84	
	Perirenal fat	-1.30	-0.85	
18:3	Inner backfat	-0.18	-0.80	
	Outer backfat	-0.17	-0.71	
	Perirenal fat	-0.22	-0.85	

Regressions were all significant ($p \leq 0.05$) except where indicated otherwise and were calculated using the results from all four pigs on all eight dietary treatments.

significant changes occurred in the inner backfat layer, whereas the fatty acid composition of the perirenal triacylglycerols appeared to be largely unaffected by diet. In the latter case the differences between treatments were only significant in a few cases, notably between maize oil-supplemented and -unsupplemented treatments, and especially with respect to the linoleic acid concentration of the triacylglycerols. This is consistent with the correlations between fatty acid concentration and oleic acid content of the triacylglycerol samples from each site (Table 3.3.2); the steepest gradients were noted with the linoleic acid *versus* oleic acid plots.

This differential effect of diet may also be explained by the varying rates of turnover of the different tissue samples. The inner backfat layer is more metabolically active than the outer layer and so has higher activities of the lipogenic enzymes (De Wilde, 1984; Sturm *et al.*, 1982). However, lipoprotein lipase activity is higher in the outer compared with the inner backfat layer (Enser, 1973) and so changes in fatty acid composition accompanying dietary changes are also apparent in this tissue owing to its greater uptake of dietary fatty acids.

The smaller response of the perirenal adipose tissue to dietary changes may be related to the effect of temperature. As discussed earlier the perirenal triacylglycerols are more saturated than those from the backfat tissue. These perirenal lipids are located in the pig abdomen and so are maintained at temperatures slightly higher than those of the backfat triacylglycerols which are subject to changes in environmental temperature. In addition, perirenal adipose tissue has a greater rate of fatty acid synthesis than the two backfat layers (Hood & Allen, 1973). Uptake of dietary fatty acids by the perirenal adipose tissue may be lower despite the greater rate of flow of blood to this

tissue. These factors lead to the formation of a more saturated fat compared with that deposited in the subcutaneous backfat. The reduced response of the perirenal adipose tissue to changes in the diet may be an attempt to maintain the lipids at the correct physiological degree of fluidity.

The changes in fatty acid composition, brought about by the feeding of different diets, lead to changes in the melting points of the triacylglycerol samples (Table 3.2.7). Within each treatment the perirenal triacylglycerols had melting points consistently higher than those of the two backfat samples. Similarly, within individual treatments the inner backfat triacylglycerol samples had higher melting points than did those of the outer backfat layer. Thus the melting points increase with increasing saturation of the triacylglycerol samples. The effect of changes in fatty acid composition on the triacylglycerol melting point will be discussed later in Section 3.3.3.3.

3.3.3.2 Effect of the source of dietary protein

Differences in triacylglycerol fatty acid composition associated with the type of protein source are described in Section 3.2.4.2. The results show that feeding soyabean diets led to slightly higher levels of linoleic acid in the pig adipose tissue triacylglycerols compared with the corresponding fishmeal treatments. This difference may be explained by the differences in fatty acid content between the two protein sources. However, calculation of the linoleic acid content of a given amount of each diet showed only a relative

small difference between analogous treatments based on different protein sources. To illustrate this, a daily intake of 2.5 kg of the S diet would supply 20.7 g of linoleic acid compared with 19.6 g from the F diet. This difference in dietary linoleic acid intake between fishmeal and soya-based treatments seems too small to account for the differences noted in adipose tissue linoleic acid content.

Another factor to be considered is the effect of the dietary protein source on lipid metabolism. Experiments on pigs and rats have shown that plant proteins lower plasma cholesterol levels to a greater extent than animal proteins (Forsythe *et al.*, 1980; Vahouny *et al.*, 1984). In addition, Diersen-Schade *et al.*, (1985) found that both plasma free fatty acid concentrations and subcutaneous lipoprotein lipase activity were higher in pigs fed soya-based diets compared with those receiving conventional diets or those based on beef protein. These last findings suggest that the contribution to the backfat from the serum lipoproteins may be greater in the pigs given the soyabean-based treatments. If a similar case were true for the experiment now reported *i.e.* the pigs given the soya diets exhibited higher subcutaneous lipoprotein lipase activity than those given the fishmeal diets, then this might account, in part, for the differences in adipose tissue triacylglycerol composition noted between pigs given different sources of protein. The higher linoleic acid content of adipose triacylglycerols from pigs on the soya treatments of this present experiment is consistent with higher uptake of this particular fatty acid by the adipose tissue.

The non-protein components present in the fishmeal and soyabean meal supplements may also contribute to the differences in the fatty acid compositions of the adipose tissue triacylglycerols. Allotta *et al.* (1985) reported that

the presence of various minerals, including copper and zinc, may contribute to the differences in lipid metabolism noted between rabbits fed diets based either on soyabean or casein protein sources.

The positional distribution of the fatty acids in the triacylglycerols was also affected by the nature of the protein source (Table 3.2.8). The backfat triacylglycerols from the pigs given the soyabean treatments had much higher quantities of palmitic and palmitoleic acids at position 2, and correspondingly higher concentrations of the 18 carbon fatty acids at the 1+3 positions.

In order to investigate the patterns of esterification in the triacylglycerols from pigs receiving different protein sources, the percentage of each fatty acid at position 2 was plotted against the total concentration of that fatty acid in the triacylglycerol samples (Christie & Moore, 1970b). Significant correlations ($p \leq 0.01$) were only found with palmitic acid; the results from the fishmeal and soyabean treatments had parallel regressions ($b = 0.52$ and 0.50 respectively). This suggests that the pattern of palmitic acid esterification is similar between treatments *i.e.* a change in the overall concentration of palmitic acid results in parallel changes at position 2 of the triacylglycerols of pigs for both the fishmeal and soyabean-based treatments.

The positional distribution of the fatty acids is determined by the specificities of the acyltransferases involved in triacylglycerol synthesis (Section 1.3.1). Substrate availability may be affected by the differences in the overall fatty acid composition of adipose tissue from pigs given different sources of protein, and this may lead to differences in the

fatty acid esterification patterns of the triacylglycerols. However, it does seem unlikely that the comparatively small differences between the fatty acid composition and content of lipids from the two protein sources have a greater effect on the positional distribution of the fatty acids in the triacylglycerols than 4% dietary maize oil.

In addition, the positional distributions of the fatty acids in the soyabean meal and fishmeal triacylglycerols were also different from each other. Soyabean oil contains approximately 70% of linoleic acid at position 2 of the triacylglycerols whereas fish oils are much less asymmetric and have mainly polyunsaturated fatty acids esterified to the 2-position of the triacylglycerols (Kuksis, 1978). These differences may have a bearing on the positional distribution patterns of the fatty acids found in the adipose tissue triacylglycerols of the pigs fed diets based on the different protein sources.

As mentioned earlier, the nature of the protein source can affect various aspects of lipid metabolism and this may partly account for the observed differences in fatty acid positional distribution noted in triacylglycerols from pigs receiving different sources of protein.

The stereospecific distribution of fatty acids in the triacylglycerols arises during the biosynthesis of phosphatidic acid from glycerol-3-phosphate (Elovson *et al.*, 1969; Yamashita *et al.*, 1981) (Section 1.2.3). In the rat the two enzymes involved, glycerol phosphate acyltransferase and monoacylglycerol acyltransferase, are located in both the microsomal and mitochondrial fractions of the adipocytes. The acyltransferases at each site are different enzymes and display different substrate specificities and k_m values (Brindley,

1985b).

Thus the relative contributions of the mitochondrial and microsomal enzymes to phosphatidate synthesis may be affected by the supply of fatty acids to the tissue. Under conditions of relatively low substrate availability the enzyme with the lower k_m will be favoured and if this has a different acyl-CoA specificity from its counterpart then a different range of triacylglycerol species will result. Similarly the synthesis of fatty acyl-CoA esters is also dependent upon fatty acid availability.

Different sources of protein are known to affect the metabolism of lipids and hence the supply of fatty acids to the adipose tissue (Forsythe *et al.*, 1980; Vahouny *et al.*, 1984). Thus, although the acyltransferases of the pig are different from those of the rat, a similar system may operate and might account, to some extent, for the differences in triacylglycerol species isolated from the backfat of pigs fed diets containing different sources of protein.

The mechanism by which the protein supplement can affect lipid metabolism is unclear. There are several reports that link the mode of action to the lysine:arginine ratio and to the availability of various minerals (Allotta *et al.*, 1985; Vahouny *et al.*, 1984). However there may be other non-protein components which are also responsible, and their action may be different from that suggested.

Differences between dietary protein sources in the present experiment also affected the melting points of the pig adipose tissue triacylglycerols (Table 3.2.7). These were slightly higher in the samples from pigs given the

soyabean compared with the fishmeal treatments despite the higher concentration of linoleic acid in the triacylglycerols from pigs on the soyabean treatments. This is contrary to expectation as an increase in unsaturation of the component fatty acids is usually associated with a decrease in the melting point of the triacylglycerols (Sections 1.2.1 and 3.3.3.3). However the presence of small amounts of highly unsaturated acids may affect the lipid characteristics of the pigs on the fishmeal-based treatments (Banks & Hilditch, 1932). These polyunsaturated acids have very low melting points and may play a part in lowering the slip point of lipids although this effect is likely to be small considering the quantities of these fatty acids involved. Both in this present experiment and in that of Banks and Hilditch (1932) there was virtually none of these polyunsaturated fatty acids in the adipose tissue triacylglycerols.

Another possible cause of this difference in the melting point results is the positional distribution of the fatty acids in the triacylglycerols. To determine how this affects the slip point, the concentrations of the various fatty acids at each position in the triacylglycerols were plotted against the slip point. At position 2 only linoleic acid concentration was found to correlate significantly with the slip point (Table 3.3.3). This may be because changes in linoleic acid concentration have a greater effect on the triacylglycerol melting point than do the other fatty acids (Section 1.2.1). The concentration of linoleic acid at the outer 1+3 positions of the triacylglycerols was also correlated with the slip point although the gradient was not as steep. In addition a significant positive correlation was noted between stearic acid concentration at the 1+3 positions and the triacylglycerol melting point (Table 3.3.3).

Table 3.3.3: Experiment 1.

Relationship between the positional distribution of stearic and linoleic acids (weight percent x) and the melting point ($^{\circ}\text{C}$, y) of triacylglycerols isolated from the inner and outer backfat of all pigs on all eight dietary treatments.

Fatty acid (x)	Triacylglycerol position	Linear regression	Significance
18:0	2	-	ns
	1 + 3	$y = 0.69x + 26.81$	$p \leq 0.01$
18:2	2	$y = -0.87x + 46.35$	$p \leq 0.01$
	1 + 3	$y = -0.24x + 42.92$	$p \leq 0.01$

These correlations may explain why the backfat triacylglycerols from the pigs on the soyabean treatments have higher slip points than those on the corresponding fishmeal treatments despite the lower linoleic acid content of the latter. Triacylglycerols from the pigs on the soyabean treatments had lower concentrations of linoleic acid at position 2 and higher concentrations of stearic acid at positions 1+3 than those from the fishmeal treatments. From the correlations given in Table 3.3.3 these differences both result in higher slip points. The linoleic acid concentration at positions 1+3 was higher in the triacylglycerols from the pigs on the soyabean treatments and this should lower the melting point. However the gradient of this regression is less steep than that at the 2-position, suggesting a smaller influence of linoleic acid at the 1+3 positions on the melting point. The reason why linoleic acid has differing effects on the melting point depending on its position in the triacylglycerols may be related to the effects of the two double bonds on the conformation of the acyl chain and their influence on packing arrangements (Section 1.2.1).

The pancreatic lipase hydrolysis technique determines the fatty acid concentration at the combined 1+3 positions of the triacylglycerol molecules, but does not distinguish between them. The presence of significant relationships for both stearic and linoleic acid concentrations at the 1+3 positions of the triacylglycerols with the slip point can be explained in several ways.

These two fatty acids may be predominantly esterified to one or other of the 1 and 3 positions of the triacylglycerols, or equally to both. In either case an increase in, for example, linoleic acid concentration would affect the melting point in a linear fashion. Christie and Moore (1970a) found that in pig backfat triacylglycerols stearic acid concentration at the 1-position

was almost four times that at the 3-position. Conversely, linoleic acid concentration at the 3-position of the triacylglycerols was approximately three times that at the 1-position.

In a different study, Christie and Moore (1970b) examined the changes in stereospecific distribution of fatty acids for a range of fat samples of differing composition. They found that the changes were smallest at position 3 of the triacylglycerols where an increase in the level of unsaturated acids caused a small decrease in the saturated fatty acid content. At position 1 stearic acid concentration decreased with increasing levels of unsaturated fatty acids. This last observation may account for the correlation between stearic acid concentration at the 1+3 positions of the triacylglycerols and the slip point. Enser (1984) also reported that stearic acid concentration is important in regulating the consistency of pig backfat triacylglycerols.

This asymmetry of the triacylglycerols implies that the different fatty acids are to some extent restricted as to the sites at which they can be esterified. This non-random pattern is thought to ensure that the triacylglycerols maintain the correct physiological states required for subsequent metabolism (Fahey *et al.*, 1985). With a less asymmetric pattern the fatty acids would be esterified in a more or less random fashion, and a wider range of triacylglycerol species would result. These latter triacylglycerols would be affected less by changes in fatty acid composition than would the less random triacylglycerol species, and this is reflected by changes in the melting points which are greater with the more asymmetric triacylglycerols. Thus the asymmetry of triacylglycerols explains why small changes in fatty acid composition may have a disproportionately large effect on the melting point (Moore *et al.*, 1969).

3.3.3.3 Effect of supplementing the diets with maize oil

The addition of maize oil to the diet resulted in major differences in the fatty acid compositions of the adipose tissue triacylglycerols (Table 3.2.6). In general, dietary maize oil greatly increased the concentration of linoleic acid (and to a lesser extent, linolenic acid) in the adipose tissue triacylglycerols at the expense of all the other fatty acids, particularly oleic and palmitic acids. This effect was observed in all valid comparisons irrespective of the type of protein or the level of dietary copper.

Linoleic acid cannot be synthesized by the pig and so is derived entirely from dietary sources. Maize oil contains nearly 60% linoleic acid (Gunstone, 1986) so accounting for the high concentrations of this acid in the triacylglycerols of pigs receiving the oil-supplemented treatments. Brooks (1967) also noted that feeding pigs diets containing high levels of linoleic acid (as supplied by 10% soyabean oil) resulted in adipose tissue concentrations of this acid three times those of the control pigs. Koch *et al.* (1966b) found that linoleic acid was deposited in the adipose tissue triacylglycerols at the expense of oleic acid, and this is also in accordance with the results of Experiment 1. The concomitant reduction in oleic acid content of the adipose tissue triacylglycerols is thought to help preserve a fairly constant level of saturated fatty acids (Christensen, 1973).

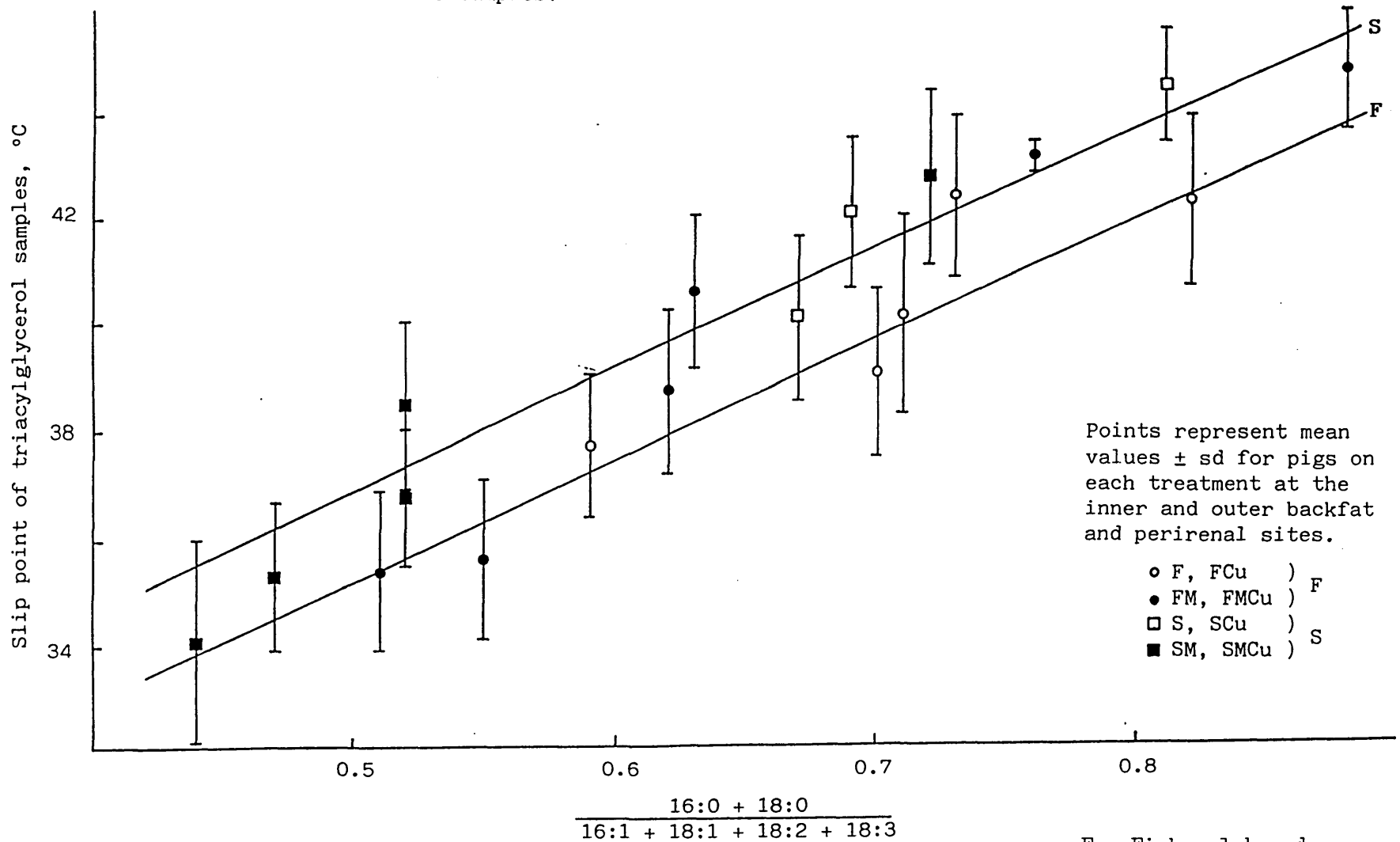
The effect of dietary maize oil in reducing the level of saturated and monounsaturated fatty acids in the triacylglycerols relative to the pigs on the basal treatment may be explained by inhibition of the lipogenic and desaturase enzymes. From their experiments on rats Jeffcoat and James (1978) suggested that within hours of feeding, dietary linoleate inhibits the net

synthesis of stearoyl CoA desaturase. This leads to an accumulation of saturated acids from *de novo* synthesis which in turn inhibit the enzymes of fatty acid synthesis: acetyl CoA carboxylase and fatty acid synthetase. Thus linoleic acid-rich diets, when fed to rats over a period of days, decrease the net synthesis of these two lipogenic enzymes.

The pigs receiving the maize oil-supplemented diets of this experiment had much lower levels of palmitic and oleic acids in the adipose tissue triacylglycerols than did their counterparts given the oil-free treatments. Palmitic acid is the major end-product of fatty acid biosynthesis and oleic acid is the main fatty acid formed by the action of the Δ^9 desaturase (Sections 1.3.2.1 and 1.3.2.2). Thus when these lipogenic and desaturase pathways are inhibited by dietary linoleate, lower levels of palmitic and oleic acids would be anticipated. The decreased levels of the other saturated and mono-unsaturated fatty acids are also a result of the lowered flux through these two pathways, but the effect on these is not as great as they are present in smaller quantities.

The increase in the concentration of polyunsaturated fatty acids in the triacylglycerols had the effect of lowering the triacylglycerol melting point (Table 3.2.7). The ratio of the concentrations of the saturated:unsaturated fatty acids in the adipose tissue triacylglycerols was found to correlate significantly with the melting point when the results from the pigs given the fishmeal and soyabean-based treatments were examined separately (Figure 3.3.3). The two regressions illustrated had similar positive gradients ($p > 0.05$) but the soyabean regression had an intercept which was significantly greater than that of the fishmeal regression ($p \leq 0.001$). This is consistent with the melting point determinations; the triacylglycerol samples from the

Figure 3.3.3: Experiment 1.. Relationship between the ratio of concentrations of saturated:unsaturated fatty acids and slip point of triacylglycerols from subcutaneous and perirenal adipose tissue samples.



F - Fishmeal based treatments
S - Soyabean based treatments

adipose tissue of pigs given the soyabean treatments generally had higher melting points than did the triacylglycerols from pigs given the corresponding diets based on fishmeal protein. These differences in the melting points of triacylglycerols from pigs given different sources of protein may be related to differences in the composition and positional distribution of the fatty acids in the adipose tissue triacylglycerols. As the differences in the fatty acid compositions of triacylglycerols of pigs given the corresponding treatments based either on fishmeal or soyabean meal protein were relatively small, the effects of the positional distribution may be of more importance.

The positive relationships were, to some extent, anticipated; Elliot and Bowland (1969) reported that a 1% increase in triacylglycerol unsaturated fatty acid content reduced the melting point by 0.87°C . However, with the results of the present experiment, a similar change in fatty acid composition appeared to decrease the triacylglycerol melting point by only $0.4 - 0.5^{\circ}\text{C}$ (Figure 3.3.3). This discrepancy may have arisen from differences in the positional distributions of the fatty acids in the adipose tissue triacylglycerols between pigs on the present experiment and those used by Elliot and Bowland (1969).

It is interesting to note that triacylglycerol samples from pigs on the maize oil treatments, containing about 20-25% linoleic acid (weight per cent of total fatty acids), melted at temperatures only a few degrees lower than those of more saturated samples containing less than 10% of this fatty acid. Dietary inclusion of copper caused a similar depression in the triacylglycerol melting point despite a much smaller change in the overall fatty acid composition.

Polyunsaturated fatty acids are expected to lower the lipid melting

/point

point to a greater extent than their monounsaturated analogues (see Table 1.2.1 for the melting points of various fatty acids). Thus an alternative ratio of fatty acids, which is biased to account for this effect, can be calculated. When this biased ratio -

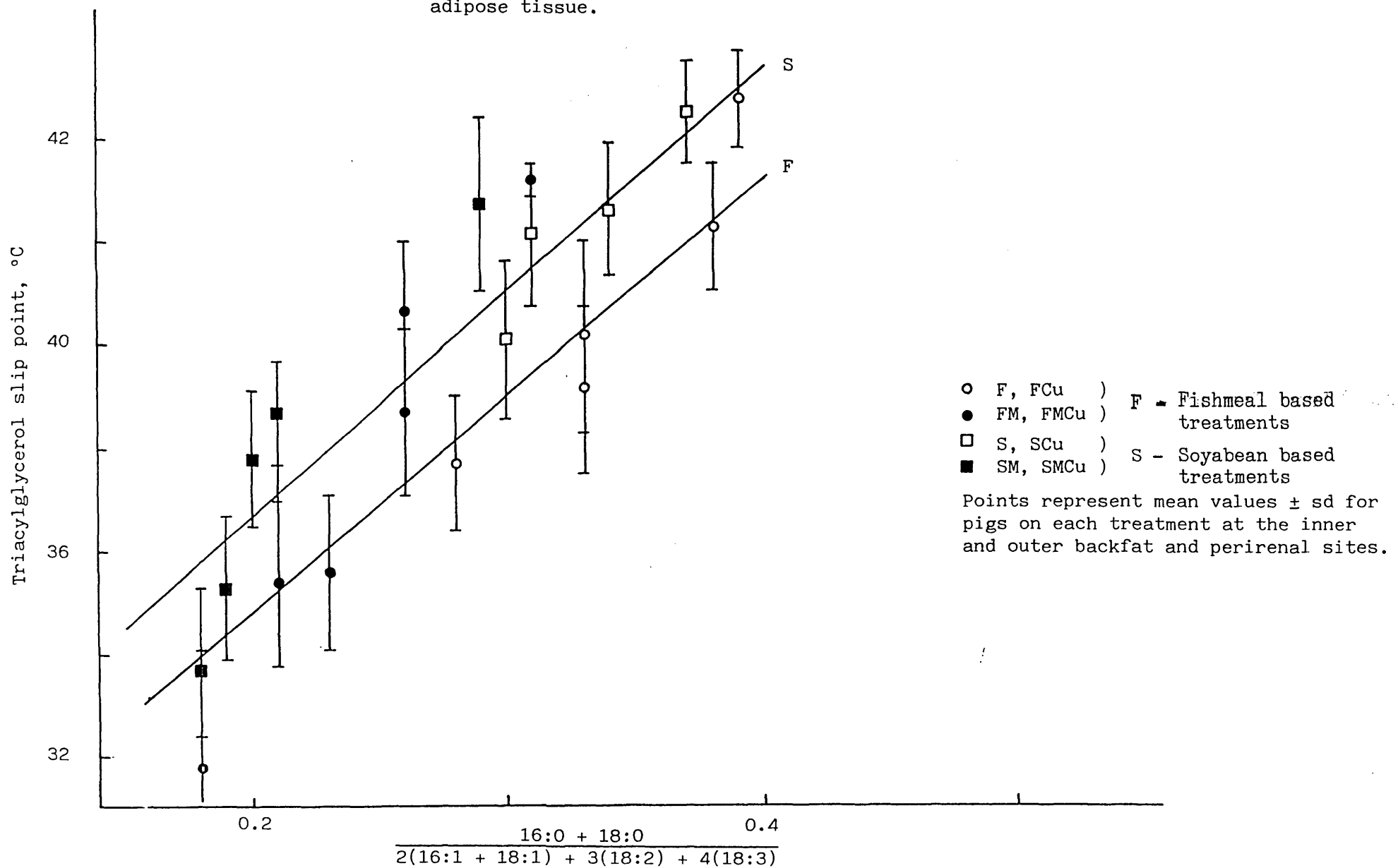
$$\frac{\Sigma \text{ saturated fatty acids}}{2(\Sigma \text{ monounsaturated}) + 3(\Sigma \text{ diunsaturated}) + 4(\Sigma \text{ triunsaturated}) \text{ fatty acids}}$$

is plotted against the slip point, similar relationships were observed (Figure 3.3.4). The two regressions, corresponding to the results from the pigs given the fishmeal and soyabean-based treatments respectively, had similar gradients ($p > 0.05$) but the intercept was significantly higher with the soya-bean regression ($p \leq 0.001$). These differences between the results from the pigs given the two different sources of protein may again be a result of differences in the positional distribution of the fatty acids in the adipose tissue triacylglycerols.

In addition these relationships had gradients which were almost twice those of the corresponding regressions calculated using the unbiased ratios of fatty acids. These steeper gradients reflect the greater effect of the more unsaturated fatty acids on the triacylglycerol melting points.

In order to investigate the effects of fatty acid positional distribution on the relationships between the ratios of (biased) saturated:unsaturated fatty acids and triacylglycerol slip points, the triacylglycerols from the adipose tissue of pigs given different sources of protein were randomly interesterified. This procedure eliminates the effects of non-random fatty acid distribution in the triacylglycerol molecules and so the melting points of triacylglycerols subjected to this procedure reflect the composition rather than the positional distribution of the component fatty acids.

Figure 3.3.4. Experiment 1. Relationship between the biased ratio of the concentrations of saturated:unsaturated fatty acids and the slip point of triacylglycerols from the backfat and perirenal adipose tissue.



A plot of the ratio of the concentrations of saturated:unsaturated fatty acids against the slip point of the randomly interesterified triacylglycerols gave a single positive relationship ($p < 0.001$) which is illustrated in Figure 3.3.5. Similarly, a single positive relationship was also obtained when the biased ratios of the concentrations of saturated:unsaturated fatty acids were plotted against the slip points of the randomly interesterified triacylglycerols ($p < 0.01$, Figure 3.3.6). These findings support the suggestion that the differences between the correlations of saturated:unsaturated ratios of fatty acids and triacylglycerol melting point between pigs given the two protein sources are a result of differences in fatty acid distribution in the triacylglycerols. In addition the gradient of the latter relationship is steeper than that of the regression calculated using the unbiased fatty acid ratio. This is in agreement with the suggestion that the more unsaturated fatty acids have a greater effect on triacylglycerol melting point than their more saturated analogues.

Enser (1984) found that the concentration of linoleic acid in the backfat triacylglycerols was linearly related to the slip point. When the results from this present experiment were examined, two significant relationships ($p < 0.001$) were obtained when the results from the pigs given the fishmeal and soyabean meal-based treatments were considered separately (Figure 3.3.7). The two linear regressions had gradients which were similar ($p > 0.05$) but intercepts which were significantly different from each other ($p < 0.001$).

This difference between the regressions with the results from the pigs given the two different sources of protein may be related to the effects of positional distribution of the fatty acids on the triacylglycerol melting point. A comparison of the positional distributions of linoleic acid in the

Figure 3.3.5: Experiment 1. Relationship between the ratio of concentrations of saturated:unsaturated fatty acids and slip point of randomly interesterified triacylglycerols from inner and outer backfat samples.

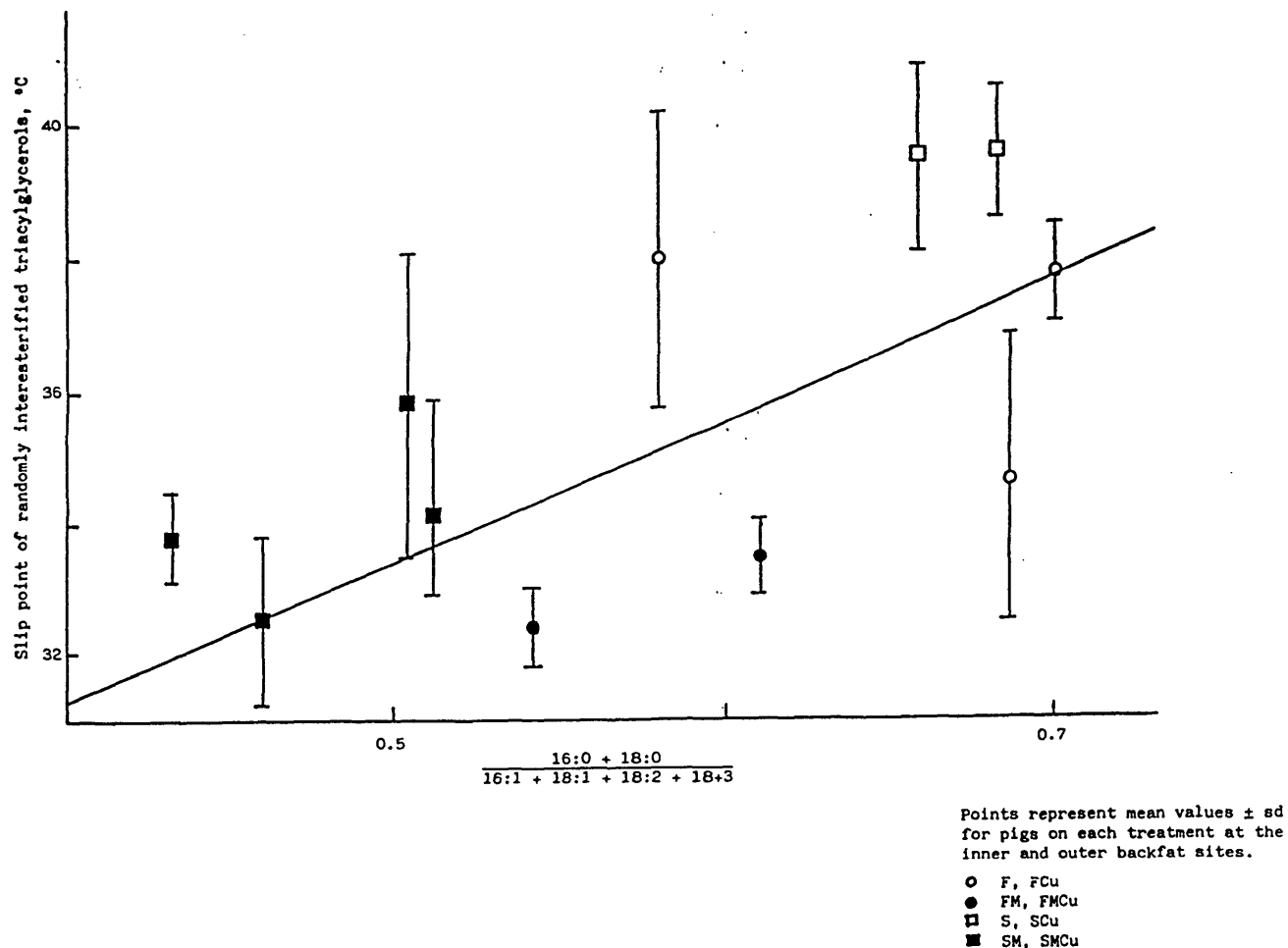
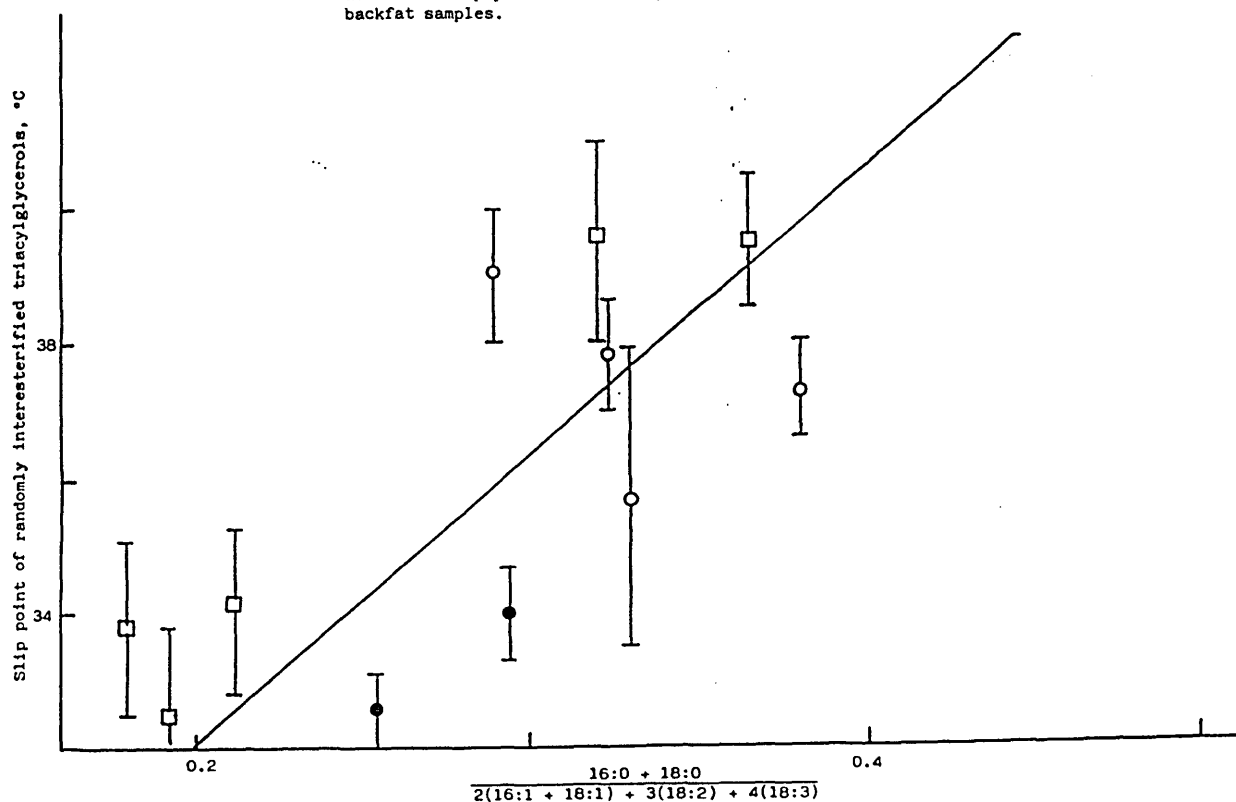


Figure 3.3.6: Experiment 1. Relationship between the biased ratio of concentrations of saturated:unsaturated fatty acids and slip point of randomly interesterified triacylglycerols from inner and outer backfat samples.



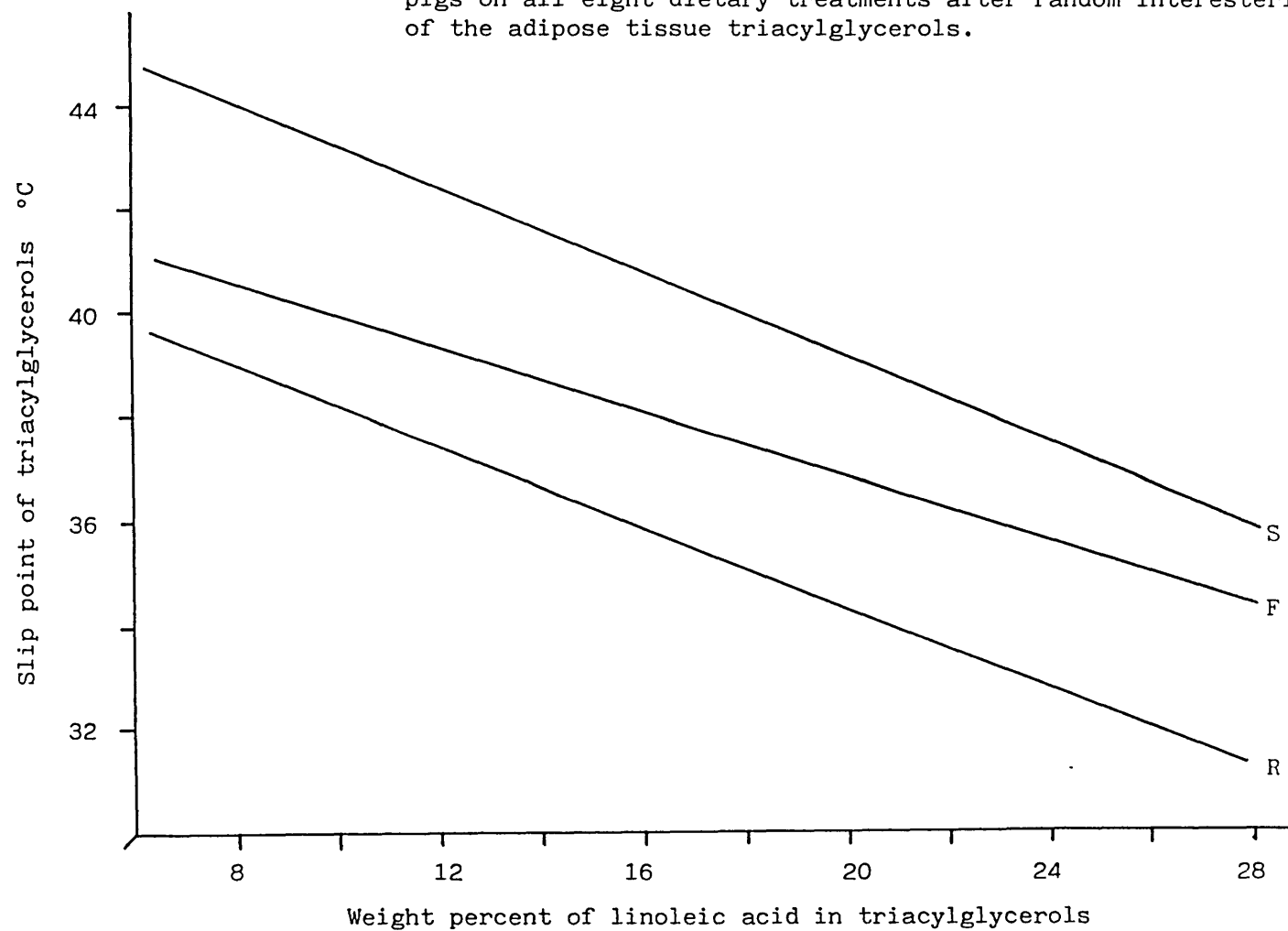
adipose tissue triacylglycerols showed differences between pigs given diets based on different sources of protein. Linoleic acid concentration was greater at position 2 but lower at the 1+3 positions of the triacylglycerols from the pigs given the treatments based on fishmeal protein. This is consistent with the earlier finding that the positional distribution of linoleic acid affects the triacylglycerol melting point, with the concentration at the 2-position having the greater effect (section 3.3.3.2).

In addition, when the concentrations of linoleic acid in the adipose tissue triacylglycerols were plotted against the slip points of the randomly interesterified triacylglycerols, a single relationship was observed ($p \leq 0.001$, Figure 3.3.7). Thus when the positional distributions of the fatty acids in the triacylglycerols were randomised no significant differences ($p > 0.05$) were noted between the results from the pigs given the two sources of dietary protein. This again supports the suggestion that the positional distribution of the fatty acids may play an important part in determining the triacylglycerol melting point.

3.3.3.4 Effect of supplementing the diets with copper

The fatty acid compositions of the adipose tissue triacylglycerols are given in Table 3.2.6. Dietary copper increased the levels of oleic and palmitoleic acids in this tissue, at the expense of the saturated fatty acids and linoleic acid. This is in accordance with the work of Elliot and Bowland (1968), Amer and Elliot (1973a) and Moore *et al.* (1969) who noted similar changes in triacylglycerol composition accompanying high levels of copper in the diet. In addition, Thompson *et al.* (1973) reported that the oleic:stearic acid ratio was greater in the triacylglycerols of pigs fed copper-

Figure 3.3.7: Experiment 1. Relationships between the concentrations of linoleic acid and the slip points of triacylglycerol samples from the subcutaneous and perirenal adipose tissue of pigs on the soyabean and fishmeal based treatments (S, F) respectively. R depicts the relationship for the results from pigs on all eight dietary treatments after random interesterification of the adipose tissue triacylglycerols.



supplemented compared with control diets.

This increase in triacylglycerol monounsaturated fatty acid content has been attributed to increased activity of the stearoyl CoA desaturase. Thompson *et al.* (1973) found an activation of the liver stearic acid desaturase system at liver copper concentrations corresponding to those of pigs fed a diet containing 250 ppm Cu. Similarly Ho and Elliot (1973) reported that dietary copper enhanced the activity of the stearoyl CoA desaturase in both hepatic and adipose tissues.

Dietary copper and maize oil have opposing effects on the Δ^9 desaturase system and as the effect of supplemental copper was still apparent when 4% maize oil was included in the diet it may be that the effect of copper is greater and possibly different from that of maize oil. As discussed earlier, maize oil inhibits desaturation of fatty acids by decreasing the net synthesis of the enzymes involved in fatty acid synthesis (Section 3.3.3.3). The mechanism by which dietary copper stimulates the Δ^9 desaturase system is less clear. Although copper is not an integral part of the desaturase, enzyme activity is stimulated by the addition of CuSO_4 to microsomal preparations *in vitro* (Thompson *et al.*, 1973). There are also reports that dietary copper affects the plasma lipid concentrations in both the pig and rabbit (Myres & Bowland, 1973; Allotta *et al.*, 1985). The possible modes of action of dietary copper are dealt with more fully in Section 1.7.

A comparison of the adipose tissues from pigs fed the basal and copper-supplemented diets for both the fishmeal and soyabean treatments of this present experiment showed increases in triacylglycerol monounsaturated fatty acid content at the expense of stearic and palmitic acids. There was little

change in the concentrations of the polyunsaturated acids suggesting that dietary copper had no major effects on the deposition of these particular dietary fatty acids. In addition there appeared to be no interaction between dietary copper and the source of protein as the changes in adipose triacylglycerol compositions associated with dietary copper were similar between pigs fed diets based on either fishmeal or soyabean protein. This is consistent with the finding that copper uptake and excretion were similar between pigs fed the same levels of dietary copper (Section 3.2.3). Thus although differences in triacylglycerol composition were noted between pigs fed diets containing different sources of protein, there appeared to be no differences in the response to dietary copper. However differences in growth rates between pigs fed the copper-supplemented treatments based on the two protein sources were apparent at 46 kg liveweight (Section 3.3.1).

These changes in the fatty acid compositions of the adipose tissue triacylglycerols were accompanied by a decrease in the triacylglycerol melting point. However it is interesting to note that whereas, in general, the slip points of the triacylglycerols were significantly different between treatments, the fatty acid compositions of the triacylglycerols from pigs on the basal and copper-supplemented treatments were not. Thus relatively small alterations in the fatty acid compositions of the triacylglycerols may lead to significant changes in the melting point. This concurs with the work of Moore *et al.* (1969), although these authors found a much greater decrease in triacylglycerol melting point ($\sim 10^{\circ}\text{C}$) when the pigs were fed diets containing 250 mg Cu/kg feed. Amer and Elliot (1973a) also found large decreases in triacylglycerol melting point associated with dietary copper but there are also reports of much smaller differences (Braude & Ryder, 1973; Walker *et al.*, 1971). This disparity between results may arise from differences in the experimental methods used.

The breed and age of the pigs as well as the nature of the diets and the scale at which they are given to the animals may all affect the response to the copper supplement.

Moore *et al.* (1969) also noted that the outer backfat layers of pigs given control diets were similar in composition to both backfat layers of pigs on copper-supplemented treatments. In accordance with this, in the experiment reported here, no major differences were noted between the fatty acid compositions of the inner backfat layers from the pigs on the FCu treatment and those of the outer backfat layers of pigs on the F treatment (Table 3.2.6). The outer backfat triacylglycerols from the pigs on the FCu treatment were slightly more unsaturated than those from the inner layer. A similar situation was observed with the animals on the corresponding soyabean-based treatments. These differences in triacylglycerol composition were reflected in the melting points. Thus the slip points of triacylglycerols from the outer backfat of pigs given the FCu (and SCu) treatment were lower than those from the inner layers of pigs on the same treatment and the outer layers of backfat of pigs given the F (and S) treatment. These differences in triacylglycerol slip points between backfat layers of pigs on the FCu (and SCu) treatment are consistent with the widely reported greater unsaturation of the triacylglycerols from the outer backfat layer (Section 3.3.3.1).

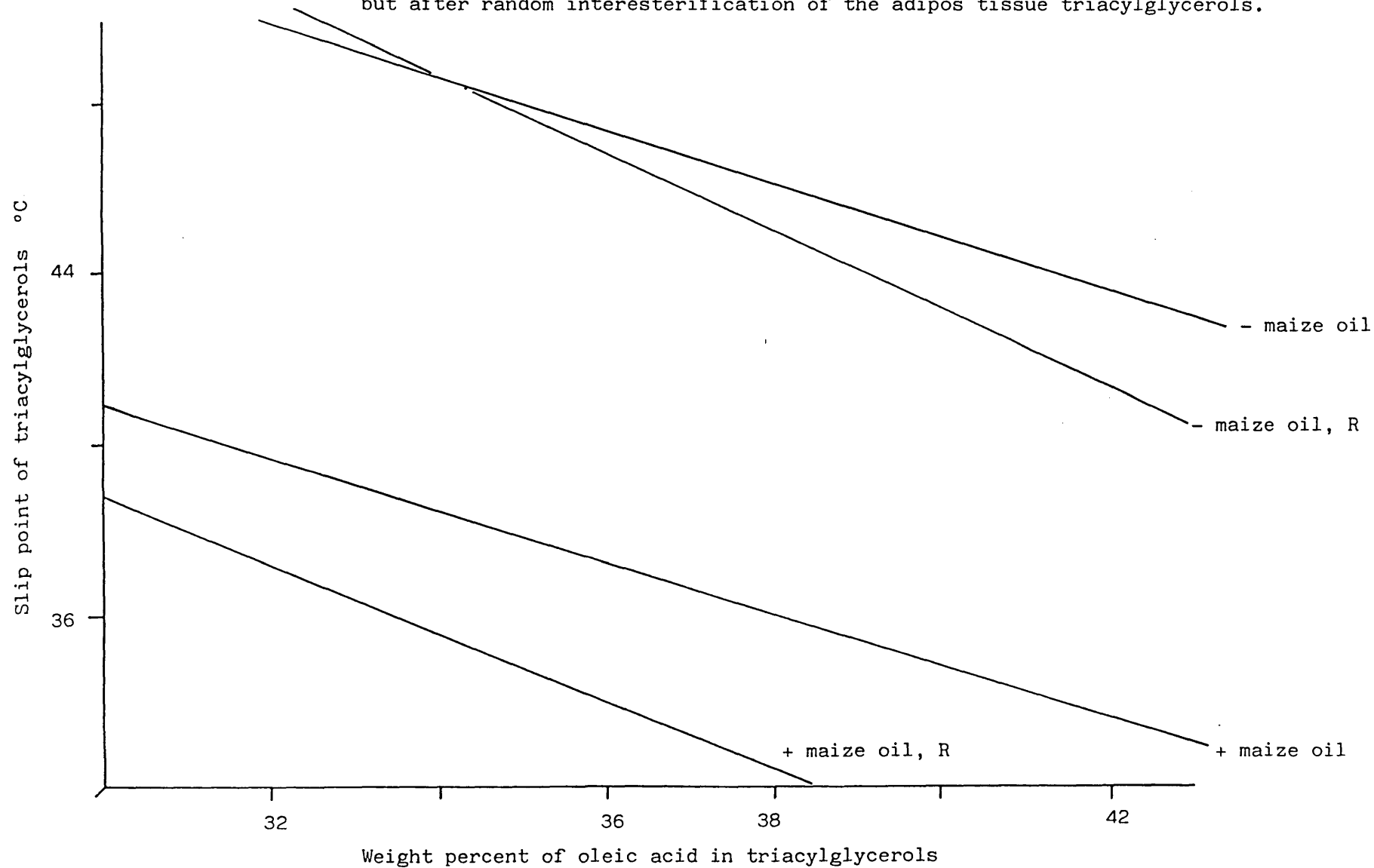
As discussed earlier, the triacylglycerol melting point is linearly related to both the ratio of the concentrations of saturated:unsaturated fatty acids and the linoleic acid concentrations of the triacylglycerol samples (Figures 3.3.3 and 3.3.7). Copper supplementation had a greater effect on the oleic acid content of the triacylglycerols and so the concentration of this fatty acid in the adipose tissue triacylglycerols was plotted against the

triacylglycerol slip point. Two very highly significant correlations ($p \leq 0.001$) were obtained, corresponding to the results from the pigs on the maize oil supplemented and maize oil lacking treatments respectively. These regressions are illustrated in Figure 3.3.8; the slopes of the two lines were not significantly different from each other at the 5% level.

These findings are not consistent with those of Enser (1984) who observed no relationships between the concentration of oleic acid in the adipose tissue and the triacylglycerol melting point. In the present experiment, oleic acid concentration was correlated with the slip point even in the presence of large amounts of linoleic acid which would have a greater effect on the lipid melting point. It may be that the concentration of oleic acid balances the level of saturated and unsaturated fatty acids in order to maintain an acceptable degree of fluidity. This is consistent with the effect of dietary fatty acids on *de novo* synthesis of fatty acids in adipose tissue. Dietary saturated and polyunsaturated fatty acids have opposing effects on the activity of stearoyl CoA desaturase which is responsible for the formation of oleic acid. The level of oleic acid in the adipose tissue may therefore be influenced, to some extent, by the nature of the dietary fatty acids.

With the results from the pigs given the treatments lacking maize oil there were no differences between pigs given different sources of protein ($p > 0.05$). This suggests that there are no major differences in the positional distribution of oleic acid between the triacylglycerols but the analysis of positional distribution between the triacylglycerols from pigs given either fishmeal or soyabean protein has shown otherwise (Table 3.2.9). The lack of a significant difference between the regressions for the results from the pigs given the F and FCu, or S and SCu treatments may, therefore, be related to the

Figure 3.3.8: Experiment 1. Relationships between the concentration of oleic acid and the slip points of triacylglycerol samples from the subcutaneous and perirenal adipose tissue of pigs on the maize oil-supplemented and unsupplemented treatments respectively. R depicts the relationships obtained for the results from the same groups of pigs, but after random interesterification of the adipos tissue triacylglycerols.



molecular species of triacylglycerols present. Although analysis of the positional distribution of the fatty acids in the triacylglycerols reveals differences in the overall patterns of esterification, it gives no indication of the esterification patterns within the individual triacylglycerol species. In order to study this the triacylglycerol samples were separated by silver nitrate thin layer chromatography into bands of triacylglycerols containing the same number of double bonds. However the resolution was poorer than expected and no consistent differences were apparent between triacylglycerol samples from pigs given different sources of protein; the results of the separations are discussed in Appendix 5.

With the regression of oleic acid concentration against triacylglycerol slip point for the pigs given the treatments supplemented with maize oil, there were no differences between pigs given different sources of protein ($p>0.05$). This may also reflect differences in the molecular species of triacylglycerols, but the presence of high levels of linoleic acid in the adipose tissue triacylglycerols may also be involved.

The two regressions, corresponding to the results from the pigs given the maize oil supplemented and unsupplemented treatments respectively had gradients which were not significantly different from each other ($p>0.05$). However the regression for the pigs on the maize oil supplemented treatments might be expected to have a shallower gradient than that for the pigs on the treatments not containing maize oil. Linoleic acid has a greater effect on the triacylglycerol melting point than does oleic acid. Thus with the pigs on the maize oil supplemented treatments, which have high levels of linoleic acid in the adipose tissue triacylglycerols, a change in the oleic acid content of the triacylglycerols might be expected to result in a smaller change in

the melting point when compared with the pigs given the treatments not supplemented with maize oil as these have much lower levels of linoleic acid in the adipose tissue triacylglycerols. However, as already mentioned, the gradients of the two linear regressions were not significantly different from each other. This discrepancy between the observed and the expected findings may be related to the definition of the melting point of the adipose tissue triacylglycerols. Although this physical property can be accurately determined, in a heterogenous mixture such as a triacylglycerol sample from pigs on the present experiment, the melting point represents the temperature at which triacylglycerols with higher melting points dissolve in those which have already melted. Thus differences in the melting point noted between triacylglycerol samples from pigs on different treatments may be a result of genuine changes in melting point or they may arise from changes in the concentration of one or two particular species of triacylglycerols which affect the solubility of the more saturated triacylglycerol types and so indirectly influence the melting point.

When the concentration of oleic acid in the adipose tissue was plotted against the slip point of the randomly interesterified triacylglycerols two correlations were also noted, corresponding to the results from the pigs on the maize oil supplemented and unsupplemented treatments respectively (Figure 3.3.8). The relationship obtained with the results from the pigs on the maize oil treatments had a slightly less steep gradient than that of the regression for the pigs on the treatments not containing maize oil. The difference between slopes was not significant at the 5% level, but this tendency to a shallower gradient when high levels of linoleic acid are present in the adipose tissue triacylglycerols is in agreement with the expected differences described above. In addition these findings support the idea that the positional

distribution of fatty acids and the range of molecular species of triacylglycerols present may both affect the triacylglycerol melting point.

Dietary copper was also associated with an increase in the concentration of palmitoleic acid in the adipose tissue triacylglycerols. However a similar plot of triacylglycerol palmitoleic acid concentration against the slip point was not significant at the 5% level.

Enser (1984) reported that stearic acid is the most important factor regulating the consistency of pig backfat triacylglycerols and so the adipose tissue concentrations of this fatty acid from pigs on the present experiment were plotted against the triacylglycerol slip points. Two significant relationships were obtained ($p \leq 0.001$), corresponding to the results from the pigs given the fishmeal and soyabean meal based treatments respectively (Figure 3.3.9). The regressions had similar gradients ($p > 0.05$) but intercepts which were significantly different from each other ($p \leq 0.001$). Palmitic acid concentration was similarly related to the slip point ($p \leq 0.001$ for both regressions, Figure 3.3.10) and the presence here of a significant positive correlation coefficient is in agreement with Enser *et al.* (1984).

With both the stearic and palmitic acid regressions the results from the pigs on the soya-based treatments had higher intercepts than those of the pigs given the treatments containing fishmeal protein. This is consistent with the higher melting points of the triacylglycerols from the adipose tissue of pigs given soya-based treatments when compared with those given the corresponding diets based on fishmeal. This difference may arise from the differences in positional distribution of the fatty acids in the triacylglycerols; the pigs given the soya-based treatments had a much more

Figure 3.3.9: Experiment 1. Relationships between concentrations of stearic acid and the slip points of triacylglycerol samples from the subcutaneous and perirenal adipose tissue of pigs on the soyabean and fishmeal-based treatments (S, F) respectively. R depicts the relationship for the results from pigs on all eight dietary treatments after random interesterification of the adipose tissue triacylglycerol.

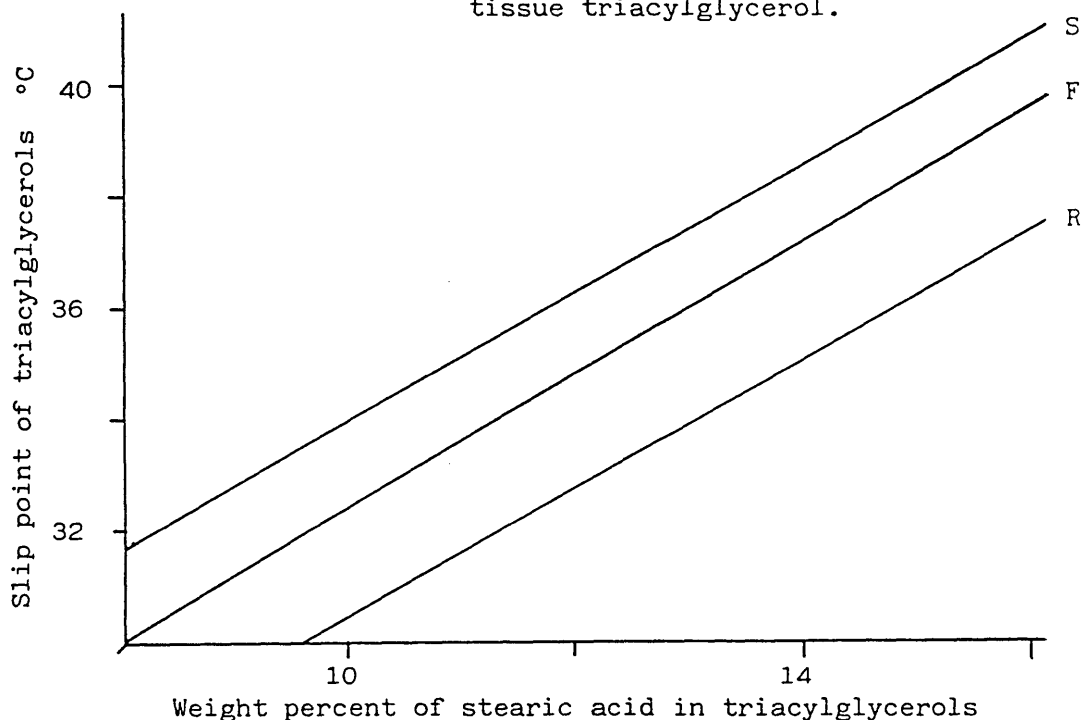
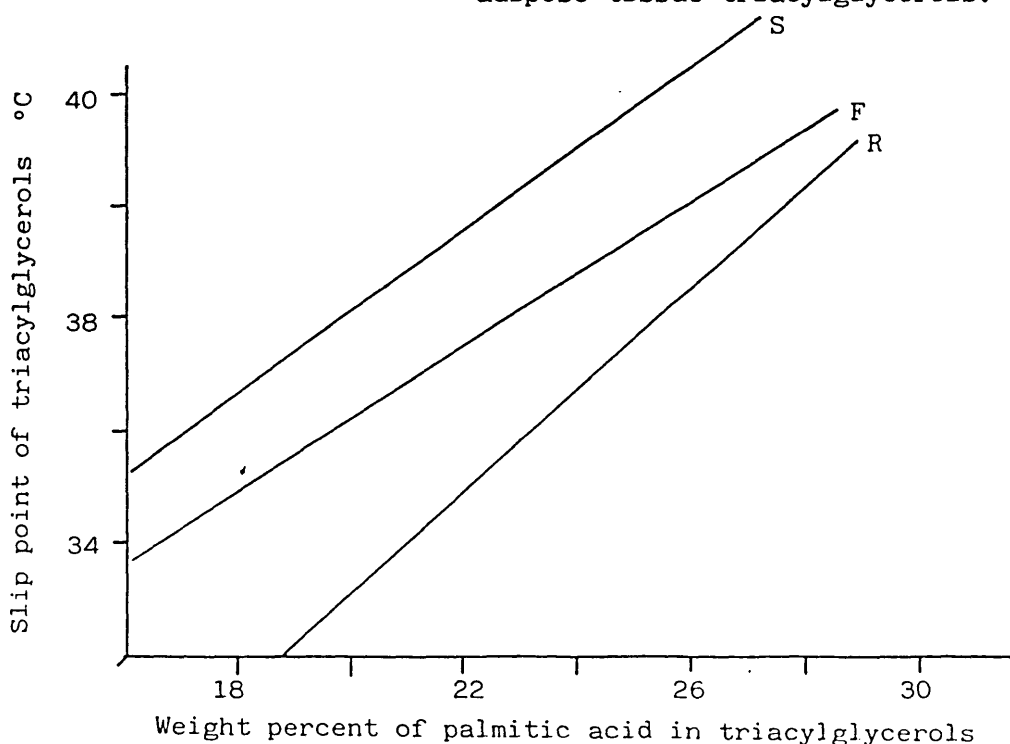


Figure 3.3.10: Experiment 1. Relationships between the concentrations of palmitic acid and the slip points of triacylglycerol samples from the subcutaneous and perirenal adipose tissue of pigs on the soyabean and fishmeal-based treatments (S, F) respectively. R depicts the relationship for the results from pigs on all eight dietary treatments after random interesterification of the adipose tissue triacylglycerols.



asymmetric fatty acid distribution of the saturated fatty acids, particularly palmitic acid, in the adipose tissue triacylglycerols (Table 3.2.8). In addition separation and analysis of the triacylglycerol species, although incomplete, suggested that there were differences in the distributions of triacylglycerol species between triacylglycerol samples taken from the adipose tissue of pigs given different sources of protein (Appendix 5).

As discussed earlier, random interesterification removes the effects of asymmetric fatty acid distribution in the triacylglycerol samples. When the concentrations of stearic acid were plotted against the slip points of the randomly interesterified triacylglycerols a single positive correlation was obtained for the results from pigs on all eight dietary treatments. This relationship is illustrated in Figure 3.3.9 and supports the suggestion that the positional distribution of the fatty acids may have a marked effect on the triacylglycerol melting point. Similarly random interesterification of the triacylglycerols eliminated the effects of positional distribution on the relationship between palmitic acid concentration and the melting point of the randomly interesterified triacylglycerols (Figure 3.3.10).

The concentrations of the three major 18 carbon fatty acids and palmitic acid in the adipose tissue triacylglycerols all appear to have a significant relationship to the melting point. This does not agree with the work of Enser (1984) who reported that the concentrations of stearic and linoleic acids in the triacylglycerols were both linearly related to the slip point but that the concentration of oleic acid was not.

In order to determine the effects of the 18 carbon fatty acids on fat consistency, the ratios of these fatty acids were plotted against the tria-

cylglycerol melting point for all pigs on all eight treatments. The relationship between stearic and oleic acid concentration and slip point is shown in Table 3.3.4. The results fell in two distinct groups corresponding to the experimental treatments containing maize oil and those lacking it. In addition with the treatments not supplemented with maize oil there was evidence of a difference between the results from pigs given different sources of protein. Two regressions were obtained and these had similar gradients ($p>0.05$), but significantly different intercepts ($p\leq 0.01$). In addition these two regressions had gradients which were similar to that of the relationship calculated using the results from the pigs given the treatments supplemented with maize oil ($p>0.05$), although the gradient of this last regression was slightly shallower. Thus when high levels of linoleic acid are present in the adipose tissue triacylglycerols, the effects of the positional distribution of stearic and oleic acids on the triacylglycerol melting point appear to be reduced. This may be because the large increase in linoleic acid concentration in the triacylglycerols is accompanied by decreases in the concentrations of oleic and stearic acids and these latter fatty acids have a smaller effect on the triacylglycerol melting point than does linoleic acid. A similar plot of the ratios of the concentrations of palmitic:palmitoleic acids against the slip points of the adipose tissue triacylglycerols revealed no clear relationship.

There were significant correlations ($p\leq 0.001$) between both the ratios of oleic:linoleic acid concentrations and stearic:linoleic acid concentrations with the triacylglycerol slip points (Table 3.3.4). In both cases two relationships were obtained, corresponding to the results from the pigs given the fishmeal and soyabean meal based treatments respectively. However, although the two relationships between the ratios of the concentrations of oleic:linoleic acids and triacylglycerol slip point were parallel ($p>0.05$),

Table 3.3.4: Experiment 1.

Relationships between the ratios of the concentrations (weight percent) of the 18 carbon fatty acids (x) and the melting points ($^{\circ}\text{C}$, y) of triacylglycerols from the inner and outer backfat and perirenal adipose tissue sites.

	Linear regression	Significance
<u>18:0/18:1 ratio</u>		
Treatments lacking maize oil		
Fishmeal treatments	$y = 34.50x + 28.44$	$p \leq 0.01$
Soyabean treatments	$y = 35.58x + 28.76$	$p \leq 0.001$
Treatments supplemented with maize oil		
Fishmeal and soyabean treatments	$y = 32.25x + 24.69$	$p \leq 0.01$
<u>18:0/18:2 ratio</u>		
Fishmeal treatments	$y = 3.19x + 33.21$	$p \leq 0.001$
Soyabean treatments	$y = 4.99x + 33.93$	$p \leq 0.01$
<u>18:1/18:2 ratio</u>		
Fishmeal treatments	$y = 0.88x + 35.12$	$p \leq 0.001$
Soyabean treatments	$y = 1.28x + 37.03$	$p \leq 0.01$

those between the ratios of stearic:linoleic acids and triacylglycerol slip points were not ($p \leq 0.001$). This suggests that the effects of differences in fatty acid positional distribution on the triacylglycerol melting point are greater with stearic and linoleic acids than with oleic acid. This is in keeping with the observation that, in contrast to the oleic:linoleic acid ratios, relatively small changes in the stearic:linoleic acid ratios were associated with quite marked effects on the melting point. With the relationships for the fishmeal and soyabean-based treatments respectively, the gradient of the stearic:linoleic acid plot is nearly four times that of the oleic:linoleic acid graph, suggesting that changes in the concentrations of stearic and linoleic acids are accompanied by greater changes in the triacylglycerol melting point than are changes in the level of oleic acid.

These findings are consistent with the work of Enser *et al.* (1984) who found that the ratio of stearic:linoleic acid concentrations in the adipose tissue triacylglycerols could be used as an indicator of fat softness. However, with the results of the present experiment the relationships between the ratios of stearic:linoleic acid concentrations and triacylglycerol slip point were different between pigs given diets containing different sources of protein. This suggests that the stearic:linoleic acid ratio, although useful, is only a valid measure of fat consistency within groups of pigs which share the same characteristics of fatty acid distribution in the adipose tissue triacylglycerols.

In the present experiment, dietary copper had no significant effects on the positional distribution of the fatty acids in the triacylglycerols (Table 3.2.8). It has previously been noted that relatively small changes in fatty acid composition can have disproportionate effects on the melting

points of pig backfat triacylglycerols (Moore *et al.*, 1969). In this present experiment, the changes in positional distribution and fatty acid composition associated with supplemental dietary copper may be sufficient to lower the triacylglycerol melting point, but not large enough to reach the 5% level of significance. In addition, as discussed in Section 3.2.4.4, dietary copper altered the concentrations of stearic and linoleic acids, both of which are esterified to the 2-position in only small quantities. The pancreatic lipase hydrolysis technique does not distinguish between the 1 and 3 positions of the triacylglycerols and so specific changes at these sites were not detected. Similarly, dietary copper was associated with increased oleic acid concentrations at the 1 and 3 positions of the triacylglycerols, but the precise pattern of esterification was not determined by the method used.

3.3.4 General summary

The growth responses of the pigs *i.e.* the efficiency of food utilization and the growth rates up to 96 kg, appeared to be largely unaffected by the dietary treatments of this experiment. In particular the lack of response to dietary copper supplementation was not anticipated as, although there are exceptions, the majority of previous work has associated high levels of dietary copper with improved growth performance (Braude, 1967; Bradley *et al.*, 1983). In some cases, the phytic acid present in soybean meal has been found to reduce copper uptake from the intestinal contents (O'Dell *et al.*, 1972; Van Campen, 1971). However the copper analyses carried out during this present experiment suggest that the copper intake is similar between animals receiving diets containing the same level of copper, irrespective of protein source. These findings are consistent with reports that pigs can digest phytic acid and that soyabean meal itself contains phytases (Taylor, 1980). Despite this

overall lack of response to dietary copper up to 95 kg liveweight, the results calculated up to 46 kg liveweight show both the anticipated effects of copper on the growth rates and the reduced response with the pigs given the soya-based treatments. Dietary maize oil was expected to increase the pig growth rates, owing to the high calorific value of the diets (Allee *et al.*, 1971). This was not seen in the experiment now reported although the daily food intakes were lower in the pigs receiving the maize oil-supplemented treatments.

The maize oil supplement was also expected to increase the thickness of the subcutaneous backfat (De Wilde, 1984; Allee *et al.*, 1971). However, there were no differences in backfat thickness between pigs on any of the eight experimental treatments. This is consistent with the growth rates which were also similar between treatments. Pigs vary in their disposition to fatness and this is illustrated by the negative correlations between adipose tissue linoleic acid concentration and backfat thickness. Two regressions were obtained corresponding to the plus and minus maize oil treatments respectively. This is in contrast with the work of Wood and Enser (1982) who found only a single correlation, although in the experiment now reported a much wider range of backfat linoleic acid concentrations were present.

The differences in the fatty acid compositions of the triacylglycerols at the three adipose tissue sites are consistent with those previously reported (Moore *et al.*, 1969; Hood & Allen, 1973). These differences are thought to be related to the lipogenic activity of the tissue and the effects of temperature on Δ^9 desaturase activity. However, in agreement with Christie and Moore (1970a), positional analysis of the fatty acids indicates that the acyltransferases involved have similar specificities at each of the three adipose tissue sites.

Dietary maize oil increased the linoleic acid concentration of the adipose tissue triacylglycerols, while dietary copper had a greater influence on the monounsaturated acid content. These findings are consistent with those reported by Brooks (1967), Moore *et al.* (1969) and others. In addition these changes in fatty acid composition were associated with decreases in the triacylglycerol melting point which correlated with the degree of unsaturation of the triacylglycerols. However this fall in triacylglycerol slip point accompanying supplementation of the diets with either copper or maize oil was lower than that reported by Moore *et al.* (1969), Amer and Elliot (1973a), Koch *et al.* (1968b) and others. Moore *et al.* (1969) have shown that this disproportionate fall in melting point may be associated with the effect of the positional distribution of the fatty acids on the melting point.

Interestingly the type of protein added to the diet had a marked effect on the positional distribution of the fatty acids in the triacylglycerols. This was surprising as even the presence of 4% dietary maize oil had a relatively small effect on positional distribution. The differences in the positional distribution of the fatty acids in the adipose tissue triacylglycerols from pigs given diets containing different sources of protein was evident in the relationships between the degree of unsaturation and the slip points of the triacylglycerol samples. However when the slip points of randomly interesterified triacylglycerols were plotted against the ratio of saturated:unsaturated fatty acids, no effects of protein source were evident supporting the suggestion that the positional distribution of the fatty acids plays an important part in determining the triacylglycerol slip point. The differences in fat content and fatty acid composition of the two protein supplements were not that great, but it has been reported that the nature of the protein source may affect various aspects of lipid metabolism such as

VLDL production, lipoprotein lipase activity and cholesterol metabolism (Vahouny *et al.*, 1984; Diersen-Schade *et al.*, 1985).

In summary the results of this experiment are in agreement with similar work undertaken by earlier workers. Although the effects of both dietary copper and maize oil on growth performance were only apparent up to 46 kg, they are consistent with previously reported findings. The changes in fatty acid composition of the adipose tissue triacylglycerols accompanying supplementation of the diets with copper and maize oil tended to agree with earlier findings. However the concomitant decreases in triacylglycerol melting point were lower than those generally reported, particularly with the triacylglycerols from the pigs on the soyabean-meal based treatments. These differences have been attributed to differences in the positional distribution of the fatty acids, but as the patterns of distribution found in the present experiment were different from those previously reported, it is suggested that differences in breed and nutrition may be involved.

Section 4
EXPERIMENT 2

SECTION 4

Experiment 2

The effect of dietary copper and maize oil on backfat quality and lipid metabolism

4.1.1 Introduction

The results of Experiment 1 showed that dietary copper increased the concentration of monounsaturated fatty acids while dietary maize oil increased the linoleic acid concentration of the adipose tissue triacylglycerols. Two different sources of protein were used; fishmeal and soyabean meal and there was some evidence to suggest that the presence of the latter reduced the response to supplemental dietary copper. In this experiment, therefore, the experimental treatments were replicates of the fishmeal diets of Experiment 1.

It has been suggested that the dietary supplements may affect the nature of the plasma lipids, so accounting for the differences in adipose triacylglycerol composition (Allotta *et al.*, 1985; Diersen-Schade *et al.*, 1985). Thus blood samples were taken during the growing period for analysis of the effects of these two dietary supplements on the concentrations and compositions of the plasma lipids. In addition, the increase in oleic acid concentration of the adipose triacylglycerols accompanying copper supplementation of the diets has been attributed to increased activity of the stearoyl CoA desaturase. Dietary maize oil is thought to have the opposite effect and so the activity of this enzyme was assayed in microsomal preparations of the adipose tissue samples. Analyses of the fatty acid compositions of the adipose triacylglycerols were also carried out as before to ascertain if the changes noted with the dietary supplementation of copper and maize oil were comparable with those found in Experiment 1.

4.1.2 Dietary treatments

There were four experimental treatments, corresponding to the fishmeal-based diets of Experiment 1 (Section 3.1.2). As before the effects of dietary copper (200 mg Cu/kg diet) and maize oil (4g oil/100g diet) were examined separately and together, *i.e.*

	<u>Treatment</u>
F	Fishmeal
FCu	Fishmeal + copper
FM	Fishmeal + maize oil
FMCu	Fishmeal + maize oil + copper

The compositions of the experimental diets are given in Table 3.1.1.

4.1.3 Animals

Sixteen Landrace pigs from the Wye College herd were used for this experiment. Allocation to the treatments was at random but there was an overall balance of sex so that two intact males and two females were assigned to each of the experimental diets.

However, details of littermates were not recorded.

4.1.4 Experimental procedure

From three to nine weeks of age, the animals received a copper-supplemented diet^{*} which was replaced by the basal fishmeal diet (F) for the following three weeks. The pigs were given the four experimental diets when they reached about 35 kg liveweight.

^{*} containing 5.5 mg Cu/kg feed

The animals were housed in individual pens to enable the food intake of each pig to be measured. The experimental diets were given wet and once daily according to a semi *ad lib.* scale (reproduced in Appendix 2) up to a maximum of 2.95 kg diet/pig. Weighing of the animals was carried out on the same day and at the same time each week.

Blood samples were taken from each pig at the start of the experiment *i.e.* before the animals received the experimental diets, at the fourth and seventh weeks of the experiment and at slaughter. Samples (approximately 7 ml) were drawn, at the same time each day (~10 a.m.) from the jugular vein of the animals into heparinized tubes.

Each pig was slaughtered on reaching a minimum of 95 kg. No food was given on the day of slaughter and ultrasonic backfat measurements were taken on the previous day. Immediately after slaughter, sections of backfat 15 cm square were taken from the middle of the shoulder and loin regions of the carcass, together with a sample of perirenal fat. The tissue samples were rapidly transferred to a deep freeze for storage at -20°C except for the portions used for enzyme analysis. These were kept on ice and microsomal fractions were prepared as rapidly as possible.

4.1.5 Methods of analysis

Blood samples were centrifuged at 3000 rpm for 15 minutes and the plasma fractions collected. The concentrations and fatty acid compositions of the triacylglycerols, cholesteryl esters and non-esterified fatty acids in the plasma were determined by the methods quoted in Section 2.2.7. Determination of the plasma cholesterol concentrations was as described in Section 2.2.8.

The adipose tissue samples taken from the carcasses were kept on ice and transferred to the laboratory as rapidly as possible. The inner and outer layers of backfat were separated and portions of these, together with samples of perirenal fat, were used for the preparation of microsomes (Section 2.2.12). Stearoyl CoA desaturase activity in the microsomal fraction was assayed using the method described in Section 2.2.14.

The remaining sections of adipose tissue were used for the analysis of triacylglycerol composition. Lipid extraction and subsequent purification of the triacylglycerols (Sections 2.2.11 and 2.2.2) was followed by gas chromatographic determination of the concentrations of the component fatty acids (Section 2.2.4). Triacylglycerol melting points were determined as described in Section 2.2.9. The positional distributions of the fatty acids in the triacylglycerols were also established, using the pancreatic lipase hydrolysis technique (Section 2.2.6).

In general, the feed, growth and carcass data together with the plasma lipid data and the fatty acid compositions, slip points and desaturase activities of the adipose tissue triacylglycerols were analysed by analysis of variance. The overall effects of copper (1df), maize oil (1df), sex (1df) and interactions (4df) were looked for. No statistically significant effects of sex were noted for any of the parameters measured ($p > 0.05$). Where statistically significant effects of maize oil or copper were noted the corresponding treatment means (F/FCu or F/FM as appropriate) were then compared by t test with 7 degrees of freedom.

Differences between the shoulder and loin sites of individual backfat layers within treatments were compared by t test (6df). None of these intrasite comparisons were statistically significant and so the results for each pig were pooled. Differences between inner and outer backfat layers and perirenal fat were also compared by t test (14 df between backfat layers and 10df between backfat layers and perirenal fat).

Changes in plasma lipid concentrations and fatty acid compositions were analysed by t test comparing consecutive time points only (df). Where linear regressions were calculated, significance was tested by reference to the correlation coefficient ($n-2df$). Differences between linear regression were calculated by t test between the intercepts and slopes.

4.2 Results of Experiment 2

4.2.1 Growth rates, food intakes and feed conversion ratios.

The growth rates and feed-related results are given in Tables 4.2.1 and 4.2.2 respectively. The differences between treatments were not significant ($p>0.05$), but with the pigs receiving the copper-supplemented treatments, there was a small trend towards an increased daily liveweight gain and, as a result, a slightly more efficient feed conversion ratio.

4.2.2 Thickness of subcutaneous backfat

The carcass fat measurements are shown in Table 4.2.3. In general, there were no significant differences between the treatments for each of the fat thicknesses although the pigs on the FM treatment tended to have slightly thinner backfat at all three sites ($p>0.05$).

4.2.3 Composition, structure and physical properties of adipose tissue triacylglycerols:

4.2.3.1 Comparisons between different adipose tissue sites

Adipose tissue samples were taken from the perirenal fat and from the shoulder and loin regions of both layers of backfat. The mean fatty acid compositions of the triacylglycerol samples for each treatment (expressed as weight per cent of fatty acids) are shown in Table 4.2.4. The backfat samples from the shoulder and loin regions of the pigs on each treatment were found to be not significantly different from each other within each layer ($p>0.05$) and so these results were pooled. However, for completeness the data for each site is listed in Appendix 6. In addition, there was no significant effect of sex on the triacylglycerol composition ($p>0.05$).

Table 4.2.1: Experiment 2. Mean weekly weights (kg) for all four pigs on each dietary treatment during the experimental trial

Dietary treatment

Week of experiment	F	FCu	FM	FMCu	LSD (7df)
1	39.3	38.8	38.8	39.0	0.8
2	45.5	44.8	46.0	43.8	2.4
3	50.5	50.3	52.0	49.5	2.6
4	55.8	55.8	57.0	55.0	2.4
5	62.8	61.0	62.5	61.5	2.3
6	69.3	69.5	70.0	68.0	2.7
7	73.5	75.5	74.8	73.8	3.2
8	81.0	82.8	80.5	82.0	2.9
9	87.5	89.3	87.3	87.3	2.8
10	91.3	95.0	90.0	92.0	5.7
11	94.0	98.3	96.0	93.7	4.7

Table 4.2.2: Experiment 2.

Mean liveweight gains, weights of food consumed and food conversion ratios for all four pigs on each of the dietary treatments.

	Dietary Treatment				
	F	FCu	FM	FMCu	LSD
Total liveweight gain (kg)	57.5	61.8	60.8	57.8	4.4
Total food consumed (kg)	212	210	222	206	18
Food consumed per day (kg)	2.95	2.94	2.95	2.94	0.4
Liveweight gain per day (g)	808	866	811	830	66
Food conversion ratio	3.7	3.4	3.7	3.6	0.4

7df

Table 4.2.3: Experiment 2.

Mean backfat thickness (mm) at the shoulder, P₂ and loin sites of all pigs on each dietary treatment.

	Dietary Treatment				
	F	FCu	FM	FMCu	LSD
Backfat thickness					
Shoulder - maximum fat thickness (mm)	34.0	32.9	31.3	33.6	3.2
P ₂ - fat thickness (mm)	13.3	13.5	12.3	13.6	1.5
Loin - maximum fat thickness (mm)	15.1	16.3	14.5	16.1	1.9

7df

Measurements were taken on the day after slaughter.

Table 4.2.4: Experiment 2.

Mean fatty acid compositions (weight percent) of triacylglycerols isolated from the inner and outer backfat and perirenal fat of all pigs on each dietary treatment.

		Dietary Treatment				LSD 7df
Fatty acid (%)		F	FCu	FM	FMCu	
16:0	Inner	24.6	23.8	22.5	21.5	2.0
	Outer	23.8	24.5	20.9	21.3	2.5
	Perirenal	25.6	25.4	22.9	23.3	1.8
	LSD(a,b)	2.0, 0.9	1.8, 0.7	1.8, 1.8	2.1, 1.1	
16:1	Inner	5.0	5.3	4.3	4.7	0.6
	Outer	5.3	4.9	4.4	4.8	0.8
	Perirenal	5.2	4.9	4.1	4.9	0.7
	LSD(a,b)	0.4, 0.4	0.5, 0.5	0.4, 0.4	0.3, 0.3	
18:0	Inner	10.9	9.9	9.8	8.9	1.0
	Outer	9.7	9.2	8.3	7.5	1.4
	Perirenal	14.0	10.3	10.6	11.3	2.1
	LSD(a,b)	2.7, 1.3	1.7, 0.8	1.8, 1.8	3.1, 1.4	
18:1	Inner	47.3	49.5	39.9	42.4	2.6
	Outer	48.0	49.9	41.6	43.5	3.1
	Perirenal	44.4	47.1	38.4	41.2	2.9
	LSD(a,b)	3.0, 1.4	2.5, 0.9	2.8, 1.9	2.4, 1.1	
18:2	Inner	9.5	9.0	21.7	20.8	4.5
	Outer	10.5	9.5	23.1	21.3	3.1
	Perirenal	8.3	11.2	21.9	17.3	1.2
	LSD(a,b)	11, 1.2	1.9, 0.8	1.3, 1.4	2.1, 1.2	
18:3	Inner	2.6	2.5	1.8	1.8	0.4
	Outer	2.8	2.0	1.6	1.6	1.0
	Perirenal	2.5	1.4	2.1	2.0	1.5
	LSD(a,b)	0.4, 0.3	1.1, 0.7	0.8, 0.4	0.5, 0.5	

Results from the inner and outer backfat layers are the means of two sample sites. Appendix 6 lists the mean compositions of triacylglycerols from each of these two sites.

LSD values between sites are with 14df between backfat layers (a) and 7df between backfat layers and perirenal fat (b).

Within each treatment there were differences in the fatty acid compositions of the triacylglycerols between the sample sites. In general the perirenal fat was the most saturated and the outer backfat layer was the least saturated. Thus, palmitic acid concentration appeared to be highest in the perirenal fat and lowest in the outer backfat triacylglycerols. However, these differences were only significant with the FM treatment ($p \leq 0.05$). This pattern was also true for the stearic acid concentration of the triacylglycerols and was significant for the F, FM and FMCu treatments ($p \leq 0.01$). The concentration of oleic acid was higher in the outer backfat compared with the perirenal triacylglycerols in all treatments except FMCu. Similarly, linoleic acid concentrations were significantly higher in triacylglycerols from both layers of backfat relative to the perirenal fat for the basal F diet ($p \leq 0.05$). There were no site differences in the palmitoleic and linolenic acid concentrations in the triacylglycerols ($p > 0.05$).

Slip points were measured in all triacylglycerol samples; the mean treatment values are given in Table 4.2.5 and the results for the shoulder and loin sites respectively are listed in Appendix 7. With all treatments the perirenal fat melted at temperatures significantly higher than those of the backfat samples ($p \leq 0.01$), reflecting the degree of saturation of the component triacylglycerols. Similarly the inner backfat had a higher melting point than the outer backfat triacylglycerols ($p \leq 0.01$) although with the FM treatment the melting points of the two layers of backfat were not significantly different from each other ($p > 0.05$).

The positional distribution of the fatty acids in the triacylglycerols is given in Table 4.2.6. Adipose tissue triacylglycerols from the pigs on all treatments had mainly palmitic acid esterified to the 2-position, with the

Table 4.2.5: Experiment 2.

Mean slip points (°C) of triacylglycerols isolated from the inner and outer backfat and perirenal fat of all four pigs on each of the dietary treatments.

	Dietary Treatment				LSD
	F	FCu	FM	FMCu	
Sample site					
Inner backfat	42.3	40.9	37.8	37.5	1.6
Outer backfat	38.8	38.0	35.3	34.9	2.7
Perirenal fat	45.4	43.3	41.3	40.9	2.1

7df

a.	2.9	1.4	4.4	2.1
b.	2.9	2.3	3.4	3.2

a. = Comparisons between inner and outer backfat 14 df

b. = Comparisons between backfat layers and perirenal fat 10 df

Appendix 7 lists the mean slip points at these two sites for each backfat layer, for all dietary treatments.

Table 4.2.6: Experiment 2.

Mean fatty acid compositions (weight percent) at the 2- and 1+3- positions of triacylglycerols isolated from the inner and outer backfat and perirenal fat of all pigs on each of the dietary treatments.

Positional distribution of fatty acids (wt.%)			Dietary Treatment				LSD (7df)
		F	FCu	FM	FCu		
16:0	Inner backfat	2	69.2	68.5	56.6	58.9	5.4
		1+3	2.8	1.8	6.4	3.5	
	Outer backfat	2	60.0	64.3	60.8	57.6	6.7
		1+3	5.5	5.6	1.0	3.7	
	Perirenal fat	2	66.4	66.4	57.4	58.2	9.6
		1+3	5.2	4.9	5.6	5.9	
16:1	Inner backfat	2	6.0	7.4	7.6	5.1	1.8
		1+3	4.5	7.8	2.6	4.8	
	Outer backfat	2	8.0	7.1	5.8	4.6	2.9
		1+3	4.2	3.1	3.8	5.0	
	Perirenal fat	2	6.0	6.3	8.7	7.4	2.8
		1+3	4.8	3.7	1.8	3.7	
18:0	Inner backfat	2	10.1	11.4	18.5	15.2	2.7
		1+3	11.3	9.1	5.7	6.3	
	Outer backfat	2	15.9	13.8	14.4	14.8	2.3
		1+3	11.3	9.1	5.7	6.3	
	Perirenal fat	2	9.9	11.3	14.6	15.6	4.8
		1+3	16.0	9.8	8.7	9.1	
18:1	Inner backfat	2	8.5	7.2	7.8	8.5	1.4
		1+3	66.9	69.8	55.0	58.3	
	Outer backfat	2	8.4	9.1	7.7	8.6	2.2
		1+3	68.4	71.0	58.4	60.6	
	Perirenal fat	2	6.4	9.2	9.2b	7.2	0.9
		1+3	63.4	66.1	52.9	58.2	
18:2	Inner backfat	2	5.4	5.5	9.5	12.3	2.9
		1+3	11.7	11.5	29.8	24.6	
	Outer backfat	2	7.6	5.7	11.2	14.4	3.7
		1+3	11.7	11.5	29.8	24.6	
	Perirenal fat	2	4.7	6.9	10.2	11.7	4.3
		1+3	10.1	13.4	27.8	20.1	

The composition of fatty acids at position 2 of the triacylglycerols was determined by the pancreatic lipase hydrolysis technique and the composition at positions 1+3 was determined by calculation.

other fatty acids esterified in this position present in much lower concentrations. At the 1+3 - positions oleic acid was the major component, followed by linoleic and stearic acids. Adipose tissue site differences within treatments were only significant between the oleic acid content at position 2 of triacylglycerols from the inner backfat and perirenal fat of pigs on the FM treatment and between the linoleic acid content at position 2 of triacylglycerols from the outer backfat and perirenal fat of pigs on the F treatment ($p \leq 0.05$). However, at positions 1+3 there was a tendency for the concentrations of stearic acid to decrease and for the concentrations of oleic acid to increase in progression from the perirenal fat to the inner and subsequently the outer backfat.

4.2.3.2 Effect of supplementing the diets with maize oil

The fatty acid compositions of the backfat and perirenal triacylglycerols from each treatment are given in Table 4.2.4. In the triacylglycerols from the pigs given the diets supplemented with maize oil (FM, FMCu), the concentrations of linoleic acid were much greater than in those from the pigs given the diets without maize oil (F, FCu); this increase in the concentration of linoleic acid was counterbalanced by decreases in the concentrations of the other fatty acids present in the triacylglycerol samples.

Irrespective of the level of copper in the diet, supplementary maize oil resulted in statistically significant decreases in the slip points of the triacylglycerols obtained from the perirenal fat and the inner and outer layers of backfat (Table 4.2.5).

The positional distributions of the fatty acids in the triacylglycerols are given in Table 4.2.6. Adipose tissue triacylglycerols from pigs on the F

and FCu treatments had mainly palmitic acid esterified in position 2 and very little of this fatty acid at the 1+3 - positions. The addition of maize oil to the diet reduced the concentration of palmitic acid at position 2 of the triacylglycerols from the perirenal and inner backfat depots of the pigs on both the copper-supplemented and basal treatments, but the palmitic acid concentration at the 1+3 - positions remained very low. The positional distribution of fatty acids in triacylglycerols from the outer layers of backfat from pigs on the FCu and FMCu treatments followed a similar pattern. However with pigs from the F and FM treatments the outer backfat triacylglycerols had similar concentrations of palmitic acid at position 2, while at positions 1+3 the concentrations of this fatty acid were lower in the pigs on the FM treatment. The distribution of palmitoleic acid between the 1+3 - and 2-positions of the triacylglycerols was fairly even and was largely unaffected by dietary maize oil.

Stearic acid concentration was also fairly evenly distributed between the 2- and 1+3 - positions of the triacylglycerols, although the concentration of this acid at the latter positions was slightly lower in the outer backfat triacylglycerols. Dietary maize oil tended to increase the concentration of stearic acid at the 2-position of the adipose tissue triacylglycerols, but it decreased the concentration of stearic acid esterified in the 1+3 positions. Oleic acid was predominantly esterified to the 1+3 positions of the triacylglycerols from the pigs on the F and FCu treatments, with very much less of this acid present at position 2. The presence of dietary maize oil had little effect on the concentration of oleic acid at position 2 but it decreased the concentration of oleic acid esterified at the 1+3 - positions of the triacylglycerols by approximately 15%. The concentration of linoleic acid esterified at the 1+3 - positions of the triacylglycerols from the pigs on the F and

FCu treatments was about twice that esterified at position 2. Supplementing the diets with maize oil resulted in a two-fold increase in the concentration of linoleic acid esterified at position 2 and a two to three-fold increase in the concentration of this acid esterified at positions 1+3 of the adipose tissue triacylglycerols.

4.2.3.3 Effect of supplementing the diets with copper

The fatty acid compositions of the adipose tissue triacylglycerols are given in Table 4.2.4.

Certain of the underlying trends were not always statistically evident. A comparison of the triacylglycerols from pigs on the F and FCu treatments showed a decrease in the concentrations of palmitic, stearic and linoleic acids and an increase in monounsaturated acid content when copper was added to the diet. These increases in triacylglycerol palmitoleic and oleic acid contents are still apparent when the results for the FM and FMCu treatments are compared despite the high linoleic acid concentrations attributable to the dietary maize oil supplement.

There was, however, no significant effect ($p>0.05$) of dietary copper on the triacylglycerol melting point (Table 4.2.5), although the slip points of triacylglycerols from the FCu (and FMCu) treatments appeared to be slightly lower than those of the triacylglycerols from the F (and FM) treatments.

Small differences due to the presence of dietary copper were noted in the positional distribution of the fatty acids in the triacylglycerols (Table 4.2.6). As described earlier (Section 4.2.3.2), triacylglycerols from the F treatment had mainly palmitic acid esterified in position 2, while oleic acid was the major component at the 1+3 positions. Irrespective of the maize oil contents of the diets, supplementation of the diets with copper resulted in increases in the concentrations of oleic acid esterified in positions 1+3 of the triacylglycerols obtained from all three types of adipose tissues.

4.2.4 Adipose tissue stearoyl CoA desaturase activity

4.2.4.1 Comparisons between different adipose tissue sites

Stearoyl CoA desaturase activity was measured in the microsomal fractions of perirenal fat and both the shoulder and loin regions of the two backfat layers. The results of the analyses are given in Table 4.2.7. The two samples *i.e.* from the shoulder and loin regions, from each backfat layer had enzyme activities which were not significantly different from each other ($p > 0.05$).

Within treatments, the inner and outer backfat layers had desaturase activities significantly different from each other in pigs from the F ($p < 0.001$), FCu ($p \leq 0.01$) and FM ($p \leq 0.05$) treatments. The outer backfat layers had higher desaturase activities than the inner layers in pigs from all treatments except FCu. Similarly, for each treatment, both backfat layers had desaturase activities higher than those of the corresponding perirenal fat samples ($p < 0.01$) except for the FM inner backfat layers, the enzyme activities of which were not significantly different from those observed for the FM perirenal fat.

Table 4.2.7: Experiment 2.

Mean stearoyl coenzyme A desaturase activities of adipose tissue microsomes (μmol oleate formed min^{-1} mg protein $^{-1}$) isolated from perirenal fat and from the shoulder and loin regions of both layers of backfat.

Adipose tissue site		Dietary Treatment					7df
		F	FCu	FM	FMCu	LSD	
Inner backfat	Shoulder	0.845	1.287	0.684	0.805	0.13	
	Loin	0.878	1.279	0.626	0.830	0.15	
Outer backfat	Shoulder	0.984	1.051	0.825	0.952	0.10	
	Loin	1.183	1.063	0.834	0.845	0.15	
Perirenal fat		0.553	0.742	0.644	0.566	0.19	
LSD a		0.12	0.16	0.13	0.16		
b		0.18	0.19	0.14	0.17		

Microsomes (0.5 mg protein) were incubated with 1.2 mM NADH and 60 μM 1- ^{14}C -stearoyl CoA (5.5 $\mu\text{Ci/mol}$) in 1 ml total volume of 0.1 M potassium phosphate buffer, pH 7.4, at 37°C for ten minutes and assayed for ^{14}C -labelled monounsaturated fatty acids.

LSD values between sites are with 14df between backfat layers (a) and 7df between backfat layers and perirenal fat (b).

4.2.4.2 Effect of supplementing the diets with maize oil

Stearoyl Coenzyme A desaturase activity was also found to be significantly altered by the presence of maize oil in the diet (Table 4.2.7). Microsomes isolated from the inner backfat of the pigs on the F and FCu treatments had higher desaturase activities than those from the inner backfat of the pigs on the FM and FMCu treatments ($p \leq 0.001$). Similarly, desaturase activities in the outer backfat layers were significantly higher for the pigs on the F and FCu treatments compared with the values obtained for the corresponding maize oil-supplemented treatments ($p \leq 0.01$). There were no significant differences in enzyme activity between any of the perirenal fat samples ($p > 0.05$). Thus, with the exception of the perirenal fat, the addition of maize oil to the diet resulted in a significant decrease in the stearoyl CoA desaturase activity of pig adipose tissue.

4.2.4.3 Effect of supplementing the diets with copper

Dietary copper appeared to significantly enhance the activity of stearoyl CoA desaturase in the inner backfat of pigs given the diets unsupplemented with maize oil *i.e.* comparison of treatments F and FCu ($p \leq 0.001$). Similarly microsomes from the inner backfat layer of the pigs on the FMCu treatment had a higher desaturase activity than those of the same tissue in the pigs on the FM treatment.

These results indicate that dietary copper and maize oil have opposing effects on the activity of stearoyl CoA desaturase. Thus, desaturase activity

in adipose tissue of the pigs on the FMCu treatment is higher than that in adipose tissue of the pigs on the FM treatment, but significantly lower than that in adipose tissue of the pigs on the FCu treatment.

4.2.5 Plasma lipids; concentration in plasma and fatty acid composition

4.2.5.1 Changes in the concentration of the plasma lipids accompanying supplementation of the diets with maize oil and/or copper.

Samples of plasma obtained during the experiment were analysed for their lipid content. The changes in the plasma concentrations of cholesterol, cholesteryl esters, triacylglycerols and non-esterified fatty acids are illustrated in Figures 4.2.1, 4.2.2, 4.2.3 and 4.2.4 respectively. At week 0, before the animals received the experimental diets, the plasma concentrations of each lipid between treatments were not significantly different from each other ($p > 0.05$). However all the plasma lipids measured showed changes in concentration both with age and between treatments.

The concentration of cholesterol in the plasma decreased with age, the greatest fall occurring between weeks 4 to 7 of the experiment (Figure 4.2.1). Compared with the pigs on the F and FCu treatments, the animals on the FM and FMCu treatments had respectively lower concentrations of plasma cholesterol at week 4 ($p \leq 0.01$) and respectively higher concentrations at slaughter ($p \leq 0.01$).

The changes in plasma cholesteryl ester concentration are given in Figure 4.2.2. The plasma cholesteryl ester concentrations in the pigs from the F and FCu treatments increased slightly with age while in the plasma of pigs given the maize oil-supplemented treatments the cholesteryl ester concentration

Figure 4.2.1: Experiment 2. Mean plasma cholesterol concentrations (mg dl^{-1}) of all pigs on each dietary treatment at weeks 0, 4 and 7 of the experiment and at slaughter.

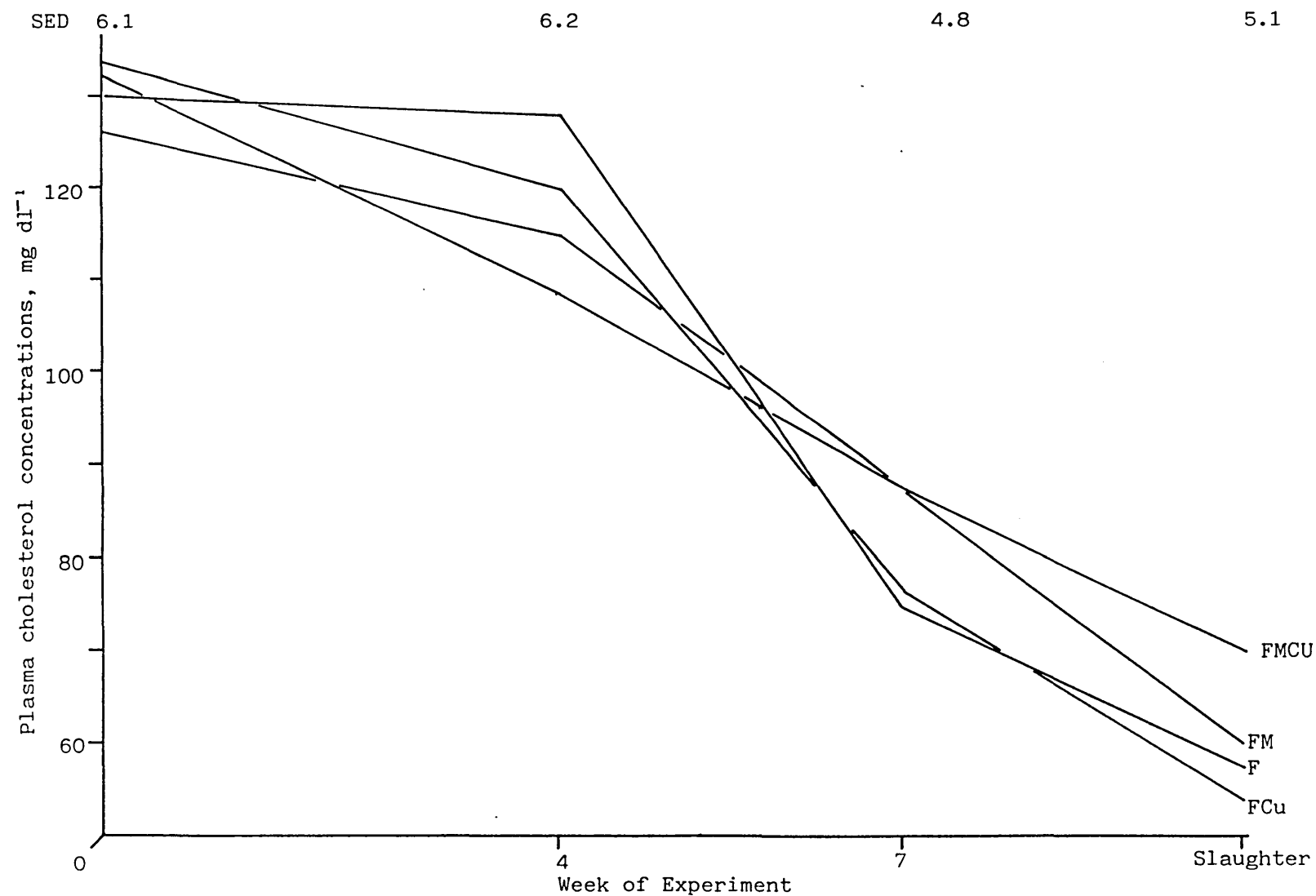


Figure 4.2.2: Experiment 2. Mean plasma cholesteryl ester concentrations (mg dl^{-1}) of all pigs on each dietary treatment at weeks 0, 4 and 7 of the experiment and at slaughter.

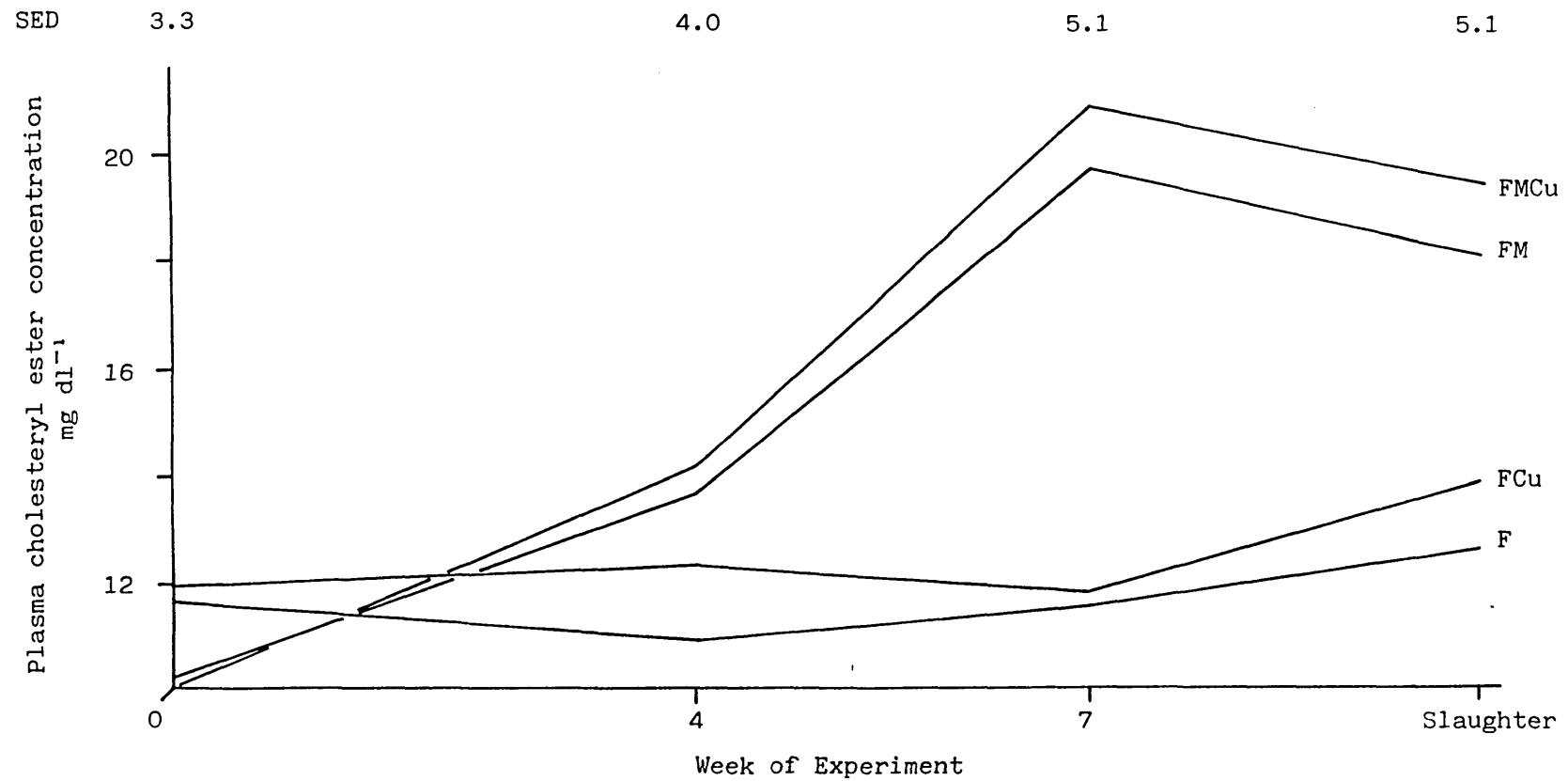


Figure 4.2.3: Experiment 2. Mean plasma triacylglycerol concentrations (mg dl^{-1}) of all pigs on each dietary treatment at weeks 0, 4 and 7 of the experiment and at slaughter.

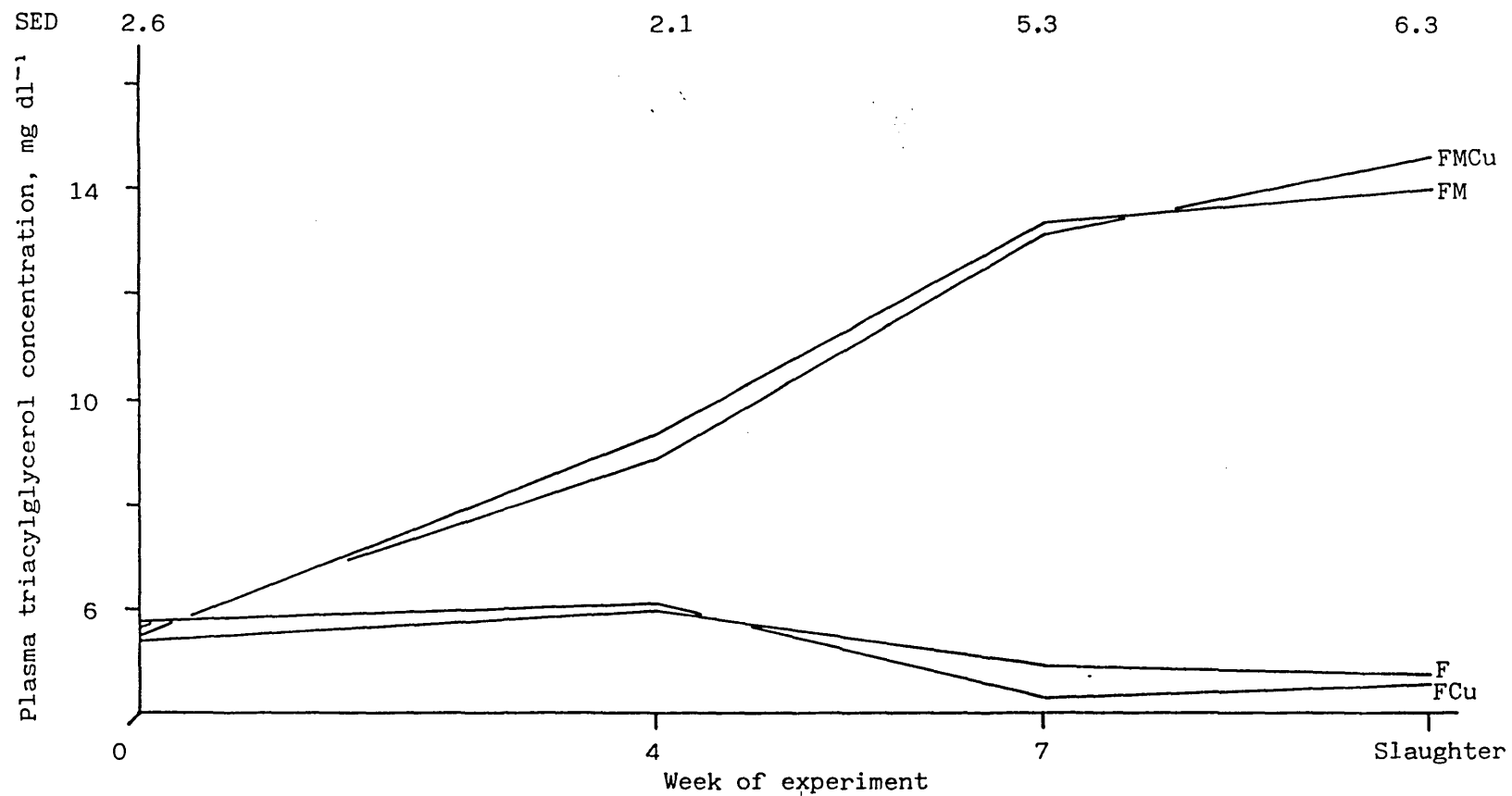
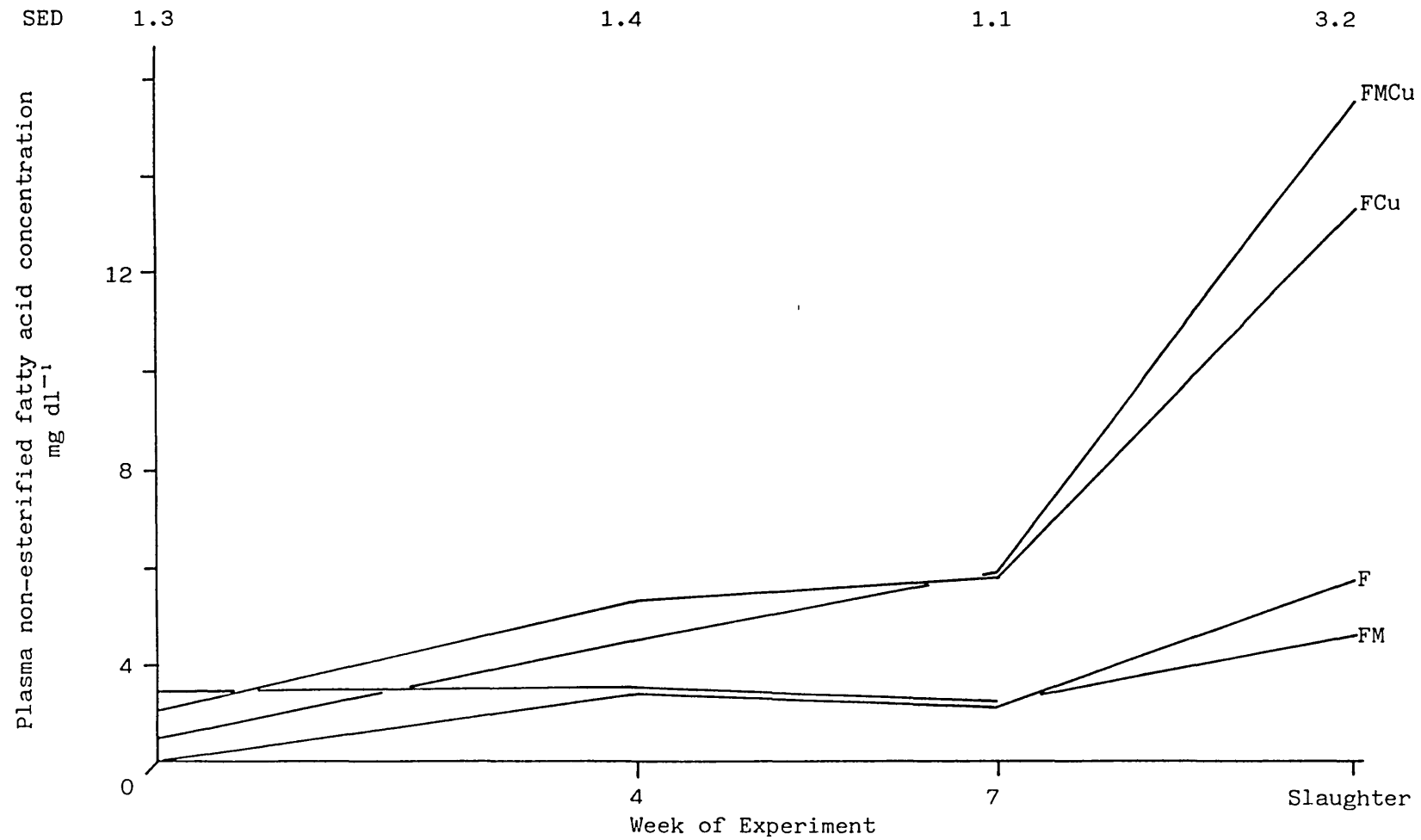


Figure 4.2.4: Experiment 2. Mean plasma non-esterified fatty acid concentrations (mg dl^{-1}) of all pigs on each dietary treatment at weeks 0, 4 and 7 of the experiment and at slaughter.



increased sharply until week 7, and then decreased slightly until slaughter.

A significant effect of dietary maize oil was also noted with the plasma triacylglycerol concentrations (Figure 4.2.3). From the beginning of the experiment until slaughter there was a marked increase in the triacylglycerol concentration of the plasma from pigs given the maize oil treatments while for the pigs on the F and FCu treatments the concentration of plasma triacylglycerols decreased slightly with age. The final concentrations at slaughter of both the cholesteryl esters and triacylglycerols were, therefore, much greater in the plasma of pigs given the maize oil-supplemented treatments ($p \leq 0.001$).

In contrast, the concentrations of plasma non-esterified fatty acids in the pigs appeared to be unaffected by the presence of maize oil in the diet. This plasma fraction was the only one the concentration of which was influenced by dietary copper (Figure 4.2.4). The concentration of unesterified fatty acids in the plasma in the pigs from the F and FM treatments increased slightly from the beginning of the experiment until slaughter, although the final values at slaughter were not much greater than those observed at the start. However, with the pigs on the two treatments in which the diets were supplemented with copper, the plasma non-esterified fatty acid concentrations were greater than those observed for the pigs on the F and FM treatments by week 4 and increased sharply from week 7 to slaughter, so that the final plasma concentrations were much higher than those of the basal F and FM treatments ($p \leq 0.001$).

The plasma concentrations of cholesterol, cholesteryl ester and triacylglycerols were largely unaffected by dietary copper. In most cases the values for the pigs on the FCu treatment were not significantly different from those of the pigs on the F treatment ($p > 0.05$). The same was also true of the

pigs on the FM and FMCu treatments.

4.2.5.2 Changes in the fatty acid compositions of the plasma lipids accompanying supplementation of the diets with maize oil and/or copper.

The fatty acid compositions of the plasma lipid classes were determined, but owing to the small size of the samples, only the concentrations of palmitic, stearic, oleic and linoleic acids were measured.

Analysis of the fatty acid compositions of the plasma cholesteryl esters at the beginning of the experiment revealed roughly equal quantities of palmitic, oleic and linoleic acids (Table 4.2.8). The concentration of stearic acid was much lower and did not alter significantly throughout the experiment. Palmitic acid concentrations decreased slightly in the plasma cholesteryl esters from pigs on all four treatments despite a large increase at week 4 in the plasma of pigs on the F treatment ($p \leq 0.01$). Also, during the latter half of the experiment the palmitic acid concentration was lower in the plasma cholesteryl esters of pigs from the two maize oil-supplemented treatments ($p \leq 0.05$).

The oleic acid content of the cholesteryl esters did not vary significantly during the experiment ($p > 0.05$), in the plasma of pigs of either the F or FCu treatments, except for an increase in concentration at week 7 in the plasma of pigs on the copper supplemented treatment, FCu ($p \leq 0.01$). The levels of oleic acid in the cholesteryl esters from the plasma of pigs on the two maize oil treatments were similar from weeks 0 to 4 and then declined until slaughter.

The linoleic acid concentration of the plasma cholesteryl esters from pigs given the F and FCu treatments decreased from weeks 0 to 7 but then increased

Table 4.2.8: Experiment 2.

Effect of dietary maize oil and/or copper on the fatty acid compositions (weight percent) of plasma cholesteryl esters from all pigs on each dietary treatment.

Dietary treatment	Fatty acid	Week of sampling			Slaughter
		Week 0	Week 4	Week 7	
F	16:0	30.1	45.3	34.9	23.1
	18:0	12.1	2.5	13.6	4.6
	18:1	32.4	30.3	31.5	30.1
	18:2	25.4	22.4	20.0	42.2
FCu	16:0	27.0	33.1	30.7	21.7
	18:0	10.0	17.8	7.7	14.7
	18:1	31.0	29.5	46.4	30.8
	18:2	32.1	19.6	15.3	32.8
FM	16:0	33.6	40.6	15.9	23.3
	18:0	14.1	5.7	14.2	6.7
	18:1	38.5	30.0	16.3	27.1
	18:2	13.8	23.8	53.3	43.0
FMCu	16:0	26.7	37.6	19.3	17.9
	18:0	13.7	4.6	3.4	4.1
	18:1	32.7	34.8	24.0	21.3
	18:2	26.9	23.0	53.4	56.8
LSD	16:0	3.8	5.4	6.6	3.0
7df	18:0	3.2	6.3	6.7	6.2
	18:1	4.4	3.4	7.0	3.5
	18:2	5.2	4.3	6.4	6.1

until slaughter ($p < 0.001$). In contrast, the linoleic acid content of the plasma cholesteryl esters from pigs on the two maize oil treatments increased throughout the experiment, with the greatest increase ($p < 0.001$) occurring between weeks 4 and 7. There was also a small decrease in linoleic acid concentration ($p < 0.05$) in the plasma cholesteryl esters of pigs on the FM treatment from week 7 to slaughter so that the final plasma concentrations were similar to those of pigs on the F treatment.

In general, the plasma cholesteryl esters from pigs on the F and FCu treatments had high concentrations of oleic and palmitic acids and lower concentrations of linoleic and stearic acids. The cholesteryl esters from the plasma of pigs on the two maize oil treatments displayed a different pattern; stearic acid concentration remained low and the amount of linoleic acid was greatly increased. The concentrations of palmitic and oleic acids were midway between the concentrations of stearic and linoleic acids.

The changes in plasma triacylglycerol composition are given in Table 4.2.9. Palmitic acid concentration in the triacylglycerols increased in all four treatments, although there were small decreases in the concentration of this fatty acid from weeks 4 to 7 in the plasma triacylglycerols of pigs on the maize oil-supplemented treatments ($p < 0.05$). The concentration of stearic acid did not change significantly within the plasma triacylglycerols of pigs from each treatment, but was greatest in the plasma triacylglycerols of pigs on the FCu treatment and lowest in the plasma triacylglycerols of pigs given the dietary treatments supplemented with maize oil. The oleic acid content of the plasma triacylglycerols did not change appreciably either with age or between treatments. Linoleic acid concentration in the plasma triacylglycerols of pigs on the F and FCu treatments also did not change appreciably and was significantly

Table 4.2.9: Experiment 2.

Effect of dietary maize oil and/or copper on the fatty acid compositions (weight percent) of plasma triacylglycerols from all pigs on each dietary treatment.

Dietary treatment	Fatty acid	Week of sampling			Slaughter
		Week 0	Week 4	Week 7	
F	16:0	29.4	53.1	45.0	41.1
	18:0	17.2	2.2	11.8	16.8
	18:1	36.3	31.6	32.5	31.0
	18:2	17.1	12.7	10.8	11.2
FCu	16:0	38.7	27.6	40.7	45.1
	18:0	16.5	24.1	15.8	13.8
	18:1	37.9	26.4	35.2	30.4
	18:2	7.0	7.1	8.3	10.7
FM	16:0	43.6	35.4	20.6	39.2
	18:0	21.4	7.0	9.1	10.7
	18:1	25.0	31.9	32.8	28.5
	18:2	9.2	25.8	37.5	21.7
FMCu	16:0	26.3	35.5	23.4	38.6
	18:0	16.4	15.6	7.9	7.1
	18:1	37.0	26.6	30.1	36.7
	18:2	20.4	22.4	38.1	17.6
LSD 7df	16:0	4.8	11.4	6.1	3.4
	18:0	3.4	6.7	3.3	3.7
	18:1	4.0	4.7	3.2	3.6
	18:2	4.2	5.3	6.9	3.4

lower than in the plasma triacylglycerols from pigs on the maize oil-supplemented treatments ($p \leq 0.01$). In these two latter treatments the linoleic acid concentration of the plasma triacylglycerols increased from week 0 to week 7 and then decreased until slaughter.

The compositions of the plasma non-esterified fatty acids are given in Table 4.2.10. Palmitic acid concentration declined very gradually in the plasma fatty acid fraction of pigs of all four treatments except for an increase ($p \leq 0.05$) at week 4 in the plasma of pigs on the F treatment. Stearic acid concentration in the plasma non-esterified fatty acids of pigs of all treatments increased steadily so that the stearic acid concentration of the plasma fatty acids at week 7 was significantly higher than at week 0 ($p \leq 0.01$), and then decreased until slaughter. A gradual increase in oleic acid content of the plasma unesterified fatty acids was observed in pigs from all four treatments with the final concentrations at slaughter being much higher in the plasma unesterified fatty acid fraction of the pigs given either of the two copper-supplemented treatments ($p \leq 0.01$). The linoleic acid concentration was the lowest of the four fatty acids measured and decreased gradually in the plasma fatty acid fraction of pigs on the F and FCu treatments. The amount of linoleic acid in the plasma fatty acids increased slightly in the pigs on the FM treatment but did not change significantly in the plasma of pigs given the FMCu treatment.

Thus, in general, dietary maize oil had a significant effect on the fatty acid compositions of the plasma cholesteryl esters and triacylglycerols, resulting in increased concentrations of linoleic acid in these plasma fractions. This was also evident, to a lesser extent, with the plasma non-esterified fatty acids. However the plasma unesterified fatty acids from pigs given the copper-

Table 4.2. 10: Experiment 2.

Effect of dietary maize oil and/or copper on the fatty acid compositions (weight percent) of plasma non-esterified fatty acids from all pigs on each dietary treatment.

Dietary treatment	Fatty acid	Week of sampling			Slaughter
		Week 0	Week 4	Week 7	
F	16:0	44.0	62.9	48.3	45.0
	18:0	21.3	15.0	30.9	17.5
	18:1	15.6	13.8	16.1	28.3
	18:2	19.1	7.9	4.7	9.3
FCu	16:0	54.2	44.3	44.0	38.6
	18:0	9.8	21.8	28.5	14.7
	18:1	20.5	15.9	20.5	40.7
	18:2	15.4	18.1	7.0	6.0
FM	16:0	43.1	43.6	37.4	37.1
	18:0	24.7	31.6	24.9	17.2
	18:1	14.8	13.2	22.4	24.5
	18:2	17.4	11.6	15.4	21.2
FMCu	16:0	39.3	46.4	38.3	33.6
	18:0	27.8	22.1	25.4	16.6
	18:1	17.3	15.9	20.2	37.6
	18:2	15.5	15.7	16.1	12.3
LSD	16:0	4.1	4.9	3.2	4.3
7df	18:0	3.9	5.1	4.8	3.9
	18:1	2.5	4.4	4.7	6.4
	18:2	3.6	4.1	3.0	5.6

supplemented diets had higher concentrations of oleic acid than did the plasma non-esterified fatty acids of pigs on either of the corresponding treatments, the diets of which were not supplemented with copper. Apart from this difference, dietary copper appeared to have no major influence on the fatty acid compositions of the plasma lipid classes measured.

4.2.6 Summary of results

In general these results suggest that, as with the previous experiment, the effect of dietary maize oil on the structure, composition and physical properties of the adipose tissue triacylglycerols of pigs is much greater than that of supplementary copper.

No differences between treatments were noted in the growth rates or carcass results and this is also consistent with the findings of Experiment 1. Analysis of the fatty acid compositions of the triacylglycerol samples shows that, within pigs of each dietary treatment, the outer backfat layer was the most and the perirenal fat the least ^{un-}saturated. Supplemental dietary copper was associated with a higher concentration of monounsaturated fatty acids even when dietary maize oil greatly increased the concentration of linoleic acid in the adipose tissue triacylglycerols. In contrast, the melting points of the adipose tissue triacylglycerols only showed differences accompanying the presence of maize oil, as dietary copper had no significant effect.

Positional differences in the fatty acid distribution of the adipose tissue triacylglycerols were also studied. In general, palmitic acid was the major fatty acid esterified in position 2 and oleic acid was the main component at the 1+3 - positions. The presence of copper in the diet was

associated with an increase in the linoleic acid concentration at the 2-position and in the overall oleic acid content of the triacylglycerols. Maize oil in the diet was associated with much greater levels of linoleic acid, particularly at positions 1+3.

Stearoyl Coenzyme A desaturase activity was markedly influenced by the nature of the diet. Enzyme activity in the adipose tissue was increased by dietary copper and reduced by dietary maize oil. Site differences were also noted; within each treatment the outer backfat layer had the highest desaturase activity and the perirenal fat the lowest. This corresponds with the degree of unsaturation of the triacylglycerols isolated from these sites.

The plasma concentrations of cholesterol, cholesteryl esters and triacylglycerols were affected by dietary maize oil whereas the concentration of plasma non-esterified fatty acids were influenced more by dietary copper. The fatty acid compositions of the plasma cholesteryl esters and triacylglycerols showed significant differences between the pigs given the basal and maize oil-supplemented treatments, mainly as a result of the increased linoleic acid concentration. Dietary copper had no significant effect on the fatty acid compositions of the plasma lipids studied.

4.3 Discussion of results of Experiment 2

4.3.1 Growth rates, food intakes and feed conversion ratios

The animals from this experiment received diets similar to those on the fishmeal-based treatments of Experiment 1. In both of these experiments the growth rates of the pigs did not appear to be affected by the presence of either copper, maize oil or both of these supplements in the diet (Tables 3.2.1. and 4.2.1). However, the pigs from Experiment 2 which received either of the diets supplemented with copper (FCu and FMCu) did show a tendency towards more efficient feed conversion ratios and greater daily liveweight gains than did the animals given the corresponding treatments the diets of which did not contain copper (F and FM). These differences were not significant at the 5% level and this lack of treatment differences may be explained by factors similar to those discussed in connection with the results obtained from Experiment 1 (Section 3.3.1).

The pigs from Experiment 1 which received the copper-supplemented treatments displayed improved growth rates up to 46 kg liveweight when compared with the animals that received the diets lacking additional copper (Table 3.3.1). However the pigs from Experiment 2 were started on the diets at a later age than those from Experiment 1, and so reached 46 kg liveweight after only two weeks on the experimental diets. This is not sufficient to calculate accurately the daily liveweight gain up to this weight.

Although the feed-related results from these two experiments are not strictly comparable, it was noted that the feed conversion ratios of pigs from Experiment 2 were much higher than those from Experiment 1. Daily liveweight gains were similar between the experiments but the animals from Experiment 1 consumed much less food per day than those from Experiment 2.

The diets from each experiment were of similar composition but were made up using different supplies of feed components. However it is unlikely that this would have such a marked effect on the feed conversion ratios. The animals were fed on the same feeding scale and so pigs at the same liveweight received similar amounts of food. However, in Experiment 1 the animals were fed twice a day and in Experiment 2, only once daily. This, together with the differences in liveweight at the start of the trial, may contribute to the differences in feed-related results. Another factor which may be significant is the breed of pig; the animals used for Experiment 1 were LW x (LR x LW) crosses, whereas pure-bred Landrace pigs were used for Experiment 2. Differences in growth rates between different breeds of pig have been noted by many authors including Johnson *et al.* (1973) who reported differences in average daily gain and daily feed intakes between pure-bred Hampshire, Duroc and Yorkshire pigs and between cross-bred pigs of these three breeds.

4.3.2 Thickness of subcutaneous backfat

The carcass shoulder, P₂ and loin backfat measurements were not significantly different at the 5% level, between pigs on the four treatments of this experiment (Table 4.2.3). This is consistent with the fact that the growth rates of the animals were also similar between treatments (Table 4.2.1). In addition these results correspond with those of Experiment 1 where supplementary copper and/or maize oil in the diets were not associated with any changes in backfat thickness. The factors which may cause this lack of differences in backfat thickness between pigs on different diets have already been considered with regard to the results of Experiment 1 (Section 3.3.2).

The subcutaneous backfat thicknesses of the pigs from Experiment 2 were

much smaller than those of the animals given the corresponding treatments of Experiment 1 (Table 3.2.3). As discussed previously, in Section 4.3.1, the feed-related results were similar between the two experiments, although the pigs from Experiment 2 consumed more food than did those from Experiment 1. This may have a bearing on the thickness of the subcutaneous adipose tissue but, as already suggested, this difference may also be a result of the heavier starting weights of the animals on Experiment 2. In addition the animals used in each experiment were of different breeds and so may have differing propensities to fat deposition. This last suggestion is consistent with work by Metz (1984) and Metz and Dekker (1981) who reported that Large White and Pietrain pigs, fed and housed under comparable conditions, have differing amounts of total bodyfat, and that the Pietrain pigs deposit less fatty acids per day than do the Large White animals. Other authors have also noted differences in fat metabolism between different breeds of pigs (Miller, 1984; Lea *et al.* 1970).

In Experiment 1, significant correlations were noted between the thicknesses of the backfat in the shoulder, P₂ and loin regions and the concentrations of both linoleic and oleic acids in the adipose tissue triacylglycerols (Figures 3.3.1 and 3.3.2). Similar plots using the results from Experiment 2 gave relationships which were not significant at the 5% level, probably owing to the small number of samples involved. Despite this lack of statistical significance, the results followed trends similar to those of Experiment 1 *i.e.* increasing backfat thicknesses at each site appeared to be associated with higher levels of oleic acid and lower levels of linoleic acid in the adipose tissue triacylglycerols. This suggests that similar relationships may exist in the pigs from both experiments, although there would be differences attributable to breed, age and other factors.

In accordance with the results of Experiment 1, no relationships, significant at the 5% level, were observed between the concentrations of the other fatty acids in the triacylglycerols and the carcass backfat thicknesses. Mersmann and MacNeil (1985) reported that the backfat thickness was correlated with the plasma concentrations of both non-esterified fatty acids and triacylglycerols. However, when the results from this present experiment were examined, no relationships were found that were significant at the 5% level. This was not entirely unexpected as a correlation between plasma triacylglycerol concentration and backfat thickness precludes endogenous fatty acid synthesis and so suggests that only dietary fat is responsible for the growth of the subcutaneous backfat layers. Similarly a significant negative correlation between plasma non-esterified fatty acid concentration and backfat thickness discounts factors such as hormonal effects and implies that circulating fatty acids derive solely from fat mobilization in the subcutaneous adipose tissue of the pig back. These correlations clearly oversimplify the factors which affect the thickness of the backfat and so it is not surprising to note that the correlations obtained by Mersmann and MacNeil (1985) were quite small in magnitude ($p \leq 0.05$) and generally tended to be significant at this level only when large numbers of pigs were included in the study.

4.3.3 Composition, structure and physical properties of adipose tissue triacylglycerols

4.3.3.1 Comparisons between different adipose tissue sites

Samples of adipose tissue were taken from the perirenal fat and the inner and outer layers of subcutaneous backfat. Table 4.2.4 gives the mean

fatty acid compositions of the triacylglycerols from each tissue site; the full results from the shoulder and loin regions of each layer of backfat are given in Appendix 6.

Differences were noted between the fatty acid compositions of the triacylglycerols at the three adipose tissue sites (Table 4.2.4). In general, the outer layer of backfat was the most unsaturated and contained the highest quantities of oleic and linoleic acids. In contrast the perirenal fat, containing the greatest amounts of palmitic and stearic acids, was the most saturated of the three adipose tissue samples.

These results are in agreement both with the work of Moore *et al.* (1969) and Santoro (1984) and with the results of Experiment 1. As discussed earlier (Section 3.3.3.1) these site differences may be accounted for by differences in the activities of the various enzymes involved in lipid metabolism and by the effects of temperature (Christie *et al.*, 1972).

Christie and Moore (1970b) studied the relationships between the various fatty acids by plotting the concentrations of each against the concentration of oleic acid in the triacylglycerols at each site. When the results from this present experiment were examined, correlations were observed for all the fatty acids and, with the exception of the palmitoleic and linolenic acid regressions, the relationships were significant at the 5% level or above (Table 4.3.1). In general, for each fatty acid the gradients of the regressions were similar at the three adipose tissue sites *i.e.* the differences between the gradients were not significant at the 5% level. However the gradients of the regressions at the perirenal site tended to be less steep than those from the backfat sites. This suggests a smaller response of the perirenal

Table 4.3.1: Experiment 2.

Relationships between the concentrations (weight percent) of various fatty acids (x) and oleic acid (y) in triacylglycerols isolated from the inner and outer backfat and perirenal fat of pigs from all dietary treatments.

Fatty acid and adipose tissue site	Linear regression	Significance
16:0		
Inner backfat	$y = 0.98x + 22.08$	$p \leq 0.05$
Outer backfat	$y = 0.96x + 24.05$	$p \leq 0.01$
Perirenal fat	$y = 1.03x + 17.77$	$p \leq 0.05$
18:0		
Inner backfat	$y = 1.13x + 33.32$	ns
Outer backfat	$y = 1.23x + 34.32$	$p \leq 0.05$
Perirenal fat	$y = 1.01x + 29.46$	$p \leq 0.01$
18:2		
Inner backfat	$y = -0.62x + 54.22$	$p \leq 0.001$
Outer backfat	$y = -0.63x + 55.15$	$p \leq 0.001$
Perirenal fat	$y = -0.59x + 50.82$	$p \leq 0.001$

Regressions were calculated using results obtained from all four pigs on each of the four dietary treatments. The regressions obtained with palmitoleic and linoleic acids were not significant at the 5% level.

adipose tissue to dietary changes, and is in agreement with the corresponding results from Experiment 1 (Section 3.3.3.1).

The gradients of the linear regressions from Experiment 2 were compared with the corresponding relationships from Experiment 1. As the dietary treatments of Experiment 1 were based on either fishmeal or soyabean meal protein the correlations between the adipose tissue triacylglycerol concentrations of oleic acid and the other fatty acids were recalculated to include only the results from the pigs given the diets based on fishmeal protein. The gradients of the linear regressions from the two experiments are shown in Table 4.3.2.

The gradients of the relationships between palmitic and stearic acid concentrations and oleic acid concentration were much steeper with the results from the triacylglycerols of the pigs on Experiment 2 ($p < 0.001$). However a change in the triacylglycerol concentration of linoleic acid appeared to be counterbalanced by a change in the oleic acid concentration which was similar between the adipose tissue of pigs on both Experiments 1 and 2 ($p > 0.05$).

Linoleic acid cannot be synthesized by the pig and so the concentration of this acid in the adipose tissue triacylglycerols is influenced by the linoleic acid content of the diet. Thus it may be that the relationships between adipose tissue linoleic acid and oleic acid concentrations reflect the need to regulate the consistency of the fat and so maintain an acceptable ratio of saturated:unsaturated fatty acids. These might be similar between pigs on different experiments as, despite any differences in lipid metabolism between different pigs, the fluidity of the adipose tissue triacylglycerols must be kept within certain physiological limits. This is consistent with

Table 4.3.2: Experiment 2

Comparison of gradients from Experiments 1 and 2 for the relationships between the concentrations of various fatty acids (x) and oleic acid (y) in triacylglycerols isolated from the inner and outer backfat and perirenal fat of pigs from all treatments, the diets of which were based on fishmeal.

Fatty acid and adipose tissue site	Gradient of regression (b)	
	Experiment 1 (F treatments only)	Experiment 2
16:0		
Inner backfat	0.52	0.98
Outer backfat	0.42	0.96
Perirenal fat	0.45	1.03
18:0		
Inner backfat	0.35	1.13
Outer backfat	0.48	1.23
Perirenal fat	0.48	1.01
18:2		
Inner backfat	-0.56	-0.62
Outer backfat	-0.73	-0.63
Perirenal fat	-0.63	-0.59

Regressions were calculated using results obtained from all four pigs on each of the four dietary treatments of Experiment 2 and those from all four pigs on each of the four fishmeal-based treatments of Experiment 1. The regressions obtained with palmitoleic and linoleic acids were not significant at the 5% level.

the findings of Experiment 1 where it was suggested that the concentration of oleic acid in the adipose tissue triacylglycerols was to some extent influenced by the nature of the dietary fatty acids as oleic acid appeared to balance the level of saturated and unsaturated fatty acids in the adipose tissue triacylglycerols.

The relationships between the concentrations of the saturated fatty acids and the oleic acid contents of the triacylglycerols from the adipose tissue of pigs on Experiment 2 had steeper gradients than the corresponding regressions obtained with Experiment 1 (Table 4.3.2). This is consistent with the overall more saturated nature of triacylglycerols from the pigs on Experiment 1. However these triacylglycerols had melting points similar to those of triacylglycerols from pigs on the corresponding treatments of Experiment 2 (Tables 3.2.7 and 4.2.5). This discrepancy may be a result of the differences in the positional distributions of the fatty acids in the adipose tissue triacylglycerols of pigs on different experiments (Tables 3.2.8 and 4.2.6); in Experiment 1 differences in positional distribution were associated with quite marked differences in triacylglycerol melting points (Section 3.3.3.3). The differences in the degree of saturation and in the positional distribution of fatty acids of the adipose tissue triacylglycerols may arise from differences in lipid metabolism between the pigs on each experiment, perhaps as a result of the use of different breeds of pig.

In the experiment now reported, the positional distribution of fatty acids in the triacylglycerols was similar between samples taken from the different adipose tissue sites. This concurs with both the results of Experiment 1 and the work of Christie and Moore (1970a) and suggests that the acyltransferases at each of the three sites have similar fatty acyl CoA specificities.

The triacylglycerols from the adipose tissue of pigs on Experiment 2 had patterns of fatty acid distribution which were different from those of the adipose tissue from pigs on the corresponding fishmeal-based treatments of Experiment 1 (Tables 4.2.6 and 3.2.8). In particular the palmitic acid concentration at position 2 of the triacylglycerols was much greater in the adipose tissue samples from the pigs on Experiment 2, mainly at the expense of oleic acid. As the fishmeal diets used in each of these two experiments were similar in composition, the differences in the positional distribution of fatty acids in the triacylglycerols from the two experiments seems likely to be an effect of breed. LW x (LW x LR) crosses were used in Experiment 1 whereas in the present experiment the pigs were of the Landrace breed. In addition, the Large White pigs used by Christie and Moore (1970a) produced triacylglycerols with fatty acid distributions different from those of both Experiments 1 and 2 reported here.

These findings are consistent with Lea *et al.* (1970) who reported that the breed of pig can affect the iodine values *i.e.* degree of unsaturation, and melting points of adipose tissue triacylglycerols. In particular, they noted that half-Hampshire crosses showed a high proportion of pigs with excessively soft fat. These authors suggested that the positional distribution of the fatty acids in the triacylglycerols may be involved in these breed differences. However little work appears to have been carried out on the structure of triacylglycerols obtained from different breeds of pig.

The melting points of the triacylglycerols were also different between samples taken from different adipose tissue sites of the pigs from Experiment 2 (Table 4.2.5). In general, within treatments the triacylglycerols from the perirenal fat tissue had the highest melting points and those from the

outer backfat had the lowest. A similar trend was observed with the triacylglycerols obtained from the pigs on the fishmeal-based treatments of Experiment 1 (Table 3.2.7). These differences between sites correspond with the degree of unsaturation of the samples, with the more unsaturated triacylglycerol samples having the lower melting points. The effect of fatty acid unsaturation on triacylglycerol melting points will be discussed later in Sections 4.3.3.2 and 4.3.3.3.

4.3.3.2 Effect of supplementing the diets with maize oil

The presence of maize oil in the pig diets had a marked effect on the fatty acid compositions of the adipose tissue triacylglycerols (Table 4.2.4). In general dietary maize oil greatly increased the linoleic acid content of the adipose tissue triacylglycerols at the expense of all the other fatty acids. These results are comparable with those of Experiment 1 (Table 3.2.6), with the exception of the linolenic acid content of the triacylglycerols. The concentration of this fatty acid was greater in the triacylglycerols from the pigs on the maize oil-supplemented diets when compared with those on the control treatments of Experiment 1, but was less than in the triacylglycerols of animals on the control treatment of Experiment 2. However the fatty acid compositions of triacylglycerols from the adipose tissue of pigs on the control treatments in the two experiments were different from each other, with those from Experiment 2 having higher levels of linolenic acid than those from Experiment 1. These slightly differing responses to dietary maize oil may be a result of differences in lipid metabolism between the pigs from the two experiments.

As described in Section 3.3.3.3, the increase in adipose tissue linoleic

acid concentration arises from the high content of this fatty acid in the maize oil supplement. Dietary linoleate suppresses both *de novo* synthesis and the activity of the Δ^9 desaturase so that the deposited triacylglycerols contain relatively more of the dietary fatty acids and, in particular, linoleic acid (Jeffcoat & James, 1978). The effects of dietary maize oil on stearoyl CoA desaturase activity will be dealt with in Section 4.3.4.2.

With Experiment 1, the ratio of saturated:unsaturated fatty acids was found to correlate significantly with the triacylglycerol melting point (Figure 3.3.3). When the results from the present experiment were examined, a significant linear relationship was also obtained ($p < 0.001$, Figure 4.3.1). However the gradient of this linear regression was steeper than that obtained using the results from the pigs on the fishmeal-based treatments of Experiment 1. Similarly, the biased saturated:unsaturated fatty acid ratios, when plotted against the triacylglycerol slip points, also gave a significant correlation ($p < 0.001$, Figure 4.3.2) which had a steeper gradient than the corresponding linear regression calculated using the results from the pigs given the fishmeal-based treatments of Experiment 1. This linear regression, in keeping with the results of Experiment 1, also had a gradient which was approximately twice that of the regression calculated using the unbiased ratios of fatty acids, reflecting the greater effect of the more unsaturated fatty acids on the triacylglycerol melting points. The differences between corresponding gradients in the two experiments may be a result of the differing compositions and positional distributions of the fatty acids in the triacylglycerols from the adipose tissue of pigs used in each of the two experiments. As the differences in fatty acid composition between triacylglycerols from pigs on analogous treatments from the two experiments were relatively small, the positional distribution of the fatty acids in the adipose tissue triacylglycerols may be of

Figure 4.3.1: Experiment 2. Relationship between the ratios of the concentrations of saturated:unsaturated fatty acids and the slip points of triacylglycerols isolated from the inner and outer backfat and perirenal adipose tissue.

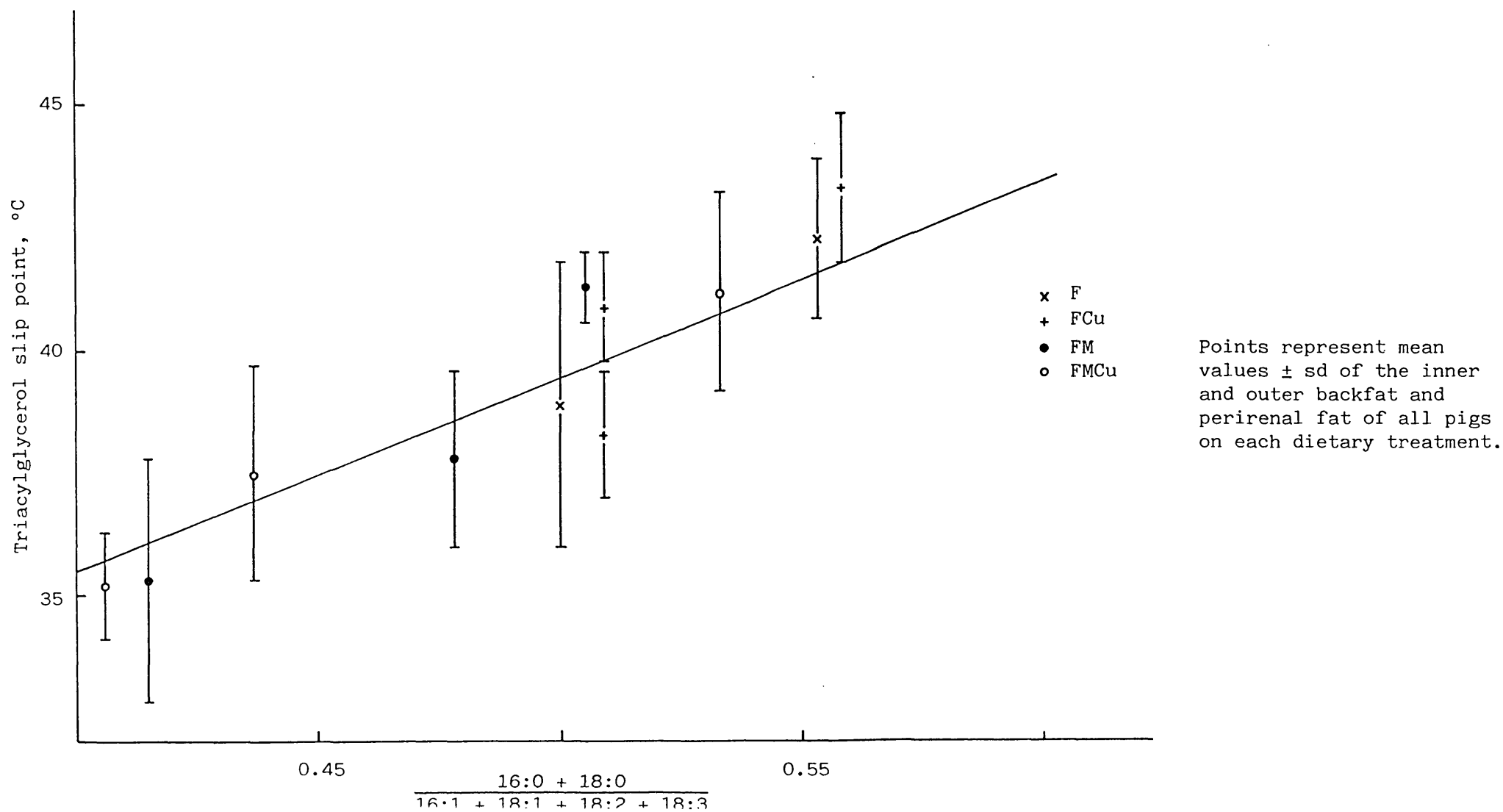
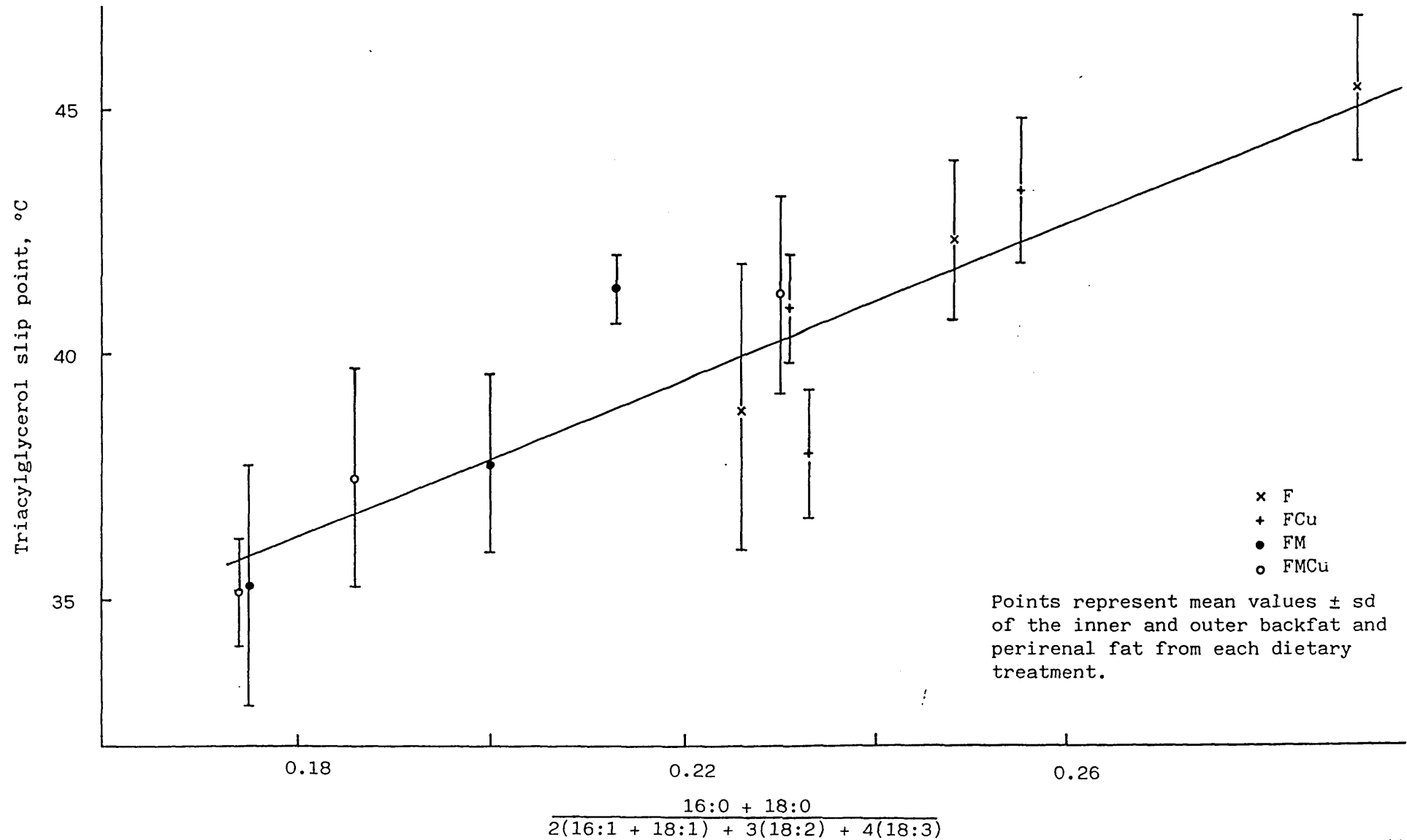


Figure 4.3.2: Experiment 2. Relationship between the biased ratios of the concentrations of saturated:unsaturated fatty acids and the slip points of triacylglycerols isolated from the inner and outer backfat and perirenal adipose tissue.



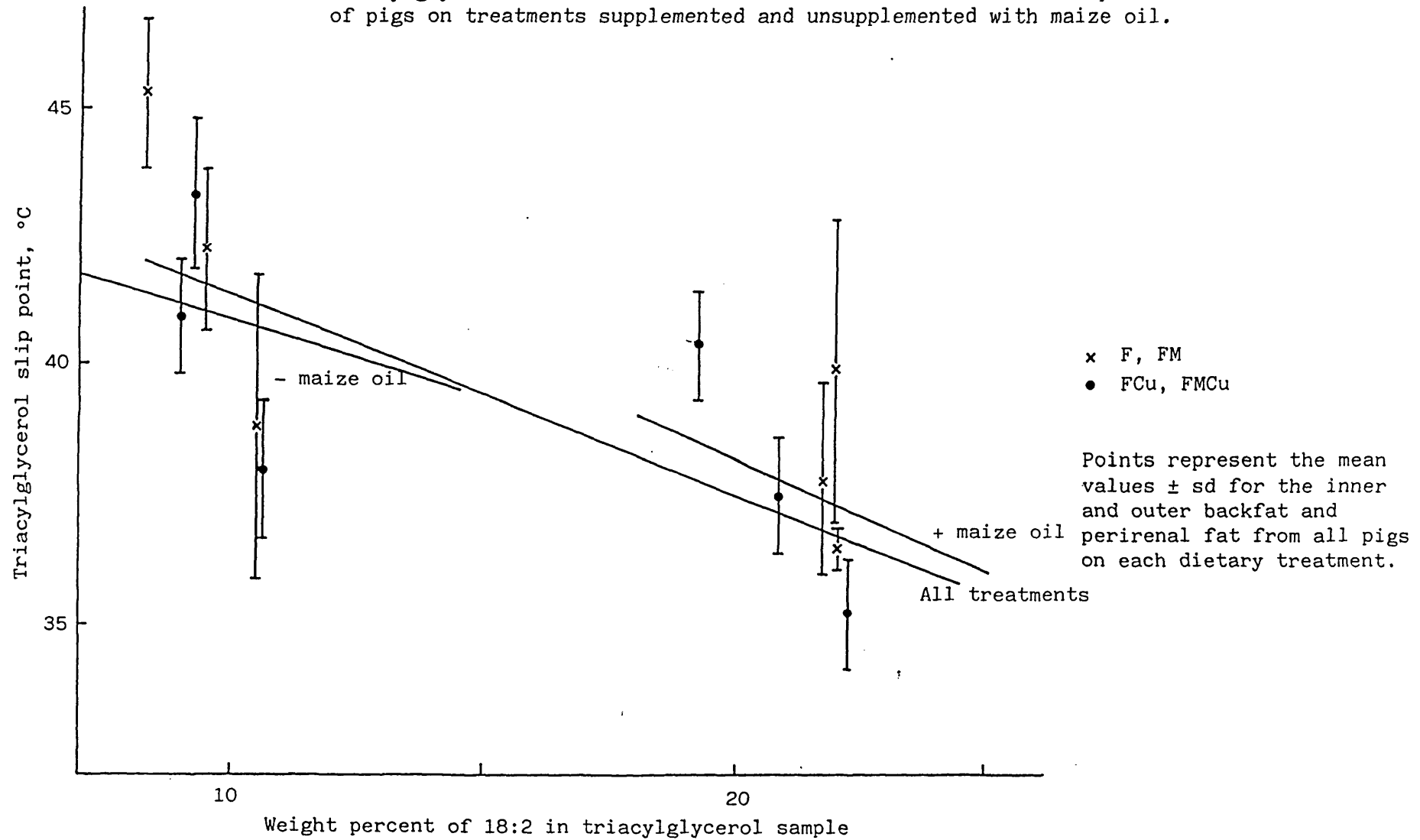
greater importance. In addition there may also be differences in the concentrations of the various triacylglycerol species present.

The higher levels of linoleic acid in the triacylglycerols from the adipose tissue of pigs given the maize oil-supplemented diets of Experiment 2 were accompanied by a lowering of the melting point when compared with the triacylglycerols from pigs on the control treatment (Table 4.2.5). In keeping with this the concentration of linoleic acid was found to correlate with the triacylglycerol slip point. Two regressions were calculated, corresponding to the results from the pigs on the maize oil-supplemented and unsupplemented treatments respectively. These relationships were not significantly different from each other ($p > 0.05$) and so when the concentrations of linoleic acid in the adipose tissue triacylglycerols of pigs from all four treatments were plotted against the triacylglycerol slip points a significant relationship was obtained ($p \leq 0.001$) and this is illustrated in Figure 4.3.3.

The relationship between the linoleic acid concentrations and the slip points of the adipose tissue triacylglycerols was steeper with the results of Experiment 2 compared with those of Experiment 1. In keeping with this, supplementation of the diets with maize oil was associated with a slightly greater decrease in triacylglycerol melting point with the animals on Experiment 2 despite a comparable increase in linoleic acid content of the adipose tissue triacylglycerols from pigs on the two experiments. This may be a result of differences in the positional distribution of fatty acids in the triacylglycerols from pigs on the two experiments or in the molecular species of triacylglycerols present.

In order to investigate further the effects of fatty acid positional

Figure 4.3.3: Experiment 2. Relationship between the linoleic acid concentration and the slip point of triacylglycerols isolated from the inner and outer backfat and perirenal fat of pigs on treatments supplemented and unsupplemented with maize oil.



distribution on triacylglycerol melting point, the concentrations of the various fatty acids at the 2- and 1+3 - positions of the triacylglycerols were plotted against the triacylglycerol melting point. At position 2 of the triacylglycerols the concentrations of palmitic and linoleic acids were found to correlate positive and negatively, respectively, with the slip point, whereas at positions 1+3 significant positive and negative relationships were only observed between the slip point and the respective concentrations of stearic and linoleic acids. The gradients and correlation coefficients of these various linear regressions are given in Table 4.3.3.

Table 4.3.3: Experiment 2.

Gradients and correlation coefficients for the linear relationships calculated between the concentrations of various fatty acids (wt. %) at the 2 and 1 + 3 positions (x) and the melting points (°C, y) of the adipose tissue triacylglycerols of pigs on all four dietary treatments.

Fatty acid	Triacylglycerol position	Gradient	Correlation coefficient	Significance
16:0	2 1 + 3	0.18 -	0.46 -	$p \leq 0.01$ ns
18:0	2 1 + 3	- 0.60	- 0.67	ns $p \leq 0.001$
18:2	2 1 + 3	-0.79 -0.24	-0.99 -0.74	$p \leq 0.001$ $p \leq 0.001$

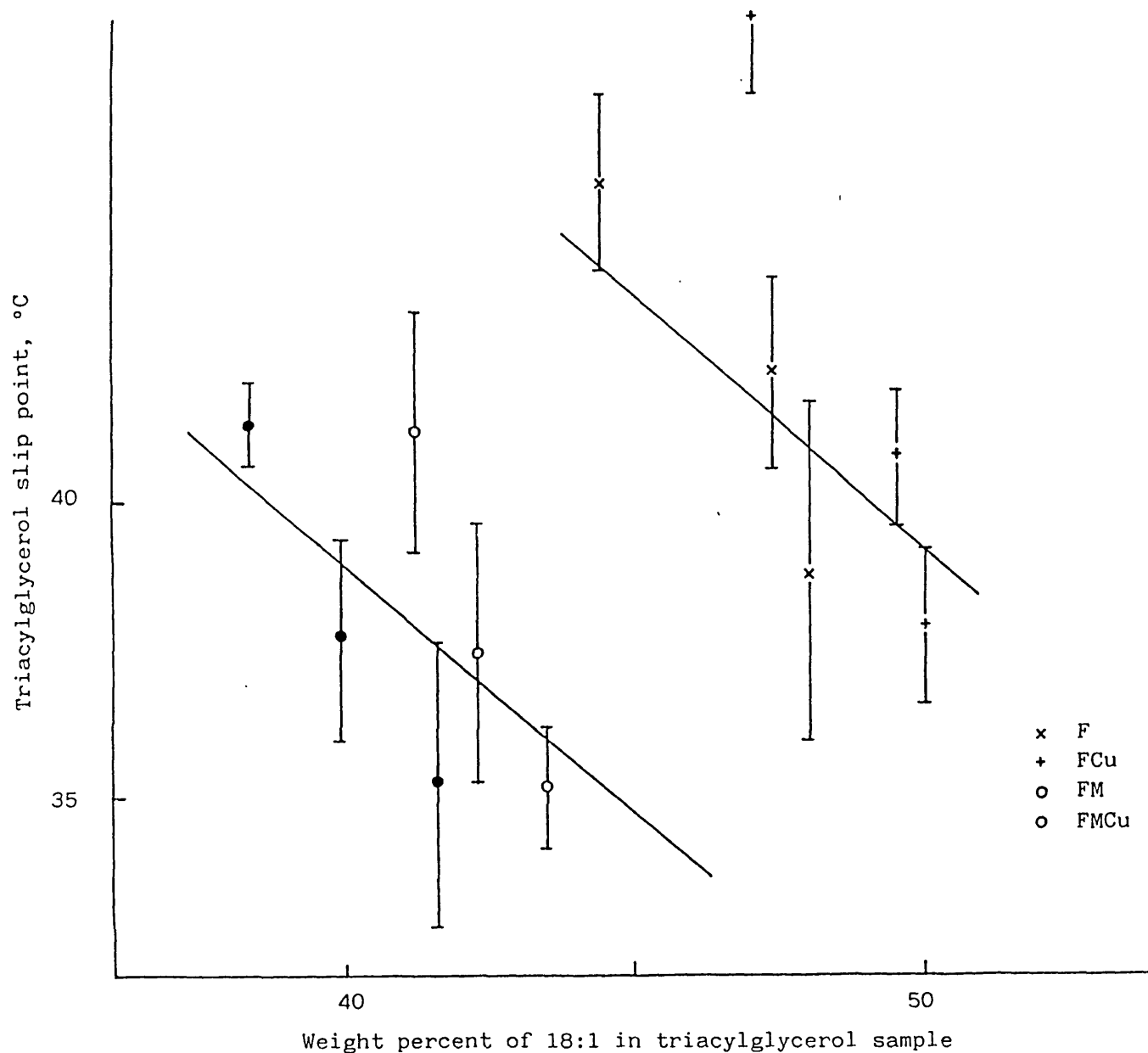
Palmitic acid concentration at position 2 of the triacylglycerols was positively correlated with the slip point. Higher levels of saturated fatty acids are associated with higher melting points and as palmitic acid is predominantly esterified in the 2-position an increase in the overall concentration of this fatty acid is accompanied by both an increase in palmitate concentration at the 2-position of the triacylglycerols and an increase in the melting point. Similarly, at positions 1+3, stearic acid concentration was positively correlated with the triacylglycerol slip point and this is in agreement with the work of Christie and Moore (1970b).

The concentrations of linoleic acid at both position 2 and positions 1+3 of the triacylglycerols were linearly related to the slip point of the triacylglycerols (Table 4.3.3). In each case an increase in linoleic acid content was associated with a decrease in the triacylglycerol melting point. As in Experiment 1 (Table 3.3.3) the gradient for the regression obtained with the linoleic acid concentrations at position 2 was much steeper than that at positions 1+3. This agrees with the suggestion that the positional distribution of linoleic acid has an effect on the triacylglycerol melting point, possibly as a result of the linoleic acid double bonds on the packing of the acyl chains.

4.3.3.3 Effect of supplementing the diets with copper

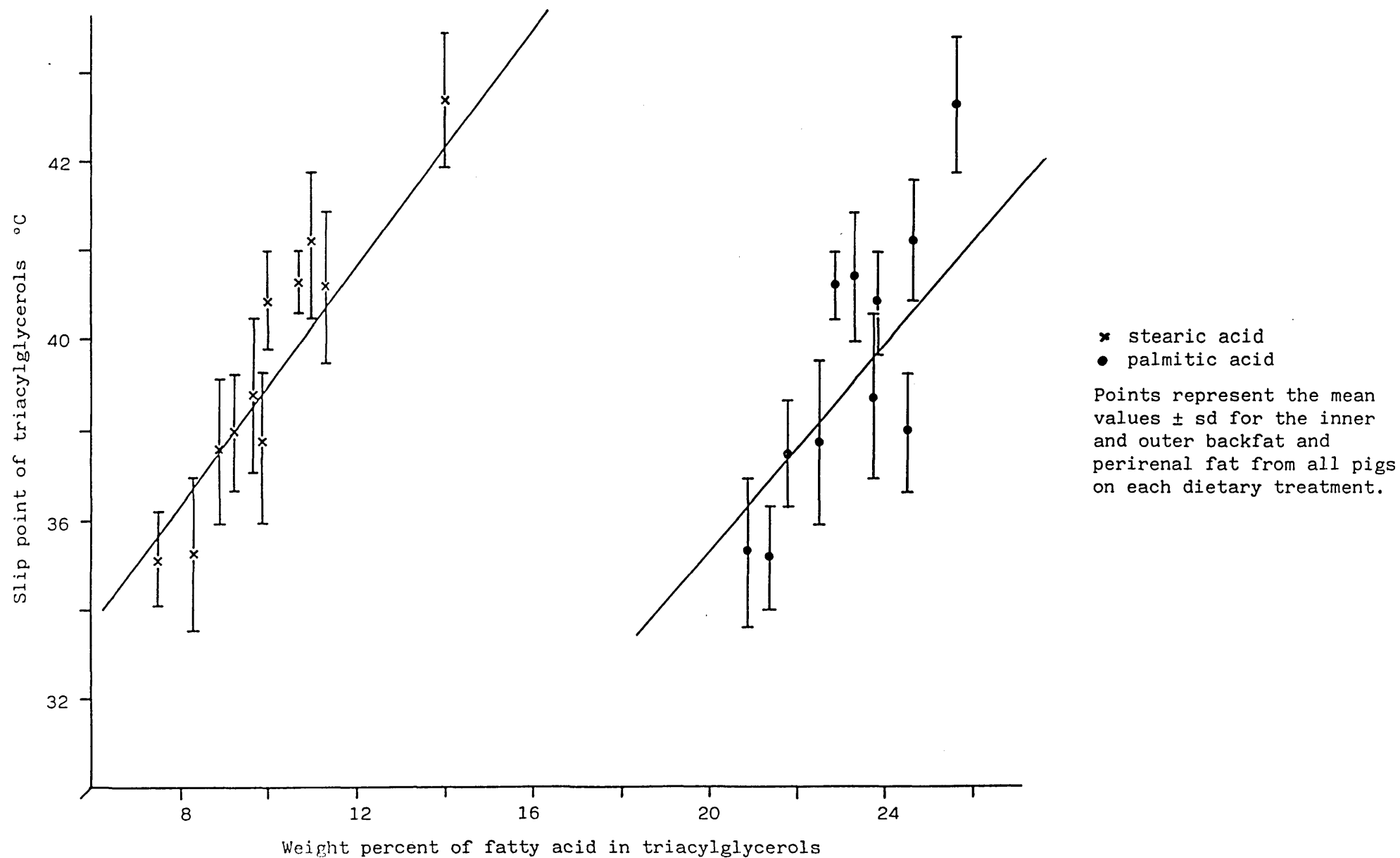
The presence of 200 mg Cu/kg feed in the pig diets was found to increase the concentrations of monounsaturated fatty acids in the adipose tissue triacylglycerols of pigs when compared with animals receiving control diets lacking this supplement (Table 4.2.4). These increases in oleic and palmitoleic acid concentrations are in accordance with the results of Experiment 1 and with

Figure 4.3.4: Experiment 2. Relationship between the oleic acid concentrations and the slip point of triacylglycerols isolated from the inner and outer backfat and perirenal fat of pigs supplemented and unsupplemented with maize oil.



Points represent the mean $\pm$ sd for the inner and outer backfat and perirenal fat from all pigs on each dietary treatment.

Figure 4.3.5: Experiment 2. Relationships between the concentrations of palmitic and stearic acids respectively and the slip points of triacylglycerols from the subcutaneous and perirenal adipose tissue of pigs on all dietary treatments.



the reports of Moore *et al.* (1969), Elliot and Bowland (1968) and others (Sections 1.5.3.3. and 3.3.3.4).

The changes in the fatty acid compositions of the triacylglycerols accompanying the addition of copper to the diet have been attributed to an activation of stearoyl CoA desaturase (Ho & Elliot, 1973; Thompson *et al.*, 1973). As the activity of this enzyme was assayed in the course of the present investigation, the effect of dietary copper on Δ^9 desaturase activity will be dealt with in a separate section (Section 4.3.4.3).

Although the ratios of saturated:unsaturated fatty acids in the adipose triacylglycerols were decreased by the addition of copper to the diet, there were no concomitant decreases in the triacylglycerol melting points which were significant at the 5% level. However the melting points of triacylglycerols from the pigs fed the FCu treatment appeared to be slightly lower than those from pigs given the F treatment.

These findings are in contrast with the results of Experiment 1 (Section 3.3.3.4) and earlier work by Moore *et al.* (1969) and others (Section 1.5.3.3) where significant depressions in triacylglycerol melting point associated with copper supplementation of the diets were reported. However in the present investigation the concentration of oleic acid in the adipose tissue triacylglycerols was found to correlate with the melting point despite the increase in oleic acid concentration associated with the presence of dietary copper. The relationships are illustrated in Figure 4.3.4 and, as with the corresponding results from Experiment 1 (Figure 3.3.6), two linear regressions were observed, representing the results from the pigs on the maize oil-supplemented and -unsupplemented treatments respectively. With the regressions from the present experiment, the gradients of the two

lines were not significantly different from each other at the 5% level, suggesting that the concentration of oleic acid has an effect on the triacylglycerol melting point, even in the presence of high tissue concentrations of linoleic acid. However, as discussed in connection with the corresponding relationships from Experiment 1 (Section 3.3.3.4), the regression for the results from the pigs given the maize oil-supplemented treatments might be expected to have a shallower gradient than that for the pigs given the F and FCu treatments, owing to the greater effect of the high levels of linoleic acid on the triacylglycerol melting point. There was no significant correlation ($p > 0.05$) between the palmitoleic acid concentration and the melting point of adipose tissue triacylglycerols from the pigs on Experiment 2.

The concentrations of palmitic and stearic acids in the adipose tissue triacylglycerols both correlated positively with the slip point ($p \leq 0.001$ for both regressions, Figure 4.3.5). Thus, as anticipated, increases in the concentrations of the saturated fatty acids were associated with increased melting points of the triacylglycerols.

These two relationships together with the correlations between the concentrations of both oleic and linoleic acids and the slip points of the adipose tissue triacylglycerols are in agreement with the results of Experiment 1 (Section 3.3.3.4) but not with the work of Enser (1984) who reported that only stearic acid concentration was important in regulating fat consistency. In Experiment 1, the various ratios between the concentrations of stearic, oleic and linoleic acids were plotted against the triacylglycerol slip point and significant relationships were noted in all cases ($p \leq 0.001$, Figures 3.3.8 and 3.3.9). Similar correlations were obtained when the results of Experiment 2 were examined so that two linear regressions were noted when the ratios

of stearic:oleic acid concentrations were plotted against the triacylglycerol melting points (Table 4.3.4). These regressions from Experiment 2 were significantly different from each other ($p \leq 0.05$) with the relationship obtained with the results from the pigs given the dietary treatments not containing maize oil having the steeper gradient. This is consistent with the greater effect of linoleic acid on the melting point of the adipose tissue triacylglycerols. The linear relationships between the triacylglycerol slip points and the ratios of the concentrations of stearic:oleic acids and oleic:linoleic acids respectively are given in Table 4.3.4. In agreement with the results of Experiment 1 the regression of the stearic:linoleic acid ratios against the triacylglycerol slip points had a steeper gradient than did the regression calculated using the ratios of oleic:linoleic acids. This again suggests that the concentration of oleic acid in the adipose tissue triacylglycerols may to some extent be determined by the concentrations of stearic and linoleic acids (Section 3.3.3.4).

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Although the addition of copper to the diet had a significant effect on the fatty acid composition of the triacylglycerols, there were no apparent effects on the positional distribution of the fatty acids (Tables 4.2.4 and 4.2.6). As discussed in Sections 3.2.4.4. and 3.3.3.4, the presence of higher levels of dietary copper were associated with higher concentrations of mono-unsaturated fatty acids and lower amounts of stearic and linoleic acids. The latter two fatty acids are only esterified at the 2-position of the triacylglycerols in small amounts and oleic acid is mainly esterified at the 1+3 positions of the triacylglycerols. Thus any changes in the positional distribution of these fatty acids attributable to the presence of dietary copper would not be determined by the analytical technique employed; pancreatic lipase hydrolyses fatty acids at both the 1- and 3-positions of the triacylglycerols and so it is not possible to distinguish between these two sites

Table 4.3.4: Experiment 2.

Linear relationships between the ratios of the concentrations of the 18 carbon fatty acids and the slip points of the triacylglycerols from inner and outer backfat and perirenal fat samples of all pigs on all four dietary treatments.

Fatty acid ratio	Linear regression	Significance
18:0/18:1		
Treatments lacking maize oil	$y = 53.69x + 29.29$	$p \leq 0.001$
Treatments supplemented with maize oil	$y = 41.95x + 28.04$	$p \leq 0.001$
18:0/18:2	$y = 5.76x + 34.69$	$p \leq 0.001$
18:1/18:2	$y = 1.15x + 34.63$	$p \leq 0.001$

x – ratios of the concentrations of the 18 carbon fatty acids

y – slip points ($^{\circ}\text{C}$) of triacylglycerols

using this method.

It has been reported that small changes in fatty acid composition may have a disproportionate effect on the triacylglycerol melting point (Moore *et al.*, 1969). It may be that, with the present study, although there was an increase in the triacylglycerol concentration of monounsaturated fatty acids associated with supplementation of the diets with copper, this was not accompanied by major changes in the positional distribution patterns. In addition the concentrations of the various triacylglycerol species may have been affected by the dietary copper supplement and this may also influence the melting point; as discussed earlier the melting point of a heterogeneous mixture of triacylglycerols represents both the melting of the more unsaturated triacylglycerol species and the dissolving in these of the more saturated triacylglycerol types (Section 3.3.3.4).

4.3.4 Adipose tissue stearoyl Coenzyme A desaturase activity

4.3.4.1 Comparisons between different adipose tissue sites

The results of the assays of stearoyl CoA desaturase activity in adipose tissue microsomes are given in Table 4.2.1. It can be seen that, with the exception of the pigs on the FCu treatment, the outer layer of backfat had the highest Δ^9 desaturase activity, while the perirenal fat samples had the lowest. These differences in stearoyl CoA desaturase activity are consistent with the degree of unsaturation of the adipose tissue samples. Thus a higher activity of stearoyl CoA desaturase in the adipose tissue is associated with higher levels of unsaturated fatty acids.

The three different adipose tissue sites may have differing Δ^9 desaturase activities as a result of differences in overall lipogenic activity between the three sites. However, although the inner backfat layer is known to have higher activities of the lipogenic enzymes than the outer layer, it does not have a correspondingly higher stearoyl CoA desaturase activity (De Wilde, 1984; Sturm *et al.*, 1982).

Although stearoyl CoA desaturase is subject to both dietary and hormonal control (Section 1.3.2.2), it has been reported that the activity of this enzyme may also be sensitive to changes in temperature. Early work has suggested that, in plant tissues, the concentration of oxygen is rate-limiting to the desaturation step (Harris & James, 1969). As the solubility of oxygen is temperature-dependent, fungi grown at higher temperatures have lower concentrations of unsaturated acids owing to the lower concentrations of available oxygen. However more recent work has suggested that the control of desaturation may be related to the physico-chemical state of the endoplasmic reticulum. Kasai *et al.* (1985) reported that the turnover rate of the palmitoyl CoA desaturation system in *Tetrahymena pyriformis* was dependent upon the temperature of the culture medium. Their results suggest that the induction of desaturase activity through increased protein synthesis may be controlled by changes in membrane viscosity. This hypothesis is consistent with the work of Lippiello *et al.* (1979) who reported that subcutaneous injection of 17β -estradiol produced marked alterations in the activity of stearoyl CoA desaturase in cockerel liver, which were accompanied by changes in both the fatty acid composition and physical properties of the microsomal membranes. These authors also noted that the changes in microsomal membrane microviscosity had no apparent effect on the efficiency of interaction of components of the microsomal electron transport chain and that increased desaturase activity is a result of increased

protein synthesis rather than an allosteric effect of the microsomal lipids.

These various systems of control have not been experimentally determined in pig adipose tissues, but the results of the present experiment are consistent with the general findings; Δ^9 desaturase activities were highest in outer subcutaneous backfat and lowest in the perirenal adipose tissue microsomes (Table 4.2.7).

4.3.4.2 Effect of supplementing the diets with maize oil

Supplementation of the diets with 4% maize oil markedly reduced the activity of stearoyl CoA desaturase in pig adipose tissue microsomes (Table 4.2.7). This was evident in the comparisons between the pigs on the F and FM treatments and between those on the FCu and FMCu treatments, although in the latter comparison the decrease in activity accompanying the presence of dietary maize oil was slightly lower. In addition, the differences in desaturase activity were not significant for the comparisons between the perirenal adipose tissue samples from pigs on different dietary treatments ($p > 0.05$).

This effect of dietary maize oil on Δ^9 desaturase activity is consistent with the findings of Jeffcoat *et al.* (1979) and Jeffcoat and James (1978). These authors reported that dietary maize oil reduced the activities of both the lipogenic and desaturase enzymes. Jeffcoat and James (1978) reported that stearoyl CoA desaturase is three to four times more sensitive to changes in dietary linoleic acid content than is fatty acid synthetase when measured in rat cell-free preparations. Jeffcoat *et al.* (1979) found a similar, but somewhat smaller, difference in the Δ^9 desaturase activity of isolated

rat hepatocytes. These combined results suggest that lowering the stearyl CoA desaturase activity affects triacylglycerol synthesis indirectly. A lower desaturase activity results in an accumulation of saturated fatty acids which inhibit both acetyl CoA carboxylase and fatty acid synthetase, so inhibiting further synthesis of fatty acids.

These findings are consistent with those of the present study. In both Experiments 1 and 2 dietary maize oil was associated with higher levels of linoleic acid in the pig adipose tissue triacylglycerols. This increase in polyunsaturated fatty acid content was at the expense of all the other fatty acids and so is consistent with reduced *de novo* synthesis and desaturation of fatty acids. However there were no significant correlations between the adipose tissue linoleic acid concentrations and Δ^9 desaturase activities ($p > 0.05$).

Contrary to these results, the relationships from Experiments 1 and 2 between backfat thickness and both oleic and linoleic acid concentrations gave separate but parallel regressions for the results from the pigs on the maize oil supplemented and unsupplemented treatments respectively (Sections 3.3.2 and 4.3.2). This may suggest that dietary linoleic acid has no major effects on endogenous fatty acid synthesis but as it is not possible to distinguish between fatty acids derived from the diet or those synthesized *de novo* it may be that a more complex relationship exists between the backfat thicknesses and the concentrations of the various fatty acids in the adipose tissue triacylglycerols. Different ratios of fatty acid concentrations were plotted against the backfat thicknesses for both experiments, but no clear relationships were evident.

The mechanism by which linoleic acid inhibits stearoyl CoA desaturase activity has not been fully elucidated. However it has been suggested that the prostaglandins or other derivatives of linoleic acid, particularly arachidonic acid, are involved (Flick *et al.*, 1977; Abraham *et al.*, 1977). In addition it also appears that the fatty acids may inhibit desaturase activity while bound to either lipid or albumin (Jeffcoat, 1979).

This inhibitory action of dietary linoleic acid on Δ^9 desaturase activity may prevent an excessive increase in tissue unsaturation. Stearoyl CoA desaturase has an important role in maintaining tissue fluidity; in experiments on cockerels, desaturase activity was high when membrane fluidity was low (Holloway, 1983). The regulation of the desaturation step appears to be by means of the synthesis and degradation of the terminal protein component rather than by a membrane-allosteric mechanism (Holloway, 1983). This cyanide-sensitive factor has a relatively short half life of three to four hours and so can be quickly degraded to enable the microsomal electron transport chain to be utilized for other purposes (Oshino & Sato, 1972).

4.3.4.3 Effect of supplementing the diets with copper

The changes in adipose tissue stearoyl CoA desaturase activity accompanying the addition of 200 mg Cu/kg feed to the pig diets are shown in Table 4.2.7. Supplemental dietary copper was associated with higher adipose tissue stearoyl CoA desaturase activities compared with the animals receiving the corresponding control diets. Thus pigs on the FMCu treatment had a higher overall adipose tissue Δ^9 desaturase activity than those on the FM treatment, but a lower Δ^9 desaturase activity than the pigs on the F and FCu treatments.

This increased Δ^9 desaturase activity accompanying the presence of dietary copper is consistent with the findings of Ho and Elliot (1975) and Thompson *et al.* (1973) who reported a stimulation of both hepatic and adipose tissue stearoyl CoA desaturase activity accompanying supplementation of the diets with 250 ppm CuSO_4 . These authors also associated the increase in Δ^9 desaturase activity with a decrease in the ratio of stearic to oleic acid concentration in the adipose tissue. When the results of the present experiment were examined, there was evidence of a nonlinear relationship between Δ^9 desaturase activity and the stearic:oleic acid ratio in adipose tissue (Figure 4.3.6). When linear and polynomial regressions were calculated, only the linear and quadratic equations were statistically significant ($p \leq 0.001$, Table 4.3.5). Of these two regressions the quadratic equation was chosen as giving the best fit to the data as analysis of variance between the deviations from the linear and curved regressions suggests that there is a significant curvilinearity in the regression ($p \leq 0.001$, Snedecor & Cochran, 1974). In addition, enzyme activity is affected by various factors including substrate concentration and so would not be expected to be linearly related to the stearic:oleic acid ratio of the adipose tissue triacylglycerols.

Various other fatty acid concentrations and ratios of fatty acid concentrations in the adipose tissue triacylglycerols were also plotted against the Δ^9 desaturase activity, but no significant correlations were observed ($p > 0.05$). However the fatty acids present in adipose tissue are mainly in the esterified form and as such may not have any major effects on the activity of the enzyme. Non-esterified fatty acids, which may be involved in the regulation of the Δ^9 desaturase, are present in adipose tissue in much smaller quantities and probably do not reflect the fatty acid compositions of the adipose tissue triacylglycerols. However, the trends observed were

Figure 4.3.6: Experiment 2. Linear and quadratic relationships between Δ^9 desaturase activity (x) and the stearic:oleic acid ratio of triacylglycerols (y) from subcutaneous and perirenal adipose tissue from pigs on each dietary treatment.

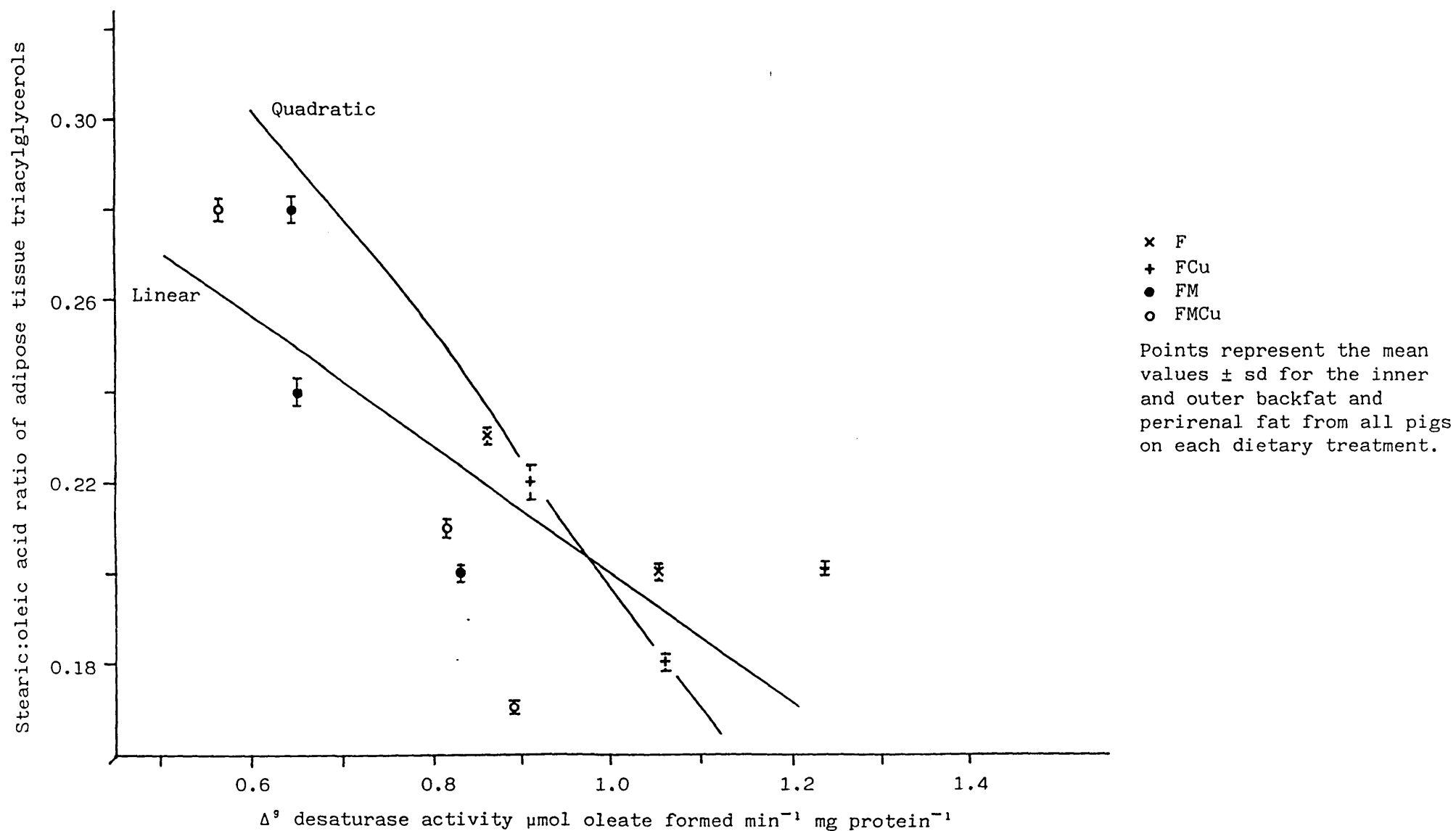


Table 4.3.5: Experiment 2.

Comparison of linear and quadratic equations for the relationship between the Δ^9 desaturase activity (μmol oleate formed min^{-1} mg protein $^{-1}$, x) and the stearic:oleic acid ratio (y) of adipose tissue from pigs on all four dietary treatments.

		Significance	r^2
Linear	$y = 0.307 - 0.0999x$	$p < 0.001$	0.27
Quadratic	$y = 0.47 - 0.47x + 0.21x^2$	$p < 0.001$	0.35

consistent with the activity of the enzyme; a lower Δ^9 desaturase activity was associated with higher linoleic acid concentrations. The results also corroborate reports that stearoyl CoA desaturase activity is stimulated by factors similar to those which enhance endogenous fatty acid synthesis (Jeffcoat, 1979).

The manner in which dietary copper stimulates stearoyl CoA desaturase activity is unclear. Experiments on rats have shown that copper is not an integral part of the enzyme and is not required for its formation, as copper-chelating agents, such as diethyldithiocarbamate, do not inhibit enzyme activity. Stearoyl CoA desaturase is an iron-containing protein and activity is inhibited by the presence of the iron-chelator bathophenanthroline sulphonate (Jeffcoat, 1979). However, Wahle and Davies (1975) demonstrated that copper-deficient rats have a reduced stearoyl CoA desaturase activity, compared with rats of normal copper status, which can be restored either by an intraperitoneal injection of copper sulphate or by supplementation of the diets with copper. Ho *et al.* (1975) noted that the addition of CuSO_4 *in vitro* to microsomal preparations from the liver and adipose tissue of pigs fed control diets slightly increased the Δ^9 desaturase activity. The increases, however, were much smaller than those expected from the *in vivo* results obtained from pigs fed copper-supplemented diets. This would suggest that the copper is not acting directly on the desaturase enzyme. It may be that other aspects of lipid metabolism are influenced by copper and that these indirectly stimulate Δ^9 desaturase activity.

With the exception of stearoyl CoA desaturase, little work appears

to have been carried out on the changes in lipid metabolism accompanying dietary copper supplementation, but much has been concentrated on the effects of dietary copper deficiency. In particular, copper-deficient rats appear to have altered cholesterol metabolism compared with rats of normal copper status (Lau & Klevay, 1982; Harvey & Allen, 1981; Tanaka *et al.*, 1988).

Copper, as Cu^{2+} ions, has also been reported to mimic some of the effects of insulin at the adipocyte membrane (Saggerson *et al.*, 1976). These authors have suggested that Cu^{2+} ions may affect the distribution of cellular Ca^{2+} ions and promote sugar transport into the fat cells, although copper does not appear to mimic all of the effects of insulin. If this is so, then the addition of copper *in vitro* would enhance *de novo* fatty acid synthesis which in turn would lead to an increase in the activity of the Δ^9 desaturase.

When both copper and maize oil are present in the diet (FMCu treatment), the activity of stearoyl CoA desaturase in the adipose tissue microsomes was lower than that of pigs given the FCu treatment, but greater than that of pigs on the FM treatment. The antagonistic effects of these two dietary supplements suggests that their modes of action may be different. As already discussed, copper is not an integral part of the enzyme and doesn't appear to act as an activating agent, although it is suggested that it may stimulate fatty acid synthesis, and hence desaturation, through an insulin-like action (Ho *et al.*, 1975; Jeffcoat, 1979; Saggerson *et al.*, 1976). In contrast, linoleic acid appears to be responsible for the inhibition of stearoyl CoA desaturase, acetyl CoA carboxylase and fatty acid synthetase by acting via a specific lipid or lipoprotein containing either linoleic acid or a derivative of this acid (Jeffcoat & James, 1978; Abraham *et al.*, 1977). Jeffcoat (1979)

has also suggested that dietary polyunsaturated fatty acids may affect desaturase activity by influencing the secretion of various hormones or by affecting the number of binding sites of hormone receptors.

4.3.5 Plasma lipids; concentration in plasma and fatty acid composition

4.3.5.1 Changes in the concentrations of the plasma lipids accompanying supplementation of the diets with maize oil and/or copper

The changes in the plasma concentrations of cholesterol in the pigs both with age and between treatments are illustrated in Figure 4.2.1. Within each treatment the plasma free cholesterol levels decreased with age and this is consistent with the report that the concentration of plasma cholesterol decreased significantly in pigs supplied with 25g soya oil daily during the growth period from 20 to 60 kg liveweight (Staun *et al.*, 1970).

The pigs receiving the maize oil supplemented treatments had higher plasma cholesterol concentrations during the latter half of the experimental period than did the animals receiving the oil-free dietary treatments. This does not agree with the generally accepted view that diets rich in polyunsaturated fatty acids are associated with lowered plasma cholesterol concentrations (Jackson *et al.*, 1984; Durrington *et al.*, 1977). However the observations made by these authors are based on differences between animals given diets differing only in the saturated:unsaturated ratio of fatty acids supplied. In the present experiment the level of fat supplied by the two maize oil supplemented treatments was greater than that present in the oil-free diets. The higher level of cholesterol may, therefore, be related to the increased production of very low density lipoproteins

(VLDL) by the liver accompanying the higher intake of dietary fat. Goh and Heimberg (1977) suggested that as cholesterol is an essential component of VLDL, increased VLDL secretion is necessarily associated with increased synthesis of cholesterol. However the increase in hepatic VLDL release may not be very large as the triacylglycerols present in the VLDL are derived from chylomicron remnants which are taken up by apo E receptor mediated transport into the liver. These remnants are formed when chylomicrons, synthesized in the intestinal enterocytes, enter the circulation and are depleted of triacylglycerol by means of the enzyme lipoprotein lipase which is located in the endothelial cells of tissues such as adipose tissue and muscle. About 80% of the triacylglycerol is removed in this manner so that the chylomicron remnants which are taken up by the liver contain comparatively small amounts of the ingested dietary fat (Fielding & Fielding, 1985). The fatty acids derived from these chylomicron remnants stimulate VLDL and hence cholesterol synthesis; but as the remnant particles are depleted in triacylglycerol content the increase in VLDL release from the liver may not be all that great. However Allee *et al.* (1971) also noted a trend towards increased plasma concentrations of cholesterol as the fat content of the pig diets was increased from 1 to 13%. Work by Forsythe *et al.* (1980) has also shown that high fat diets (42% of metabolizable energy as fat), whether with a high or low saturated:unsaturated ratio of fatty acids lead to a higher plasma unesterified cholesterol concentration in pigs than do control diets containing 8% of the metabolizable energy as fat.

These findings are also consistent with the increased plasma cholesteryl ester concentrations observed with the pigs receiving the maize oil supplemented treatments of the present experiment (Figure 4.2.2). These higher levels of plasma cholesteryl esters compared with the animals given the treatments

not containing maize oil may be a result of increased lecithin:cholesterol acyltransferase (LCAT) activity in plasma (Fielding & Fielding, 1985). This enzyme catalyses the acylation of free cholesterol derived from both cell membranes and plasma lipoproteins, particularly VLDL and LDL, and is required for cholesterol transport between tissues (Dobiasova, 1983).

Dietary copper had no apparent effect on plasma free and esterified cholesterol concentrations (Figures 4.2.1 and 4.2.2). There are some reports that copper deficiency in rats is associated with decreased LCAT activity and hypercholesterolaemia, but there is no comparable evidence that copper supplementation of the diets has a hypocholesterolaemic effect (Harvey & Allen, 1981; Tanaka *et al.*, 1988; Lau & Klevay, 1982).

The changes in plasma triacylglycerol concentrations occurring with age and between pigs on different dietary treatments are illustrated in Figure 4.2.3. The animals receiving the maize oil supplemented diets had much higher plasma triacylglycerol concentrations than did the pigs given the corresponding treatments which did not contain maize oil. These findings are consistent with reports that higher fat diets are associated with increased triacylglycerol transport in the blood (Forsythe *et al.*, 1980; Diersen-Schade *et al.*, 1985).

The concentrations of triacylglycerols in the plasma of the pigs were much lower than those obtained by other authors. Forsythe *et al.* (1980) and Diersen-Schade *et al.* (1985) reported triacylglycerol concentrations in the range of 31 to 72 mg triacylglycerols/100 ml plasma. The cause of this discrepancy is unclear but this plasma fraction is the one most affected by the time of sampling and, in addition, the nature of the diet and genetic variations between pigs may also be involved. Thus differences between

experimental methods may to some extent explain the lower plasma triacylglycerol levels noted in the present experiment, although even with these factors taken into account the values appear much lower than anticipated.

The presence of copper in the experimental diets had no apparent effect on the concentration of triacylglycerols in the plasma of pigs from the present experiment.

In contrast with the other plasma lipid fractions dietary maize oil had no apparent effect on the pig plasma unesterified fatty acid concentrations (Figure 4.2.4). This is contrary to the findings of Allee *et al.* (1971) who reported that plasma non-esterified fatty acid concentrations increased linearly with increasing levels of dietary fat from 1 to 13%. However, in the present study, the concentrations of unesterified fatty acids were greater in the plasma of the pigs which received either of the copper supplemented diets. This is in agreement with the findings of Myres and Bowland (1973) who reported that pigs given diets containing 250 ppm CuSO_4 had plasma unesterified fatty acid levels which were significantly greater than those of pigs given control diets lacking the copper supplement.

This increase in circulating unesterified fatty acid levels may result either from an increased rate of release of fatty acids from adipose tissue stores or from a decreased rate of clearance from the blood. The effects of dietary copper on these is unclear; copper deficient rats have been shown to have reduced lipoprotein lipase activity (Lau & Klevay, 1982), but there is no comparable evidence that dietary copper supplements influence the lipoprotein lipase activity of pig tissues.

4.3.5.2 Changes in the fatty acid compositions of the plasma lipids accompanying supplementation of the diets with maize oil and/or copper

The changes in the fatty acid compositions of the plasma cholesteryl esters from pigs on all four experimental treatments are shown in Table 4.2.8. The cholesteryl esters isolated from the plasma of pigs given either of the maize oil supplemented treatments had higher concentrations of linoleic acid than did those obtained from the pigs which received either of the diets which lacked the maize oil supplement. The concentrations of the other fatty acids measured, palmitic, stearic and oleic acids were all correspondingly lower in the plasma cholesteryl esters of the pigs given the FM and FMCu treatments.

The cholesteryl esters in the plasma are formed by the esterification of fatty acids to free cholesterol molecules, a reaction catalysed by the enzyme lecithin:cholesterol acyltransferase. The fatty acids are derived from circulating lipoproteins, VLDL in particular, or from cell membranes (Fielding & Fielding, 1985). With the pigs given the maize oil supplemented treatments, slightly greater quantities of VLDL may be secreted by the liver in response to the higher intake of dietary fat compared with the pigs given the oil free diets (Goh & Heimberg, 1977). These lipoproteins have greater concentrations of linoleic acid owing to the high concentration of this fatty acid in the diet. As the VLDL are a source of fatty acids for the esterification of free cholesterol, then the plasma cholesteryl esters of pigs given the maize oil supplemented diets would be expected to have greater concentrations of linoleic acid than the plasma cholesteryl esters of pigs given the corresponding treatments which lack the maize oil supplement.

Dietary copper had no major effects on the fatty acid compositions of the plasma cholesteryl esters. Copper deficiency has been shown to reduce

LCAT activity in rats (Harvey & Allen, 1981) but neither the concentration in plasma nor the fatty acid composition of the cholesteryl esters of the pigs in the present study appears to be influenced by the high level of copper supplied by the FCu and FMCu treatments.

The changes in the fatty acid compositions of the plasma triacylglycerols are shown in Table 4.2.9. As anticipated the concentration of linoleic acid was greater in the plasma triacylglycerols of the pigs which received the maize oil supplemented treatments and this was accompanied by lower concentrations of stearic and palmitic acids.

The plasma triacylglycerols which occur in the VLDL and chylomicrons are cleared from the circulation by means of the enzyme lipoprotein lipase. This enzyme, located on the endothelial walls of the tissue capillary network, hydrolyses the triacylglycerols and the fatty acids are taken up principally by adipose tissue and muscle (Section 1.3.3). The fatty acids derived from the plasma triacylglycerols which are taken up by muscle are mainly oxidised whereas those taken up by the adipose tissue are esterified and stored as triacylglycerols. It is therefore possible that the fatty acid composition of the plasma triacylglycerols may be related to that of the triacylglycerols stored in the adipose tissue.

In order to investigate this, the concentrations of the various fatty acids were plotted against those of the same fatty acid in the subcutaneous adipose tissue triacylglycerols. However as the fatty acid compositions of the adipose tissue triacylglycerols were only determined for samples taken at slaughter, then these correlations were calculated using the fatty acid

compositions of blood samples taken from the animals at slaughter. The relationships obtained are shown in Table 4.3.6. The concentrations of the saturated fatty acids in the plasma triacylglycerols did not correlate significantly ($p > 0.05$) with the concentration of the same fatty acid in the adipose tissue triacylglycerols. This may be a result of the endogenous synthesis of palmitic and stearic acids in the adipose tissue but the relatively small range of concentrations of the saturated fatty acids in the adipose tissue may also partly account for the poor correlation coefficients obtained. Oleic acid concentration in the plasma triacylglycerols, however, was negatively correlated with the oleic acid content of the adipose tissue triacylglycerols. This suggests that *de novo* synthesis of oleic acid in adipose tissue decreases as increasing amounts of oleic acid are taken up from the circulation. In contrast, linoleic acid concentration in the plasma triacylglycerols was very highly correlated with the concentration of linoleic acid in the adipose tissue triacylglycerols ($p \leq 0.001$). The positive correlation coefficient suggests that increasing concentrations of this acid in the plasma triacylglycerols are associated with increasing concentrations of linoleic acid in the adipose tissue triacylglycerols. This was, to some extent, anticipated as linoleic acid cannot be synthesized by the pig and so is derived entirely from dietary sources.

The changes in the fatty acid compositions of the plasma unesterified fatty acids are shown in Table 4.2.10. In keeping with the changes in the concentrations of the fatty acids in plasma, the composition was influenced by the presence of copper in the diet. Thus the oleic acid concentration of the plasma unesterified fatty acids was greater in the plasma of pigs given the FCu and FMCu treatments. Dietary maize oil also had an effect,

Table 4.3.6: Experiment 12.

Relationships between the concentrations of various fatty acids in the plasma triacylglycerols (x) and the concentrations of the same fatty acid in the adipose tissue triacylglycerols (y).

Fatty acid	Linear regression	Significance
16:0	$y = 0.12x + 19.12$	ns $p > 0.05$
18:0	$y = 0.07x + 7.66$	ns $p > 0.05$
18:1	$y = -0.43x + 59.38$	$p < 0.05$
18:2	$y = 0.5x + 8.25$	$p < 0.001$

Regressions were calculated using the results obtained from the analyses of the blood samples taken at slaughter and the mean fatty acid compositions (weight percent) from the backfat of individual pigs on each experimental diet.

Table 4.3.7: Experiment 2.

Relationship between the concentrations (weight percent) of various fatty in the plasma unesterified fatty acids (x) and the concentrations (weight percent) of the same fatty acid in the adipose tissue triacylglycerols (y)

Fatty acid	Linear regression	Significance
16:0	$y = 0.15x + 17.14$	$p < 0.05$
18:0	$y = 0.009x + 8.43$	ns
18:1	$y = 0.23x + 36.55$	$p < 0.05$
18:2	$y = 0.41x + 10.37$	$p < 0.05$

Regressions were calculated using the results obtained from the analyses of the blood samples taken at slaughter and the mean fatty acid compositions (weight percent) from the backfat of individual pigs on each experimental diet.

the linoleic acid content of the plasma unesterified fatty acids was generally greater with the pigs given the maize oil supplemented treatments, and particularly with the pigs which received the diet containing maize oil, but no copper *i.e.* the FM treatment.

These findings are consistent with the fatty acid compositions of the adipose tissue triacylglycerols from which the plasma unesterified fatty acids are derived. Thus when the concentrations of each of the plasma unesterified fatty acids were plotted against the concentrations of the same fatty acid in the adipose tissue triacylglycerols, significant correlations were noted in all cases except for stearic acid concentration (Table 4.3.7). The poor correlation obtained with the stearic acid regression may be a result of the small range of stearic acid concentrations in the adipose tissue triacylglycerols *e.g.* SEM for stearic acid concentration in outer backfat triacylglycerols = ± 0.69 . The significant correlations noted with the other fatty acids all had positive gradients, suggesting that greater concentrations of a particular fatty acid in the adipose tissue were associated with a greater release of that fatty acid into the circulation. The steepest gradient was that of the linoleic acid regression. Morrisett *et al.* (1977) reported that the more fluid unsaturated triacylglycerols were hydrolysed more rapidly by lipoprotein lipase than were the more saturated analogues, presumably as a result of a more efficient interaction between the lipase and the more fluid lipid. It may be that the lipases involved in the mobilization of fatty acids from the adipose tissue, although distinct from lipoprotein lipase, have similar properties.

Myres and Bowland (1972) also studied the effects of dietary copper on the composition of the plasma unesterified fatty acids. They reported

that the difference between the compositions of the plasma unesterified fatty acids of pigs given either basal diets or diets supplemented with 250 ppm copper were similar to those noted with the adipose tissue triacylglycerols, although the differences were much smaller and on the whole not statistically significant ($p > 0.05$). These findings are consistent both with subsequent work by the same authors (Myres & Bowland, 1973) and with the observations of the present study.

4.3.6 General summary

The growth rates, up to slaughter, of the pigs on this experiment did not appear to be affected by the nature of the dietary treatment. The efficiencies of food utilization were also similar between animals on different treatments. These findings are consistent with those of Experiment 1, but not with the work of Braude (1967) and others who reported that high levels of dietary copper improved the overall growth performance of pigs, or with Allee *et al.* (1971) who noted that dietary maize oil increased pig growth rates.

The maize oil and copper supplements also had no apparent effect on the thickness of the subcutaneous backfat. This is in agreement with the growth rates of pigs on the different dietary treatments^m which were also not significantly different from each other. As with the results of Experiment 1, the concentration of linoleic acid in the triacylglycerols was correlated with the thickness of the subcutaneous adipose tissue. Two relationships were obtained, corresponding to the results from pigs on the maize oil supplemented and unsupplemented treatments respectively. In contrast, Wood and

Enser (1982) noted only a single correlation although these authors used results which covered a narrower range of backfat linoleic acid concentrations.

The fatty acid compositions of the triacylglycerols from the three adipose tissue sites of each pig differed from each other with the perirenal fat being the most saturated and the outer backfat the least saturated. This is in agreement with the results of Experiment 1 and with previous reports (Christie *et al.*, 1972; Moore *et al.*, 1969; Hood & Allen, 1973). The differences are thought to arise from differences in the activities of the lipogenic and Δ^9 desaturase enzymes at each site. In keeping with this suggestion the stearoyl CoA desaturase activities at the three adipose tissue sites were consistent with the degree of unsaturation of the triacylglycerol samples. Thus outer backfat samples had the highest Δ^9 desaturase activity, and perirenal fat had the lowest. This is in agreement with the work of Enser (1973) and may be a result of differences in the microviscosities of the microsomal lipids (Kasai *et al.*, 1985; Lippiello *et al.*, 1979).

Dietary maize oil was associated with an increased concentration of linoleic acid in the adipose tissue triacylglycerols while dietary copper appeared to increase the triacylglycerol concentration of oleic acid. In addition these two dietary supplements had opposing effects on the Δ^9 desaturase activity of adipose tissue. Thus enzyme activity was enhanced by dietary copper, so accounting for the increase in the oleic acid content of the adipose tissue triacylglycerols, and was decreased by dietary maize oil. These findings are consistent with previous reports (Jeffcoat, 1979) and with the fatty acid compositions of the adipose tissue triacylglycerols.

The melting points of the adipose tissue triacylglycerols were also

affected by the nature of the dietary treatment. Both dietary copper and maize oil were associated with decreases in the triacylglycerol melting point compared with the triacylglycerols from pigs on the control treatment, although these decreases were much lower than anticipated, particularly with the triacylglycerols from pigs given the FCu treatment. This has been attributed to the effects of the positional distribution of the fatty acids on the triacylglycerol melting point (Moore *et al.*, 1969).

The positional distributions of the fatty acids in the adipose tissue triacylglycerols were markedly different from those of pigs receiving the corresponding treatments of Experiment 1. As the diets used in each experiment were comparable, it seems likely that the differences are an effect of breed.

Analysis of the blood lipids showed that the concentrations of cholesterol, cholesteryl esters and triacylglycerols in plasma were all increased by the presence of maize oil in the diet. The cholesteryl ester and triacylglycerol plasma fractions from pigs receiving the FM and FMCu diets also had higher concentrations of linoleic acid owing to the high concentration of this acid in the maize oil supplement. In contrast the plasma concentration of unesterified fatty acids appeared to be influenced more by the level of copper in the diet. Pigs receiving the FCu and FMCu treatments had greater concentrations of plasma unesterified fatty acids than did the animals receiving the F and FM diets. These findings are in agreement with the work of Myres and Bowland (1972, 1973) who noted similar changes accompanying supplementation of the diets with 250 ppm copper.

In summary the results of this present experiment support those of

Experiment 1 as well as work undertaken by previous authors. The absence of differences in the growth performance and backfat thicknesses of pigs given different dietary treatments is consistent with the results of Experiment 1. The changes in the fatty acid compositions of the adipose tissue triacylglycerols and the plasma lipids associated with the different diets are also consistent with earlier findings and are also in agreement with the activities of adipose tissue stearoyl CoA desaturase. However the changes in the melting points of the adipose tissue triacylglycerols accompanying supplementation of the diets were lower than expected and this may be a result of the patterns of fatty acid distribution in the adipose tissue triacylglycerols.

The positional distribution of the fatty acids in the triacylglycerols were different from those of the pigs given the corresponding fishmeal-based treatments of Experiment 1 and this may be a result of the use of different breeds of pig.

Section 5
EXPERIMENT 3

Section 5

Experiment 3

Investigation of the structure and physical properties of triacylglycerols isolated from the adipose tissue of different breeds of pig.

5.1.1 Introduction

The results of Experiments 1 and 2 revealed differences in both the compositions and positional distributions of the fatty acids isolated from the adipose tissue triacylglycerols of the pigs receiving the fishmeal-based treatments. These animals received analogous barley-based diets and so differences between the two experiments cannot be wholly attributed to the nature of the feedstuff. However the pigs from Experiment 1 were LW X LR X LW crosses while those from Experiment 2 were pure-bred Landrace pigs. In addition, Moore *et al.* (1969) reported large differences between the melting points of adipose tissue triacylglycerols from pure-bred Large White pigs that were given diets containing 0 or 250 ppm copper. These findings suggest that the response to copper and other dietary supplements may be influenced by the breed of pig involved. If this is so, then certain breeds of pig may have a greater disposition than others to produce soft fat, and this has obvious implications for the bacon manufacturing industry. Thus, in this experiment, the structure and composition of adipose tissue triacylglycerols from eleven breeds of pig receiving identical diets were investigated. The effects of breed on growth and carcass characteristics were also determined.

5.1.2 Dietary treatments

A single diet was employed in this experiment as the aim was to investigate the effects of breed rather than those of diet. The feedstuff was a standard wheat-based grower ration: Pig Supergrow Pellets No. 304 (West of England Farmers); the overall composition is shown below:

Protein (soya, fishmeal)	21%
Oil	5.25%
Fibre	4%
Ca	0.9%
P	0.6%
Salt	0.4%
MJDE	13.8

The feed was given to the animals *ad libitum*.

5.1.3 Animals

The pigs, a minimum of six from each breed, were from the AFRC Institute at Langford, Bristol, where the experiment was carried out. The eleven breeds used were:-

		Number of pigs
Large White	LW	9
Landrace	LR	11
Tamworth	T	8
Hampshire	H	8
Duroc	D	11
Gloucester Old Spot	GOS	9
Pietrain	P	10
Saddleback	S	9
Oxford Sandy and Black	OSB	6
Large Black	LB	10
Berkshire	B	9

5.1.4 Experimental procedure

The animals were fed the grower ration *ad libitum* until slaughter at 65 kg. During the experimental period the pigs were weighed weekly so that growth rates could be ascertained. After slaughter small sections of both backfat layers of subcutaneous adipose tissue were taken from each pig and stored at -20°C until analysis. The P_2 fat thickness of each carcass was also measured after slaughter.

5.1.5 Methods of analysis

The samples of backfat were separated into the constituent inner and outer layers. Triacylglycerols were isolated from each of these layers by extraction of the adipose tissue lipids (Section 2.2.11) and subsequent purification of the triacylglycerol fraction (Section 2.2.2). The fatty acid composition was determined both in the whole triacylglycerol extract (Section 2.2.4) and at position 2 and positions 1+3 separately of the triacylglycerols (Section 2.2.6). The melting points of the triacylglycerol extracts were also established, as described in Section 2.2.9. In addition pooled samples of triacylglycerols from the inner backfat of all pigs from each breed group were randomly interesterified (Section 2.2.11) and their melting points determined.

Differences between treatment means were compared by multiple range t test ($n_1 + n_2 - 2$ df for each comparison). The 5% level of probability was taken as significant. Where linear regressions were calculated, significance was tested by reference to the correlation coefficient ($n-2df$). Differences between linear regressions were compared by t test of both the gradients and slopes.

5.2 Results of Experiment 3

5.2.1 Growth rates and thickness of subcutaneous backfat

The mean growth rates and the P_2 fat thicknesses at slaughter of the eleven breeds of pig are shown in Table 5.2.1. The growth rates of the Tamworth pigs tended to be lower than those of all the other breeds of pig, although in certain instances the differences were not significant at the 5% level. The greatest growth rates were noted with the Berkshire pigs. These differences suggest that the different breeds of pig have characteristic rates of growth when given the same diet *ad libitum*.

The P_2 fat thicknesses, taken at slaughter, were smallest in the Large White and Pietrain animals and greater in the Large Blacks. The Hampshire, Saddleback, Oxford Sandy and Black and Berkshire pigs had similar P_2 fat thicknesses, as did the pigs of the Duroc and Gloucester Old Spot breeds.

The Large White and Landrace pigs, which had two of the greater growth rates, had much lower P_2 backfat thicknesses than did the slower growing Tamworth, Oxford Sandy and Black, and Large Black animals ($p \leq 0.001$). However this is not a consistent trend as the Berkshire pigs had the greatest growth rates but P_2 fat thicknesses significantly greater than those of the Large White and Landrace pigs ($p \leq 0.001$).

5.2.2 Composition, structure and physical properties of adipose tissue triacylglycerols

5.2.2.1 Comparisons between different adipose tissue sites

Triacylglycerols from the inner and outer backfat samples from each pig were analysed for their fatty acid compositions. The mean compositions

Table 5.2.1: Experiment 3. Mean rates of growth (g/day) and carcass P₂ backfat thicknesses (mm) of eleven breeds of pig fed identical diets *ad libitum*.

	Breed of Pig											Range SED
	LW	LR	T	H	D	GOS	P	S	OSB	LB	B /	
Number of pigs in group (n)	9	11	8	8	11	9	10	9	6	10	9	
Growth rate g/day	786 ^a	771 ^b	514 ^{abcdef}	714 ^c	729 ^d	786 ^e	629 ^h	600	729 ^f	771 ^{gh}	871 ^{gh}	32-67
P ₂ fat thickness (mm)	10.8 ^{abcde}	12.9 ^{fgj}	18.7 ^{afi}	14.7 ^a	15.6 ^{bj}	15.7 ^{cj}	10.8 ^{ijklm}	14.7 ^k	14.9 ^{dl}	23.1 ^{bhm}	14.7 ^{cm}	0.4-2.3

(n₁+n₂-2)df

Results shown are the means of n pigs.

Values bearing the same superscript within rows are significantly different from each other ($p \leq 0.05$)

for each breed of pig are shown in Table 5.2.2. Within each breed, significant differences ($p \leq 0.05$) between triacylglycerols from the two backfat layers were noted for the concentrations of all fatty acids measured except palmitoleic and linolenic acids. However there were no statistical differences in fatty acid composition between the triacylglycerols from the inner and outer backfat layers of the Large White pigs ($p > 0.05$).

Palmitic acid concentrations were greater in the triacylglycerols from the inner compared with the corresponding outer layers of backfat of pigs of all breeds. However the differences were only significant for the Oxford Sandy and Black, Gloucester Old Spot and Berkshire pigs ($p \leq 0.01$). Similar differences were observed with the concentrations of stearic acid in the triacylglycerols ($p \leq 0.05$ for all pigs except Large Whites). This greater concentration of saturated fatty acids in the inner backfat triacylglycerols was reflected in the slip points of the triacylglycerol samples (Table 5.2.3). With each of the eleven breeds of pigs, triacylglycerols from the inner backfat layer had significantly greater melting points than did those of the corresponding outer backfat samples ($p \leq 0.05$).

In contrast to the saturated fatty acids, the concentrations of oleic acid in the pig adipose tissue triacylglycerols were greater in the outer backfat layers, although again these differences were not always statistically significant ($p \leq 0.05$ for the LR, T, D, LB and B breeds of pigs). Linoleic acid concentrations were also greater in the triacylglycerols from the outer compared with the inner backfat of all breeds except for the Landrace pigs where the pattern was reversed. These differences were significant for the Large Black, Saddleback, Gloucester Old Spot and Berkshire pigs ($p \leq 0.01$).

Table 5.2.2: Experiment 3. Mean fatty acid compositions (wt. %) of the inner and outer backfat triacylglycerols of pigs of differing breeds fed the same diet *ad libitum*.

		Breed of Pig										Range	
		LW	LR	T	H	D	GOS	P	S	OSB	LB	B	SED
Number of pigs in group (n)		9	11	8	8	11	9	10	9	6	10	9	
16:0	Inner	21.5 ^a	22.9 ^b	25.0 ^{ac}	21.1 ^c	21.8 ^c	23.3 ^d	20.9 ^{cde}	22.6 ^c	23.3 ^f	24.7 ^e	27.5 ^{abcdef}	0.2-1.1
	Outer	19.6 ^a	21.2 ^b	23.7	20.0 ^c	21.0 ^d	21.7 ^e	19.9 ^f	21.6 ^g	21.0 ^d	3.0	24.6 ^{abcdef}	0.3-1.1
16:1	Inner	2.4 ^{abcde}	3.5	4.4 ^a	3.9	4.3 ^b	4.7 ^c	4.0	4.2 ^d	4.0	3.4	4.8 ^e	0.2-0.8
	Outer	3.1 ^a	4.0	4.5	4.8	4.3	4.7	4.8	4.5	4.7	3.7	5.5 ^a	0.5-1.4
18:0	Inner	10.6	11.0	12.7	10.9	12.8 ^a	10.8	10.0 ^{abcd}	12.0 ^b	14.3 ^c	12.8	12.5 ^d	0.4-1.1
	Outer	9.1	9.0 ^{ae}	9.8	8.8 ^b	11.0 ^{abcd}	8.3 ^{cf}	8.3 ^{dg}	9.8	10.9 ^f	9.9	10.8 ^{eg}	0.7-1.3
18:1	Inner	45.1	45.0 ^a	45.0 ^b	47.1	45.2 ^c	47.0 ^d	44.6	46.4 ^e	45.0 ^{af}	46.3 ^g	42.5 ^{bcdefg}	0.5-1.4
	Outer	47.6	48.0 ^{ab}	47.2	48.7 ^{cd}	46.8	48.1 ^e	44.9 ^{acefg}	47.7 ^f	47.3	48.9 ^{gh}	45.1 ^{bdh}	0.5-1.5
18:2	Inner	17.1 ^{abcde}	16.2 ^{fgh}	11.9 ^{af}	14.8 ^{ij}	14.3	13.4 ^b	18.5 [*]	13.3 ^c	12.3	11.1 ^{dgi}	11.3 ^{ekj}	0.5-1.4
	Outer	17.5 ^{abc}	15.9 ^d	13.3 ^{ae}	15.9 ^f	15.4 ^g	15.6 ^h	20.4 ^{deghij}	14.8 ⁱ	14.6	13.0 ^{bj}	12.2 ^{cdfhi}	0.2-0.8
18:3	Inner	3.3	2.1 ^a	1.1 ^b	2.1 ^{abc}	1.7	1.3	2.0	1.6	1.1 ^c	1.7	1.4	0.1-1.0
	Outer	3.1	1.9	1.6	1.8	1.5 ^a	1.5 ^b	2.1 ^a	1.7	1.5 ^b	1.5	1.8	0.1-1.0

Values bearing the same superscript within rows are significantly different from each other ($p \leq 0.05$)

* Significantly greater ($p \leq 0.001$) than all other treatments except LW and LR.

($n_1 + n_2 - 2$) df

Table 5.2.3: Experiment 3. Mean slip points (°C) of triacylglycerols isolated from the inner and outer backfat of pigs of differing breeds given the same diet *ad libitum*.

	Breed of Pig											Range SED
	LW	LR	T	M	D	GOS	P	S	OSB	LB	B	
Number of pigs (n)	9	11	8	8	11	9	10	9	6	10	9	
Melting points °C												
Inner backfat triacylglycerols	31.0 ^{bcd}	33.1 ^{ef}	38.3 ^{agh}	33.5 ^{gijk}	37.0 ^{bi1}	33.7	33.5 ^{hlmn}	36.1 ^{cj}	37.4	38.4 ^{dehm}	39.1 ^{cfjn}	0.6-2.3
Outer backfat triacylglycerols	27.1 ^{abcd}	29.9 ^e	33.0 ^{fg}	27.2 ^{fhijk}	31.7 ^{ah}	29.4 ¹	29.3 ^{glmn}	32.9 ^{bim}	34.8 ^{cej}	32.5 ^{dkn}	35.1	1.0-3.1

(n₁ + n₂ - 2)df

Values bearing the same superscript within rows are significantly different from each other ($p \leq 0.05$)

The compositions of fatty acids at position 2 and positions 1+3 of the adipose tissue triacylglycerols were determined by the pancreatic lipase hydrolysis technique (Section 2.2.6) and the results obtained are given in Table 5.2.4.

In general, palmitic acid was the major fatty acid esterified in position 2 of the triacylglycerols, followed by oleic acid, and with the other fatty acids present at concentrations of less than 10% each of the total. At the 1+3 - positions, oleic acid was the predominant fatty acid, followed by linoleic and stearic acids. The 16 carbon fatty acids were present at these 1+3 positions at concentrations of less than 5% each of the total.

Differences in fatty acid composition at position 2 of the triacylglycerols between the backfat layers of individual breeds of pig were small and generally not significant ($p > 0.05$). However, palmitic acid concentrations in position 2 of the adipose tissue triacylglycerols were greater in the inner compared with the outer backfat layer in the Tamworth, Duroc, Large White and Saddleback pigs although the differences were only significant with the latter two breeds ($p \leq 0.05$ and $p \leq 0.01$ respectively). A similar difference was observed in the stearic acid content of backfat triacylglycerols from the Gloucester Old Spot animals ($p \leq 0.001$). In contrast, the concentrations of linoleic acid at position 2 of the triacylglycerols were higher in the outer relative to the inner backfat layer of the Duroc pigs ($p \leq 0.05$).

5.2.2.2 Comparisons between different breeds of pig

Differences in the fatty acid compositions of the adipose tissue triacylglycerols from the various breeds of pig are given in Table 5.2.2.

The Pietrain, Hampshire and Large White pigs appeared to have the most unsaturated backfat triacylglycerols. The concentrations of palmitic and stearic acids in the adipose tissue triacylglycerols from these particular breeds of pig were among the lowest, and the adipose tissue concentrations of the unsaturated fatty acids were among the highest. In comparison, the Large Black, Berkshire, Tamworth and Gloucester Old Spot pigs tended to have more saturated adipose tissue triacylglycerols, as measured by the relative proportions of saturated and unsaturated fatty acids.

The slip points of the adipose tissue triacylglycerols are given in Table 5.2.3 and show differences between the different breeds of pig. Triacylglycerols which were more unsaturated *i.e.* those from the adipose tissue of the Large White, Hampshire and Pietrain pigs, had the lowest melting points. Conversely the more saturated adipose tissue triacylglycerols obtained from the Berkshire, Tamworth and Large Black pigs had the higher melting points.

The positional distribution of the fatty acids in the adipose tissue triacylglycerols of pigs from each breed is shown in Table 5.2.4. Significant differences in palmitic acid concentration at position 2 of the triacylglycerols were evident between pigs from several of the breeds. The Large White, Large Black, Berkshire and Saddleback pigs had over 70% of the total amount of this acid present esterified in position 2 of the backfat triacylglycerols and very low amounts at positions 1+3. In contrast the Landrace and Duroc pigs had less than 60% of palmitic acid at position 2 of the triacylglycerols but comparatively more of this acid at the 1+3 positions than did the other breeds of pig.

Table 5.2.4: Experiment 3. Mean distribution of fatty acids (weight percent) between positions 2 and 1+3 of adipose tissue triacylglycerols from the inner and outer backfat of pigs of differing breeds given the same diet ad libitum.

			LW	LR	T	H	D	GOS	P	S	OSB	LB	B	Range SED
16:0	Inner backfat	2	70.2	57.8*	71.6	66.2	66.7 ^d	65.6 ^{ac}	67.6 ^b	72.8 ^{bcde}	66.1 ^e	72.0 ^a	71.5	1.3 - 4.8
		1+3	-2.8	5.4	1.7	-1.4	-0.7	2.1	-2.5	-2.5	2.0	1.0	5.5	
	Outer backfat	2	66.2	57.4	64.9	69.7	57.6	64.8	67.1	68.3	67.5	70.2	71.7	1.7 - 5.2
		1+3	-3.7	3.1	3.0	-4.9	2.7	0.2	-3.7	-1.7	-4.6	-0.7	1.0	
16:1	Inner backfat	2	2.6 ^{abcd}	4.2 ^e	2.6 ^{gh}	7.4 ^{befh}	7.4 ^{bfb}	5.6 ^{agij}	3.6	5.4 ^{dj}	5.5 ^{ci}	3.5 ^f	5.7	0.5 - 1.6
		1+3	2.3	3.1	5.3	2.2	2.7	3.7	4.2	3.5	3.3	3.4	4.5	
	Outer backfat	2	2.3 ^{abcd}	3.8 ^e	2.7 ^{ghi}	5.6	7.5 ^{cefhj}	6.5 ^{bg}	3.6 ^j	6.3 ^{di}	4.7 ^c	3.9 ^f	8.4 ^a	0.4 - 1.8
		1+3	3.5	4.1	5.4	4.4	2.7	3.9	5.3	3.6	4.8	3.7	4.1	
18:0	Inner backfat	2	5.5 ^a	5.5	8.3	6.4	5.2 ^{fh}	7.5 ^{adfg}	8.7 ^{bc}	4.4 ^{cgi}	8.2 ^{ehi}	7.1	4.2 ^{bde}	0.5 - 2.0
		1+3	13.1	13.8	14.9	13.2	16.7	12.4	10.6	15.8	17.3	15.6	16.7	
	Outer backfat	2	5.1 ^{hij}	5.6 ^{klm}	10.8 ^{abhl}	5.8 ^e	6.2 ^{bc}	5.1 ^{bdg}	8.9 ^{cim}	7.0	7.4 ^{fgjk}	6.5 ^a	3.9 ^{acdef}	0.5 - 2.2
		1+3	11.1	10.6	9.3	10.3	13.4	9.9	8.0	11.1	12.6	11.6	14.3	
18:1	Inner backfat	2	15.9 ^a	20.8 ^{abcdef}	10.8 ^a	15.4	14.6 ^{eg}	14.7 ^d	10.9 ^{eg}	11.6 ^a	13.7 ^f	12.5 ^b	12.7 ^c	1.1 - 3.2
		1+3	59.7	57.1	62.1	63.0	60.5	63.1	61.4	63.8	60.7	63.1	57.5	
	Outer backfat	2	17.8 ^{abcd}	20.7 ^{efghi}	11.4 ^{ae}	14.2	20.6	15.9	12.7 ^f	12.5 ^{di}	11.9 ^{ch}	13.9	10.7 ^{bg}	1.1 - 3.4
		1+3	62.5	61.7	65.2	66.0	59.9	64.2	61.1	65.3	65.0	66.4	62.4	
18:2	Inner backfat	2	6.2 ^a	11.2 ^{abcdef}	7.8	4.6 ^{eg}	6.2 ^a	5.9 ^d	9.4 ^{gh}	5.7 ^{fh}	6.5	4.8 ^b	5.9 ^c	0.5 - 2.0
		1+3	22.6	18.7	13.9	19.9	18.3	17.1	23.1	17.1	15.2	14.2	13.9	
	Outer backfat	2	7.8	10.9 ^{abcde}	9.0	4.6 ^{dgh}	9.1 ^{fhijk}	6.6 ^{ci}	7.7 ^g	6.0 ^{ej}	6.4	6.1 ^{af}	5.4 ^{bh}	0.6 - 2.0
		1+3	22.3	18.3	15.4	21.6	18.6	20.2	26.8	19.2	18.7	16.4	15.5	
(n ₁ +n ₂ -2) df														

($n_1 + n_2 - 2$) df

Values bearing the same superscript within rows are significantly different ($p < 0.05$) from each other.

* The concentration of palmitic acid at position 2 of the triacylglycerols from the inner backfat of Landrace pigs was significantly lower than that of all the other breeds of pig ($p < 0.001$)

The distribution of palmitoleic acid between positions 2 and 1+3 of the backfat triacylglycerols was fairly even for the Large White, Landrace, Hampshire, Pietrain, Oxford Sandy and Black and Large Black breeds of pig, but was more asymmetric with the other breeds of pig. With these latter pigs the concentration of palmitoleic acid was greater at position 2 of the adipose tissue triacylglycerols in all but the Tamworth pigs.

Stearic acid concentrations were approximately two to three times greater at positions 1+3 than at position 2 of the adipose tissue triacylglycerols. The major exceptions were the triacylglycerols from the backfat of the Pietrain pigs where the distribution was more or less equal and with the Berkshire animals where the concentration of stearic acid at positions 1+3 of the adipose tissue triacylglycerols was about four times greater than that at position 2.

Oleic acid was asymmetrically distributed between positions 2 and 1+3 of the backfat triacylglycerols from all pigs with the concentration being much greater at the 1+3 positions. Despite the range of breeds, the oleic acid concentrations at the 1+3 - positions of the triacylglycerols differed by less than 10% between the eleven groups of pigs. The concentration of oleic acid at the 2 - position of the adipose tissue triacylglycerols was more variable, but tended to be greater in the adipose triacylglycerols of the Landrace and Duroc pigs. These were the breeds of pig with the lowest levels of palmitic acid at this same position.

Linoleic acid was also asymmetrically distributed in the backfat triacylglycerols, but not to such a large extent as oleic acid. The concentration of linoleic acid at positions 1+3 of the triacylglycerol was approximately

two to three times that at position 2. The pigs which had the most unsaturated adipose tissue triacylglycerols *i.e.* the Pietrain, Hampshire, Large White and Landrace breeds, also had the highest concentrations of linoleic acid at the 1+3 - positions of the triacylglycerols. In comparison, the more saturated triacylglycerols, obtained from the adipose tissue of the Berkshire, Large Black, Tamworth and Gloucester Old Spot pigs had much lower levels of linoleic acid at these 1+3 triacylglycerol positions. At position 2 linoleic acid concentrations were greatest in the triacylglycerols from the pigs which had the lowest levels of palmitic acid esterified in this same position *i.e.* the Landrace and Duroc pigs.

Pooled samples of triacylglycerols from the inner backfat tissue of pigs of each breed were randomly interesterified and their melting points determined after this procedure. The melting points of the triacylglycerol samples both before and after random interesterification are shown in Table 5.2.5. It can be seen that with each breed of pig the melting points of the randomly interesterified triacylglycerols were much lower than those of the original triacylglycerol samples ($p < 0.001$). The fall in melting point accompanying random interesterification of the triacylglycerol samples ranged from 5.5°C with the samples from the Berkshire pigs to 14.5°C with the samples from the Tamworth pigs.

5.2.3 Summary of results

In summary, the results suggest that the breed of pig may affect aspects of growth and metabolism. Growth rates varied considerably, being lowest in the Tamworth and greatest in the Berkshire pigs. Backfat thickness at the P_2 site was also influenced by breed, with the slower growing pigs tending

Table 5.2.5: Experiment 3. Mean melting points (°C) of pooled samples of inner backfat triacylglycerols of pigs of different breeds both before and after random interesterification of the triacylglycerol samples.

	Breed of Pig											
	LW	LR	T	M	D	GOS	P	S	OSB	LB	B	SED
Number of pigs (n)	9	11	8	8	11	9	10	9	6	10	9	
Melting point of sample (°C)												
Before random interesterification	31.0	33.1	38.3	33.5	37.0	33.7	33.5	36.1	37.4	38.4	39.1	1.5
After random interesterification	22.6	26.7	23.8	23.8	24.9	22.5	19.9	26.5	28.4	32.8	33.6	1.0

4df

to have thicker backfat.

Analysis of the backfat triacylglycerol composition suggested the presence of three main groups of pigs. The Berkshire, Tamworth, Large Black and Gloucester Old Spot pigs had adipose tissue triacylglycerols which were significantly more saturated than those of the Large White, Hampshire and Pietrain pigs. The remaining breeds of pig had backfat characteristics in between those of the other two groups. The fatty acid compositions were reflected in the slip points, with the more saturated triacylglycerols melting at higher temperatures. Random interesterification had the effect of decreasing the melting points of pooled samples of inner backfat triacylglycerols.

The analysis of the positional distribution of fatty acids in the adipose tissue triacylglycerols showed that palmitic acid was the major fatty acid esterified in position 2, followed by oleic acid. Breed appeared to affect the level of palmitic acid at this 2 - position and also the amount of unsaturated 18 carbon fatty acids at the 1+3 - positions of the triacylglycerols.

5.3 Discussion of results of Experiment 3

5.3.1 Growth rates and thickness of subcutaneous backfat

The mean rates of growth of the different breeds of pig are shown in Table 5.2.1. Although some breeds of pig had similar growth rates ($p>0.05$), the difference in growth rates between pigs displaying the two extreme values *i.e.* the Tamworth and Berkshire pigs, was 2.5 kg per week. However as the pigs were given the grower ration *ad libitum* and the experiment was carried out elsewhere, no information was available on daily feed intakes and so feed conversion ratios cannot be calculated. Nevertheless, although there may be differences in the efficiencies of feed conversion and the appetites of pigs of differing breeds, it appears that for whatever reason some breeds of pig gain weight more rapidly than others.

Experiments carried out on the effects of breed on rates of growth have tended to concentrate on two or three breeds of pig and their crosses. Thus, for example, Landrace pigs are reported to have greater average daily gains than Pietrain pigs when offered food *ad libitum* while LR x P crosses have average daily gains similar to those of the pure-bred Landrace animals (Lean *et al.*, 1972). However, these findings and similar reports by other authors are not particularly valuable for comparison with the present experiment as they only consider two or three pure breeds of pig. In addition, as a result of cross-breeding and selection for improved performance, some of the breeds of pig used in the present study are no longer produced commercially. Published data referring to the growth rates of these animals is, therefore, not available. However, with the exception of the Gloucester Old Spot pigs, the rates of growth of the Large White, Landrace, Duroc, Gloucester Old Spot, Hampshire and Saddleback pigs from the present experiment follow trends similar to those listed by the

Meat and Livestock Commission (1978) *i.e.* the rates of growth followed the order $LW > LR > D > H > S$ pigs, although the actual rates of growth and the differences between them for pigs of different breeds were not always comparable.

The thickness of backfat, measured at the P_2 site, was also found to vary with the breed of pig (Table 5.2.1). Although only the P_2 backfat thickness was measured this is still a useful indicator of overall fatness as changes in the fat thickness at this particular site reflect those at the shoulder and loin regions of the subcutaneous backfat (Section 3.3.2).

The breeds of pig which had the greater growth rates also tended to have thinner backfat (Table 5.2.1). However this is not a consistent trend as the Tamworth pigs, which displayed the lowest rates of growth, had backfat which was thicker than that of all but the Large Black animals. Thus when the rates of growth were plotted against the P_2 backfat thicknesses, no significant correlations ($p > 0.05$) were observed either between pigs of the same breed or between pigs of different breeds. Thus pigs of different breeds which display similar rates of growth when kept under the same conditions may have differing thicknesses of backfat. These findings are in agreement with Walstra *et al.* (1984) and Wiseman (1986) who also reported that the carcass backfat thickness appeared to vary with the breed of pig.

In Experiment 1 the P_2 backfat thickness was found to correlate negatively with the concentration of linoleic acid in the adipose tissue triacylglycerols (Section 3.3.2). When the results of the present experiment were examined, negative relationships were noted for each breed of pig, although the correlations were not always significant at the 5% level, possibly owing to the small number of pigs within some of the breed groups. The linear regressions which were

significant at the 5% level are shown in Table 5.3.1.

The linear regressions for the Landrace, Large Black, Duroc and Saddleback pigs were not significantly different from each other ($p > 0.05$), with the exception of the intercepts from the Landrace and Duroc regressions ($p \leq 0.05$). However, the other regressions, which were not significant ($p > 0.05$), had gradients which varied markedly with the breed of pig, suggesting that breed differences may occur.

The Large Black pigs had much thicker backfat than did the Landrace, Saddleback and Duroc pigs. The animals did not receive a very high fat diet and so the deposited fat may be derived mainly from *de novo* synthesis of fatty acids. As pigs cannot synthesize linoleic acid, the higher rate of endogenous fat synthesis is accompanied by a lower concentration of linoleic acid in the adipose tissue compared with pigs of the other breeds which have a lower propensity to fat deposition. These findings are consistent with those of Experiment 1 where similar relationships between P_2 backfat thickness and adipose tissue linoleic acid concentration were noted with pigs receiving the same level of dietary linoleic acid (Figure 3.3.1).

The relationships between the P_2 backfat thicknesses and the concentrations of oleic acid in the adipose tissue triacylglycerols did not reach the 5% level of significance with any of the breeds of pig. This may be a result of the small number of pigs within each breed group. However the relationships did follow trends similar to those of the pigs from Experiment 1 (Figure 3.3.2) *i.e.* thicker layers of backfat were associated with higher adipose tissue concentrations of oleic acid owing to increased synthesis of this fatty acid in the adipose tissue.

Table 5.3.1: Experiment 3

Relationships between the backfat thicknesses (mm) at the P₂ site (x) and the concentration of linoleic acid (weight percent) in the adipose tissue triacylglycerols (y) of pigs of the Landrace, Large Black, Duroc and Saddleback breeds.

<u>Breed</u>	<u>Linear Regression</u>	<u>Significance</u>
Landrace	$y = 19.24 - 0.36x$	$p < 0.05$
Large Black	$y = 16.73 - 0.21x$	$p < 0.05$
Duroc	$y = 20.57 - 0.4x$	$p < 0.05$
Saddleback	$y = 21.10 - 0.42x$	$p < 0.05$

Relationships for the other breeds of pig did not reach 5% level of significance.

The concentrations of the other fatty acids in the adipose tissue triacylglycerols of pigs of the same breed did not correlate significantly with the backfat thickness at the P₂ site ($p > 0.05$).

5.3.2 Composition, structure and physical properties of the adipose tissue triacylglycerols

5.3.2.1 Comparisons between different adipose tissue sites

The triacylglycerols extracted from the subcutaneous adipose tissue samples were significantly more saturated in the inner compared with the outer backfat layers of animals of all breeds, except for the Large White pigs (Table 5.2.2). These differences are in keeping with previously reported work (De Wilde, 1984; Sturm *et al.*, 1982; Koch *et al.*, 1986a) and with the results of both Experiments 1 and 2 (Sections 3.2.4.1 and 4.2.3.1). However, in contrast to the findings of the present study, Christie *et al.* (1972) noted differences between the fatty acid compositions of triacylglycerols from the two backfat layers of pure-bred Large White pigs. De Wilde (1984) and Sturm *et al.* (1982) attributed these differences in the fatty acid compositions of the backfat layers to the greater activity in the inner backfat layer of the lipogenic enzymes which synthesize fatty acids from carbohydrate. In addition the results of Experiment 2 suggested that Δ^9 desaturase activity tended to be greater in the outer compared with the inner backfat layer and this would also contribute to the greater unsaturation of the outer layer of backfat (Section 4.2.4.1). It has also been reported that lipoprotein lipase activity is greater in the outer compared with the inner layer of pig backfat (Enser, 1973). These differences in the relative contributions of endogenous synthesis and uptake of dietary fatty acids between the two backfat layers may account for the differences in overall fatty acid composition noted between these two adipose tissue sites

in each breed of pig.

The outer backfat triacylglycerols from each pig had melting points lower than those of the corresponding triacylglycerol samples of the inner backfat. This is also consistent with previous reports as greater amounts of unsaturated fatty acids are generally associated with lower triacylglycerol melting points (Section 1.2.1). However, the Large White pigs had inner and outer backfat triacylglycerols which had similar fatty acid compositions ($p > 0.05$, Table 5.2.2) but melting points which were significantly different from each other ($p \leq 0.05$, Table 5.2.3). It seemed that this difference may be related to the positional distributions of the fatty acids in the triacylglycerols from each backfat layer. However no significant differences were noted in the patterns of fatty acid distribution in the triacylglycerols from each backfat layer from individual breeds of pig (Table 5.2.4), but it has been reported that even small differences in the distribution of the fatty acids in the adipose tissue triacylglycerols may cause marked differences in the triacylglycerol melting points (Moore *et al.*, 1969). It is also possible that the distributions of the various triacylglycerol species are different between the two layers of backfat.

The positional distributions of the fatty acids were similar between the inner and outer layers of backfat from pigs of the same breed (Table 5.2.4). This suggests that the specificities of the acyltransferases involved in triacylglycerol synthesis are similar at each of the two sites and this is consistent with the findings of both Experiments 1 and 2 and with the work of Christie and Moore (1970a).

5.3.2.2 Comparisons between different breeds of pig

The mean fatty acid compositions of the inner and outer backfat triacylglycerols from each of the eleven breeds of pig are shown in Table 5.2.2. Differences in composition were noted between all of the breeds of pig, with some animals having much more unsaturated backfat and hence lower triacylglycerol melting points than others. Lea *et al.* (1970) noted that, in a study of cross-bred pigs, most of the triacylglycerol samples which had high concentrations of unsaturated fatty acids and hence very low melting points were obtained from half-Hampshire crosses. In accordance with this, adipose tissue triacylglycerols from Hampshire pigs on the present experiment were among the most unsaturated and had some of the lowest melting points when compared with triacylglycerols from animals of other breeds (Tables 5.2.2 and 5.2.3). In addition, Metz (1984) noted that Large White and Pietrain pigs had backfat triacylglycerols of similar fatty acid composition and this is also in agreement with the findings of the present study.

In both Experiments 1 and 2, previously reported, the degree of saturation was found to correlate with the melting point of the adipose tissue triacylglycerols (Sections 3.3.3.3 and 4.3.3.2). Similarly, in the present experiment, with each breed of pig the ratio of saturated:unsaturated fatty acids was positively correlated with the melting point of the adipose tissue triacylglycerols ($p < 0.01$, Table 5.3.2). In addition these linear regressions were found to be similar between some of the breeds of pig ($p > 0.05$) such that three groups of animals were evident. The first group comprised the Large White, Tamworth, Gloucester Old Spot, Pietrain and Oxford Sandy and Black pigs; the second group comprised the Landrace and Duroc pigs and the remaining pigs constituted the third group of animals. Significant linear relationships were

Table 5.3.2: Experiment 3

Relationships between the ratios of the concentrations (wt %) of saturated:unsaturated fatty acids (x) and the melting points ($^{\circ}\text{C}$, y) of adipose tissue triacylglycerols for each breed of pig.

<u>Breed</u>	<u>Linear Regression</u>	<u>Significance</u>
LW	$y = 20.27x + 29.37$	$p \leq 0.05$
T	$y = 24.67x + 22.76$	$p \leq 0.05$
GOS	$y = 23.44x + 20.45$	$p \leq 0.05$
P	$y = 23.39x + 21.55$	$p \leq 0.05$
OSB	$y = 24.40x + 23.36$	$p \leq 0.01$
LR	$y = 14.02x + 24.49$	$p \leq 0.05$
D	$y = 14.95x + 27.48$	$p \leq 0.05$
H	$y = 54.19x + 7.55$	$p \leq 0.01$
S	$y = 54.02x + 6.85$	$p \leq 0.001$
LB	$y = 56.54x + 3.60$	$p \leq 0.001$
B	$y = 61.79x - 0.59$	$p \leq 0.05$

also obtained between the ratios of saturated:unsaturated fatty acids and the melting points of the adipose tissue triacylglycerols of pigs from these three groups. The three relationships obtained were significantly different from each other ($p \leq 0.05$).

Similarly the biased ratio of saturated:unsaturated fatty acids was significantly correlated with the melting point of the adipose tissue triacylglycerols for each breed of pig (Table 5.3.3). This biased ratio takes into account the greater effect of the more unsaturated fatty acids on the triacylglycerol melting point although the equation used is arbitrary with respect to the effects of the number of double bonds on the melting points of the triacylglycerols. As with the relationships calculated using the unbiased ratio, some of the linear regressions obtained were not significantly different from each other ($p > 0.05$) and the same three groups of pigs were noted. When the results from pigs of these three groups were examined, significant linear relationships were obtained for each group between the biased saturated:unsaturated ratio of fatty acids and the slip point of the adipose tissue triacylglycerols ($p \leq 0.01$, Figure 5.3.2). These three linear relationships were significantly different from each other ($p \leq 0.05$).

It is interesting to note that the three groups of pigs do not correspond to the breeds of pig which have similar fatty acid compositions but rather to those which exhibit similar patterns of fatty acid distribution in the adipose tissue triacylglycerols (Tables 5.2.2 and 5.2.4). This is in keeping with the results of Experiment 1 where similar findings were reported (Section 3.3.3.3); pigs given diets which were based on the same source of protein had similar relationships between the degree of saturation and the melting points of their adipose tissue triacylglycerols despite the differences in overall triacyl-

glycerol fatty acid composition arising from the different dietary treatments. In contrast the linear relationships were different between pigs given diets based on either fishmeal or soyabean meal respectively as the adipose tissue triacylglycerols from these pigs had differing patterns of fatty acid distribution. However whereas the relationships for the three groups of pigs from the present experiment were significantly different from each other ($p \leq 0.05$) those on the fishmeal and soyabean based treatments respectively of Experiment 1 were parallel. This difference may be an effect of breed; the pigs used in Experiment 1 were of the same breed whereas the three groups of animals used in Experiment 3 were combinations of different breeds of pig.

These findings from both Experiments 1 and 3 support the suggestion that the positional distribution of the fatty acids can have a major effect on the melting point of triacylglycerols. This suggestion can be studied further by the use of the random interesterification technique which hydrolyses triacylglycerols and then re-esterifies the fatty acids in a random fashion. This procedure^{*} in melting points between triacylglycerol samples are attributable to differences in fatty acid composition alone.

Pooled samples of inner backfat triacylglycerols from each of the eleven breeds of pig were randomly interesterified as described in Section 2.2.6, and the melting points before and after random interesterification were compared (Table 5.2.5). With each breed of pig the melting point of the randomly interesterified triacylglycerols was lower than that of the original sample, suggesting that with these samples non-random distribution of the fatty acids had the effect of maintaining a higher melting point. The fall in triacylglycerol melting point accompanying random interesterification was greater with some breeds of pig than with others, suggesting that different patterns

* eliminates the effects of positional distribution so that the differences

Table 5.3.3: Experiment 3

Relationships between the biased ratios of the concentrations (wt %) of saturated:unsaturated fatty acids (x) and the melting points ($^{\circ}\text{C}$, y) of adipose tissue triacylglycerols for each breed of pig.

<u>Breed</u>	<u>Linear Regression</u>	<u>Significance</u>
LW	$y = 39.13x + 20.88$	$p \leq 0.05$
T	$y = 49.88x + 24.12$	$p \leq 0.05$
GOS	$y = 50.27x + 21.07$	$p \leq 0.05$
P	$y = 50.11x + 22.42$	$p \leq 0.05$
OSB	$y = 50.59x + 23.77$	$p \leq 0.05$
LR	$y = 24.55x + 26.16$	$p \leq 0.05$
D	$y = 23.54x + 29.66$	$p \leq 0.05$
H	$y = 126.65x + 7.23$	$p \leq 0.001$
S	$y = 112.5x + 8.88$	$p \leq 0.001$
LB	$y = 118.96x + 5.53$	$p \leq 0.01$
B	$y = 111.58x + 6.97$	$p \leq 0.01$

Figure 5.3.1: Experiment 3. Relationship between the saturated:unsaturated ratios of fatty acid concentrations (x) and the slip points ($^{\circ}\text{C}, y$) of adipose tissue triacylglycerols from three groups of pigs.

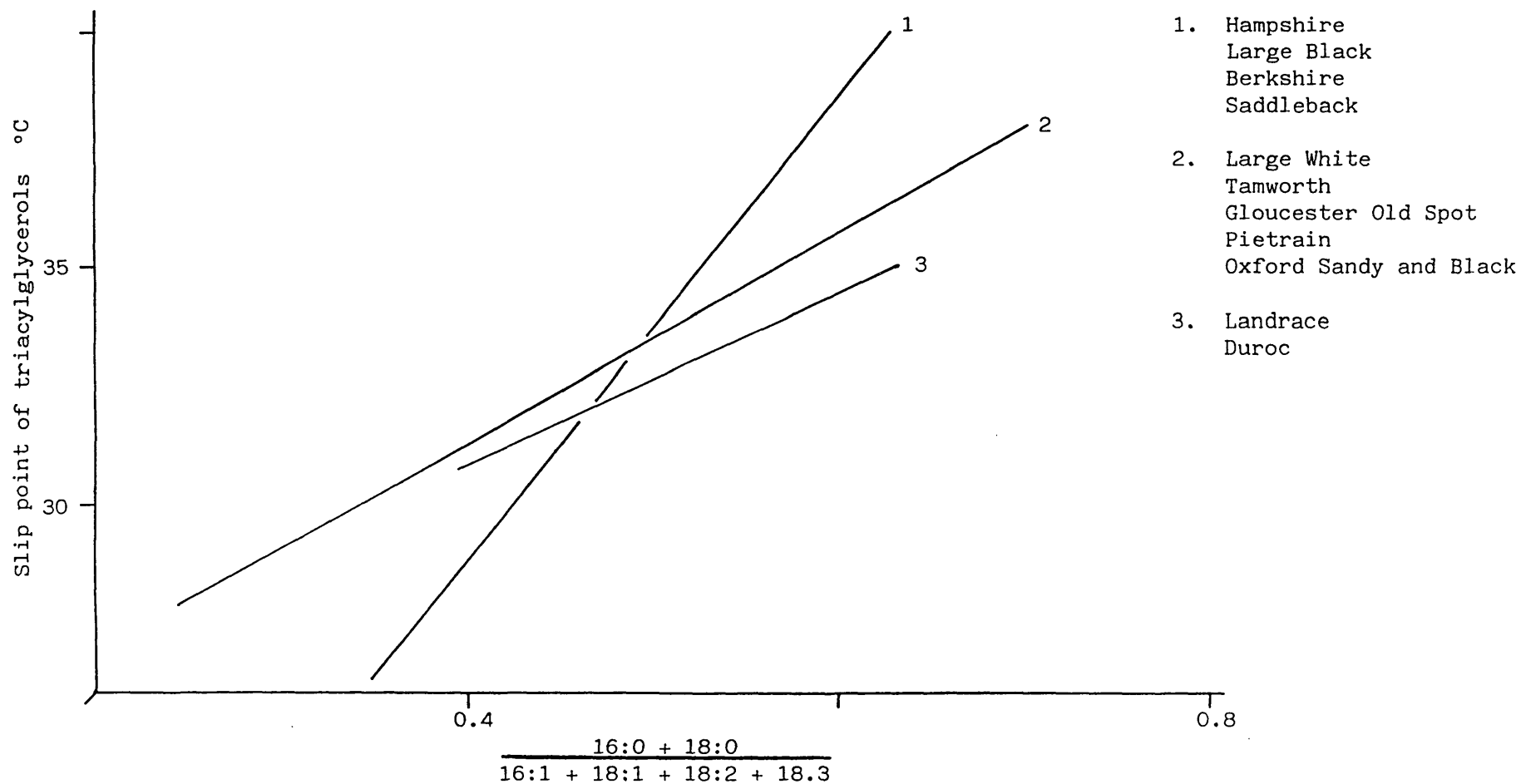
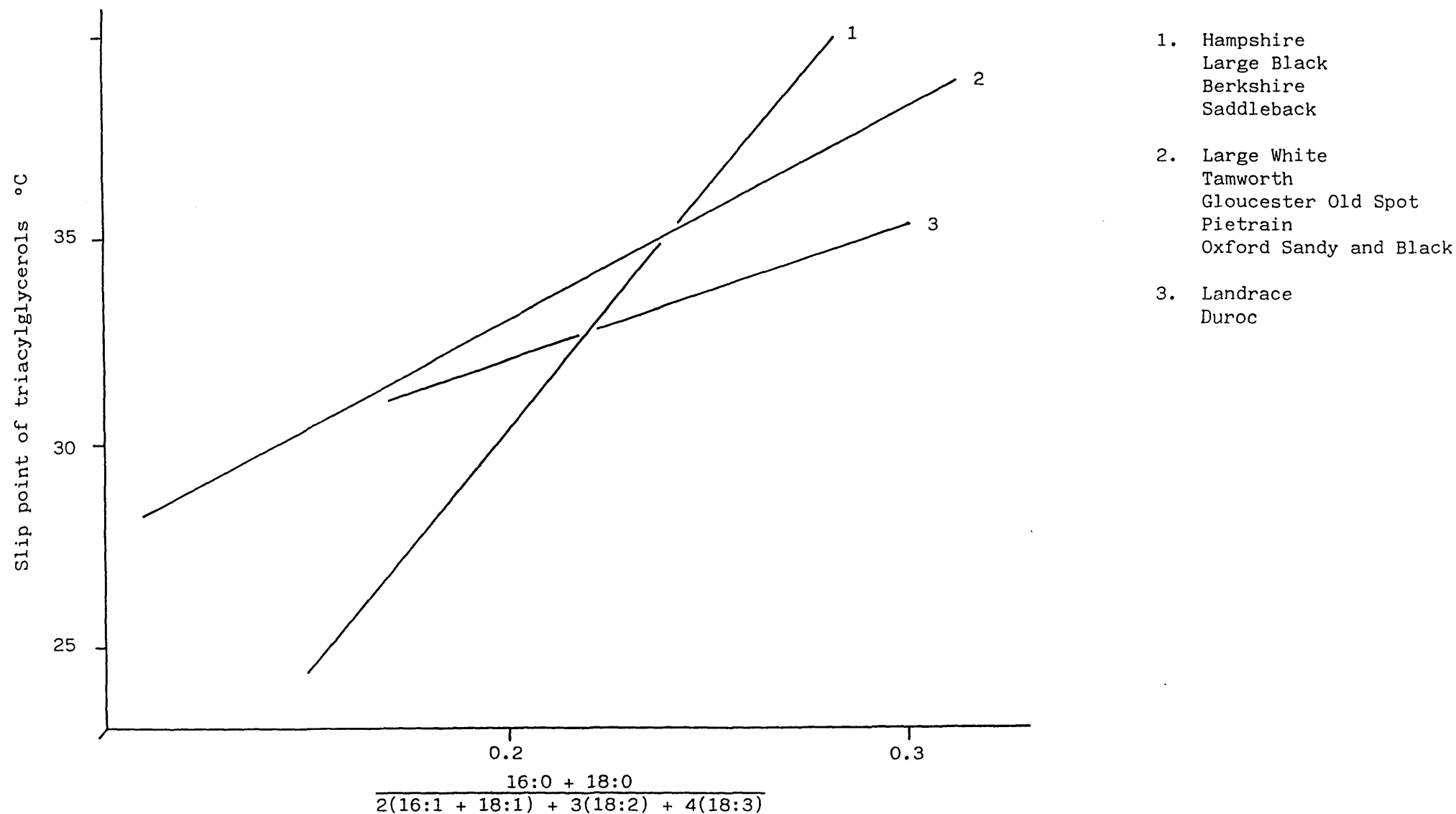


Figure 5.3.2: Experiment 3. Relationships between the biased saturated:unsaturated ratios of fatty acid concentrations (x) and the slip points ($^{\circ}\text{C}$, y) of adipose tissue triacylglycerols from three groups of pigs.



of positional distribution were associated with different melting points although differences in the overall fatty acid compositions of the triacylglycerols are also involved. When the ratio of saturated:unsaturated fatty acids was plotted against the melting point of the randomly interesterified triacylglycerols a single relationship was observed with the results from all eleven breeds of pig ($p \leq 0.001$, Figure 5.3.3). Similarly a single significant correlation was also observed when the biased ratio of saturated;unsaturated fatty acids was used ($p \leq 0.001$, Figure 5.3.4).

Enser *et al.* reported that the concentration of linoleic acid in the adipose tissue triacylglycerols was a good indicator of fat softness and that, in addition, the concentrations of stearic and palmitic acids respectively were correlated with the triacylglycerol melting points. When the concentrations of the various fatty acids were plotted against the melting points of the adipose tissue triacylglycerols from pigs of each breed from the present experiment, significant correlations were noted with most breeds for all but the linolenic acid relationships. The gradients of the linear relationships which were significant at the 5% level or above are shown in Table 5.3.4.

The lack of significant correlations with several of the breeds of pig may be a result of the small number of pigs involved. However it can be seen that the groups of pigs which display similar patterns of fatty acid distribution in their adipose tissue triacylglycerols did not always have similar gradients for the relationships between the concentrations of a particular fatty acid and the triacylglycerol melting point. This was not as expected, but it may be that although the positional distribution of fatty acids was similar between position 2 and positions 1+3 of the

Table 5.3.4: Experiment 3. Gradients of linear regressions calculated between the concentrations (weight percent) of each fatty acid (x) and the melting points ($^{\circ}\text{C}$) of the adipose tissue triacylglycerols (y) for all eleven breeds of pig.

Breed of Pig	n	Fatty Acid					
		16:0	16:1	18:0	18:1	18:2	18:3
LW	9	0.96	1.92	1.04	-0.88	ns	ns
T	8	1.22	ns	0.85	-1.13	ns	ns
GOS	9	1.82	ns	1.89	ns	-1.15	ns
P	10	0.90	ns	1.82	-1.19	ns	ns
OSB	6	ns	ns	0.90	ns	-0.76	ns
LR	11	ns	ns	0.88	-0.94	ns	ns
D	11	ns	ns	1.21	ns	ns	ns
H	8	2.09	-1.23	1.36	ns	-1.44	ns
S	9	1.04	-2.88	0.95	-0.55	ns	ns
LB	10	0.66	ns	1.02	-0.81	-0.84	ns
B	9	0.92	ns	0.87	ns	-1.81	ns

ns = $p > 0.05$

Figure 5.3.3: Experiment 3. Relationship between the ratios of the concentrations of saturated:unsaturated fatty acids and the melting points of randomly interesterified triacylglycerols from pooled samples of triacylglycerols from eleven breeds of pig.

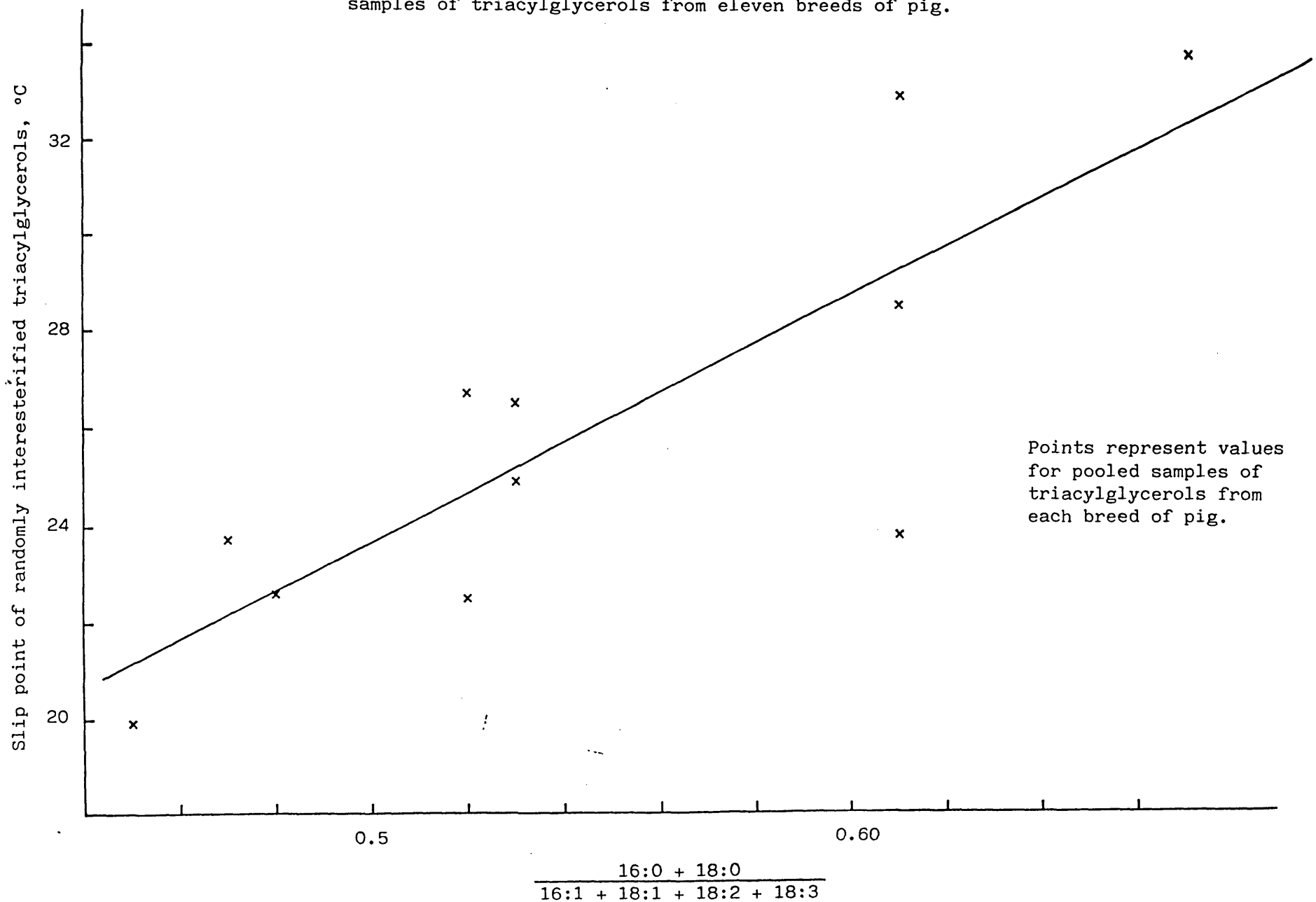
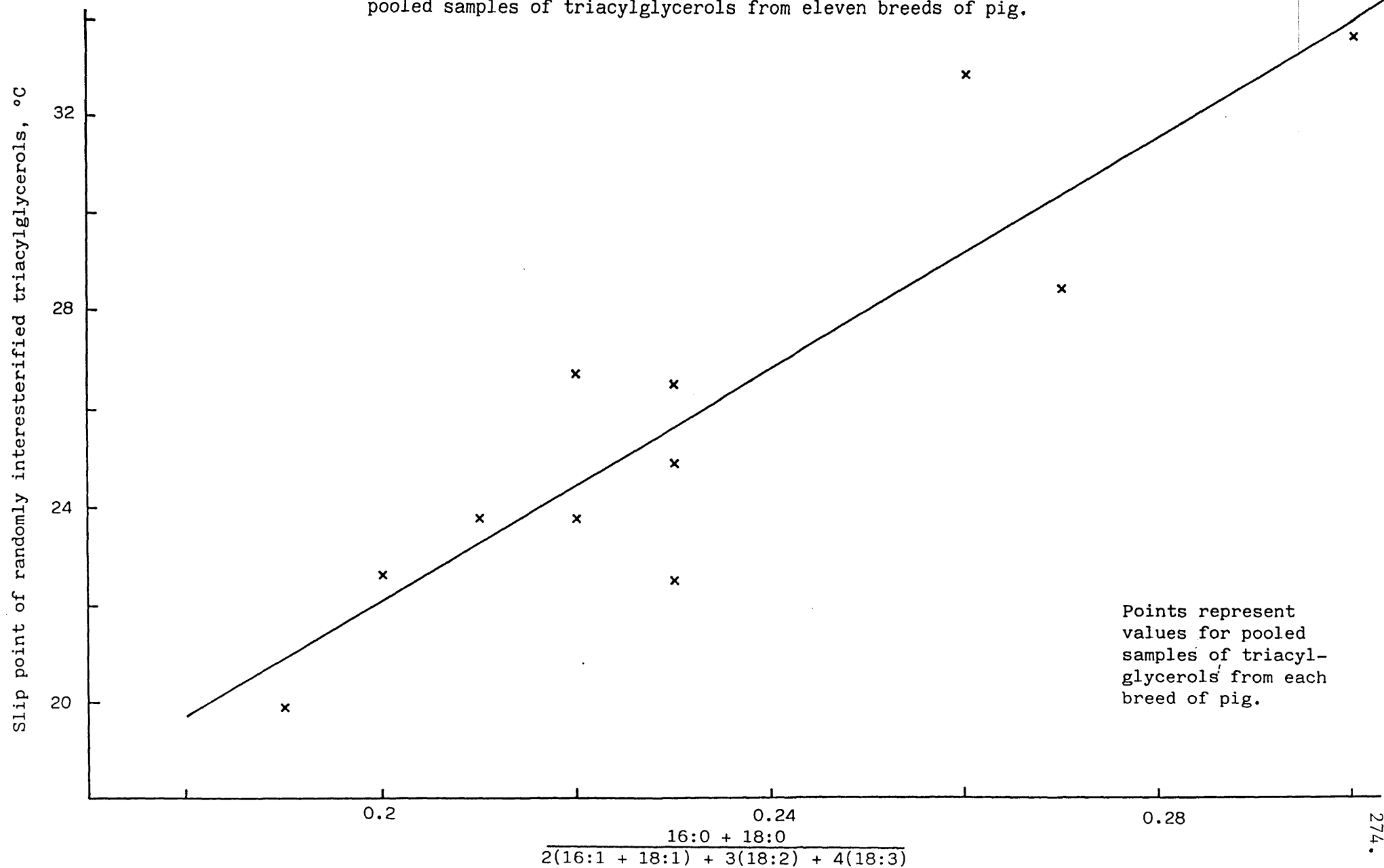


Figure 5.3.4: Experiment 3. Relationship between the biased ratios of the concentrations of saturated:unsaturated fatty acids and the melting points of randomly interesterified triacylglycerols from pooled samples of triacylglycerols from eleven breeds of pig.



triacylglycerols from the pigs of different breeds, the distribution at positions 1 and 3 respectively may have been different. In addition, the different breeds of pig may not have corresponding ranges of the molecular species of triacylglycerols. Thus an increase in the concentration of a particular fatty acid may alter the distribution of triacylglycerol species in a manner which may be different for each breed of pig and this in turn may affect the overall triacylglycerol melting point. Despite this, the linear relationships obtained tended to follow the expected trends *i.e.* increases in the concentrations of the saturated fatty acids were associated with an increase in the triacylglycerol melting point while the opposite was found to accompany an increase in the unsaturated fatty acid content of the adipose tissue triacylglycerols.

The lack of consistent results between pigs with similar patterns of fatty acid distribution in their triacylglycerols may also be explained by the fact that changes in the concentration of a single fatty acid are not necessarily indicative of changes in the overall fatty acid composition of the adipose tissue triacylglycerols. For example, a decrease in oleic acid concentration may be accompanied by an increase in the concentrations of either stearic or linoleic acids or both of these, and in each case a different effect on the triacylglycerol melting point would be noted. It was, therefore, decided to plot the various ratios of the concentrations of the 18 carbon fatty acids against the melting points of the adipose tissue triacylglycerols. The relationships between the oleic linoleic acid ratios and the melting points of the adipose tissue triacylglycerols for each breed of pig were not significant at the 5% level. However the gradients of the linear regressions for the stearic:oleic and stearic:linoleic acid ratios respectively against the triacylglycerol melting points for each breed of pig are shown in Table 5.3.5. The gradients of these regressions were not significantly different between

Table 5.3.5: Experiment 3.

Gradients of the relationships between the ratios of the concentrations (weight percent) of stearic:oleic acids and stearic:linoleic acids respectively (x) and the melting points ($^{\circ}\text{C}$) of the adipose tissue triacylglycerols (y) for each of the eleven breeds of pig.

Breed of Pig	n	x variable	
		18:0/18:1	18:0/18:2
LW	9	50.24	14.80
T	8	51.84	13.05
GOS	9	52.20	13.42
P	10	52.09	12.15
OSB	6	52.62	12.54
LR	11	45.90	9.89
D	11	39.90	10.28
H	8	63.43	15.54
S	9	56.64	15.30
LB	10	68.13	15.65
B	9	54.92	18.46

different breeds of pig at the 5% level but despite this lack of statistical evidence the gradients tended to be similar for pigs which had similar patterns of fatty acid distribution in their adipose tissue triacylglycerols. These findings are consistent with those obtained when the saturated:unsaturated ratios of fatty acids were plotted against the melting points of the adipose tissue triacylglycerols (Table 5.3.2).

Table 5.3.5 shows that the steepest gradients were generally obtained for both the stearic:oleic and stearic:linoleic acid ratios with the results from the pigs that had the more asymmetric distribution of fatty acids in the adipose tissue triacylglycerols *i.e.* the animals of the Hampshire, Saddleback, Berkshire and Large Black breeds. This is in agreement with the findings of Experiment 1 (Section 3.3.3.2) where it was noted that the changes in melting point accompanying the changes in overall fatty acid composition were greatest with the triacylglycerols which had the most asymmetric pattern of fatty acid distribution. The less asymmetric triacylglycerols have a more random pattern of fatty acid esterification which in turn leads to a wider range of triacylglycerol species. The melting point of a heterogeneous mixture of triacylglycerols in effect represents both the melting of triacylglycerols with low melting points and the dissolving in these of the triacylglycerols which normally melt at higher temperatures. Thus the melting points of samples of triacylglycerols which contain a wider range of triacylglycerol species may be affected less by changes in overall fatty acid composition as the distribution of fatty acids in these triacylglycerols is more random than with comparable samples of triacylglycerols whose ranges of triacylglycerol types are more limited. When the fatty acid composition of the triacylglycerols is altered, those triacylglycerol samples with the more random pattern of fatty acid distribution may be less affected, with regard to changes in

melting point, as the fatty acids are esterified in much the same pattern. However with the more asymmetric triacylglycerols changes in fatty acid composition may have the effect of decreasing the asymmetry of the triacylglycerols as the pattern of fatty acid distribution becomes more random. This change in the pattern of fatty acid distribution may have a considerable effect on the melting point of the triacylglycerols.

The findings of the present experiment are not in full agreement with those of Enser *et al.* (1984) who noted that the stearic:linoleic acid ratio could be used as an index of fat softness. It has been shown, both in the present experiment and with the results of Experiments 1 and 2, that the positional distributions of the fatty acids in the adipose tissue triacylglycerols may have a marked effect on the melting point of the triacylglycerols. Thus for a particular stearic:linoleic acid ratio, there may be a considerable range of melting points depending on the pattern of fatty acid distribution in the triacylglycerol sample. This can be illustrated by reference to the pigs used in the present experiment. Although the fatty acid compositions of the adipose tissue triacylglycerols from, for example, the Large White and Duroc pigs were not significantly different from each other ($p > 0.05$), their melting points differed by as much as 6°C (Tables 5.2.2 and 5.2.3).

The importance of the positional distribution of the fatty acids in determining the melting point of the triacylglycerols suggests that the various fatty acid ratios which have been put forward as indices of fat softness are only valid when the triacylglycerols from the pigs under consideration have the same general pattern of fatty acid distribution between positions 1, 2 and 3. This is supported by the results of the present experiment; the ratio of saturated:unsaturated fatty acids correlated with the slip point

of the adipose tissue triacylglycerols from pigs which had the same overall pattern of fatty acid esterification, irrespective of their fatty acid compositions (Figure 5.3.1). However, after random interesterification of the triacylglycerols, a procedure which eliminates the effects of positional distribution, a single correlation was observed with the results from all eleven breeds of pig (Figure 5.3.3).

The effects of the positional distribution of fatty acids on the triacylglycerol melting point may also be studied by plotting the concentrations of the individual fatty acids at the 2- and 1+3 - positions respectively against the slip points of the adipose tissue triacylglycerols. Unfortunately, when the results for each breed of pig were considered separately only a few correlations were found to be significant at the 5% level. This may be a result of the small number of samples employed, but as they were insufficient for further study, the linear regressions are not reproduced here.

Although this further investigation into the effects of positional distribution on the melting points of adipose tissue triacylglycerols was not possible, it is nevertheless evident that different breeds of pig have characteristic patterns of fatty acid distribution in their adipose tissue triacylglycerols and that these play an important part in determining the triacylglycerol melting point. In an earlier study, Lea *et al.* (1970) reported that when different cross-bred pigs were given similar diets, the half-Hampshire crosses tended to have softer fat than the other animals. It may be that these animals have a highly asymmetric distribution of fatty acids in their adipose tissue triacylglycerols, as noted with the pure-bred Hampshire pigs of the present experiment, although in addition these latter pigs had backfat triacylglycerols which were amongst the most unsaturated of all the breeds

of pig studied (Tables 5.2.4 and 5.2.2).

As discussed earlier the more asymmetric triacylglycerols types may be subject to greater changes in melting point when the overall fatty acid composition of the triacylglycerols is altered. Similarly the feeding of a particular diet to pigs of different breeds may result in markedly differing adipose tissue triacylglycerol melting points. These findings would suggest that certain breeds of pig, such as the Hampshire animals which display a more asymmetric pattern of fatty acid distribution and tend to have more unsaturated adipose tissue triacylglycerols may be more prone to produce soft fat than other breeds of pig whose adipose tissue is affected less by changes in the overall fatty acid composition. With this in mind it may be possible to reduce the occurrence of excessively soft fat by selecting pigs on the basis of their triacylglycerol types in addition to the more usual feed and growth-related criteria. The source of dietary protein has also been shown to affect the positional distribution of fatty acids in triacylglycerols (Section 3.3.3.2), and so careful selection of the diet may also serve to prevent the occurrence of soft fat in pigs whose adipose tissue triacylglycerols are highly asymmetric in nature.

5.4 General summary

The results of the present experiment suggest that some breeds of pig gain weight more rapidly than others when fed the same diet *ad libitum*. These differences in growth rates between breeds of pig are well-established and the differences noted between some of the breeds on the present experiment are consistent with those previously reported (MLC, 1978).

The backfat thicknesses also tended to vary with the breed of pig and this is in accordance with the findings of Walstra *et al.* (1984) and Wiseman (1986). However pigs of different breeds with similar growth rates did not necessarily have comparable backfat thickness measurements. The P_2 backfat thickness was found to correlate negatively with the concentration of linoleic acid in the adipose tissue triacylglycerols and although not all of the relationships were statistically significant it appears that breed differences may exist. Wood and Enser (1982) reported a similar relationship with pigs of the same breed and with a limited range of backfat linoleic acid concentrations.

With each pig, triacylglycerols from the outer backfat layer were more unsaturated and had lower melting points than did the triacylglycerols from the corresponding inner layer of backfat. These findings are consistent with those of Moore *et al.* (1969), Hood and Allen (1973) and others. However for each breed of pig the positional distribution of fatty acids was similar between triacylglycerols from both of these sites. This is in agreement with Christie and Moore (1970a) who suggested that the acyltransferases in the two layers of backfat had similar specificities.

Differences between the fatty acid compositions of the adipose tissue

triacylglycerols were apparent between the different breeds of pig. However although some breeds of pig such as the Large White and Duroc animals had triacylglycerol compositions which were not significantly different from each other, the triacylglycerol melting points were not comparable. This difference may be a result of the differences in the positional distributions of the fatty acids in the triacylglycerols which were noted between the different breeds of pig. In keeping with this the ratio of saturated:unsaturated fatty acids was correlated with the slip point of the triacylglycerols for each breed of pig. In addition, these relationships were similar between those breeds of pig whose patterns of fatty acid distribution in positions 1, 2 and 3 of the adipose tissue triacylglycerols were similar. Three such groups of pigs were noted; the Large White, Tamworth, Gloucester Old Spot, Pietrain and Oxford Sandy and Black animals comprised one group; the Landrace and Duroc pigs comprised the second group and the third group was composed of the animals of the remaining four breeds of pig. The three relationships obtained were significantly different from each other. However, after random interesterification of the triacylglycerols, which removes the effects of non-random positional distribution, a similar plot using the results from each breed of pig gave a single line. This supports the suggestion that the positional distribution of fatty acids in the triacylglycerols plays an important part in determining the melting point of the adipose tissue triacylglycerols (Moore *et al.*, 1969).

The ratios of stearic:oleic and stearic:linoleic acids respectively were correlated with the melting points of the adipose tissue triacylglycerols for each breed of pig. As with the saturated:unsaturated ratios of fatty acids the gradients of these relationships were similar between pigs whose adipose tissue triacylglycerols displayed similar patterns of fatty acid

distribution. In addition the steeper gradients were obtained with the results from the pigs which had the more asymmetric distribution of fatty acids in their adipose tissue triacylglycerols. This is in keeping with the results of Experiment 1 where it was noted that the more asymmetric triacylglycerol types were associated with greater changes in melting point when the overall fatty acid composition of the triacylglycerols was altered by changes in the diet (Section 3.3.3.2).

Thus, in summary, the results of the present experiment suggest that, when given the same diet *ad libitum*, pigs of different breeds may have different rates of growth and different backfat thickness measurements. In some cases a greater growth rate was associated with thinner backfat but this is not a consistent trend. Both the composition and the positional distribution of the fatty acids in the adipose tissue triacylglycerols were affected by breed and differences in the latter may account for the differences in melting point noted between triacylglycerol samples with similar overall compositions, but from pigs of different breeds.

Section 6
EXPERIMENT 4

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Experiment 4

The effects of dietary copper and sodium salicylate on pig backfat quality

6.1.1 Introduction

During the initial experiments which studied the beneficial effects of copper on pig growth rates, a dietary supplement of 250 ppm CuSO_4 was found to give the optimum results (Braude, 1967). However since then the level of dietary copper present in standard pig rations has been reduced to 200 mg Cu/kg feed and, more recently, EEC directives have lowered the permissible level of copper to 175 mg Cu/kg diet (CEC, 1984). These reductions in the level of supplemental copper are an attempt to reduce both liver copper concentrations and the level of copper excreted. The resulting lower copper concentration in pig slurry has helped to reduce the toxic effects of this heavy metal on the environment. Sheep, in particular, are susceptible to the high levels of copper found in pastures to which pig slurry has been applied (Meyer & Kroger, 1973).

These lower levels of supplemental dietary copper have resulted in a smaller overall improvement in pig growth performance. It may be possible to offset this fall in growth performance by supplementing the diet with a second growth-promoting agent. Earlier studies by Barber *et al.* (1955, 1974) have shown that dietary copper and sodium salicylate (2-hydroxy benzoic acid, sodium salt) have a synergistic beneficial effect on pig growth rates.

The aim of this present experiment, therefore, was to determine whether

the addition of sodium salicylate to the diet could offset the fall in growth response expected with the lower levels of dietary copper. The effects of these supplements on the structure and physical properties of the adipose tissue triacylglycerols were also investigated as, in contrast to dietary copper, there have been no reports of sodium salicylate adversely affecting adipose tissue consistency.

6.1.2 Dietary treatments

There were four dietary treatments in this experiment, two containing copper, one containing copper and sodium salicylate, and a basal treatment lacking both of these supplements. The detailed compositions of the diets are given in Table 6.1.1, but a summary of the treatments is shown below:

<i>Treatment</i>		<i>Diets</i>
1	Basal	A
2	High copper	B
3	Low copper	C,D
4	Low copper + sodium salicylate	E,F

Animals on the basal treatment and the high copper treatment* (treatments 1 and 2) received the relevant diets (A and B) throughout the experimental period. Pigs assigned to treatment 3 received diet C, containing 175 mg Cu/kg diet, until they reached sixteen weeks of age. After this they were given diet D, containing 100 mg Cu/kg diet, until slaughter. Similarly, the pigs receiving the sodium salicylate-supplemented treatment (treatment 4) were changed from diet E to diet F at sixteen weeks of age. Thus these animals received 175 mg Cu/kg diet and 700 mg sodium salicylate/kg diet

* 200 mg Cu/kg diet

Table 6.1.1: Experiment 4. Composition (kg/100 kg) of the experimental diets.

Diets	A	B	C	D	E	F
<u>Composition of diets (kg/100 kg)</u>						
Barley meal	735.1	735.1	735.1	735.1	735.1	735.1
Soyabean meal	240.0	240.0	240.0	240.0	240.0	240.0
Limestone flour	2.9	2.9	2.9	2.9	2.9	2.9
Dicalcium phosphate	9.5	9.5	9.5	9.5	9.5	9.5
Mineral/vitamin supplement**	12.5	12.5	12.5	15.5	12.5	12.5
CuSO ₄ .5H ₂ O (g/1000 kg)	-*	768.0	668.0	368.0	668.0	368.0
Sodium salicylate (g/1000 kg)	-	-	-	-	700.0	700.0
<u>Calculated composition (g/100 g)</u>						
Crude protein	17.9	17.9	17.9	17.9	17.9	17.9
Lysine	0.92	0.92	0.92	0.92	0.92	0.92
Calcium	0.78	0.78	0.78	0.79	0.78	0.78
Phosphorus	0.60	0.60	0.60	0.60	0.60	0.60
MJDE/kg	12.8	12.8	12.8	12.8	12.8	12.8

* Analysed Cu content of basal diet 8 mg/kg

** See Appendix 1 for composition

until sixteen weeks when they were given diets containing the same level of sodium salicylate, but only 100 mg Cu/kg diet. For the last seven days before slaughter, these animals on treatment 4 were given diet D, containing 100 mg Cu/kg diet but no supplemental sodium salicylate.

6.1.3 Animals

At six weeks of age, the animals (LW x LR x LW crosses) were selected in twentysix littermate groups of four. These groups were balanced overall for sex so that there were thirteen intact males and thirteen females available for each treatment. From six to eight weeks of age the pigs were dry fed the basal diet, A, and at eight weeks they were gradually changed to wet feeding and accustomed to individual feeding troughs.

When they reached nine weeks of age the pigs were randomly allocated to the four dietary treatments. The pigs in any one pen were all on the same treatment in order to prevent contamination via the faeces.

6.1.4 Experimental procedure

The feed was given wet (2.5 : 1 ratio) and twice daily in amounts the animals just cleared up in the two thirty minute feeds per day. If on two successive days the feed was cleared up completely then the allowance was increased by 0.1 kg feed/pig up to a daily maximum of 2.95 kg feed/pig. Refusals were measured each day and adjustments to the feed allowance were made to ensure that the animals just cleared up their ration each day. Pigs were fed individually.

The animals on treatments 3 and 4 had their diets changed when they

reached exactly sixteen weeks of age. In addition, the pigs receiving treatment 4 were changed to diet D on the weekly weighing day exactly seven days before slaughter.

Weighing of the animals was carried out on the same day and at the same time each week. The pigs on treatments 3 and 4 were also weighed at sixteen weeks of age when their diets were changed.

The animals were slaughtered on the day following the weekly weighing at which they reached a minimum liveweight of 87.5 kg. No food was given on the day of slaughter. At slaughter the liver was weighed and a strip of backfat from loin to shoulder was taken from all pigs and stored at -20°C until analysis. On the day following slaughter the maximum shoulder, loin and P_2 fat thicknesses of the carcass were measured.

6.1.5 Methods of analysis

The strips of subcutaneous backfat were separated into their component inner and outer layers. Small portions of tissue were taken from both the shoulder and loin regions of each of these two layers. The lipids were then extracted from each of these tissue samples by the Folch wash method (Section 2.2.1.1). The triacylglycerols in the lipid extract were purified by thin layer chromatography (Section 2.2.2) and the fatty acid composition of the triacylglycerol fraction was then analysed by gas-liquid chromatography of the fatty acid methyl esters (Section 2.2.4). The melting points of the adipose tissue triacylglycerol extracts were established, using the method described in Section 2.2.9. In addition the pancreatic lipase hydrolysis

technique was used to determine the positional distribution of the fatty acids in the adipose tissue triacylglycerols (Section 2.2.6).

Although the study design ensured that the dietary treatments were balanced for both sex and littermate, details of these were not recorded at the time of the experiment and so statistical analysis of any effects of these two variables cannot be taken into account.

Differences between treatment means for each parameter were compared by multiple range t test (50 df for feed, growth and carcass data, 102 df for fatty acid compositions and slip points of the adipose tissue triacylglycerols). Differences between the triacylglycerol fatty acid compositions and slip points of the shoulder and loin sites of individual backfat layers within treatments were compared by t test (52df). None of these intersite comparisons were statistically significant and so the results for each pig were pooled. Differences between inner and outer backfat layers were also compared by t test (102 df between backfat layers and 76df between backfat layers and perirenal fat).

The significance of linear regressions were calculated by using the correlation coefficient ($n-2df$). Differences between linear regressions were compared by t test of both the gradients and the intercepts.

6.2 Results of Experiment 4

6.2.1 Growth rates, food intakes and feed conversion ratios

The daily liveweight gains, feed conversion ratios and other feed-related results are given in Table 6.2.1. The pigs which received the control diet (treatment 1) ate significantly more food than did those on the other experimental treatments ($p \leq 0.05$) and had significantly greater feed conversion ratios ($p \leq 0.05$). The presence of sodium salicylate in the diet had no significant effect on feed consumption or feed conversion ratios when compared with the results from the animals given the same level of dietary copper ($p > 0.05$).

Daily liveweight gains were greatest in the pigs receiving 200 mg Cu/kg feed and lowest in the pigs given the treatment containing both copper and sodium salicylate *i.e.* those on treatments 2 and 4 respectively ($p \leq 0.05$). However the mean weekly weights of the pigs on each treatment, shown in Table 6.2.2, suggest that the pigs which received treatment 1 had slower rates of growth than did the animals on the other three experimental treatments, although this difference was not significant at the 5% level. There were no apparent differences ($p > 0.05$) in overall growth performance between pigs on the two copper-supplemented treatments *i.e.* treatments 2 and 3, suggesting that the lower copper level supplied by treatment 3 was sufficient to cause the growth-promoting effect.

6.2.2 Thickness of subcutaneous backfat, carcass length and liver weights

The fat thicknesses at the shoulder, P_2 and loin regions of the subcutaneous adipose tissue, the carcass lengths and the liver weights of the

Experiment 6.2.1: Experiment 4.

Mean liveweight gains, weights of food consumed and food conversion ratios for all pigs on each of the four dietary treatments.

	Treatment				SED
	1	2	3	4	
Total liveweight gain (kg)	72.8	73.5	73.6	72.2	1.6
Total feed consumed (kg)	199.7 ^{abc}	190 ^a	191 ^b	190 ^c	3.3
Food consumed/day (kg/day)	2.3 ^a	2.2	2.2	2.2 ^a	0.03
Liveweight gain/day (g/day)	834	864 ^a	847	828 ^a	19
Feed conversion ratio	2.7 ^{abc}	2.6 ^a	2.6 ^b	2.6 ^c	0.03

50df

Values bearing the same superscript within rows are significantly different from each other ($P \leq 0.05$).

Table 6.2.2: Experiment 4. Mean weekly weights (kg) of the animals on each of the four dietary treatments during the experimental trial.

Dietary Treatment	Week of Experiment												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	16.6	19.7	22.8	26.8	32.0	38.3	44.1	50.1	58.0	65.0	72.2	78.3	84.3
2	16.7	20.3	24.6	29.5	35.1	41.5	47.7	54.2	61.8	70.1	76.9	82.6	87.7
3	16.9	20.8	24.9	29.2	34.8	41.2	47.7	53.5	61.6	68.6	74.9	79.7	83.7
4	16.9	21.2	25.0	29.4	34.0	40.8	47.4	53.6	61.6	68.8	74.8	78.9	85.3
SED	0.74	1.42	1.55	2.19	2.60	3.35	2.99	3.62	3.15	3.38	4.0	3.5	2.37

50df

animals on each treatment are shown in Table 6.2.3. There were no significant differences between pigs on different treatments for any of these carcass measurements ($p>0.05$). However the pigs on treatments 3 and 4 did have slightly lower liver weights than those on treatments 1 and 2.

6.2.3 Composition, structure and physical properties of the adipose tissue triacylglycerols

6.2.3.1 Comparison between different adipose tissue sites

Samples of adipose tissue were taken from the shoulder and loin regions of both the inner and outer layers of backfat from each pig and analysed for the fatty acid composition of the adipose tissue triacylglycerols. No significant differences ($p>0.05$) were noted within treatments between the fatty acid compositions of the triacylglycerols from the shoulder and loin regions of each layer of backfat and so these results were pooled and are shown in Table 6.2.4; the separate results for the shoulder and loin regions of each layer of backfat are given in Appendix 8.

Differences in triacylglycerol composition between the inner and outer backfat layers of pigs on each treatment were significant in several cases. Palmitic acid concentration was greater in the triacylglycerols from the inner compared with the outer backfat layer for pigs on both treatments 1 and 4 ($p\leq 0.05$). Stearic acid concentration, however, was significantly greater in the adipose tissue triacylglycerols of the inner relative to the outer backfat layers of pigs from all treatments ($p\leq 0.001$). The concentration of oleic acid was lower in the triacylglycerols of the inner compared with

Table 6.2.3: Experiment 4.

Mean backfat thicknesses (mm) at the shoulder, P₂ and loin sites, mean carcass length (mm) and mean liver weights (g) of all pigs on each dietary treatment.

	Dietary Treatment				SED
	1	2	3	4	
Length (mm)	787	785	785	790	7
Shoulder (mm)	31.4	32	32.6	32.7	1.5
P ₂ (mm)	17.5	17.6	17.2	17.1	1.1
Loin (mm)	14.9	15.8	15.7	15.4	1.2
Liver weight (g)	1626	1619	1599	1594	64

50df

Table 6.2.4: Experiment 4.

Mean fatty acid compositions (wt. %) of triacylglycerols isolated from the inner and outer backfat of all pigs on each dietary treatment.

Fatty acid (%)		Dietary Treatment				SED
		1	2	3	4	
16:0	Inner	23.7 ^a	22.3 ^{ab}	22.8	23.4 ^b	0.3
	Outer	22.1	22.2	22.3	22.5	0.2
16:1	Inner	3.0	2.8	3.2	2.9	0.1
	Outer	3.0	2.8 ^a	3.3 ^a	3.0	0.1
18:0	Inner	18.3 ^{ab}	16.5 ^a	15.7 ^b	14.7 ^a	0.4
	Outer	15.1 ^{ab}	13.3 ^a	12.9 ^a	12.2 ^a	0.4
18:1	Inner	43.3 ^{abc}	46.4 ^a	45.8 ^b	46.0 ^c	0.4
	Outer	46.3 ^a	45.9	47.2	47.7 ^a	0.3
18:2	Inner	10.8	10.9	11.3	11.0	0.3
	Outer	12.5	13.2	13.0	12.7	0.3
18:3	Inner	1.6	1.4	1.6	1.8	0.1
	Outer	2.2	1.7	1.8	1.9	0.2
						102df

Values bearing the same superscript within rows are significantly different from each other ($p \leq 0.05$).

the outer layer for treatments 1 ($p \leq 0.001$), 3 ($p \leq 0.05$) and 4 ($p \leq 0.01$). Similarly linoleic acid concentration in the adipose tissue triacylglycerols was lower in the inner compared with the outer backfat layer, but here the differences were significant for all treatments ($p \leq 0.01$). Differences in palmitoleic and linolenic acid concentrations between the triacylglycerols from the inner and outer layers of backfat from pigs on the same treatment were not significant at the 5% level.

The mean slip points of the triacylglycerols from the two layers of backfat of pigs on each treatment are shown in Table 6.2.5. The full results for the shoulder and loin regions of each layer are given in Appendix 9. With pigs on all four experimental treatments the triacylglycerols from the inner backfat layers had greater melting points than did those from the corresponding outer layers of backfat ($p \leq 0.001$). This is in keeping with the degree of saturation of the samples (Table 6.2.4).

The distributions of the fatty acids between positions 2 and 1+3 of the adipose tissue triacylglycerols from the inner and outer backfat layers of pigs on each dietary treatment are shown in Table 6.2.6. In general, palmitic acid was the major fatty acid esterified at position 2 of the backfat triacylglycerols, with very little of this fatty acid present at positions 1 and 3. Palmitoleic acid concentration was also greater at position 2 than at positions 1+3 of the adipose tissue triacylglycerols. In contrast, the 18 carbon fatty acids were generally present at greater concentrations of the 1+3 positions of the adipose tissue triacylglycerols. With both stearic and linoleic acids, the concentration at positions 1+3 was approximately two to three times that at position 2. However, oleic acid was the major fatty acid esterified at positions 1+3, and the concentration at these

sites was five to six times greater than that at position 2 of the triacylglycerols.

Comparisons between the fatty acid compositions at position 2 of the triacylglycerols from the inner and outer backfat of pigs on the same dietary treatment showed no differences that were significant at the 5% level. However the inner backfat triacylglycerols tended to have slightly greater oleic acid and lower palmitoleic acid concentrations at position 2 than did the corresponding outer backfat triacylglycerols. At the combined 1+3 - positions, differences between inner and outer layers of backfat were also noted. Triacylglycerols from the inner backfat layer of pigs on each treatment had greater concentrations of palmitic and stearic acids at these sites and, consequently, lower concentrations of the unsaturated fatty acids compared with triacylglycerols from the corresponding outer backfat layer.

6.2.3.2 Comparison between different dietary treatments

The differences in the fatty acid compositions of the adipose tissue triacylglycerols of pigs receiving the four experimental treatments are shown in Table 6.2.4. In general, the pigs which received treatment 1, lacking dietary copper and sodium salicylate, had inner backfat triacylglycerols that contained greater concentrations of saturated fatty acids and lower concentrations of oleic acid than did the triacylglycerols from the corresponding layer of pigs which received the other dietary treatments ($p \leq 0.05$). The concentrations of the other fatty acids in the inner backfat triacylglycerols were not significantly different between treatments ($p > 0.05$).

Differences between the fatty acid compositions of the outer backfat

Table 6.2.5: Experiment 4

- Mean slip points (°C) of triacylglycerols isolated from the inner and outer layers of backfat from all pigs on each of the dietary treatments.

	Dietary Treatment				SED
	1	2	3	4	
<u>Slip point °C</u>					
Inner backfat	44.9 ^{ab}	42.5 ^a	41.7 ^b	41.3 ^a	1.4
Outer backfat	39.8 ^{ab}	37.9 ^a	37.5	37.0 ^b	0.9

102df

Values bearing the same superscript within rows are significantly different from each other ($p \leq 0.05$).

triacylglycerols of pigs on different treatments were only significant in a few cases. Stearic acid concentration was significantly greater in the outer backfat of pigs on treatment 1 compared with pigs on the other experimental treatments ($p \leq 0.05$). Pigs on treatments 2 and 4 respectively also had outer backfat triacylglycerols with differing stearic acid contents ($p \leq 0.05$). Oleic acid concentration was greater in the outer backfat triacylglycerols of pigs on treatment 4 compared with those on treatment 1 ($p \leq 0.05$). There were no major differences between the fatty acid compositions of the adipose tissue triacylglycerols from pigs on the two copper-supplemented treatments (treatments 2 and 3), although there was a slightly higher palmitoleic acid concentration in the outer backfat of pigs on treatment 3 ($p \leq 0.05$). Similarly, triacylglycerols from the backfat of pigs receiving treatments 3 and 4 had fatty acid compositions which were not significantly different from each other ($p > 0.05$).

In accordance with these findings the triacylglycerols from the backfat of pigs on treatment 1, which had the greatest degree of saturation, also had the greatest melting points (Table 6.2.5). This difference was evident with both layers of backfat ($p \leq 0.05$). There were no significant differences in the melting points of triacylglycerols from either layer of backfat between pigs from treatments 3 and 4 which received the same level of supplemental dietary copper, despite the presence of dietary sodium salicylate in treatment 4 ($p > 0.05$). In addition there were no differences in adipose tissue triacylglycerol melting points between pigs on treatments 2 and 3, receiving the high and low copper supplements respectively. The melting points of inner backfat triacylglycerols from pigs on treatment 2 were slightly greater than those of the inner backfat of pigs on treatment 4 ($p \leq 0.05$), but there were

no significant differences between the melting points of triacylglycerols from the corresponding outer layers of backfat ($p>0.05$).

Differences in the positional distribution of fatty acids in the adipose tissue triacylglycerols of pigs on different dietary treatments were not significant at the 5% level, although some trends were in evidence (Table 6.2.6). Pigs given the basal treatment (treatment 1) had higher concentrations of oleic and linoleic acids at position 2 of the triacylglycerols from both layers of backfat when compared with the triacylglycerol samples from pigs on the other three experimental treatments. There were higher concentrations of palmitic and stearic acids and a correspondingly lower concentration of oleic acid esterified to the 1+3 - positions of the adipose tissue triacylglycerols from pigs given treatment 1 relative to those from pigs which received any of the other dietary treatments. There were no major differences in the distribution of fatty acids between the triacylglycerols from the subcutaneous adipose tissue of pigs which were given any of the copper-supplemented treatments *i.e.* those on treatments 2, 3 and 4 ($p>0.05$).

6.2.4 Summary

In this experiment the presence of supplemental copper in the diet slightly increased the overall growth performance of pigs compared with animals receiving only the trace copper requirement. The two copper-supplemented diets, containing 200 mg Cu/kg feed and 175-100 mg Cu/kg feed respectively, had similar effects on growth parameters suggesting that the lower rate of inclusion in the diet is sufficient to elicit the growth-promoting response. Sodium salicylate had no apparent effect on the rates of growth of the pigs.

Table 6.2.6: Experiment 4.

Mean distribution of fatty acids (wt. %) between positions 2 and 1 + 3 of adipose tissue triacylglycerols from the inner and outer backfat of all pigs on each of the four dietary treatments.

Fatty acid	Sample Site		1	2	3	4	SED
16:0	Inner backfat	2	64.7	63.2	62.4	65.3	1.6
		1 + 3	3.5	2.4	3.6	2.4	
	Outer backfat	2	65.7	66.7	69.0	65.4	1.2
		1 + 3	1.1	-0.2	-1.4	0.6	
16:1	Inner backfat	2	5.2	5.9	5.9	6.0	0.3
		1 + 3	2.4	1.5	2.1	1.3	
	Outer backfat	2	5.6	7.4	6.8	8.2	1.5
		1 + 3	2.5	1.0	1.8	0.4	
18:0	Inner backfat	2	9.2	9.4	8.5	11.0	1.7
		1 + 3	22.3	19.6	19.4	15.7	
	Outer backfat	2	10.2	10.2	8.8	9.6	0.8
		1 + 3	15.8	13.5	14.3	12.7	
18:1	Inner backfat	2	13.2	10.4	12.4	10.8	1.7
		1 + 3	56.8	62.6	60.9	63.3	
	Outer backfat	2	10.6	9.3	9.9	10.1	0.5
		1 + 3	64.2	66.3	65.9	66.6	
18:2	Inner backfat	2	7.8	7.6	7.4	6.8	1.3
		1 + 3	13.5	13.8	13.7	14.0	
	Outer backfat	2	7.9	6.4	5.6	6.7	1.5
		1 + 3	15.9	17.4	18.1	16.8	

102df

Analysis of triacylglycerols from the subcutaneous adipose tissue of the animals also showed no major differences in fatty acid composition between the pigs on treatments 2 and 3, attributable to different levels of dietary copper, or between pigs on treatments 3 and 4 owing to the presence of sodium salicylate in the diet. However the pigs which were given the basal treatment had significantly greater concentrations of saturated fatty acids in the triacylglycerols from both layers of backfat compared with the corresponding triacylglycerol samples from the backfat of pigs on the other three experimental treatments. These findings were reflected by the melting points which were greatest for the adipose tissue triacylglycerols of the pigs on treatment 1. In addition, the triacylglycerols from the inner layer of backfat were more saturated than those of the corresponding outer layer for pigs on the same dietary treatment and in accordance with this the inner backfat triacylglycerols had the greater melting points.

Positional analysis of the fatty acids in the adipose tissue triacylglycerols of pigs on all treatments showed that palmitic acid is the major fatty acid esterified in position 2 of the triacylglycerols while oleic acid is the predominant acid at positions 1+3. Differences between treatments were only apparent between the triacylglycerols of pigs on the basal treatment and those of animals given any of the three copper-supplemented treatments. The adipose tissue triacylglycerols of pigs given the basal treatment (treatment 1) contained greater quantities of saturated fatty acids at positions 1+3 and higher levels of oleic acid at the 2-position of the triacylglycerols when compared with those from pigs given treatments 2, 3 or 4.

6.3 Discussion of results of Experiment 4

6.3.1 Growth rates, food intakes and feed conversion ratios

The rates of growth of the pigs and feed-related results are shown in Tables 6.2.1 and 6.2.2. The animals which received the control treatment, which lacked both copper and sodium salicylate, ate more food and gained less weight than did the pigs which received the copper-supplemented diets. This improved growth response is consistent with the work of Braude (1967) and others, although the increase in growth rates accompanying the presence of dietary copper was not significant in the present experiment. The smaller response to dietary copper, in comparison with the results obtained by Braude (1967), may be related to the use of soyabean meal as the protein source in the diets of the present experiment. Soyabean meal contains phytic acid and this has been shown to chelate copper in the gastrointestinal lumen, so reducing the uptake of this metal (O'Dell *et al.*, 1972; Van Campen, 1971). However in both Experiments 1 and 2 dietary copper supplements of 200 mg Cu/kg diet failed to increase the pig growth rates even when a different source of protein was employed, suggesting that more complex factors may also be involved.

The pigs which received the diets containing 200 mg Cu/kg feed and 175–100 mg Cu/kg feed respectively had similar rates of growth and ate similar amounts of food. This is not in agreement with Braude and Ryder (1973) who noted that pigs fed 150, 200 or 250 mg Cu/kg feed all displayed improved growth rates compared with pigs given a control diet lacking copper, with the growth performance improving with increasing dietary intakes of copper. However, Elliot and Amer (1973) reported that there were no differences in average daily liveweight gain between pigs fed different levels of dietary

copper ranging from 0 to 250 mg Cu/kg feed, although levels of 175 or 200 mg Cu/kg feed did slightly stimulate the average daily gains of the animals. Bradley *et al.* (1983) also observed no differences in the average daily liveweight gains of pigs given diets containing different levels of dietary copper.

The pigs on treatment 4, which received both dietary copper and sodium salicylate had slightly, but not significantly, lower daily liveweight gains than did those receiving the same level of dietary copper but no sodium salicylate ($p > 0.05$). These findings are consistent with the work of Barber *et al.* (1971) who reported that pigs given diets supplemented with both 250 mg Cu/kg feed and 200 mg sodium salicylate/kg feed had growth rates which were lower, but not significantly so, than those obtained with pigs which received either supplement alone. However, in a subsequent experiment, Barber *et al.* (1974) reported that pigs given diets containing both 700 mg sodium salicylate/kg feed and 250 mg Cu/kg feed had greater growth rates than did those which received the dietary treatment supplement^{ed} with copper alone.

Elliot and Amer (1973) reported that the growth promoting effects of dietary copper were only apparent up to 46 kg liveweight. In order to study this further, the daily liveweight gains of the animals, on the experiment reported here, were calculated from the start of the experiment up until week 7 by which time most of the animals had reached this weight. The results of those calculations are shown in Table 6.3.1 and are similar to those obtained up to 87.5 kg liveweight. The pigs given the control diet had significantly lower rates of growth than did the pigs given the other three experimental treatments ($p < 0.05$). Daily liveweight gains were similar between

animals receiving the copper-supplemented diets. However, large differences between the growth rates of pigs from treatments 2 and 3 were not expected as the dietary copper intakes of these two groups of pigs, up to sixteen weeks of age, differed by only 25 mg Cu/kg feed. The growth rates were slightly higher with the animals given treatment 4, the diets of which contained sodium salicylate as well as the lower level of dietary copper. These findings are in agreement with Barber *et al.* (1974) who found that dietary copper and sodium salicylate together had a significantly greater effect on pig growth rates than did either of these supplements alone. However the results of the present experiment are not consistent with those of Elliot and Amer (1973) who reported that the growth promoting effects of dietary copper were only apparent up to 46 kg liveweight.

The pigs used in the present experiment were of the same breed (LW x LR x LW) and from the same herd as those of Experiment 1. In addition, the diets used in Experiment 4 were fairly similar in composition to those of the soyabean-based treatments of Experiment 1 (Tables 3.1.1. and 6.1.1). It was therefore anticipated that the animals on both of these experiments, which received comparable diets, would respond in a similar manner to the dietary copper supplement. However, as reported above, the growth promoting effect of dietary copper was evident up to slaughter with the animals on Experiment 4, while the pigs given the S and SCu treatments of Experiment 1 had growth rates which were not significantly different from each other, although there was a tendency to an improved growth rate up to 46 kg liveweight with the animals on the SCu treatment (Table 3.3.1). In keeping with this the feed-related results show that the differences in daily liveweight gains between pigs on the basal and copper-supplemented treatments of the same experiment were greatest with the animals on Experiment 4. The reason

for this discrepancy is unclear but the feed conversion ratios and daily feed intakes were slightly different between pigs on the corresponding treatments of Experiments 1 and 4 (Tables 3.2.2 and 6.2.1). In addition the diets were slightly different in composition and the supplies of the various feed-stuff components may also have differed.

The aim of this present experiment was to investigate whether or not a dietary supplement of sodium salicylate could offset the fall in growth response expected with pigs given the lower level of dietary copper. However the results suggest that a dietary supplement of 175 mg Cu/kg feed, later reduced to 100 mg Cu/kg feed at sixteen weeks of age, is just as effective as a continual dose of 200 mg Cu/kg feed in improving the rate of gain of pigs when compared with animals receiving a basal diet. Sodium salicylate also tended to increase the growth rate, but this was only evident in conjunction with a dietary copper supplement and up to 46 kg liveweight.

The modes of action of both dietary copper and sodium salicylate in increasing daily liveweight gains have not been fully elucidated. It has been suggested that dietary copper may affect the gut microflora or may increase pepsin activity (Hawbaker *et al.*, 1961; Lenis, 1981; Kirchgessner, 1976). In addition the effects of dietary copper on stearoyl CoA desaturase activity and hence adipose tissue lipid unsaturation are well-documented (Sections 1.5.3.3 and 1.7). In contrast, sodium salicylate is generally used as an antipyretic and analgesic agent and its effect on pig growth rates is unclear. However it has been reported that short term administration of sodium salicylate has a moderate stimulatory effect on the adreno-cortical function of piglets and prostaglandin synthesis may also be affected (Raszyk and Dvořák, 1976).

6.3.2 Thickness of subcutaneous backfat

The carcass fat thicknesses are shown in Table 6.2.3. The backfat was thickest at the shoulder and thinnest at the loin while the P_2 backfat thickness was slightly greater than that at the loin site. Variations in backfat thickness along the length of the pig have been reported (Mersmann & Leymaster, 1984), but in contrast to the findings of the present experiment these authors noted that the P_2 site was the thinnest region of backfat.

Differences in backfat thickness at each site, between pigs on different treatments, were not significantly different ($p>0.05$), despite the greater growth rates observed with the pigs receiving the copper-supplemented diets. These findings are in agreement with those of Experiments 1 and 2, where pigs receiving different dietary treatments also had similar backfat thicknesses at each of the three adipose tissue sites (Tables 3.2.3 and 4.2.3).

Wood and Enser (1982) reported a relationship between the P_2 backfat thickness and the concentration of linoleic acid in the adipose tissue triacylglycerols. When the results from the present experiment were examined the correlations were only significant for the fat thickness measurements from the shoulder and loin regions of subcutaneous adipose tissue of pigs on all four treatments and these regression equations are given in Table 6.3.2. The gradients of these two regressions were similar ($p>0.05$) but the intercepts were not ($p<0.001$).

The animals which were given the copper-supplemented treatments of the present experiment ate similar amounts of food and so received comparable amounts of dietary linoleic acid. However pigs with a tendency to thicker

Table 6.3.1: Experiment 4.

Average daily liveweight gains (g/day) of all pigs on each dietary treatment up to 46 kg liveweight

	Dietary Treatment				SED
	1	2	3	4	
Daily liveweight gain (g/day)	566 ^{abc}	610 ^a	628 ^b	658 ^c	46

Values sharing the same superscript are significantly different from each other ($P \leq 0.05$) 50df

Table 6.3.2: Experiment 4.

Relationships between the backfat thicknesses (mm) at the shoulder, P₂ and loin sites (x) and the linoleic and oleic acid concentrations (weight percent, y) of the adipose tissue triacylglycerols of all pigs on all four experimental treatments.

Backfat site	Linear regression	Significance
<u>Linoleic acid</u>		
Shoulder	$y = -0.19x + 18.07$	$p \leq 0.05$
Loin	$y = -0.22x + 14.74$	$p \leq 0.01$
<u>Oleic acid</u>		
P ₂	$y = 0.43x + 36.84$	$p \leq 0.001$

backfat have increased rates of endogenous fatty acid synthesis in adipose tissue and this higher rate of synthesis effectively reduces the adipose tissue concentration of linoleic acid. The animals on treatment 1 ate smaller amounts of food compared with the animals on the other three experimental treatments. However the backfat thicknesses and the adipose tissue concentrations of linoleic acid were not significantly different between pigs on different treatments and so a similar correlation between these two parameters would be expected with pigs on each of the experimental treatments. Dietary sodium salicylate also appeared to have no effect on the thickness of the subcutaneous backfat. This is consistent with Barber *et al.* (1974) who also noted no significant differences between the carcass measurements of pigs fed basal diets or those given diets supplemented with 250 mg Cu/kg diet and/or 700 mg sodium salicylate/kg diet.

The regressions between the shoulder and loin backfat thicknesses and the concentrations of linoleic acid in the adipose tissue triacylglycerols of the present experiment had gradients that were similar to those of the regressions obtained with the results from the pigs given the treatments of Experiment 1, based on either fishmeal or soyabean meal but which did not contain maize oil (Figure 3.3.1). Thus changes in linoleic acid uptake appear to be associated with similar changes in backfat thickness between the pigs on Experiments 1 and 4. This was, to some extent, anticipated as the animals used in these two experiments were of the same breed and were given diets which were comparable with respect to energy, protein and linoleic acid content.

The intercepts of the regressions at both the shoulder and loin sites were significantly different between the results of Experiments 1 and 4

($p \leq 0.001$). This is a result of the overall differences in backfat thickness between pigs on the different experiments which may arise from the differences in feed consumption and daily liveweight gains. In addition, the regressions from Experiment 1 include the results from the pigs given both fishmeal and soyabean-based treatments, whereas the diets used in Experiment 4 were based solely on soyabean meal as the protein source. However the regressions from Experiment 1 were used as the backfat thicknesses of pigs given the soyabean-based treatments of Experiment 1, i.e. the S and SCu treatments only, did not correlate significantly with the adipose tissue linoleic acid concentration owing to the small number of samples.

The concentrations of the other fatty acids in the adipose tissue triacylglycerols were also plotted against the backfat thicknesses at the shoulder, loin and P_2 sites. The only significant correlation was that between the oleic acid concentration of the adipose tissue triacylglycerols and the P_2 backfat thickness ($p \leq 0.001$, Table 6.3.2), the gradient of this relationship was positive, so that increasing synthesis and deposition of oleic acid was associated with thicker layers of backfat. These linear regressions at the P_2 site were not significantly different between Experiments 1 and 4 ($p > 0.05$). Similar trends were noted at the shoulder and loin regions of backfat, but at these sites the correlations were not statistically significant ($p > 0.05$).

6.3.3 Composition, structure and physical properties of adipose tissue triacylglycerols

6.3.3.1 Comparison between different adipose tissue sites

The fatty acid compositions of the inner and outer backfat triacylglycerols of pigs on each dietary treatment are shown in Table 6.2.4. In agreement

with previous reports (Moore *et al.*, 1969; Christie *et al.*, 1972; Santoro, 1984) and with the results of Experiments 1, 2 and 3, the triacylglycerols from the outer backfat tissue were significantly more unsaturated than those from the inner layer of subcutaneous adipose tissue. As discussed in Section 3.3.3.1, these differences between the compositions of the triacylglycerols from the two backfat layers may be a result of differences in the rates of uptake and synthesis of fatty acids at the two adipose tissue sites. Earlier work has shown that lipogenic activity is greater in the inner backfat layer but lipoprotein lipase activity is greater in the outer layer of backfat (De Wilde, 1984; Sturm *et al.*, 1982; Enser, 1973). In addition the results of Experiment 2 suggest that stearoyl CoA desaturase activity is greater in the outer compared with the inner layer of backfat and this is also consistent with the observed greater unsaturation of the triacylglycerols from the outer layer of subcutaneous adipose tissue (Section 4.2.4.1).

The greater concentration of unsaturated fatty acids in the outer compared with the inner backfat triacylglycerols of pigs on the same dietary treatment was accompanied by a lower triacylglycerol melting point. This is consistent with previous reports and with the results of Experiments 1, 2 and 3, reported here (Sections 1.2.1; 3.3.3.1; 4.3.3.1 and 5.3.2.1).

In addition the positional distribution of fatty acids was not significantly different between triacylglycerols from the inner and outer backfat layers of pigs on the same dietary treatment. This suggests that the specificity of the acyltransferases at each adipose tissue site are similar and is consistent with the findings of Christie and Moore (1970a). However there was a tendency to greater unsaturation at the 1+3 positions of the triacylglycerols from the outer compared with the inner backfat layer. This may be a result of

the overall greater concentration of unsaturated acids in the triacylglycerols of the outer backfat layer.

The patterns of fatty acid distribution in the adipose tissue triacylglycerols of the pigs on the present experiment were different from those of the pigs which received the S and SCu treatments of Experiment 1 (Tables 3.2.9 and 6.2.6). This difference may be a result of the slightly differing diets used in each experiment and the differing supplies of feed components.

6.3.3.2 Comparison between different dietary treatments

The differences between the fatty acid compositions of adipose tissue triacylglycerols from pigs on different treatments are shown in Table 6.2.4. The pigs which received the basal treatment (treatment 1) had greater concentrations of saturated fatty acids in the adipose tissue triacylglycerols compared with the pigs given any of the other experimental treatments, all three of which contained supplemental copper.

With the results of Experiment 1, significant increases in the concentrations of monounsaturated fatty acids in the adipose tissue triacylglycerols were associated with supplementation of the diets with copper (Section 3.2.4.4). In the present experiment there were no significant differences in palmitoleic acid concentration between the adipose tissue triacylglycerols from pigs on all four experimental treatments. However, triacylglycerol oleic acid concentration was lower in the adipose tissue of pigs given treatment 1 which lacked the copper supplement. This is also in agreement with the work of Moore *et al.* (1969), Thompson *et al.* (1973) and Amer & Elliot (1973). In keeping with this the backfat triacylglycerols from the pigs on treatment 1

had greater melting points than did those from the pigs on the other three experimental treatments.

The fatty acid compositions of the adipose tissue triacylglycerols of pigs on treatments 2 and 3, which received different levels of dietary copper, were not significantly different from each other. Consistent with this, the melting points of the adipose tissue triacylglycerols from pigs on these two treatments were also similar ($p > 0.05$). This suggests that the lower level of copper, as supplied by the diets of treatment 3, is sufficient to cause the increase in oleic acid content associated with the presence of copper in the diet. However, Walker *et al.* (1971) noted that the mono-unsaturated:saturated ratio of fatty acids in the outer backfat triacylglycerols was decreased by increasing levels of dietary copper, but that there was no comparable effect on the inner backfat triacylglycerols.

The pigs on treatments 3 and 4 received the same level of dietary copper (175 mg Cu/kg diet reduced at 16 weeks of age to 100 mg Cu/kg diet), but those on the latter treatment also received 700 mg sodium salicylate/kg diet. However there were no apparent differences in the fatty acid compositions of triacylglycerols from these two groups of pigs, suggesting that dietary sodium salicylate did not affect the uptake or synthesis of fatty acids in adipose tissue. In keeping with this, there were no differences between the carcass lengths or backfat thicknesses of pigs on either of these two treatments. The melting points of backfat triacylglycerols from pigs on these two treatments were also similar.

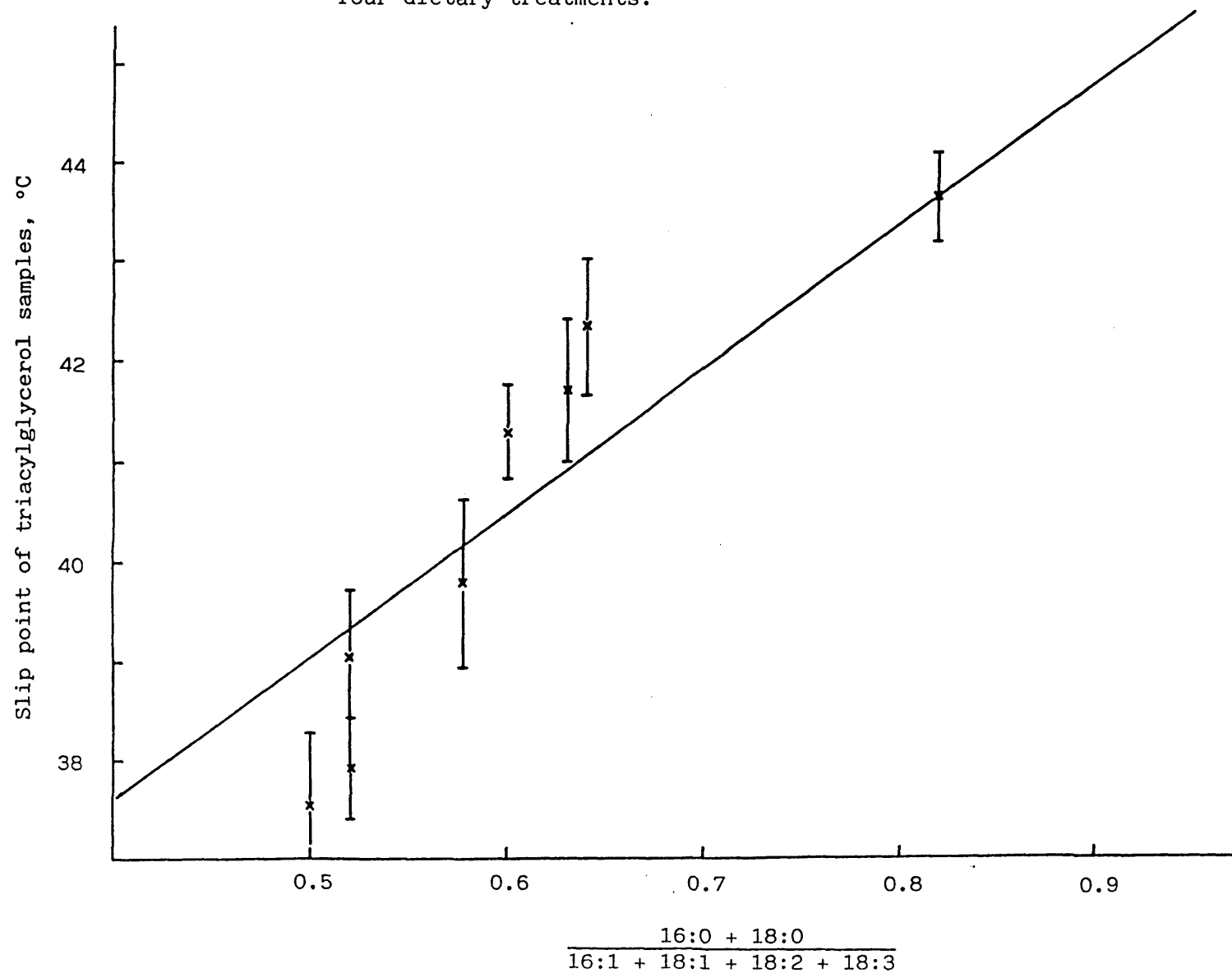
The fatty acid compositions of the adipose tissue triacylglycerols from pigs on treatments 2 and 4 were slightly different. Stearic acid concentration

was greater in the triacylglycerols from both layers of backfat of pigs on treatment 2 while palmitic acid concentration was greater in the inner backfat triacylglycerols of pigs on treatment 4. The reason for this difference is unclear, but is reflected by the melting points of the adipose tissue triacylglycerols. The inner backfat triacylglycerols of pigs on treatment 2 had greater melting points than did those from the corresponding backfat layers of pigs given treatment 4. However, the triacylglycerols from the outer backfat layers of pigs on both treatments had similar melting points.

With Experiments 1, 2 and 3 the ratios of the concentrations of saturated:unsaturated fatty acids were found to correlate with the melting points of the pig adipose tissue triacylglycerols (Section 3.3.3.3, 4.3.3.2 and 5.3.2.2). When the results from pigs on all four dietary treatments of the present experiment were examined, a similar relationship was obtained and this is illustrated in Figure 6.3.1 ($p < 0.001$). As with the previous experiments the regression had a positive gradient so that increasing saturation of the adipose tissue triacylglycerols was associated with a greater melting point. Similarly the biased concentration of saturated:unsaturated fatty acids was related to the melting point of the adipose tissue triacylglycerols ($p < 0.001$, Figure 6.3.2). In keeping with the findings of Experiments 1, 2 and 3 the gradient of this regression was almost twice that of the unbiased regression, reflecting the greater effect of the more unsaturated fatty acids on the triacylglycerol melting point.

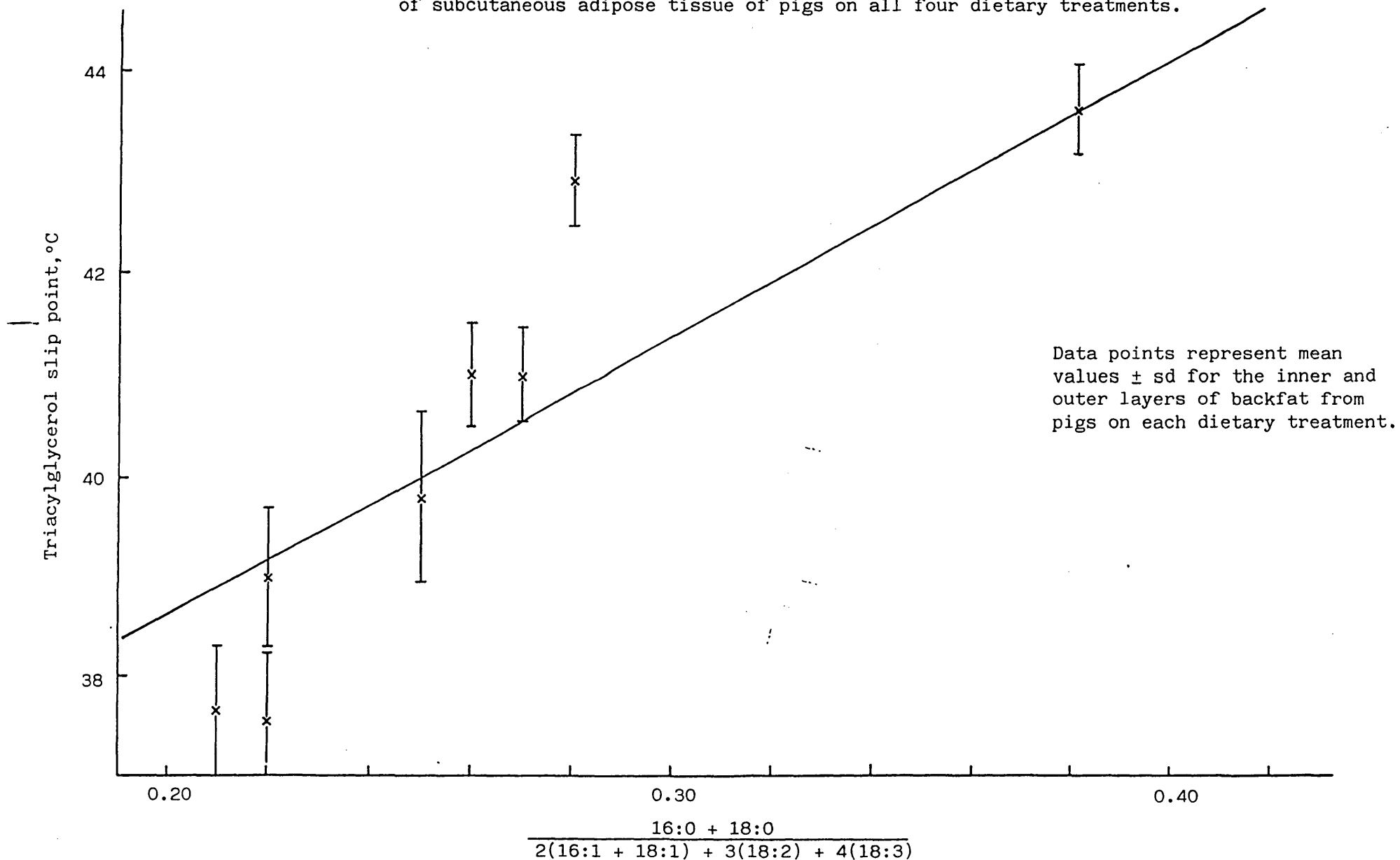
The concentrations of the individual fatty acids were also plotted against the melting points of the adipose tissue triacylglycerols of pigs on all four dietary treatments. Significant correlations were noted with palmitic, stearic, oleic and linoleic acids (Table 6.3.3). The gradients of the regressions

Figure 6.3.1: Experiment 4. Relationship between the ratio of the concentrations of saturated:unsaturated fatty acids and the slip point of triacylglycerols from the subcutaneous adipose tissue of pigs on all four dietary treatments.



Points represent mean $\pm$ sd for inner and outer layers of backfat respectively from pigs on each dietary treatment.

Figure 6.3.2: Experiment 4. Relationship between the biased ratio of the concentrations of saturated:unsaturated fatty acids and the melting point of triacylglycerols from the inner and outer layers of subcutaneous adipose tissue of pigs on all four dietary treatments.



for adipose tissue oleic and linoleic acid concentrations respectively against the triacylglycerol melting point were negative and this is in agreement with the earlier finding that greater concentrations of unsaturated fatty acids are associated with lower triacylglycerol melting points (Section 1.2.1). Consistent with this the relationships between the concentrations of the saturated fatty acids and the melting points of the adipose tissue triacylglycerols in the present experiment had positive gradients.

These relationships between the various fatty acid concentrations and the melting points of the adipose tissue triacylglycerols were compared with the corresponding regressions obtained with the results from the pigs given the S and SCu treatments of Experiment 1 (Table 6.3.3). The correlations between the concentrations of stearic and linoleic acids respectively against the triacylglycerol melting point were similar between the two experiments ($p > 0.05$) whereas with the oleic acid regression the gradients were similar ($p > 0.05$) but the slopes were significantly different from each other ($p \leq 0.05$). These findings again show that the pigs from Experiment 4 and those from Experiment 1 which received comparable diets had adipose tissue with similar characteristics despite the differences in backfat thicknesses between animals on each experiment.

The results of Experiment 1 suggested that the concentration of oleic acid in the adipose tissue triacylglycerols was to some extent influenced by the concentrations of the other major 18 carbon fatty acids, stearic and linoleic acids (Section 3.3.3.4). This was investigated further, in the present experiment, by plotting various ratios of the 18 carbon fatty acids against the slip points of the adipose tissue triacylglycerols. The relationships between the stearic:oleic acid and stearic:linoleic acid ratios and

Table 6.3.3: Experiment 4.

Relationships between the concentrations (weight %) of various fatty acids (x) and the melting points (°C, y) of adipose tissue triacylglycerols from pigs on all four dietary treatments. For comparison, the gradients from the corresponding regressions obtained using the results from the pigs given the S and SCu treatments of Experiment 1 are also shown.

Fatty acid	Linear regression	Significance	Gradient of corresponding regression for S and SCu treatments of Experiment 1
16:0	$y = 0.47x + 29.66$	$p \leq 0.05$	0.38
18:0	$y = 0.63x + 31.25$	$p \leq 0.001$	0.58
18:1	$y = -0.82x + 77.79$	$p \leq 0.001$	-0.51
18:2	$y = -0.48x + 46.41$	$p \leq 0.01$	-0.41

Table 6.3.4: Experiment 4.

Relationships between the ratios of the concentrations (weight %) of the 18 carbon fatty acids (x) and the slip points (°, y) of triacylglycerols from the inner and outer backfat samples of pigs on all four dietary treatments. For comparison the gradients of the corresponding regressions obtained using the results from the pigs given the S and SCu treatments of Experiment 1 are also shown.

Fatty acid ratio	Linear regression	Significance	Gradient of corresponding regression S and SCu treatments of Experiment 1.
18:0/18:1	$y = 35.45x + 29.68$	$p \leq 0.001$	35.58
18:0/18:2	$y = 3.09x + 36.68$	$p \leq 0.001$	3.26
18:1/18:2		ns	

triacylglycerol melting point were significant ($p < 0.001$) but that for the ratio of oleic:linoleic acid concentrations against the triacylglycerol melting points was not ($p > 0.05$). The former two correlations are given in Table 6.3.4, together with the gradients of the corresponding regressions calculated using the results from the pigs from Experiment 1 which received diets similar to those of the present experiment *i.e.* those on the S and SCu treatments. With both the stearic:oleic and stearic:linoleic acid regressions there were no significant differences between the relationships obtained using the results from both Experiments 1 and 4 ($p > 0.05$). This was to some extent anticipated as the animals from both experiments were of the same breed and the S and SCu treatments of Experiment 1 were fairly similar in composition to the diets used in Experiment 4.

The positional distributions of the fatty acids in the triacylglycerols are also known to affect the melting points of the adipose tissue triacylglycerols. However when the concentrations of the various fatty acids at positions 2 and 1+3 respectively were plotted against the triacylglycerol melting points no relationships were obtained which were significant at the 5% level.

6.4 General summary

The results of this experiment indicated that a dietary supplement of 175 mg Cu/kg diet reduced at sixteen weeks of age to 100 mg Cu/kg diet was sufficient to cause a small increase in the rate of growth when compared with pigs receiving diets lacking in copper. These findings are in keeping with the widely reported beneficial effects of copper on pig growth rates. However the pigs which received diets supplemented with different levels of copper (treatments 2 and 3) had similar rates of growth. This is incon-

sistent with the work of Braude and Ryder (1973) who reported that increasing concentrations of copper in the diet were associated with increasing rates of growth. The results of the present experiment are also not in agreement with those of Experiments 1 and 2 or with the work of Elliot and Amer (1973) and Bradley *et al.* (1983) where copper supplementation of the diets had no apparent effect on the rates of pig growth. The addition of sodium salicylate to the dietary treatment containing the lower level of copper had no apparent effect on the pig growth rates up to slaughter although there was a tendency to an improved growth rate up to 46 kg liveweight. The results up to slaughter at 87.5 kg liveweight are in disagreement with Barber *et al.* (1974) who noted that the growth promoting effects of dietary copper and sodium salicylate were additive.

There were no significant differences in backfat thickness between pigs on any of the four dietary treatments despite the slower rates of growth of the animals given the basal treatment (treatment 1). As with the results of Experiments 1 and 2 the backfat thickness was found to correlate with the adipose tissue concentrations of both linoleic and oleic acids respectively. This is in contrast with Wood and Enser (1982) who only noted significant correlations with linoleic acid. In addition the gradients of the regressions were not significantly different between the results from the pigs on Experiment 1 which did not receive the maize oil supplement and those from the pigs on all four dietary treatments of Experiment 4.

In keeping with previous reports the outer layer of backfat from all pigs was more unsaturated than the corresponding inner layer (Moore *et al.*, 1969; Hood & Allen, 1973; Christie *et al.*, 1972). Consistent with this,

triacylglycerols from the outer layer of backfat had lower melting points than those from the corresponding inner layer.

Pigs on the basal treatment had backfat triacylglycerols which were significantly more saturated than those of pigs on any of the other experimental treatments. This greater unsaturation of the triacylglycerols from pigs on the copper-supplemented treatments is largely a result of the greater concentration of oleic acid in the triacylglycerols compared with the pigs on the basal treatment. This is in keeping with previous findings and with reports that pigs given copper-supplemented diets have greater stearoyl CoA desaturase activity in both liver and adipose tissue (Moore *et al.*, 1969; Ho & Elliot, 1973). The greater concentrations of oleic acid in the backfat triacylglycerols of the pigs given the copper-supplemented treatments were associated with lower triacylglycerol melting points. Consistent with this both the biased and unbiased ratios of saturated:unsaturated fatty acids in the adipose tissue correlated positively with the triacylglycerol melting points. In keeping with the results of Experiments 1 and 2 the concentrations of palmitic, stearic, oleic and linoleic acids respectively in the adipose tissue were also linearly related to the triacylglycerol melting points.

The results of this present experiment suggest that the lower level of dietary copper had the same effect on the fatty acid composition of the adipose tissue triacylglycerols as did the higher rate of inclusion of copper in the diet. Dietary sodium salicylate, however, had no apparent effect on adipose tissue triacylglycerol characteristics. The positional distribution of the fatty acids in the adipose tissue was also apparently unaffected by the nature of the dietary treatment. This last observation is in keeping with the findings of Experiment 1, where pigs given diets containing different

levels of copper but based on the same source of dietary protein had similar patterns of fatty acid distribution in their adipose tissue triacylglycerols.

Thus, in summary, both levels of dietary copper appeared to increase slightly the growth rates of pigs when compared with animals fed the control diet. Dietary sodium salicylate, however, in conjunction with the lower level of dietary copper only improved pig growth rates up to 46 kg liveweight when compared with pigs receiving the copper supplement alone. Backfat thickness was not significantly altered by dietary treatment.

The inner layer of backfat from each pig was more saturated than the corresponding outer backfat layer. This greater degree of saturation was accompanied by a higher triacylglycerol melting point. The pigs given the copper-supplemented diets had more unsaturated backfat and hence lower adipose tissue triacylglycerol melting points than did those which received the control diet. Dietary sodium salicylate did not appear to affect the nature of the backfat triacylglycerols. The positional distribution of the fatty acids in the triacylglycerols was also not significantly affected by the nature of the dietary treatment.

Section 7

GENERAL CONCLUSIONS

Section 7

GENERAL CONCLUSIONS

The initial aims of the experiments now reported were to investigate some of the factors that affect the composition, structure and physical properties of the triacylglycerols in pig adipose tissues. In particular, the effects of dietary copper, maize oil and protein source were studied, as earlier work had suggested that the effects of these dietary constituents are not merely related to changes in the fatty acid compositions of the adipose tissue triacylglycerols (Section 1.5).

From the studies now reported several major conclusions may be drawn.

1. The presence of Cu in the diet (200 mg/kg) was associated with an increase in the activity of the microsomal stearoyl CoA desaturase in the inner layer of subcutaneous adipose tissue. This was accompanied by a decrease in both the stearic:oleic acid ratio and the melting point of the constituent triacylglycerols.
2. The presence of maize oil in the diet (4 g/100g) decreased the activity of the microsomal stearoyl CoA desaturase in both layers of subcutaneous adipose tissue. The linoleic acid content of the constituent triacylglycerols was greatly increased but there was a surprisingly small decrease in the triacylglycerol melting point.
3. In contrast to the effects of dietary copper and maize oil, the nature of the dietary protein had little effect on the fatty acid composition of the adipose tissue triacylglycerols; however it had a marked effect on the positional distributions of the fatty acids in the triacylglycerol

3.(Ctd.)

molecules. These differences in positional distribution were associated with differences in the melting points between triacylglycerol samples which had similar overall fatty acid compositions. However, random interesterification, a technique which eliminates the differences in positional distribution, also removed the differences in melting point noted between triacylglycerol samples with similar fatty acid compositions but with differing positional distributions of the constituent fatty acids. This finding supports the contention that the positional distribution of the constituent fatty acids plays an important part in determining the physical properties of triacylglycerols.

4. Different breeds of pig, given the same diet *ad libitum*, exhibited characteristic patterns of fatty acid composition in the subcutaneous adipose tissue triacylglycerols. However, although some breeds of pig had adipose triacylglycerols with very similar fatty acid compositions the melting points of the triacylglycerol samples differed considerably. Correlations between the saturated:unsaturated fatty acid ratios and the melting points of the adipose tissue triacylglycerols were found to be similar between groups of pigs which displayed similar patterns of fatty acid positional distribution in their backfat triacylglycerols. Three distinct correlations were noted and this suggests that there are substantial breed differences in the extent to which a given change in fatty acid composition affects the physical properties of the adipose tissue triacylglycerols. Thus, for a given dietary induced change in the saturated:unsaturated fatty acid ratio in the subcutaneous adipose tissue triacylglycerols, the effect on the melting point of the backfat of Hampshire pigs, for example, would be much greater than on the

/melting

4.(Ctd.)

melting point of the backfat of Large White pigs. After random inter-esterification of the triacylglycerol samples from each of the eleven breeds of pig a plot of the melting point against the saturated:unsaturated fatty acid ratio of the adipose tissue triacylglycerols revealed only a single correlation.

5. In general, adipose tissue triacylglycerols with a more asymmetric distribution of the constituent fatty acids exhibited greater changes in melting point with a given change in fatty acid composition than did those triacylglycerols with a more random pattern of fatty acid distribution.

In summary these findings all suggest that the positional distribution of the fatty acids in the adipose tissue triacylglycerols plays an important part in determining the melting point. Further studies are needed to help elucidate the manner in which these changes in the positional distribution of the fatty acids in the triacylglycerols affects the melting point. In Experiment 1 an attempt was made to investigate differences in the molecular species of triacylglycerols between samples of triacylglycerols from pigs of the same breed given diets differing in the source of protein and in the amounts of copper and maize oil present. The analysis using the technique of silver nitrate chromatography proved ineffective as separation of the triacylglycerol species on the thin layer plates was incomplete. However, the use of a more sophisticated technique such as hplc may provide a more complete separation. Such information may provide an insight into whether the differences in melting point between triacylglycerol samples which have similar overall compositions are simply a result of the different patterns

of fatty acid distribution or else represent differences in the nature of the molecular species of triacylglycerols present. Once this has been established further experiments may help to explain the mechanism by which dietary constituents such as the source of protein can affect the nature of the adipose tissue triacylglycerols.

An insight into the factors which affect the physical properties of pig subcutaneous adipose tissue triacylglycerols may help to prevent the occurrence of excessively soft fat which is unsuitable for the bacon manufacturing industry. The results of these experiments, now reported, suggest that soft fat may arise either as a result of the effects of the diet or of the breed of pig or both on the adipose tissue triacylglycerols.

APPENDICES

Appendix 1. *Composition of the mineral/vitamin supplement used in Experiments 1, 2 and 4.*

	g/kg or MIU/kg
Vitamin A	0.48 MIU
Vitamin D3	0.08 MIU
Vitamin E	0.48 g
Vitamin B1	0.08 g
Vitamin B2	0.24 g
Vitamin B6	0.08 g
Vitamin B12	0.0013 g
Vitamin K	0.08 g
Nicotinic acid	0.96 g
Pantothenic acid	0.72 g
Iron	20.00 g
Cobalt	0.08 g
Manganese	2.50 g
Copper	8.00 g
Zinc	8.00 g
Iodine	0.08 g
Selenium	0.008 g
Calcium	250.00 g
Salt	200.00 g

Inclusion rate - 12.5 kg/tonne.

Appendix 2. *Semi-ad libitum feeding scale used in Experiments 1 and 2*

Liveweight (kg)	Meal/day (kg)	Water/day (l)
17.5-18.5	1.00	2.50
20.5-21.5	1.10	2.75
25.0-26.0	1.40	3.50
29.5-30.5	1.70	4.25
34.0-35.0	1.95	4.75
38.5-39.5	2.10	5.25
44.5-45.5	2.25	5.75
49.0-50.0	2.35	5.75
55.0-56.0	2.50	6.25
59.5-60.5	2.60	6.75
65.5-66.5	2.75	6.75
70.0-71.0	2.85	7.25
74.5-75.5	2.90	7.25
76.0 to slaughter	2.95	7.25

The table above is part of the semi-*ad libitum* feeding scale used in Experiments 1 and 2. The amount of food given to each pig was increased up to a daily maximum of 2.95 kg feed/day.

Appendix 3: Experiment 1. Fatty acid composition (wt. %) of triacylglycerols isolated from the shoulder, middle and loin regions of the subcutaneous backfat layers of pigs from all eight dietary treatments.

Fatty acid (%) Inner backfat layer sample site		Dietary Treatment								SED
		F	FCu	FM	FCu	S	SCu	SM	SMCu	
16:0	Shoulder	27.2	25.2	21.8	21.5	26.2	25.1	19.8	19.0	1.5
	Middle	27.2	25.0	21.9	20.7	25.7	25.0	20.3	19.6	1.7
	Loin	27.4	25.1	23.1	20.8	25.1	24.9	19.2	19.0	2.4
16:1	Shoulder	3.2	3.6	1.9	2.0	2.0	2.5	1.7	0.9	0.7
	Middle	3.3	3.8	1.6	2.1	1.7	2.6	1.3	1.1	0.5
	Loin	2.9	3.1	1.7	1.8	1.9	2.3	1.1	1.0	0.5
18:0	Shoulder	17.2	16.3	15.5	13.6	19.0	16.8	12.7	15.0	1.3
	Middle	16.2	14.9	15.4	12.0	18.4	16.0	14.8	14.3	1.4
	Loin	17.4	16.8	15.8	12.1	18.5	16.9	12.1	15.0	1.4
18:1	Shoulder	43.2	45.7	35.1	39.4	41.2	42.3	35.9	36.7	1.4
	Middle	44.4	48.0	36.3	39.6	44.1	45.1	37.9	37.7	1.7
	Loin	43.2	50.5	36.0	40.7	43.7	44.8	35.8	38.4	2.1
18:2	Shoulder	7.1	6.3	21.0	21.7	9.6	9.8	25.4	25.2	1.6
	Middle	7.1	6.6	20.5	22.1	8.6	9.3	21.7	24.5	2.2
	Loin	7.1	6.4	20.7	21.4	9.7	9.2	28.7	23.9	2.0
18:3	Shoulder	0.5	0.6	2.4	2.5	1.4	1.1	2.5	2.4	0.4
	Middle	0.4	0.5	2.2	2.3	0.7	0.6	2.3	2.0	0.4
	Loin	0.7	0.6	2.4	2.2	0.6	0.7	3.1	2.2	0.4

(Appendix 3 continued)

Fatty acid (%) Outer backfat layer sample site		Dietary Treatment								SED
		F	FCu	FM	FMCu	S	SCu	SM	SMCu	
16:0	Shoulder	26.8	23.8	21.1	20.3	24.4	26.8	19.9	17.8	2.0
	Middle	24.2	23.7	21.5	20.5	23.8	24.4	19.3	18.0	1.7
	Loin	25.2	22.7	22.4	18.0	25.0	26.7	18.6	18.4	2.1
16:1	Shoulder	3.3	4.0	1.7	2.3	2.2	3.2	1.7	1.2	0.7
	Middle	3.4	4.5	1.8	2.5	2.1	2.6	1.7	1.3	0.6
	Loin	3.2	3.5	1.9	1.8	1.8	2.4	1.3	1.0	0.7
18:0	Shoulder	15.0	13.5	12.3	10.1	14.9	12.1	11.0	12.1	1.3
	Middle	15.3	12.8	12.7	9.6	16.3	13.8	11.5	11.8	1.5
	Loin	16.1	13.7	13.8	11.7	17.2	14.2	13.0	12.7	1.5
18:1	Shoulder	45.0	48.9	38.1	42.1	44.9	41.1	38.3	39.5	2.4
	Middle	46.3	48.8	37.4	41.8	44.9	44.2	37.3	37.0	1.9
	Loin	45.2	50.9	36.1	42.0	43.5	44.4	36.1	37.8	1.9
18:2	Shoulder	8.5	8.3	21.6	21.6	12.1	13.7	24.7	25.7	1.7
	Middle	10.0	8.0	22.2	22.2	11.0	12.5	25.7	28.4	1.9
	Loin	8.1	7.3	20.9	22.9	10.8	10.3	26.1	26.8	1.7
18:3	Shoulder	0.4	0.6	2.0	2.3	1.0	0.9	2.0	2.7	0.5
	Middle	0.6	0.8	2.4	2.5	0.8	0.9	2.6	2.9	0.4
	Loin	0.5	0.8	2.7	2.4	1.0	0.7	2.3	2.9	0.4

The values shown are the means of the results taken from all four pigs on each treatment.

Appendix 4: Experiment 1. Slip points of triacylglycerol samples isolated from the shoulder, middle and loin regions of the inner and outer backfat layers of pigs on each dietary treatment.

Adipose tissue sample		Dietary Treatment								SED
		F	FCu	FM	FMCu	S	SCu	SM	SMCu	
Inner backfat layer	Shoulder	42.1	38.0	38.9	36.4	44.4	42.9	38.6	37.8	1.7
	Middle	41.7	39.3	37.9	35.2	44.4	41.5	38.6	37.5	1.9
	Loin	43.0	40.1	39.4	35.3	44.7	43.2	38.9	38.1	1.6
Outer backfat layer	Shoulder	39.2	37.8	34.7	33.4	41.5	39.5	33.5	33.7	1.9
	Middle	40.7	37.4	34.7	31.1	42.0	39.7	35.3	32.9	1.9
	Loin	40.6	37.9	37.0	32.2	42.9	41.1	34.7	34.6	1.5

Values given are the means of the triplicate determinations from all four pigs on each treatment.

Appendix 5

Separation and analysis of adipose tissue triacylglycerols from the pigs on Experiment 1

Determination of the fatty acid composition at the 2-position of triacylglycerols gives an indication of major trends in positional distribution. However it does not give an insight into any preferential esterification patterns within the individual triacylglycerol species. To investigate this the triacylglycerols should, ideally, be separated into single molecular species. As this is not practical, groups of triacylglycerols with similar characteristics of unsaturation were obtained for further study.

Pooled samples of inner backfat triacylglycerols from the four pigs on each of the dietary treatments of Experiment 1 were separated into bands of triacylglycerols by silver nitrate thin layer chromatography (Section 2.2.10). This technique separates the triacylglycerols on the basis of the number of double bonds as the silver ions form complexes with the unsaturated bonds. Triacylglycerols containing a fatty acid with two double bonds will complex more strongly with the silver ions than triacylglycerols containing two monounsaturated fatty acids. Trisaturated triacylglycerols, therefore, migrate the furthest.

In general, eight or nine bands were distinguishable although, in one instance, only six bands could be clearly identified. A typical chromatogram is illustrated in Figure 3.2.1, with the triacylglycerol bands numbered from the base line to the solvent front. The lipid standard contained a mixture of tristearin, triolein, trilinolein and trilinolenin. However only three spots could be seen, indicating that the last two of these *i.e.* the tria-

Figure 3.2.1: Experiment 1.

Typical AgNO_3 thin layer separation of pooled samples of inner backfat triacylglycerols from the four pigs on each of the dietary treatments.

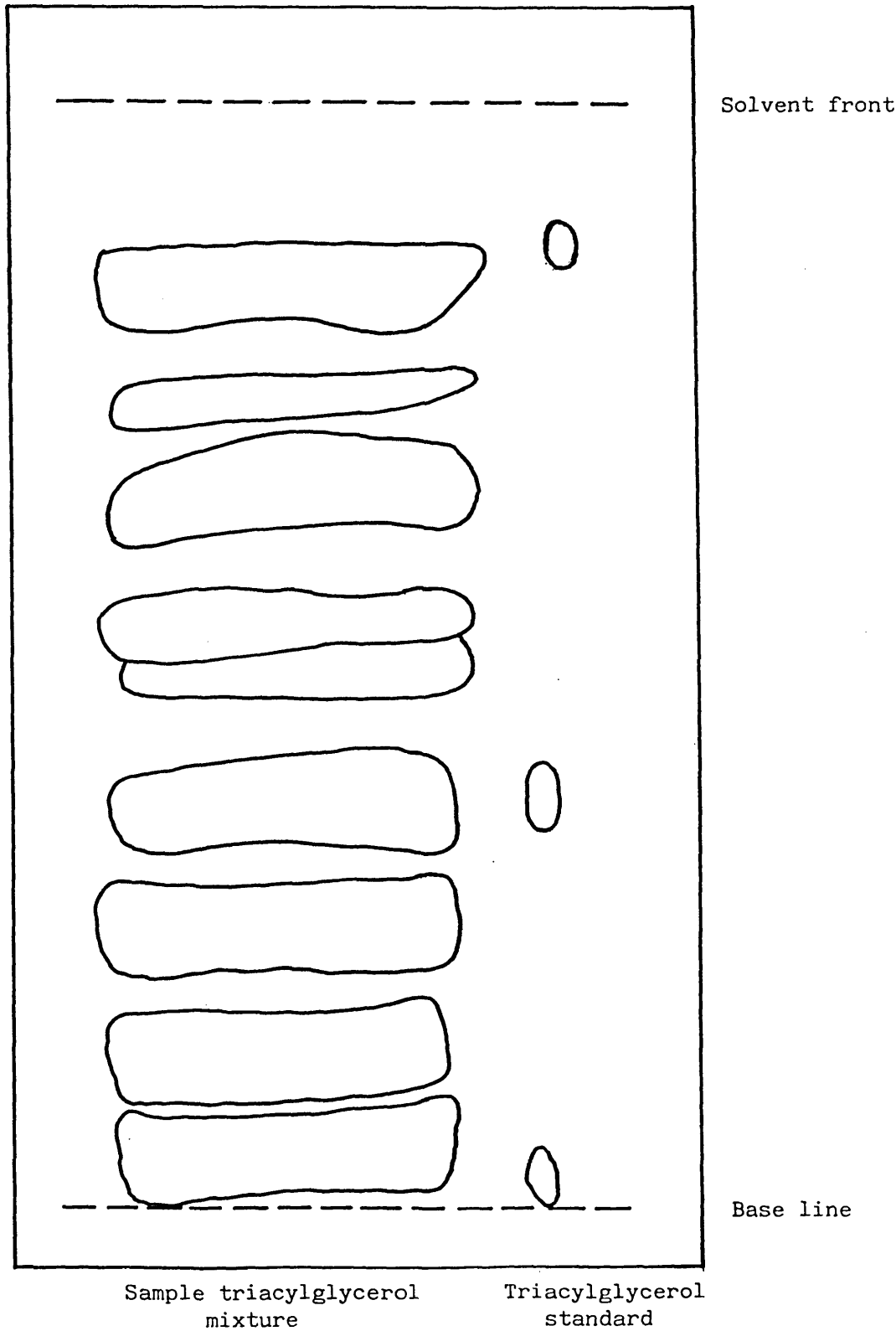


Table 3.2.10: Experiment 1. Mole percent of saturated, monounsaturated and polyunsaturated fatty acids in each band of triacylglycerols obtained by Ag/NO ₃ thin layer chromatography of pooled samples of inner subcutaneous adipose tissue of all ³ pigs on each dietary treatment.																									
Sum of saturated (S), monosaturated (M) and polyunsaturated (P) fatty acids in each band	Dietary Treatment																								
	F			FCu			FM			FMCu			S			SCu			SM			SMCu			
	S	M	P	S	M	P	S	M	P	S	M	P	S	M	P	S	M	P	S	M	P	S	M	P	
Triacylglycerol band																									
1	33.4	34.0	32.5	41.4	38.1	20.4	20.2	19.1	60.7	18.9	29.8	51.3	29.9	40.8	29.3	33.1	36.3	30.6	15.8	20.2	64.0	14.0	25.6	60.3	
2	24.4	34.0	41.5	43.0	23.6	33.4	21.2	31.0	47.8	24.2	41.3	34.5	38.5	24.2	37.3	40.9	27.8	31.3	16.4	32.9	50.7	29.6	20.9	49.5	
3	33.2	12.2	54.6	22.1	44.9	32.9	30.2	9.4	60.4	46.1	29.2	24.7	13.5	56.7	29.8	13.3	57.5	29.2	33.4	8.9	57.7	13.0	51.4	35.6	
4	7.6	57.7	34.8	28.3	38.7	33.0	19.1	43.3	37.7	33.7	61.3	5.0	28.6	39.4	32.0	29.6	38.8	31.6	17.3	44.2	38.5	32.0	42.3	25.7	
5	30.7	34.7	34.6	13.0	77.8	9.2	27.1	38.3	34.6	62.7	36.3	1.0	22.5	63.8	13.7	35.6	42.9	21.5	25.1	39.4	35.5	50.9	44.1	5.0	
6	35.1	42.7	22.0	44.3	27.1	28.6	46.4	24.1	29.5	81.5	15.5	3.0	55.2	24.8	19.9	34.1	62.3	3.7	45.7	24.4	29.9	31.0	66.3	2.7	
7	27.5	69.3	3.2	30.0	66.3	3.7	38.3	58.9	2.8				30.8	63.6	5.6	62.0	34.8	3.2	33.2	63.8	3.0	56.3	41.4	2.4	
8	58.1	40.3	1.6	65.2	32.4	2.4	62.9	36.2	0.8				61.3	37.8	0.8	95.3	3.9	0.7	63.3	35.9	0.8	84.1	14.5	1.5	
R _f 9	86.0	7.9	6.1	84.1	10.6	5.2							92.9	4.3	2.8				89.3	8.8	1.9				

Table 3.2.11: Experiment 1.

Weight percents of the bands obtained by the AgNO_3 thin layer separation of pooled samples of triacylglycerols from the inner subcutaneous adipose tissue of pigs on each dietary treatment, together with the suggested species composition of each band.

TG Species	Dietary Treatment							
	F	FCu	FM	FMCu	S	SCu	SM	SMCu
rest	5.1	6.3	9.5	31.3	4.0	20.5	5.3	11.2
SMT	3.5	3.3	6.7		2.5	4.9	8.9	9.5
MDD								
SST	2.8	5.5	17.3	19.4	4.5	7.6	2.9	13.3
SDD	6.6							
MMD	13.7	6.6	12.4		11.8	16.3	12.9	17.2
SMD	16.2	6.3	20.1	15.6	5.1	17.8	11.2	16.7
MMM		5.3			10.8			
SSD	27.1	27.0	19.7	13.5	26.0	19.5	22.1	15.6
SMM	20.6	33.3	12.1	16.0	24.6	12.4	22.8	13.6
SSS	4.6	6.4	2.2	4.3	10.7	5.3	6.3	3.3

R
f
↓

cylglycerols with six and nine double bonds were not separated from each other.

The individual bands, containing triacylglycerols which are similar with respect to their degree of unsaturation, were analysed for their fatty acid composition. The weight per cent of each of the bands was also measured (Section 2.2.10). For ease of presentation the fatty acid compositions of each of the triacylglycerol bands are given as the sums of the saturated, monounsaturated and polyunsaturated fatty acids. These results are given in Table 3.2.10. Each band was tentatively identified with respect to its component triacylglycerol species. This was achieved by reference to the standards and to the order in which the triacylglycerol bands are known to migrate in the solvent system used (Christie, 1982). The suggested band compositions, together with their weight per cent values, are given in Table 3.2.11.

In general the concentration of saturated fatty acids in the triacylglycerol bands increased with the R_f value of the band. Thus the triacylglycerol band which migrated the furthest consists predominantly of palmitic and stearic acids, presumably in the form of SSS triacylglycerols (see Abbreviations section for triacylglycerol nomenclature). In contrast, the polyunsaturated fatty acid content was higher in the two or three bands of triacylglycerols nearest the base line and was very much lower in the bands which migrated further.

Although these trends in separation were apparent, the triacylglycerol bands did not resolve as well as expected. The two polyunsaturated triacylglycerol standards were not distinguishable from each other so that

triacylglycerols containing six or more double bonds formed a single band on the thin layer plate. This was particularly evident with triacylglycerol samples obtained from pigs on the maize oil-supplemented treatments. With the more saturated triacylglycerols there were differences between treatments in the separations of the species. As an example, with the triacylglycerols from the backfat of pigs on the FCu treatment, the SSD and MMD triacylglycerols appeared to migrate together (band 3) whereas the same species from the adipose tissue triacylglycerols of pigs on the F treatment formed separate bands (bands 3 and 4 respectively). These differences may be a result of the differing amounts of each species present. Further evidence of contamination of bands by other species was noted with the SSS triacylglycerols. The SSS band should contain only saturated fatty acids whereas both mono- and polyunsaturated fatty acids were detected in this band. This was observed with the results from pigs on all eight treatments.

It is possible to isolate and rechromatograph the individual bands to obtain a more accurate picture of the molecular species of triacylglycerols forming the samples from pigs on each treatment. However although repeated separations would have increased the qualitative precision of the technique, this would have been at the expense of quantitative precision as small losses of lipid would have occurred with each separation step. In addition the length of time required to carry out this more detailed investigation was prohibitive, and so with both these factors in mind this particular study was not pursued further.

Despite the inconsistent separations, the results suggest that all three dietary variables: copper, maize oil and source of protein, appear to affect the composition of the adipose tissue triacylglycerols. Dietary

supplementation of copper and maize oil was accompanied by increases in the concentrations of the more unsaturated triacylglycerols and this was particularly evident with the latter supplement. In contrast, soyabean meal protein in the diet, as opposed to fishmeal protein, was associated with higher levels of the more saturated triacylglycerol types, even in a comparison of samples from pigs on the maize oil-supplemented treatments. These results are consistent with the effects of the dietary supplements on the triacylglycerol melting points.

Appendix 6: Experiment 2. Fatty acid composition (wt.%) of triacylglycerols isolated from the shoulder and loin regions of both layers of backfat from all pigs on all four experimental treatments.

Fatty acid (%)	Sample Site	Dietary Treatment									
		Inner backfat layers					Outer backfat layers				
		F	FCu	FM	FMCu	SED	F	FCu	FM	FMCu	SED
16:0	Shoulder	24.9	24.1	23.1	22.0	1.2	23.7	25.2	20.9	21.7	1.7
	Loin	24.4	23.6	24.9	21.6	1.1	23.8	23.8	21.0	21.1	0.9
16:1	Shoulder	5.0	5.1	4.3	4.9	0.4	5.4	4.4	4.5	4.8	0.6
	Loin	5.0	5.5	4.3	4.5	0.4	5.3	5.4	4.4	4.7	0.3
18:0	Shoulder	10.9	9.9	10.0	9.3	0.7	9.5	8.8	7.8	7.6	0.8
	Loin	11.0	9.9	9.6	8.6	0.9	9.8	9.5	8.8	7.5	0.7
18:1	Shoulder	47.4	48.9	39.3	41.7	1.5	48.4	50.4	41.5	43.2	1.0
	Loin	47.3	50.1	40.5	42.9	1.7	47.6	49.6	41.7	43.7	1.4
18:2	Shoulder	9.3	9.7	21.5	21.1	1.0	10.3	9.6	23.6	21.1	2.0
	Loin	9.8	8.4	21.9	20.6	1.0	10.6	9.4	22.7	21.3	1.0
18:3	Shoulder	2.6	2.4	1.9	1.7	0.5	2.7	1.7	1.7	1.6	0.6
	Loin	2.6	2.5	1.9	1.8	0.7	2.9	2.2	1.6	1.6	0.4

The values shown are the means of the results taken from all four pigs on each treatment.

Appendix 7 Experiment 2.

Mean slip points of triacylglycerols isolated from the shoulder and loin regions of both layers of backfat from all pigs on all four experimental treatments.

Sample Site		F	Dietary Treatment			SED
			FCu	FM	FMCu	
Inner backfat	Shoulder	41.8	41.1	38.1	37.0	1.5
	Loin	42.7	40.7	37.5	37.9	1.6
Outer backfat	Shoulder	37.9	37.9	33.1	35.0	2.9
	Loin	39.6	38.1	30.6	35.4	2.3

Values given are the means of the triplicate determinations from all four pigs on each treatment.

Appendix 8: Experiment 4. Fatty acid composition (wt %) of triacylglycerols isolated from the shoulder and loin regions of both layers of backfat from all pigs on all four experimental treatments.

Fatty acid (%)	Sample site	Inner backfat layers					Outer backfat layers				
		1	2	3	4	SED	1	2	3	4	SED
16:0	Shoulder	23.9	22.7	23.2	23.4	1.4	22.6	22.1	22.1	22.2	0.9
	Loin	23.7	22.3	22.7	23.4	0.8	21.7	22.5	22.8	22.8	1.3
16:1	Shoulder	3.3	3.0	3.4	2.9	0.4	3.6	3.1	3.5	3.0	0.4
	Loin	3.1	2.8	3.1	2.9	0.4	2.8	2.7	3.2	3.0	0.6
18:0	Shoulder	17.9	16.2	15.8	14.2	1.7	13.9	12.4	12.4	11.7	1.6
	Loin	18.4	14.1	15.9	15.2	1.4	15.5	13.9	13.1	12.8	1.3
18:1	Shoulder	42.3	45.2	44.7	45.8	1.6	46.3	47.3	47.2	47.8	1.4
	Loin	43.1	47.0	46.4	46.1	0.8	45.6	46.8	46.9	47.6	1.2
18:2	Shoulder	11.6	11.8	11.6	11.6	1.3	13.2	13.7	14.0	13.4	1.3
	Loin	10.7	10.6	11.3	10.5	1.0	12.7	12.7	12.3	12.0	1.2
18:3	Shoulder	1.5	1.5	1.6	1.7	0.3	2.1	1.7	1.7	2.0	0.4
	Loin	1.7	1.4	1.6	1.9	0.3	2.3	1.8	1.9	1.8	0.5

Values shown are the means of results from all four pigs on each treatment.

Appendix 9: Experiment 4.

Slip point (°C) of triacylglycerol samples isolated from the shoulder and loin regions of the inner and outer backfat layers of pigs on each dietary treatment.

Adipose tissue sample		Dietary Treatment				SED
		1	2	3	4	
Inner backfat layer	Shoulder	44.3	42.1	41.6	40.8	1.4
	Loin	45.8	43.0	41.8	41.8	1.3
Outer backfat layer	Shoulder	39.0	38.3	37.1	36.7	1.8
	Loin	40.7	37.3	37.9	37.3	1.4

Values given are the means of the triplicate determinations from all pigs on each treatment.

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