# EFFECT OF SUPPLEMENTAL COPPER AND VITAMIN E ON THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF SWINE DEPOT LIPIDS

by

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ABSTRACT

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EFFECT OF SUPPLEMENTAL COPPER AND VITAMIN E ON THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF SWINE DEPOT LIPIDS

Continuous supplemental dietary copper or copper plus vitamin E improved average daily gain (ADG) and feed conversion (FC) of pigs, although the observed differences in ADG were not statistically significant. Liver copper concentration was significantly ($P < 0.01$) increased by increasing the level of copper in the diet. Removal of copper from the diet at 23, 46 or 69 kg liveweight depressed ADG and resulted in a significant ($P < 0.01$) reduction in liver copper concentration.

Continuous copper supplementation of the diet at levels from 125 to 250 ppm exerted a softening effect on the porcine backfat attributable to significant ($P < 0.01$) increases in the proportions of unsaturated fatty acids (UFA) and of the triunsaturated ($U_3$) triglyceride species present in the fat. Elimination of copper from the diet resulted in a fat which did not differ in its chemical or physical properties from that of control pigs.

Evidence regarding the identification of the dienoic 18 carbon acid whose concentration in porcine depot fat increased significantly ($P < 0.01$) in the presence of supplemental dietary copper indicated that this acid was 18:2$\omega$6,9.

The presence of supplemental copper in the diet significantly ($P < 0.01$) increased the oxidative susceptibility of the fat. The addition of 22 or 44 I.U./kg of supplemental vitamin E in combination with 200 ppm copper significantly ($P < 0.01$) improved the backfat stability as compared to backfat from pigs receiving the supplemental copper alone.

Résumé

Docteur en Philosophie Science animal (Nutrition)

Mohamed A. Amer

Effet de l'apport de cuivre et de vitamine E à la ration sur les caractéristiques physiques et chimiques des dépôts lipidiques du porc.

L'apport régulier de cuivre ou de cuivre et vitamine E à la ration améliorent le gain journalier moyen (GJM) et l'efficience alimentaire chez le porc, même si les différences de gain observées n'ont pas été statistiquement significatives. La concentration en cuivre du foie a été significativement ($P < 0.01$) augmentée par l'augmentation du niveau de cuivre dans la ration. La suppression du cuivre de la ration à 23, 46 ou 69 kg de poids vif a diminué le GJM et a causé une réduction significative ($P < 0.01$) de la concentration du cuivre dans le foie.

L'apport régulier de cuivre à la ration à des niveaux de 125 à 250 ppm a causé un amollissement du gras dorsal attribuable à des augmentations significatives ($P < 0.01$) des proportions d'acides gras insaturés et des triglycérides $U_3$ présents dans la graisse. L'élimination du cuivre de la ration a donné une graisse dont les propriétés physiques et chimiques étaient semblables à celle des animaux témoins.

L'acide diénoique (18C), dont la concentration dans les dépôts adipeux du porc a été augmentée significativement ($P < 0.01$) par l'apport de cuivre à la ration, a été identifié comme étant 18:2W6,9.

La présence du cuivre dans la ration a augmenté significativement ($P < 0.01$) la susceptibilité des graisses à l'oxidation. L'apport de 22 ou 44 U.I. de vitamine E par kg en combinaison avec 200 ppm de cuivre a amélioré la stabilité du gras dorsal comparativement à l'apport de cuivre seul.

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## CLAIMS TO ORIGINAL RESEARCH

1. The first report, as far as the author is aware, on the negative correlation between the glyceride species $U_3$ and melting point on one hand and the positive correlation between the glyceride species $S_3 + S_2U$ and melting point on the other, when 250 ppm supplemental copper is added to swine diets.

2. The first report, as far as the author is aware, describing a significant ($P < 0.01$) multiple correlation between melting point (dependent variable) and UFA and $U_3$ (independent variables) when 250 ppm of supplemental copper is added to the swine diet.

3. The data showed conclusively, that in order to obtain changes in the physical and chemical characteristics, the copper supplement should be included in the diet for the entire growing-finishing period. This observation does not appear to have been previously reported.

4. The first demonstration, as far as the author is aware, of an increase in the phospholipids fraction of swine backfat when 250 ppm of supplemental copper is added to a swine diet.

5. The first reported studies that levels of supplemental copper below 250 ppm may cause significant reduction in melting point and significant increase in unsaturated fatty acids (125 ppm to 250 ppm levels were studied).

6. The provision of evidence that the observed increase in apparent linoleic acid in the backfat of pigs fed supplemental copper was, in fact, an increase in linoleic acid 18:2$\omega$6,9 and not an increase in the positional isomer 18:2$\omega$9,12.

7. The feeding of 22, 44 or 88 I.U./kg of supplemental dl-$\alpha$-tocopherol to improve stability of pork fag from pigs fed a high level os supplemental copper has not been previously reported in the literature.

8. The first studies on the possible reduction in melting point when 22 or 44 I.U./kg of supplemental dl-$\alpha$-tocopherol and 200 ppm of supplemental copper were added to a swine diet and the possible increase in melting point when 88 I.U./kg of supplemental dl-$\alpha$-tocopherol and 200 ppm of supplemental copper were added.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

# INTRODUCTION

The normal growth and development of vertebrates requires the presence of a small amount (5-8 ppm) of copper in the diet. In studies with swine, this element has been shown to improve the rate of gain when added to the diet at levels (125-250 ppm) higher than the metabolic requirement (Braude, 1965). Recently, interest has centered around alterations in the physical and chemical properties of porcine depot fat resulting from the presence of supplemental copper in the diet and the effect of these changes on carcass quality.

Elliot and Bowland (1968, 1970); Moore et al. (1968) and Christie and Moore (1970) have noted that the backfat of pigs fed diets supplemented with 250 ppm copper was softer than that of control pigs as indicated by a decreased melting point. The British workers (Christie and Moore, 1970) have attributed the observed reduction in melting point to alterations in the positional distribution of fatty acids in the component triglycerides, while Canadian investigators (Elliot and Bowland, 1968, 1969 and 1970) have attributed the change in melting point to major alterations in the proportions of saturated and unsaturated fatty acids present in the depot fat.

The mechanism by which copper supplementation of the diet influences the fatty acid composition or the triglyceride

structure of porcine depot fat, is by no means clear. Copper has not, to date, been implicated in fatty acid biosynthesis or desaturation.

Due to the discrepancies between Canadian and British reports regarding the causes responsible for the significant ($P < 0.01$) change in melting point, it was thought that the examination of the fatty acid composition, triglyceride structure, and, more specifically, the positional linkage of the fatty acids in the depot fat triglycerides of control and copper-fed pigs, might throw some light on the means by which copper supplementation alters the consistency of the depot fat under Canadian conditions.

# LITERATURE REVIEW

## Part I. Supplementary Copper for Swine

### 1. Historical

The first experimental evidence that copper is an essential element was provided in 1928 when Hart and his co-workers (Hart et al. 1928) at the University of Wisconsin showed that hypochromic anemia developed in rats fed a diet deficient in copper even though adequate amounts of iron were present in the diet. Weanling white rats were made deficient in both copper and iron by feeding a milk diet which carefully avoided contamination with metals. The animals developed a hypochromic anemia which did not respond to iron therapy unless copper was also provided. The amounts necessary were exceedingly small; 5 micrograms per day were sufficient to initiate and maintain a steady formation of hemoglobin (Hb) but a more rapid response was obtained with larger amounts. It has been shown in this study that although iron can be absorbed and stored normally in the absence of copper, it cannot be utilized by the bone marrow unless the latter element is present.

The exact role of copper in effecting an increased Hb synthesis is not known. It is not, in itself, a component of the Hb molecule nor of the structural material of erythrocytes. The

most acceptable explanation seems to be that offered by Schultze (1941). He found that in pure copper deficiency, the cytochrome oxidase activity in bone marrow was low, whereas, in animals which received adequate copper but were deficient in iron, the cytochrome oxidase activity in the marrow was increased. He postulated, therefore, that the stimulating effect of copper on iron utilization might be due to its function in the formation of cytochrome oxidase and the maintenance of its activity.

In the late 1920's some indications were found that the addition of copper sulfate in small quantities to swine diets resulted in improved weight gain (Evvard, Nelson and Swell, 1928). No further work appears in the literature for some 15 years until Braude (1945) reported that the copper rings surrounding iron pipes in pig pens to prevent rusting were chewed and licked by pigs. He fed weaned pigs 50 milligrams (equivalent to 20 to 25 ppm) of copper (as copper sulfate) daily until market weight, with no significant response in terms of weight gain.

The literature on the growth promoting properties of supplemental dietary copper has become extensive since the first reports of a positive effect on the growth rate of pigs (Barber, Braude and Mitchell, 1955a). Barber et al. (1957) presented an extensive study and cited others concerning the effect of high level (250 ppm) copper supplementation of diets for growing-

finishing swine. This work indicated no permanent deleterious effect from feeding 5 or 10 times the effective level (250 ppm Cu) for short periods of time (19 and 36 days, respectively). It also indicated that if the diet contains excessive copper in the sulfate form the pigs will compensate for this by reducing their feed intake. The response to high levels of supplemental dietary copper has been attributed to a chemotherapeutic effect similar to that of the antibiotics. Hawbaker et al. (1961) found that copper had a marked influence on the microbial population of the intestinal tract of the pig. The presence of supplemental copper sulfate in the diet significantly increased the numbers of coliforms and decreased the numbers of lactobacilli, total aerobes, total anaerobes and streptococci present in fecal samples in comparison with fecal samples from pigs receiving the control diet. This work also showed that antibiotics (Oleandomycin and Oxytetracycline) significantly increased the coliforms, molds and yeasts; and that Oleandomycin significantly lowered the lactobacilli, total aerobes, total anaerobes and streptococci. It was obvious, therefore, that copper sulfate exerted an effect on the fecal microflora which might account for the improved performance of these animals. Several other experiments (Barber, Braude and Mitchell, 1955b; Bellis, 1961; Wallace et al., 1960) failed to show that the effects of chlorotetracycline and copper were additive, while others (Barber, Braude and Mitchell, 1960a; Lucas and Calder,

1957b) showed that the effects of copper and either the tetracyclines, procaine penicillin, or oleandomycin were additive. These findings indicate that inorganic (copper) as well as organic (antibiotics) compounds may improve the rate of gain and feed efficiency of swine and that this improvement could be due to an effect of these feed additives on the intestinal microflora.

For some unexplained reason, the addition of supplemental copper to swine diets has resulted in very different results under American conditions. Small growth responses were reported when 250 ppm copper was included in the diets (Hoefer et al., 1960; Hawbaker et al., 1961 and Ritchie et al., 1961). Bass et al. (1956) found that the addition of 250 ppm copper sulfate to a maize-soybean meal diet impaired pig performance. Wallace et al. (1960) failed to obtain a positive response to copper supplementation. The findings of Hawbaker et al. (1959, 1961), however, were in agreement with British reports; these workers also found evidence of synergism between copper and antibiotic supplementation of diets.

When Wallace, Houser and Combs (1966) offered pigs simultaneous access to meal mixtures containing 0, 125, 250, 500 and 1000 ppm of supplemental copper, the pigs ate more of the meal mixture with no supplemental copper and ate progressively less of the various mixtures as copper level increased, an

level of copper has been most effective is a bit clouded, however, it can be said that the most appropriate level is probably not less than 125 ppm and not more than 250 ppm.

(b) Forms of Copper Used

Numerous experiments have been conducted in an attempt to ascertain whether it was the copper ion or the sulfate radical in the copper sulfate that was effective in promoting daily gain and feed conversion efficiency. Several other forms (other than copper sulfate, $CuSO_4 \cdot 5H_2O$) such as copper chloride ($CuCl_2$), copper sulphide (CuS), copper oxide (CuO), copper carbonate ($CuCO_3$), copper nitrate ($CuNO_3$), and copper methionine have been studied as copper supplements in the swine diet. Hawbaker et al. (1959) noted that $Cu^{++}$ radical and not the sulfate ($SO_4^{=}$) radical was responsible for the improvement in average daily gain (ADG) and feed conversion efficiency (FCE). They also indicated that copper chloride added to the diet resulted in equally as good a response in terms of ADG and FCE as copper sulfate. Copper carbonate was also equally effective as copper sulfate in improving performance, but copper sulfide was not because the response is related to the amount of soluble copper in the gut (Barber et al.; 1961a, Bowland et al., 1961, Allen et al., 1961). Using $^{64}Cu$ S, an average of 1.7 per cent of the dose was absorbed compared with 5.1 per cent when $^{64}Cu\ SO_4$ was given orally (Bowland et al., 1961). Bunch et al. (1961) also reported that copper sulfate, copper carbonate or copper

methionine resulted in significantly faster and more efficient gains when added to the swine diet (copper sulfate and copper methionine promoted a better feed conversion (FC) than did copper carbonate). Lucas et al. (1961) showed that the response to copper was not related to impurities found in these salts as it occurs even when copper sulfate of a very high purity was employed. Braude (1965) recommended the use of copper sulfate in preference because this compound has a very bitter taste, and pigs will refuse to consume diets containing copper in this form considerably in excess of the recommended level of 250 ppm of Cu. This provides an automatic safeguard against overdosing which might result from an error in mixing the diets, thus preventing toxicity.

Throughout the text, copper supplementation refers to the addition of $CuSO_4 \cdot 5H_2O$, unless otherwise indicated.

## 3. Physiological Effects

### (a) Copper Metabolism

The principal sites of copper absorption in the pig are the small intestine and colon (Bowland et al., 1961) and in the rat, the stomach and, to a slightly lesser extent, the duodenum (Van Campen and Mitchell, 1965). These investigators have indicated that acid conditions favor copper absorption, however, this could represent merely a solubility factor rather than a true absorptive effect. Nevertheless, this could explain

why the stomach and the upper small intestine are the main absorptive sites since acid conditions do occur in these segments of the gastrointestinal tract. Studies with radioactive copper (Bowland et al. 1961) have demonstrated that only 2-10 per cent, dependent on form used, of the ingested copper is absorbed by the pig, nearly all of the remainder is excreted in the faeces with a small fraction excreted in the urine. They indicated that the bile accounted for up to 40 per cent of the excretion of the absorbed copper. Buescher, Griffin and Bell (1961) noted that there was a larger amount of copper excreted in the urine after copper carbonate had been fed than after feeding copper oxide or copper sulfate. Copper must pass through the liver by way of the portal vein. The normal liver rapidly concentrates the metal with an affinity comparable to that of the thyroid for iodine (Simek et al., 1961). The liver takes up the metal from the plasma albumin, and indirectly, from the extracellular fluid, at the same time excreting copper in the bile or returning it to the plasma as ceruloplasmin. These workers also reported that the amount of copper circulating in the blood of the pig is relatively small (0.12-0.15 mg/100 ml).

(b) Copper Accumulation in Animal Tissue

Copper is widely distributed in animal tissues, with the highest concentrations occurring in the liver, kidney and heart. Castell and Bowland (1968b) reported the

following amounts of copper in ppm of dry weight of tissues; liver, kidney, heart, spleen, muscle and hair to be 31, 43, 15, 6.7, 2.5 and 9 for basal-fed and 113, 51, 14, 6.4, 2.4 and 19 for copper-fed (250 ppm) pigs, respectively. The liver, therefore, appears to function as a filter and prevents excessive accumulation of copper in other tissues. Castell and Bowland (1968b) observed a reduction in the concentrations and total copper content of kidney and spleen in supplemented pigs over the final growth period (70-90 kg live-weight). These authors also noted that copper levels of heart muscle were not affected by level of dietary copper supplementation. Kirchgessner, Wesser and Friesecke (1963a) have reported that the proportion of ingested copper retained in the body is related to the level of copper in the diet. When a control diet is fed with only traces of copper, 12.3 per cent of copper was retained, but on diets supplemented with either 0.05 or 0.1 per cent of copper sulfate, 4.8 and 10.6 per cent, respectively, of the copper was retained. The amount of copper stored in the liver of pigs which have received 250 ppm of copper in their diet has been reported to vary from 2 to 22 times the amount present in the livers of the control pigs (Braude, 1965). At 125 ppm dietary Cu, liver copper concentrations were between 1.3 and 6 times those of controls (Allen et al., 1961).

Some work has been published on the relationship of protein level and source on copper concentration in the liver tissue. With a high level protein diet (17%), the addition of 0.1 per cent $CuSO_4 \cdot 5H_2O$ to the diet from weaning led to reduced concentration of copper in the liver as compared with that in the liver of pigs fed a 14 per cent protein diet (Lucas, Livingstone and Boyne, 1962). Combs et al. (1966) also reported that pigs accumulated significantly more copper in the liver when fed casein than when fed soybean meal as a protein source. Wallace et al. (1966) observed that fishmeal diets induced higher retention of copper in the liver than diets containing soybean meal. This observation has been confirmed by Drouliscos, Bowland and Elliot (1970).

Withdrawal of copper from the diet at 46-57 kg body weight permitted liver copper concentrations to return to normal by slaughter weight (Barber, Braude and Mitchell, 1962). It has also been reported by this group of workers that copper sulfate caused greater increases in liver copper than copper oxide. Barber et al. (1960a) found that addition of zinc, at 250 ppm, and "nutritional dosage" levels of tetracyclines to copper-fortified diets significantly reduced liver copper content. This level of zinc addition is well within the tolerance limit of 1.218 g/kg reported by Lucas (1957b). Bunch et al. (1961) observed that high level copper supplementation resulted in

decreased liver iron concentrations. The work of Suttle and Mills (1966a) emphasized the beneficial effect of liberal dietary iron in the presence of a toxic level of copper. As with zinc, it appears that attention to iron supplementation is of special importance when high levels of copper are fed.

(c) Nitrogen Retention

Kirchgessner and Giessler (1961) noted that feeding 250 ppm of copper to pigs increased significantly digestibility and retention of nitrogen. As explained by Braude (1965) the significant increase in percentage of the apparently digested nitrogen may be due to the fact that copper prevents the breakdown of essential amino acids in the gut, thereby improving the balance of amino acids absorbed, which, in turn, enables anabolic processes to proceed more efficiently. In another balance test, Braude (1965) found a marked growth response associated with the observed increase in apparent digestibility of nitrogen and nitrogen retention only in pigs receiving 250 ppm Cu.

(d) Interaction with Dietary Protein Level and Source

Several reports have shown an apparent relationship between response to copper supplementation and level of protein in the swine diet. With low protein diets the inclusion of 0.1 per cent copper sulfate from weaning onwards might lead to reduced performance between 46 and 92 kg (Lucas et al. 1962). King (1964) reported that in barley-fishmeal diets with low

(12.5%) protein, copper supplementation resulted in improved average daily gain and feed efficiency when compared with the basal diet (14.5% protein). Pigs fed a high (18.1%) protein diet did not show this response. Castell and Bowland (1968a) noted that the beneficial effects of copper supplementation were more evident when diets of relatively high protein content (17%) were fed. Meanwhile, Hanrahan and O'Grady (1968) reported that the performance of pigs fed a low (12.9%) protein diet supplemented with copper was significantly reduced while that of pigs fed a high (16.5%) protein, copper supplemented diet was only slightly reduced. Bunch et al. (1961) failed to show that dietary protein level affected the response to supplemental dietary copper.

Wallace et al. (1960) determined the efficacy of adding high levels of copper (100, 150, 200 or 250 ppm) to corn-soybean meal diets which are commonly fed to growing pigs in the United States. An analysis of the growth data showed a highly significant ($P<0.01$) adverse effect due to the copper and a highly significant ($P<0.01$) interrelationship between the copper and protein level. As the protein level increased (from 15, 20 to 25%) the copper toxicosis was less pronounced. Several studies have indicated that the type of protein supplement (animal vs. vegetable) used in the diet might affect the response to supplemental dietary copper. Barber et al. (1962) reported that the response to a copper supplement was greater

in diets containing fishmeal than in diets containing soybean meal: ADG + 14.5% *vs.* + 5.1% and FC + 5.2% *vs.* + 2.1%, respectively. However, Lucas et al. (1962) found that there was no evidence of a different effect of copper supplementation of barley-fishmeal diets as compared with corn-soybean meal diets provided the level of protein in the diet was adequate. Combs et al. (1966) fed high levels of copper with two sources (soybean or casein) and two levels (14 or 22%) of dietary protein. The source of protein and the level of copper significantly influenced ADG and FC more than the level of protein. Daily gain and daily feed consumption were adversely affected when casein supplied the supplemental protein and when the diet contained 500 ppm of copper. Castell and Bowland (1968a) reported that the beneficial effects of copper were more evident in pigs fed fishmeal than in pigs fed soybean meal as the source of supplemental protein.

Source of supplemental protein had no effect on feed intake, dressing percentage, carcass length or R.O.P. score, however, source of protein significantly affected ADG, FC, age to market and total backfat thickness (Elliot and Bowland, 1970).

(e) Reproduction

Dammers and Van de Grift (1963) fed high levels of supplemental copper to breeding sows continuously for three years.

Neither adverse nor beneficial effects were observed. Wallace et al. (1966) fed sows 250 ppm copper commencing three days pre-farrowing through the first 14 days of lactation to study the influence of copper on the incidence of scouring in baby pigs. The over-all performance of copper supplemented sows was slightly better than control sows, but the copper did not prevent periodic outbreaks of bacterial scours in the suckling pigs.

(f) Interrelationships with Other Metals and Copper Toxicity

Evidence has been obtained in in vitro studies that an excess of one element can cause the displacement of other elements from binding sites on protein molecules (Kirchgessner and Wesser, 1963). The presence of copper at high levels in the diet might influence the metabolism of iron and zinc by causing liver and kidney dysfunction and by generally altering the permeability of the cell wall (Suttle and Mills, 1966). The interrelationships between Cu, Zn and Fe demonstrated in their studies might, therefore, involve competition between elements with similar affinities for binding sites on proteins in the digesta or in the tissues.

In the United States, the addition of large quantities of copper to the diet of the pig could conceivably create an imbalance of minerals within the animal and so impair function. Wallace et al. (1960) reported severe toxicity in one experiment involving 250 ppm copper and failed to obtain improvement in

feedlot performance at levels of 100, 150 and 200 ppm in ensuing trials. The basal diets used in these trials were not supplemented with the liberal quantities of Zn and Fe now deemed important. Ritchie et al. (1963) observed that supplemental Zn reduced the copper levels and alleviated symptoms of copper toxicity in pigs fed 250 ppm of supplemental copper. The addition of 500 ppm Zn or 750 ppm Fe in the presence of 750 ppm Cu eliminated jaundice and produced serum Cu and aspartic transaminase concentrations similar to control values after 4 weeks (Suttle and Mills, 1964). Barber et al. (1960a) reported responses to copper in the absence of supplemental zinc in the diet, and also observed that supplemental zinc resulted in lower concentrations of copper in the livers. Wallace (1968) recommended the addition of 100 ppm Zn when high levels of supplemental copper are included in swine diets.

(g) Quality of Carcass

Little is known of the chemical composition of the carcass of pigs given copper supplemented diets and whether it differs in any respect from that of carcasses of pigs given the basal diets. However, the effect of supplemental copper on certain physical carcass measurements has been studied. Barber et al. (1960b) reported a tendency for copper-supplemented pigs to have a higher dressing percentage. Lucas et al. (1961) failed to find any effect on dressing percentage. Shorter carcass length as well as greater thickness of backfat in copper-supplemented pigs has

also been reported (Barber et al., 1961a). Barber et al. (1957) observed that 59 out of 77 carcasses from two experiments graded as A. There was a reduction in both experiments in triple-A carcasses from the pigs given Cu but this reduction was not significant. Combined results from three experiments by Barber et al. (1960b) showed that copper sulfate significantly increased dressing percentage by 1.7 per cent and significantly reduced carcass length by 2.0 per cent. There were no marked or consistent treatment differences in commercial grades apart from a tendency for pigs that received supplemented copper to yield fewer AA+ carcasses and more AA and A grade carcasses, a function of the reduction in carcass length of the animals on this treatment.

The effects of supplementary copper on the carcass quality of pigs have not been consistent in four experiments conducted by Castell and Bowland (1968a). Increases in dressing percentage were observed in only one experiment and a decrease in dressing percentage was the common occurrence in the three other experiments. Average backfat thickness was lower in all copper-supplemented groups except for those pigs on the higher level of soybean meal. This is in contrast to the reports by Barber et al. (1961a,1962) who found that increases in backfat thickness occurred in pigs receiving supplementary copper. Wallace et al. (1966) observed no significant effects on carcass measurements when 125 ppm or 250 ppm of copper was fed. Houser

(1966) reported that copper caused no significant effect on dressing percentage, carcass length, backfat thickness and percent lean cut out.

Barber et al. (1961a) noted a trend for Cu supplementation of the diet to cause some increase in moisture content of most of the organs and tissues. Scott et al. (1966) indicated that copper fed at 200 ppm as a growth stimulant had no material effect on the meat, except for a darkening effect on the raw, uncured hams, and had no effect on the organoleptic properties after curing.

(h) Hemoglobin Level

With the use of high levels of supplemental copper in the diet, the Hb level of pigs was depressed, however, the depression was not as great at the level of 125 or 250 ppm as at the level of 375 ppm (Bunch et al., 1961). Ritchie et al. (1963), Simek et al. (1961) and Wallace et al. (1960) have reported that Hb concentration was depressed with increased copper levels although in the studies by Wallace et al. (1960) it was found that supplemental zinc failed to alter the subnormal Hb levels observed in pigs fed 200 ppm of copper. This was in contrast to studies by Castell and Bowland (1968b) where supplementary dietary copper had no consistent effect upon the Hb levels at 9, 47 and 90 kg liveweight where average values were 11.0, 12.7 and 12.7 g Hb/100 ml of blood, respectively.

Wallace et al. (1963) have reported that pigs have the capacity to adapt and recover somewhat in Hb level after the initial depression. The report by Suttle and Mills (1966a) demonstrated that a high dietary level of iron (750 ppm) afforded protection against anemia when a toxic level of copper (750 ppm) was fed.

4. Biochemistry of Copper

Copper is thought to be involved in a wide variety of biochemical systems as part of several metalloenzymes and metal enzyme complexes. The subject has been recently reviewed by Peisach, Porter and Goldstein, (1966). Enzymes involved in the oxidation of mono- and di-amines, uric acid, cytochrome C, and galactose are all copper containing proteins. Amine oxidase apparently is also involved in the oxidative deamination of $\epsilon$ amino group of elastin. In addition to these cuproenzymes, a number of enzymes such as ureidosuccinase bind copper less specifically, i.e., form metal enzyme complexes.

Ceruloplasmin, erythrocuprein and hepatocuprein are similar or possibly identical bluish-green soluble proteins containing about 0.35 per cent copper (Porter, 1966). These enzymes have no known enzymatic activity or physiologic function although they are thought to play a role in copper metabolism.

There is recent evidence that dopamine β-hydroxylase from the adrenal medulla is a copper enzyme and converts dopamine to norepinephrine (Goldstein, 1966).

Ribonucleic acid isolated from a number of biologic sources contains significant amounts of firmly bound copper in addition to other metals. Significant concentrations of copper have also been shown to be present in isolated and purified viruses, perhaps through association with nucleic acid.

Copper influences erythropoiesis; its deficiency apparently reduces iron absorption and decreases heme synthesis.

Like iron, copper plays a part in certain oxidation-reduction enzyme systems of the cell ($Cu^{++} \rightleftharpoons Cu^{+}$). This role as a component of enzyme complexes is probably of greater significance than its role in hemoglobin formation.

In contrast to calcium and potassium, but like magnesium, copper appears to inhibit the myosin ATP'ases. Tyrosinase is found in both animal and plant tissues. It oxidizes various phenolic compounds such as tyrosine, phenol, catechol and cresol. For example: Catechol + $O_2$ $\xrightarrow{\text{tyrosinase}}$ o-Quinone + $H_2O$. Water, not hydrogen peroxide, is always formed as a result of oxidation by this enzyme, and gaseous oxygen is used as the hydrogen acceptor. Copper is an integral part of the tyrosinase molecule.

Although iron has replaced copper as an oxygen carrier relatively late in evolution, it has not displaced this metal from its key role in the respiratory chain. The activity of cytochrome C oxidase present in the respiratory chain (the electron transport mechanism from which high-energy phosphate bonds are derived) is markedly reduced in the heart and liver (Gubler et al., 1957) of copper deficient swine.

Glycolysis is known to be activated by copper and is accelerated in anemia (Weissberger and Luvalle, 1944). The oxidation of glutathione is accelerated in the presence of small amounts of copper (Voegtlin et al., 1931) and it has been observed that during recovery from hemorrhagic anemia the glutathione content of the blood is increased (Bichel, 1944).

Copper deficiency results in a marked underdevelopment of the myelin sheath throughout the central nervous system in guinea pigs (Everson et al., 1968) and lambs (Underwood, 1966). Characteristic biochemical changes are associated with this form of nervous disorder (ataxia). Blood and liver copper levels are usually subnormal in the mother and in the affected lamb. Brain copper and brain cytochrome oxidase levels are invariably subnormal in lambs with ataxia (liver contains less than 10 ppm Cu on dry basis and the whole brain copper level has no more than 2.8 ppm Cu on the dry basis).

## Part II. Component Fatty Acids of Porcine Depot Lipid

### 1. Fatty Acid Composition

#### (a) Constituent Fatty Acids

Prior to 1937, pig depot fats were described in terms of palmitic (16:0)[1], stearic (18:0) and oleic (18:1) acids with smaller amounts of myristic (14:0) acid and polyethenoid $C_{18}$, $C_{20}$ and $C_{22}$ acids. Later work revealed the presence of approximately 3 mole% of hexadecenoic acid (16:1) (Hilditch and Shorland, 1937) together with traces of lauric (12:0) and tetradecenoic (14:1) acids (Hilditch et al., 1939). Hilditch and Stansby (1935) examined the fats of pigs grown on diets of low but variable fat content. Their results show that, under these conditions, the 16:0 acid content of pig depot fats was remarkably constant at 28-30 mole% tending to be slightly less in the case of the most unsaturated fats. The combined contents of $C_{18}$ acids were also fairly constant at 65-70 mole%.

In studying the composition of pig fat, Sink et al. (1964) found the minor fatty acids: lauric, myristic, pentadecanoic, heptadecanoic, heptadecenoic, linolenic and arachidonic to be present in greater abundance in pigs weighing 34 kg liveweight. They stated that the presence of odd-carbon fatty acids indicated that they should not necessarily be considered as non-

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[1] Number before colon indicates number of carbon atoms, number after colon reveals number of double bonds.

physiological compounds. Stinson, de Man and Bowland (1967) as well as Elliot and Bowland (1968,1970) have suggested that arachidic acid might also be a minor component of porcine depot fat.

(b) The Effect of Diet on the Component Fatty Acids of the Swine Depot Fat

The composition, characteristics and consistency of pork fat can vary according to the composition of the pigs' diet. It is common knowledge that swine fed starchy cereals develop a hard fat, whereas, peanut-fed swine develop a liquid lard (Hilditch, 1964). The interrelationships between composition of dietary fat and the composition of swine depot fat have been studied repeatedly by empirical feeding trials involving different fats (Callow, 1935a; Hilditch et al., 1939; Hilditch, 1964; Sink et al., 1964; Koch et al., 1968b).

Accurate fatty acid analysis of the depot fat of pigs fed diets low in fat have been made by Hilditch (1964). Comparing pigs on different planes of nutrition, he concluded that "on a restricted diet the deposition of fat is slower and the fat produced is softer, owing to increases in the proportions of linoleic acid, with some increase in the proportion of oleic acid." The result with respect to linoleic acid content is consistent with the growth-rate theory of Callow (1935a). In regard to oleic acid, the differences are small and not completely regular. Results obtained by Shorland and de la Mare

(1945) have shown that restriction of a diet supplemented with maize meal is associated with the production of fat with a high degree of unsaturation and more linoleic acid, consistent with the growth-rate theory as applied to dietary linoleic acid.

It has been shown by Shorland and de la Mare (1945) that restriction of intake of a skim or buttermilk diet apparently produces no significant changes in the consistency of porcine depot fats. Further, the results of Hilditch et al. (1939) are inconclusive in showing whether, considering the same depot fats of pigs on different planes of nutrition, a decrease in oleic acid content is associated with faster growth.

Feeding cottonseed oil was found to increase the stearic acid content of the depot fat (Hilditch, 1964). As cottonseed oil contains only 2 mole% of stearic acid, the observed increase in stearic acid content of the depot fat may have been the result of hydrogenation of dietary or synthesized oleic acid. In this study, the fat of pigs fed diets which contained ground nuts or soybeans, and which were similar to the cottonseed oil diet as regards to oil content and percentage of stearic acid, no increase in stearic acid content was observed.

A further complication affecting the fatty acid composition of deposited fats arises from the possibility that one dietary acid may modify the metabolism of another. Eckstein

(1929b), and Powell, (1930) have shown that in the rat, addition of 25 per cent tricaproin or tricaprylin to an otherwise fat-free diet results in the deposition of a fat with lower iodine value than that of rats fed a control diet. This result suggests an increased synthesis of palmitic and stearic acids in the experimental as compared with the control animals.

The unchanged palmitic acid content of the fat of pigs on a copra (highly saturated fat) diet contrasts strikingly with the changes in the palmitic acid content recorded by Ellis and his co-workers (Ellis et al., 1931) when large amounts (more than 4 per cent) of unsaturated oils were included in the diet.

Fat from hogs fed safflower oil (high in unsaturated fatty acids) contained significantly more linoleic acid and less oleic acid than the fat from control pigs, Koch et al. (1968b). Feeding tallow (high in saturated fatty acids) in their experiments tended to restore the fatty acid pattern of safflower oil-fed hogs to that of control animals.

The fatty acid composition of the intramuscular fat from the Longisimus dorsi muscle was affected much less by diet than was that of the leaf fat or backfat. Their data suggest that the inner layer of backfat exhibited a more extensive turnover of fatty acids than the outer layer. In earlier studies, it has been demonstrated that the inner backfat layer

normally contains a higher level of palmitic acid and a lower level of linoleic acid (Sink et al., 1964; Shorland and de la Mare, 1945). Although this was true for the control hogs in the studies by Koch et al. (1968b), the safflower oil-fed hogs contained a lower level of palmitic acid, and a higher level of linoleic acid in the inner backfat layer. This also indicates a greater amount of change in the inner backfat layer.

(c) Fatty Acid Composition of Swine Depot Fat as Affected by Copper Supplementation of the Diet

Recently, it has been reported that supplementation of a practical swine diet with 250 ppm copper may result in significant alteration in the composition of the depot fat and sometimes appears to be responsible for the production of a carcass with soft fat. Barber et al. (1961) and Bellis (1961) reported that copper supplementation of the diet adversely affected the carcass grades of market pigs. Taylor and Thomke (1964) observed that dietary 250 ppm copper resulted in a softening of the depot fat as indicated by a significant ($P < 0.01$) increase in the iodine value and a significant decrease in the fat consistency (mm Hg). These workers speculated that dietary copper might affect fat absorption or transport, or that the high level of copper found in the liver of pigs on copper-supplemented diets might affect the normal fat metabolism of this tissue. However, Braude (1965) reported that an attempt to produce soft fat by feeding high level copper was unsuccessful.

Experiments at the University of Alberta confirmed the report by Taylor and Thomke (1964) (Bowland and Castell, 1964,1965; Castell and Bowland, 1968a). Elliot and Bowland (1968) have reported that copper supplementation (250 ppm Cu) of the diet resulted in a significant ($P < 0.01$) increase in the proportion of unsaturated fatty acids, 16:1 and 18:1, in the outer backfat, inner backfat and perinepheric fat at 26, 47 and 70 kg liveweight. Their results also showed a corresponding significant ($P < 0.01$) decrease in the proportion of saturated fatty acids, 16:0 and 18:0. No significant differences were observed in fats from pigs at 90 kg liveweight. These investigators (Elliot and Bowland, 1970) further indicated that in comparison with pigs receiving the basal diets, copper supplementation of fishmeal, meat meal and soybean meal-supplemented diets resulted in a significant increase in the proportion of unsaturated fatty acids (UFA) in the backfat at 34, 45, 57, 68, 79 and 88 kg liveweight. Copper supplementation of barley-rapeseed meal diet did not result in similar changes.

Moore et al. (1968) reported the results from a study of the effect of dietary copper on the fatty acid composition of the swine backfat. Their results revealed a somewhat lower concentration of 16:0 and a somewhat higher concentration of 18:1 in the backfat of pigs receiving the copper-supplemented diets; the concentrations of the other fatty acids were unaltered.

(d) Effect of Area Sampled

The outer layers of the backfat of pigs are somewhat more unsaturated in character (higher oleic acid content) than the inner layers, whilst the perinephric fat of the animal is still more saturated and contains the greatest content of stearic acid. Henriques and Hanson (1901) (cited by Hilditch, 1964) concluded that the determining factor was the temperature at the site of the fat deposit in the animal (e.g., back of pig; 1 cm deep, 33.7 C, 4 cm deep, 39.0 C, rectum, 39.9 C). Further support for this hypothesis was obtained by maintaining three pigs from the same litter at different temperatures, one at 30-35 C, one at 0 C, and one at 0 C but covered with a sheepskin coat; the iodine values of the outermost layer of the backfat from these animals, after two months, were respectively 69.4, 72.3 and 67.0. The detailed analysis of Dean and Hilditch, (1933) affords general confirmation of Henriques and Hanson's views, but indicates that the increase in softness (i.e., unsaturation) is confined to the outermost layer of the outer backfat.

Sink et al. (1964) concluded that saturated fatty acids are preferentially deposited in perirenal tissues as compared to the subcutaneous area, and in this latter area, saturated fatty acids are deposited in the inside layer rather than the outside layer. Stinson, de Man and Bowland (1967)

showed that all of the fatty acids present in the fat, except myristic, arachidic and gadoleic acids, showed highly significant differences between sites (inner and outer shoulder, inner and outer loin and kidney). In most cases, kidney fat was found to be significantly different from the other sample sites; this is particularly evident in 16:0, 18:0 and 18:1 acids. When considering the depot fats taken from the shoulder and the loin, there seemed to be more difference between samples taken from the inner and outer layers than between samples taken from the shoulder and the loin. These authors have concluded that differences in fatty composition observed between sample sites are the result of differential deposition of 18:0 and 18:1 acids rather than 18:2 acid. These observations are in full agreement with those of Imaichi et al. (1965).

(e) Effect of Sex

Lush et al. (1936) indicated that the backfat of gilts had higher iodine values (1.7 units) than that of littermate barrows. Johns (1941) reported that iodine values of backfat ranged in decreasing order from boars to gilts, barrows and spayed gilts. He reported that the iodine value varied inversely with the quantity of backfat present. This same author observed that the backfat from boars contained a higher level of linoleic acid than gilts, barrows or spayed gilts. Koch et al. (1968a) found that backfat from barrows contained more palmitic

and stearic acids and less linoleic acid than that of gilts. The leaf fat of barrows and gilts behaved similarly to the backfat. However, the fatty acid composition of the intramuscular fat did not differ between barrows and gilts. They attributed these sex effects to hormonal differences or possibly to the actual differences in amount of backfat deposited. With regard to the latter supposition, the full-fed barrows in one experiment had an average backfat thickness of 1.62 inches while the gilts averaged 1.38 inches. In other experiments, the average backfat thickness for boars, barrows, gilts and spayed gilts was 1.04, 1.31, 1.16 and 1.28 inches, respectively.

(f) Effect of Age and Liveweight

Callow (1935a) has shown that, in the case of pigs fed diets low in fat, the fat content of fatty tissues increased with age, while the fat becomes more saturated. It was subsequently shown (Callow, 1937) that for pig flare fat the rate of deposition of fat is correlated with the iodine number. Pigs which grow slowly produce a more unsaturated fat than pigs which grow rapidly. At each age, the outer backfat is more unsaturated than the inner backfat, which, in turn, is more unsaturated than the flare fat. Differences are also found in the unsaturation of different parts of the outer and inner backfat.

From the above facts, Callow (1935a) developed a theory which will henceforth be described as the "growth-rate

theory." This theory is expressed by him in the following terms; ".... it appears probable that the faster the rate at which fat is deposited in fatty tissues, the more saturated the fat becomes." This would be expected in view of the fact that fats in such deposits can either be formed from the fats and oils in the diet, or from carbohydrate by synthesis. Since the fats and oils in the diet are limited in amount, the rate of deposition of fat in a rapidly growing pig may be so great that a considerable proportion of fat must be synthesized from carbohydrate. This leads to an increase in the saturation of the deposited fat, because fat synthesized from carbohydrate is relatively saturated, with an iodine number of 50 to 60, whereas, the fat deposited from the oils in the diet is relatively unsaturated, owing to the fact that the oils in the diet usually have an iodine number of over 100 (Hilditch, 1964). The growth-rate theory (Callow, 1935a) predicts that those tissues which grow slowly will contain dietary acids in proportions greater than those found in fast-growing tissues. As compared with fat synthesized by the pig, dietary fats in general contain more linoleic acid and less oleic (Callow, 1935b; Hilditch et al., 1939). The growth-rate theory therefore predicts that as compared with the depot fats of slow-growing pigs, the depot fats of fast-growing pigs should contain more oleic acid and less linoleic acid.

In studying the composition of the pig fat, Sink et al. (1964) found qualitative and quantitative changes in the pattern

of fat deposition at approximately the 59.1 kg or 120-day stage of life of the pig. The selective deposition of the saturated fatty acids increased with increasing liveweight. Sink et al. (1965) suggested that some metabolic changes occurred in the subcutaneous depot lipid of the pig at 56.8 kg liveweight. Allen, Bray and Cassens (1967) noted that much of the increase in saturation of intramuscular lipid that occurs after about 55.0 kg liveweight can be attributed to the ratio of fatty acid 18:1 to 18:0. This change was suggested to be probably dependent upon sex hormones and this is restricted to the neutral lipid fraction.

2. Fatty Acid Biosynthesis

The problem of how fatty acids are synthesized in the animal system has been approached in different ways by chemists and biochemists. The chemist has concerned himself with the end product (the fat) and the question of how such a compound might be produced; the biochemist has given more attention to the possible precursors which could be used in fat synthesis.

That animals do, in fact, synthesize fatty acids and do not acquire them solely from dietary sources has been shown by carefully controlled feeding experiments from which it was clear that the fat deposited has exceeded that supplied in the diet. Experiments carried out by Hilditch and his colleagues (Hilditch et al., 1937) involving a comparison of the component

acids of the dietary fat with those deposited by pigs led to the conclusion that biosynthesis of fats containing 16:0, 18:0 and 18:1 acids had occurred to a marked extent and that 16:1 acid and possibly 14:0 acid had also been synthesized to a lesser extent. It was also concluded that pigs, like rats, are unable to synthesize 18:2 acid.

Biochemists, (Gibson et al., 1957; Wakil, 1964; Masoro, 1968) mainly by the use of isotopic compounds, have obtained many interesting and illuminating results. It is now fairly clear that short- and long-chain saturated acids are synthesized from a two-carbon unit. This $C_2$ unit is known to be acetyl-coenzyme A identical with the product of fat catabolism. Investigations have so far failed to establish the presence of an intermediate other than acetate in the biosynthesis of fatty acids in animals. This is readily available from the metabolic reactions of proteins, carbohydrates, or fats. Whilst some amino acids (leucine, alanine) afford acetate directly, others are known to be glycogen formers and thus yield acetate by the same route as carbohydrates. Isotopic studies have shown the following to be the course whereby glucose is converted to fatty acids (Figure 1).

Two separate enzyme systems may be involved in fatty acid synthesis:

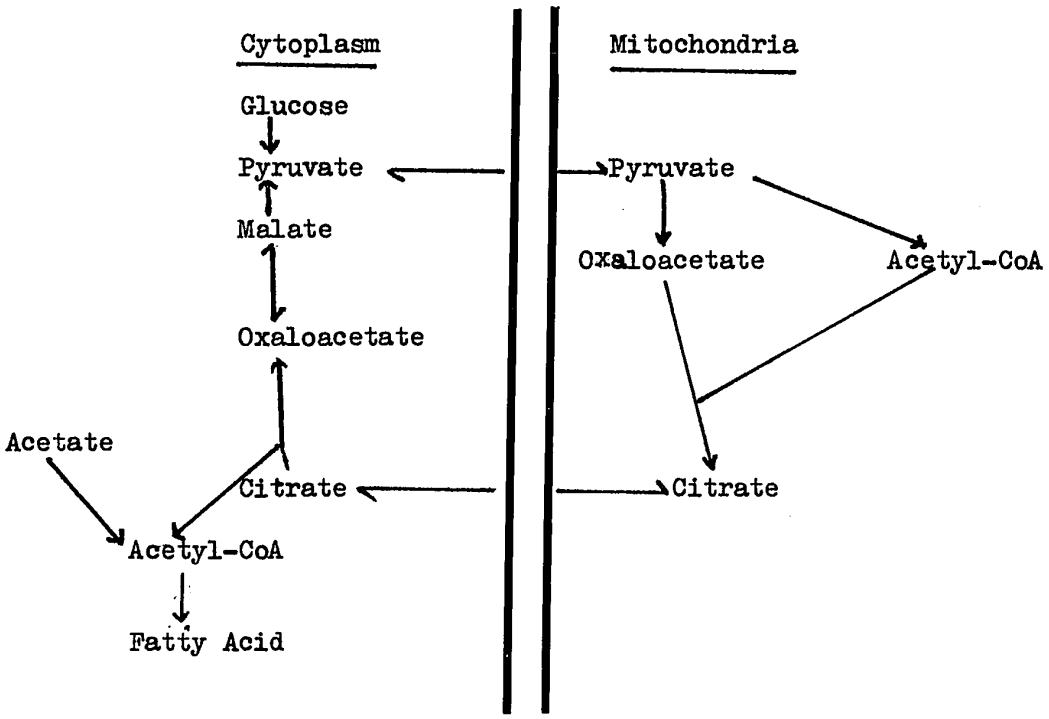



Figure 1. Intracellular location of the reaction sequences involved in the formation of fatty acid from glucose or acetate. The enzymes catalyzing the cleavage of citrate (ATP-citrate lyase) and the conversion of malate to pyruvate (malic enzyme) are absent in the ruminant animal. (Wakil et al., 1964).

(a) A mitochondrial system which may involve some enzymes of the β-oxidation pathway working in reverse, and which may affect further elongation of fatty acids already present by adding $C_2$ units (e.g., of palmitic to stearic acid). Synthesis by this mechanism is much less prominent or rapid than that by system (b) (Gibson et al., 1957).

(b) A non-mitochondrial system, located in the cytoplasm of cells, which catalyzes conversion of acetyl-CoA to higher fatty acids in the presence of adensione triphosphate (ATP), manganous ions, bicarbonate ions and nicotinamide adenine dinucleotide phosphate (NADPH) according to the following reactions:

(1) $7\ \text{acetyl-CoA} + 7HCO_3^- + 7ATP \xrightarrow[Mg^{++}]{\text{Biotin-dependent acetyl-CoA carboxylase}} 7\ \text{Malonyl-CoA} + 7ADP + 7\ Pi$

(2) $7\ \text{Malonyl-CoA} + 1\ \text{acetyl-CoA} + 14NADPH + 14H^+ \xrightarrow[\text{Synthetase}]{\text{Fatty Acid}} \text{Palmitic Acid (16:0)} + 14NADP + 7CO_2 + 8HSCoA + 6H_2O$

Bressler and Wakil (1962) stated that malonyl-CoA appears to provide the $C_2$ units for condensation (possibly in multiple units of four pre-condensed malonyl-CoA groups). All studies of the Wakil school involved enzyme systems isolated from animal sources, and the results are consonant with the observed presence of palmitic acid as the predominant saturated acid in animal fats. The proposed mechanism of biosynthesis of saturated fatty acids

is in harmony with the observation that the predominating saturated acid is accompanied by small proportions of the acids containing two more and two less carbon atoms in their molecules.

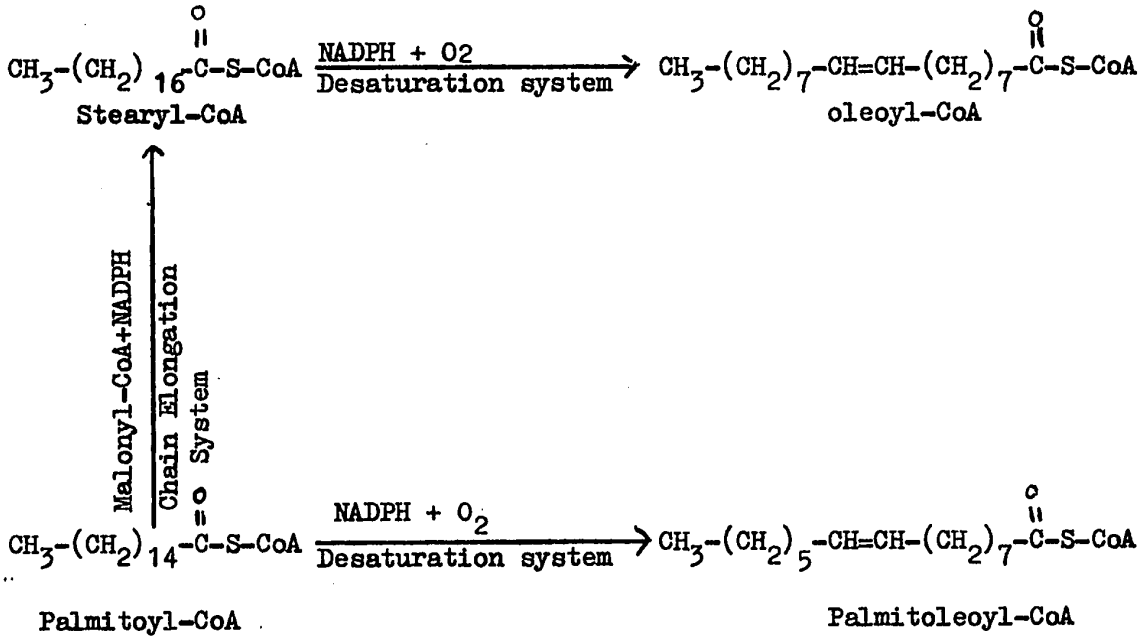
It is believed that the steps of fatty acid biosynthesis involve an acyl carrier protein, and yield palmitate or stearate (Vagilos et al., 1966). The factors which terminate synthesis at 16 or 18 carbons are unknown.

3. Formation of Unsaturated Fatty Acids

In the reserve fats of most land animals, the unsaturated acids (largely 18:1, but often with significant proportions of 18:2) amount to about 60 per cent of the total fatty acids. Handwerck and Fouarger (1959) working with rats, deduced that only a part of the monoethenoid acids was formed directly from the corresponding saturated acids. Leathes and Raper (1925) expressed the opinion forty-seven years ago that it seems improbable that the saturated acids are the intermediate and the unsaturated acids the final products of biosynthesis. These authors added that "it is almost certain that a process of "desaturation" takes place when reserve fat is called upon to yield up its energy."

Recent reports indicated that the biosynthesis of unsaturated fatty acids in the animals is produced by aerobic desaturations and elongations of fatty acids previously formed

or provided in the diet (Wakil, 1964). These reactions take place in the microsomes and the desaturating enzymes are tightly bound to the endoplasmic reticulum and require $O_2$ and NADH or NADPH, NADH being a little more effective than NADPH (Brenner, 1971). The following scheme has been proposed to describe fatty acid elongation and desaturation (Brenner, 1971):




This oxygen-dependent mechanism has been found to operate in many organisms from bacteria to mammals (Wakil, 1964). Unlike the anaerobic pathway, the $O_2$-dependent pathway is completely separate from the fatty acid synthesizing system. In this reaction, two hydrogens are removed from a saturated fatty acid (usually the coenzyme A ester of stearic or palmitic acid) to form a cis-double bond in the 9-position. Further oxygen-dependent desaturation of

pre-existing unsaturated acids yields poly-unsaturated fatty acids. The new double bonds are introduced to yield (with rare exceptions) all cis-1,4-polyenes, i.e., fatty acids in which successive double bonds are isolated by single methylene groups. It is known for many years that animals are incapable of desaturating toward the terminal methyl group. Thus, in the rat or man, oleic acid could give rise to 6,9-octadecadienoic but not to linoleic acid (Walker,1966).

## Part III. Component Glycerides

### A. Methods of Determining Glyceride Structure

The properties of fats, whether natural or synthetic, are properly related to glyceride structure as well as to fatty acid composition. To determine the constitution of a fat, it is necessary to know not only its component acids but also the way in which these are distributed within glyceride molecules. Originally, it was thought that natural fats were mixtures of simple triglycerides (Chevreul, 1823) (cited by Coleman, 1963) so that a fat containing palmitic, oleic and linoleic acid consisted of a mixture of tripalmitin, triolein, and trilinolein. Early attempts to isolate some of these simple glycerides cast doubt on this view, but very little progress was made in determining glyceride composition until the work of Hilditch and co-workers during the period 1926-1951. They devised new methods and collected sufficient information to observe a pattern

of acyl group distribution which, although no longer accepted, provided a basis for all subsequent investigations. So many methods of glyceride analysis have been proposed at one time or another that this discussion will be restricted to those which have been widely employed. These include methods based on chromatographic separations, enzymatic processes and combinations of these techniques. The subject has been reviewed recently by several workers (Coleman, 1965; Jurriens, 1968).

1. Oxidation Procedures

The first application of oxidation methods to the study of glyceride composition was made by Hilditch and Lea (1927). They described a method for determining trisaturated ($GS_3$) glycerides by oxidation of the fat in acetone solution with powdered permanganate, followed by aqueous potassium carbonate washes, which remove the acidic products from the unchanged $GS_3$. Attempts have been made by several investigators (Kartha, 1953a; Youngs, 1961) to extend the oxidation method for the determination of disaturated ($GS_2U$), diunsaturated ($GSU_2$), and triunsaturated ($GU_3$) glycerides by modifying the original oxidation method of Hilditch and Lea (1927). Kartha (1953a) used a mixture of potassium permanganate in acetone and acetic acid to oxidize the unsaturated glycerides in the fat. Youngs (1961) used a catalytic amount of potassium permanganate and an excess of periodate to carry out the oxidation. By combining this technique with that of pancreatic

lipase hydrolysis, he was able to determine positional isomers of $GS_2U$ (GSUS and GSSU) and $GSU_2$ (GUSU and GSUU).

2. Separation Procedures

The separation of glyceride mixtures into their individual components which can subsequently be identified and estimated is still an ideal not fully realized although useful progress has been made. Separation techniques applied for qualitative and quantitative analysis of natural triglyceride mixtures include the now classical crystallization method (Hilditch and Williams, 1964), counter-current distribution (Dutton and Scholfield, 1963), adsorption chromatography on columns and thin layers (Privett and Blank, 1961), liquid-liquid partition chromatography (Vereshchagin, 1965) and gas liquid chromatography (Kuksis, 1967). Only the techniques which have given the most useful results will be discussed.

(a) Thin-Layer Argentation

The ability of olefinic compounds to form $\pi$ -complexes with silver ions provides a means of separating compounds differing in unsaturation and this has been particularly effective as a thin-layer technique to separate glycerides (Mahadevan, 1967). Fully saturated glycerides travel farthest up the plate followed by glycerides of increasing unsaturation. After separation, the glycerides can be recovered quantitatively from the separated bands, and the relative weight and component acids of each fraction

determined. This is obtained by gas liquid chromatography of the fatty acid methyl esters. Each band usually contains one major glyceride (< 80%) accompanied by smaller amounts of other glycerides, and its composition can be computed from its component acids and from the known order of elution of glycerides.

This method of analysis has given useful information, particularly of oils rich in di- and tri- ethenoid acids confined almost entirely to the $C_{18}$ series (Litchfield, 1968). On its own, it gives no information concerning isomeric glycerides, little about the distribution of saturated acyl groups, and is less useful for oils containing acids of widely differing chain length. Additional information can be obtained by combining this separation with other analytical techniques such as lipolysis or gas liquid chromatography of glycerides.

(b) Liquid-Liquid Chromatography

Glycerides can be separated by a partition process. In partition chromatographic methods, a stationary liquid phase is fixed as a thin film over a finely divided, inert solid support. A second phase (an inert gas) called the mobile phase, flows over the stationary phase and this is described as gas-liquid partition chromatography (Keller and Giddings, 1967). If the mobile phase is liquid, the process is described as liquid-liquid partition chromatography. Both partition chromatographic methods have resulted in major progress in the

area of triglyceride studies by providing very efficient and relatively simple separation techniques.

Depending on whether the immobile phase is polar or essentially non-polar, these methods are divided into partition and reversed phase partition methods. Most of the liquid-liquid partition chromatographic methods applied to triglyceride quantitative separation belong to the reversed phase partition type in which the mobile phase is the more polar one. This technique is valuable for separating triglycerides on the basis of the degree of unsaturation (number of double bonds) and carbon number (Vereshchagin, 1965).

(c) Gas Liquid Chromatography

This method is valuable when applied to fats such as milkfats, coconut oil, nut oils and fish oils which contain acids of several chain lengths (Kuksis, 1967; Bezard, Bugaut and Clement, 1971). It is less useful for the many fats and oils containing only palmitic and $C_{18}$ acids where all the glycerides have 48, 50, 52, or 54 carbon atoms. Additional information can be obtained when this method is combined with other separation processes such as thin-layer chromatography.

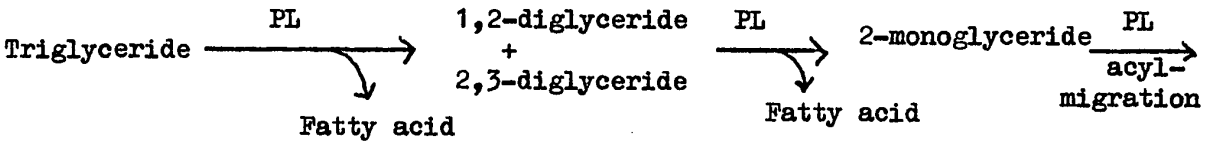
B. Enzymatic Procedures

The use of enzymes to study glyceride structure is a recent development which has given new insight into the problem.

Pig pancreatic lipase (PL) has been extensively used with interesting results and a new approach by Brockerhoff (1966) gives even more detailed information.

(a) Lipase Hydrolysis

During the period 1950-1955, it was shown (Mattson and Beck, 1956) that pig pancreatic lipase (PL) catalyses the stepwise hydrolysis of triglycerides to glycerol and fatty acids via di- and mono-glycerides by the following general equation:




glycerol + fatty acid

Acyl groups attached to the two primary hydroxyl groups are hydrolyzed much more readily than those attached to the secondary hydroxyl group which is not hydrolyzed by pancreatic lipase, i.e., the final hydrolysis step is slower than the preceeding ones and the content of 2-monoglyceride series rises to a maximum value and then declines. The apparent hydrolysis of the 2-monoglyceride by PL is the result of acyl migration to the 1 and 3 positions followed by hydrolysis.

The 2-monoglyceride fraction can be isolated chromatographically on columns or thin layers of silica gel and its

component acids converted to fatty acid methyl esters which are then identified by gas liquid chromatography. By comparison with the component esters of the triglycerides, it is possible to calculate the composition of acids at C(1) and C(3). Thus, for the first time, we have a method of distinguishing acyl groups attached to specific positions, and this has provided a new approach to the whole concept of acyl distribution.

The glyceride type distribution of the fat can be calculated from the data derived from the pancreatic lipase hydrolysis and subsequent fatty acid analyses using the method suggested by Vander Wal, (1960). It was recognized in his studies that the 2-position in the triglyceride molecule of natural fats is generally occupied by a proportion of acyl groups different from those of the 1,3-positions. Vander Wal (1960), as well as Coleman (1961), have drawn the attention to the fact that the interpretation of experimental results gives figures for isomeric glycerides. Two difficulties arise in connection with this theory, particularly as expounded by Coleman and Fulton (1961). The first experimental error arises from the sensitivity of the calculated triglyceride composition to small changes in the monoglyceride composition in fats such as cocoa butter, where the 2-positions are almost exclusively occupied by one type of acid. The second difficulty is a matter of interpretation, and is a question of whether the minor amounts (less than 0.5%) of

the various triglycerides calculated to be present are merely artifacts of the calculation.

(b) Stereospecific Analysis

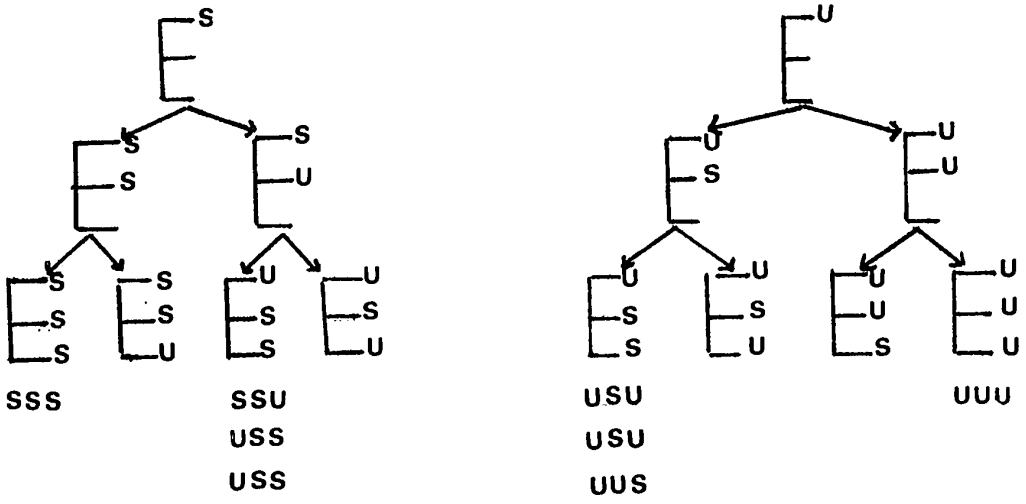
Brockerhoff (1965) has developed an enzymic procedure to determine the composition of acyl groups attached specifically at C(1), C(2) and C(3).

Triglycerides are hydrolyzed with pancreatic lipase for such a time as will give a good yield of 1, 2- and 2,3- diglycerides. The glycerides, isolated by chromatography on silica gel, are treated with phenyl dichlorophosphate to convert them to L- and D- phosphatidyl phenols, respectively. Of these, only the L- isomer is deacylated (at the 2- position) by phospholipase A and the products - lysophosphitide, unchanged D- phosphatidyl phenol, and fatty acids - are separated by thin-layer chromatography. Component acids of the several products are determined by gas liquid chromatography of their methyl esters and from these, it is possible to compute the acids present at each position and check the general accuracy of the analytical procedures.

2. Glyceride Structure of Swine Depot Fat

Studies have demonstrated that in animal fats, the 2- position of the glyceride molecules are occupied by unsaturated acyl groups in higher proportions than saturated acyl groups, and the 1,3- positions in higher proportions by saturated acyl groups

than by unsaturated acyl groups (Brockerhoff et al., 1966). The one known fat which does not conform to this pattern is pork fat (Mattson, Volpenhein and Lutton, 1964). In this fat, there is a predominance of the saturated acyl groups in the 2- position and unsaturated acyl groups in the 1,3- positions. The proposed distribution scheme for swine depot fat was described by Coleman (1963) and can be summarized as follows:




Mattson et al. (1964) found that the unusual distribution pattern in the triglycerides of pig adipose tissue is also found in the wild boar and peccary but not the hippotamus, these authors discussed the evolutionary aspects of this distribution.

Anatomical variation in glyceride type distribution has not been studied extensively. Savary, Flanzy and Densuelle (1957) showed that the preferential attachment of saturated acyl groups at the 2- position of glyceride molecules of pork fat is not

affected by anatomical location, even though the fatty acid composition does differ. A study by Chacko and Perkins (1965) on the glyceride type distribution of pork fat samples showed definite variations in glyceride types according to the area from which the fat was obtained. Backfat contained a higher proportion of SSU type than fats taken from other positions, perinephric fat contained considerably less UUU than did fat from other sample sites. Hanahan (1960) suggested that "there is the distinct possibility, particularly in the animal glycerides, that the distribution may vary not from one species to another but from one tissue to another."

3. Alterations in Glyceride Structure

The structure of endogenous animal fat is a result of the specificity of the acylating enzymes and of the nature and relative proportions of available fatty acids. The possible influence on depot fat structure of exogenous (dietary) fat, of the dynamic state of the glycerides, and of the homostatic mechanism were discussed by Reiser and Reddy (1959). These workers fed a male pig for three months a diet containing 1 per cent fat and 0.2 per cent dienoic acid. The backfat was fractionated into glyceride types by crystallization methods, and the fatty acid composition of each was determined by the spectrophotometric procedure. They found that the relative amounts of the glyceride types perfectly fitted the hypothesis that there is

an oleic acid in the 1- position of each glyceride molecule, that the residual oleic acids were in the 3- position, that the linoleic acid was in the 2- position of the resultant dioleins, and that the saturated acids complete the structure. Mattson and Lutton (1958) have reported that 47 per cent of the 2-monoglyceride acids and 59 per cent of the total acids were dienoic when they fed a male pig a diet containing 25 per cent safflower seed oil (highly unsaturated oil). These two findings emphasize the effect of the relative amounts of fatty acids in the diet on the order of the arrangement of fatty acids in the pig triglyceride.

Privett, Blank and Verdino (1965) studied the effect of dietary fat on the triglyceride structure in the rat. They showed that the triglyceride composition of the tissues (adipose tissue, liver and kidney) had no direct relationship to that of the dietary fats and oils (corn oil, lard and menhaden oil). Quantitative differences in the triglyceride composition of the tissues were produced by different dietary fats as related to their fatty acid composition. The structural analysis of the triglycerides of all tissues indicated that a competitive interrelationship existed for the 2- position, increasing in order with palmitic, oleic and linoleic acids. These results were supported by Flanzy, Rerat and Francois (1965) who found that the glyceride structure of the pig depot fat was affected

only by the fatty acid composition of the dietary fat and not by the structure of the corresponding glycerides. Mattson and Volpenhein (1962) concluded that the distribution of fatty acids in the synthesis of triglycerides in the intestinal mucosa, which involves the acylation of absorbed monoglycerides or the phosphatidic acid pathway, is believed to follow closely a random pattern. However, the studies by Privett et al. (1965) indicated that there is a preferential incorporation of fatty acids into specific positions of the triglycerides.

Although complete retention of dietary glyceride structure has not been demonstrated in mammals according to the foregoing review, partial retention of fatty acids in the 2-position has been demonstrated in lobster, cod and trout by Brockerhoff, Hoyle and Ronald (1964).

Moore et al. (1968) reported that small changes in the fatty acid composition of swine adipose tissue can have disproportionate effects on the chemical structure and physical properties of the component triglycerides. In the course of several nutritional experiments (Moore et al., 1968; Christie and Moore, 1970), in which the effect of supplemental 250 ppm copper has been studied, they found a rather unusual distribution of fatty acids between the 1-, 2- and 3- positions in porcine triglycerides as a result of the inclusion of copper in the

diet. Detailed analysis of the glyceride structure of the backfat from control and copper-fed pigs revealed significant ($P<0.01$) alterations in the triglyceride species ($S_3$, $S_2U$, $SU_2$ and $U_3$) which significantly affected the physical properties of the backfat.

4. The Synthesis of Glycerides

Triglycerides are known to be synthesized in mammalian tissue primarily from $\alpha$ -glycerophosphate via phosphatidic acid and their diglycerides (Weiss, Kennedy and Kiyasu, 1960; Kennedy, 1961). In vitro studies with rat liver slices (Hill, Husbands and Lands, 1968) and in vivo studies with rats (Elovson, Akesson and Arridson, 1969) have shown that there is sufficient positional fatty specificity in the biosynthesis of phosphatidic acid to account for the known variations in the distribution of fatty acids in natural triglycerides and glycerophosphatides. Diglycerides with a highly specific fatty acid distribution, may also be formed by reversal of phosphatidyl choline and phosphatidyl ethanolamine synthesis (Lands and Hart, 1964), and incorporated into triglycerides. It is not known how important this pathway is quantitatively but a mechanism of this type could be invoked to explain why the 2- position of pig liver triglycerides contains mainly unsaturated fatty acids, whereas, in adipose tissue, where the $\alpha$ -glycerophosphate pathway must predominate, the 2- position of the triglycerides contains

largely palmitic acid. The phenomenon could also be explained if the acyl transferase enzyme involved in phosphatidic acid synthesis in each organ had quite different fatty acid specificities but the solution to the problem must await further study.

Weiss and Kennedy (1956) showed that neutral glycerides are formed by a system similar to that giving rise to phospholipids, both proceeding via glycerophosphate and phosphatidic acid, by the following reaction steps:

1- glycerophosphate + 2 palmityl-SCoA $\longrightarrow$ phosphatidic acid

2- phosphatidic acid $\longrightarrow$ diglyceride + phosphate

3- diglyceride + palmityl-SCoA $\longrightarrow$ triglyceride

4- diglyceride + phosphoryl-choline $\longrightarrow$ choline-phospho-lipid

Part IV. Dietary Tocopherol and Tissue Lipid Stability

It has been recognized for some time that diets containing significant quantities of polyunsaturated fatty acids (PUFA) but not containing appropriate levels of $\alpha$ -tocopherol can promote destructive processes in various animal tissues (Machlin, 1963). A number of investigators (Gordon and Nitowsky, 1960; Kummerow, 1964; Roels, 1967) logically proposed that autoxidation of unsaturated fatty acids in tissues of animals deficient in tocopherol or other suitable antioxidants produce toxic substances and cause damage to lipoprotein structures which initiate the pathological changes seen.

Studies with in vitro systems by McCay et al. (1971) indicated that peroxidation of lipid does occur, however, it appeared not to be a random autocatalytic process, but one promoted by the highly localized production of free radicals formed as part of the mechanism of action of certain enzymes. When there was adequate tocopherol in the tissues to react with them, such radicals apparently produced very little alteration in the lipids of structures around the site of their origin.

The pioneer studies of Evans and Burr, (1927) in which the rat bioassay test was applied to tissues from rats, cattle, pigs and sheep indicated that vitamin E is widely distributed in the animal body but is never present in very great concentration in any one organ tissue. Musculature, body fat and liver, in the order named, contained appreciably more vitamin E than other tissues. Mason (1942) confirmed the conclusions of Evans and Burr (1927) relative to the wide distribution of vitamin E in the body of the rat but indicated that musculature and body fat, which the latter workers considered to possess the greatest concentration of the vitamin, actually contained an amount similar to or less than that present in other tissues and organs, whether previous intake of the vitamin was low or high.

A number of investigators have tried to increase the tocopherol content of animal tissues by increasing the level of

tocopherol ingestion. Lundberg et al. (1944) and Barnes et al. (1943) demonstrated clearly the ability of the rat to store, in its abdominal fats, excess tocopherol from a diet which had been supplemented with alpha-tocopherol. To improve the keeping qualities of meat to be used for human consumption, others have tried by the same means to increase the tocopherol content of the meat. In most of these investigations, the resistance of the fat to rancidification was used as a measure of its tocopherol content. In the experiment of Dammers, Stolk and Wieringen (1958) a relatively greater increase in the vitamin E level in the fat was caused by adding vitamin E to a ration poor in the vitamin.

Watts, Cunha and Major (1946) working with swine, found that tocopherol supplementation of a purified ration increased the stability of porcine fats only slightly, while no increase was obtained in the case of a ration composed of naturally occurring feed stuffs. In contrast with these latter findings, Carpenter and Lundberg (1949) reported that the induction period,[1] as measured by an oxygen absorption method at 100 C, of the fats from pigs that did not receive supplemental tocopherols during the post-weaning period was much shorter than the induction period of fats from pigs fed supplementary tocopherols. Major and Watts (1947) found that when high levels of tocopherols were fed to or injected into rabbits on a purified

[1] Induction period, a period of time elapses during which there is little oxygen uptake by a fat, followed by oxygen uptake that rapidly accelerates in rate when it once has started.

diet, protection from the development of rancidity in the meat and fat was shown. On a natural ration, no protection from the development of rancidity was shown when the animals were injected with tocopherols or tocopherol phosphate three weeks before slaughter.

Criddle and Morgan (1964) used both chemical and bio-assay methods to show that the tocopherol content of turkey tissues increased significantly as a result of tocopherol feeding. They found that the tocopherol deposits, which were widely distributed throughout the tissues but most concentrated in the liver and heart, represented only a small fraction of the vitamin which had been fed.

It has been shown by several workers that the addition of tocopherol to the pig ration counteracts the unfavourable effects of the unsaturated fatty acids on fat stability (Carpenter and Lundberg, 1949; Hove and Seibold, 1955; Dammers et al., 1958; Leat, 1961). This effect of tocopherol was also found by Hvidsten and Astrup (1963).

## GENERAL EXPERIMENTAL

### A. Pigs and Their Management

Pigs of mixed breeding (out of crossbred dams and Poland China sires) from the Macdonald College Livestock Farm were used in these studies. Piglets were identified by ear notch at birth and "black" teeth were removed. For prevention of anemia, a 1 ml intramuscular injection of Pigdex-100 (an iron dextran compound containing 100 mg Fe/ml) was given at three days of age. Male pigs were castrated at six days of age. The animals were weaned at three weeks of age and fed with a commercial ration until they were placed on feeding experiments.

The pigs were allocated to treatments and a uniform distribution with respect to weight and sex was made in so far as this was possible. Pens (with concrete floor) represented treatment units and pigs within a pen received the same diet (treatment). Feed and water were available at all times and no copper tubing or wire were used in the pens.

### B. Source and Handling of Experimental Samples

#### 1. Feed Samples

A composite sample was taken from each experimental diet for determination of dry matter, gross energy, crude protein and crude fat.

2. Liver Samples

At slaughter, a sample of liver tissue adjacent to the bile duct (Barber et al., 1962, established that the copper content of such a sample approximated closely to the mean copper content of the whole liver) was obtained and stored under nitrogen at -20 C for future analysis.

3. Fat Samples

Immediately after slaughter, a strip of the backfat, approximately 4 cm wide and 6 cm long was taken from the right shoulder of the carcass, adjacent to the midline. These were stored in plastic bags under nitrogen at -20 C for future analysis.

C. Preparation of Experimental Samples for Analysis

1. Feed and Liver Samples

An adaptation of the digestion procedure outlined by Hoffman (1970) for copper analysis in feed and liver samples was employed. A 1 gram sample of feed or liver was placed in a beaker and 10 ml of concentrated nitric acid added. The beaker was covered with a watch glass and the contents allowed to digest on a hot plate until the digestion mixture appeared homogenous. The cover glass was removed and rinsed with distilled water into the beaker containing the digestion mixture. The contents were left to simmer for 20 minutes and then transferred with washing

into a 50 ml graduated centrifuge tube. To avoid contamination with copper, glassware and syringes were soaked in 10 per cent nitric acid and well rinsed with deionized water.

2. Fat Samples

(a) Extraction of Lipids

Lipids were extracted with 2:1 (v/v) chloroform-methanol according to the method described by Folch et al. (1957). The extract was washed with distilled water, dried over sodium sulfate anhydrous, filtered and evaporated to dryness. The residue was dissolved in chloroform and filtered on sintered glass.

(b) Transmethylation

Transmethylation was affected by dissolving 50 mg lipid in one ml of benzene in a screwcap tube. Five ml of 10 per cent borontrifluoride in methanol was added and the mixture heated in a water bath at 70 C for 90 minutes (Morrison and Smith, 1964). Water (10 ml) was added to the mixture and lipid material extracted with 20 ml of petroleum ether. The methyl esters were separated from dimethyl acetals and sterol derivatives by thin-layer chromatography on silica gel G with benzene as the developing solvent.

(c) Lipase Hydrolysis

Lipid samples were subjected to pancreatic lipase hydrolysis to facilitate the determination of fatty acid distribution in the glyceride molecules. The digestion mixture contained 5 g of triglycerides, 20 mg of bile salt, 700 mg of pancreatic lipase (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.), 2 ml of calcium chloride and sufficient water to bring the volume to 60 ml. Preliminary experiments indicated that these were the optimum conditions for pork fat hydrolysis and are similar to the conditions described by Desnuelle et al. (1961). The mixture was continuously agitated with a high speed magnetic stirrer and the pH maintained between 8.0-8.5 by addition of 0.1N NaOH during hydrolysis with the aid of an automatic titrator (Metrohm Improved Model 3D, Switzerland). Temperature was thermostatically controlled at 37 $\pm$ 0.1 C by circulation of heated water through the jacketed reaction vessel. Hydrolysis was allowed to proceed until 15 per cent of the fat had been hydrolyzed, as calculated from the saponification value (determined on representative samples following the method outlined by Mehlenbacher, 1960) of the samples.

Enzyme action was stopped by addition of 10 ml of ethanol and heating to 70 C for 10 minutes. The reaction mixture was evaporated to dryness and lipids were extracted with petroleum ether-diethyl ether (1:1 v/v) and this solution dried with

anhydrous sodium sulfate. The solution was evaporated to dryness under nitrogen and the lipids dissolved in 1 ml of hexane.

(d) Column Chromatography

Florisil (60-100 mesh) was used as the adsorbent for separation of lipid classes in the hexane extract. The lipid material was added to 12 g of florisil packed in a glass column, 17 mm I.D. Fractions were collected by using stepwise elution with the following solvents: hexane-ether (85:15 v/v), 150 ml; hexane-ether (50:50 v/v), 150 ml; ether-methanol (98:2 v/v), 15 ml and ether-glacial acetic acid (96:4 v/v), 150 ml. The major lipid classes in the four fractions eluted by these solvents were tentatively identified by thin-layer chromatography as shown in Figure 2: triglycerides; diglycerides; monoglycerides and free fatty acids.

D. Analysis of Experimental Samples

1. Feed and Liver Samples

Copper analysis of feed and liver samples was performed with the aid of an Atomic Absorption Spectrophotometer (Unicam Sp-90). The digestion mixture was aspirated directly into the atomic absorption flame and the amount of copper in each sample was calculated by comparison with a standard curve.

The nitrogen content of feed was determined by the Kjeldahl method of analysis (AOAC, 1965) and reported as crude protein calculated by multiplying % nitrogen x 6.25.

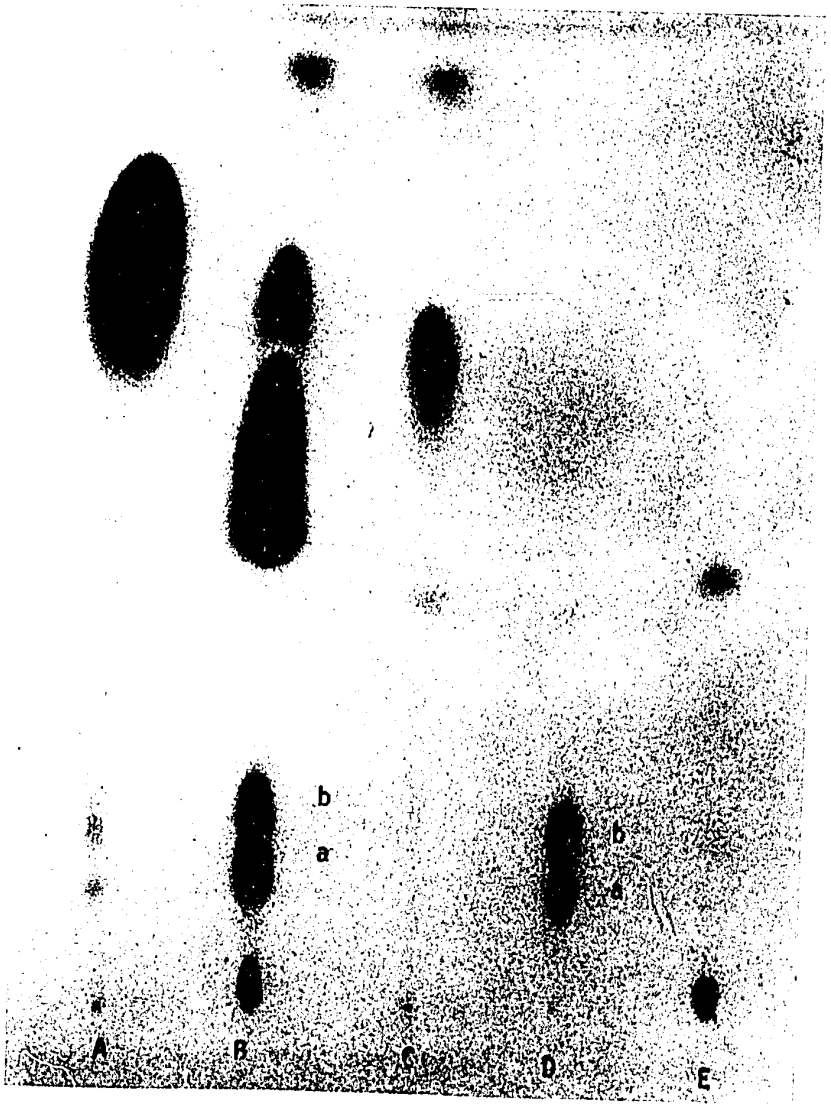



Figure 2. Thin-layer chromatogram of swine back-fat before and after hydrolysis.
Sample A - Triglyceride before hydrolysis; B - Triglyceride after hydrolysis; C - Triglyceride fraction of hydrolyzed fat; D - Diglyceride fraction. a) 1,2 diglyceride and b) 1,3 diglycerides; E - Monoglyceride fraction.
Condition of analysis: As described in experimental procedure.

Gross energy of feed samples was determined using a Parr Oxygen Bomb Colorimeter equipped with an electronic recorder.

Crude fat was determined using the direct method 22.023 (AOAC, 1965). The extraction period was 4 hr at condensation rate of 5-6 drops per second.

2. Fat Samples

(a) Melting Point

The melting point determination was carried out by both the capillary tube method, 26.012 (AOAC, 1965) and the Wiley Method, 26.011 (AOAC, 1965). The mean of triplicate determinations on each sample was taken.

(b) Silicic Acid Column

Extracted lipids were fractionated into their phospholipid and neutral lipid fractions on a silicic acid column by the method of Horstein, Crowe and Heimberg (1961). In the present study, phospholipids were collected as a single fraction.

(c) Characterization of Lipid Materials by Thin-Layer Chromatography

Separation of the lipid clesses by thin-layer chromatography on silica gel G plates (Privett, Blank and Lundberg, 1961) was used to check the purity of the fractions eluted from the florisil column. The plates (20 x 20 cm) were prepared by spreading a well-stirred mixture of 7 g acetone washed silica gel G and 17 ml distilled water with spreader adjusted to give a layer thickness of 0.25 mm.

The plates, after brief air-drying, were activated by heating in an oven at 110 C for 2 hr and cooled in a desiccator. The samples in 1:10 dilution in chloroform were applied dropwise with a microsyringe in a series of spots 1 cm apart. The developing solvent employed was petroleum ether-diethyl ether (60:40) to which 1.6 per cent formic acid was added. The developing solvent was allowed to travel to within 3.0 cm of the top of the plate; this took approximately 40 minutes. The spots were made visible with iodine vapour as described by Sims and Larose (1962).

(d) Gas-Liquid Chromatography

Analysis of the methyl esters was carried out using a Perkin-Elmer 881 Gas Chromatograph equipped with dual stainless steel columns of 2 mm I.D., and dual hydrogen flame ionization detectors. The column packings employed were 80-100 mesh Gas-Chromasorb P coated with 15 per cent, by weight, of diethylene glycol succinate (DEGS) in columns 152 cm in length and operated at 190 C; and 80-100 mesh Gas-Chromasorb W with 15 per cent, by weight, of SE-30 in columns 61 cm long and operated at 200 C. The carrier gas flow rate was 30 ml per minute. The injection temperature was 270 C, detector 240 C and the flow rate of hydrogen gas, 50 ml per minute.

(e) Identification of Acids

The fatty acids were identified by comparing their retention times with those obtained by chromatography of appropriate standard (14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0 and 20:4) methyl esters and by determination of equivalent chain length as described by Hofstetter, Sen and Holman (1965).

The results obtained with the polar DEGS and non-polar SE-30 columns were plotted (log of retention time vs. carbon number and by log of retention time vs. number of double bonds) to determine if the familiar straight line relationship (Magidman et al. 1962) existed for both the saturated and unsaturated methyl esters (Figure 3). The weight per cent of the various methylated fatty acids was determined from the peak areas (peak area = height x width at one-half the height).

(f) Calculation of Glyceride Composition

The weight per cent of a particular fatty acid esterfied at the 2- position was calculated as described by Mattson and Volpenhein (1961 ) (per cent in 2- position = per cent in monoglyceride/3 x per cent in triglyceride). The theory of Vander Wal (1960) was employed as a means of calculating glyceride sturcture of the fat. This procedure is useful in calculating not only the $S_3$, $S_2U$, $SU_2$ and $U_3$ but also the isomeric forms of $S_2U$, which are SSU and SUS, and those of $SU_2$, which are UUS and USU. The method is based on the following two

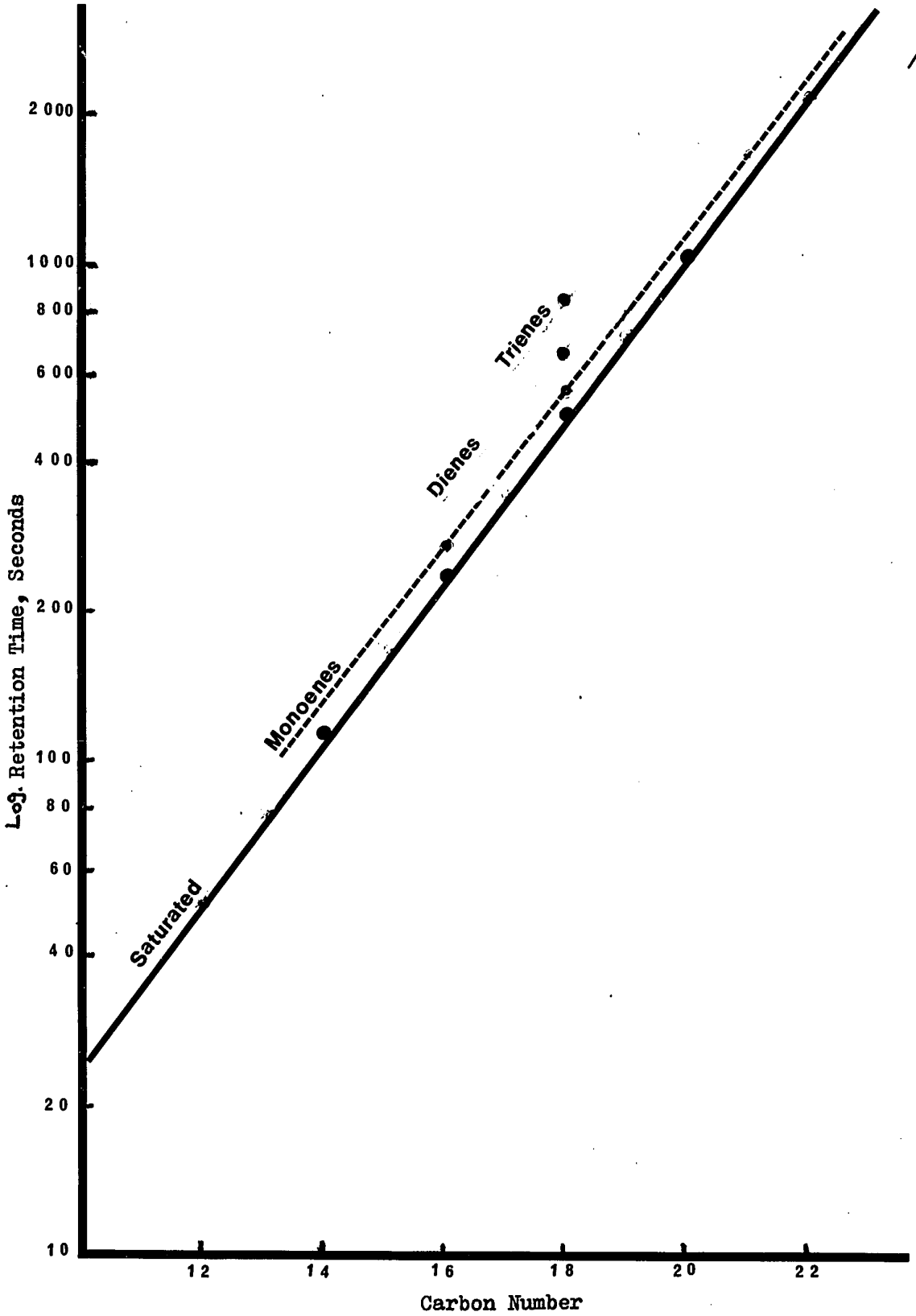



Figure 3. Linear log plot of Carbon number vs. retention time.

postulates: whatever proportions of S and U occupy the collective 1- positions, the collective 2- positions and the collective 3- positions in the triglycerides of a natural fat, they are dispersed therein at random; whatever proportions of S and U occupy the collective 1- positions, equal proportions occupy the collective 3- positions.

E. Identification of Linoleic Acid

(a) Fractionation of Methyl Esters According to Unsaturation

Thin-layer plates (20 cm square and 0.25 mm thick) impregnated with silver nitrate, prepared by using a slurry composed of 25 g of silica gel G and 50 ml of 11 per cent aqueous $AgNO_3$ solution were used for analytical separation of methyl ester fatty acids according to their degree of unsaturation. The plates were dried at room temperature for 10 minutes and then activated at 110 C for 2 hr. Between 20 and 25 mg of sample was applied in a band on the plates and chromatograms were developed in the dark at 10 C in tanks with a mixture of toluene-diethylether (94:6 v/v). After the plates had been sprayed with Rhodalamine 6G, the bands were visualized under U.V. light and scraped off using a razor blade into small vials. Three bands corresponding to the saturated, monounsaturated and diunsaturated fatty acid methyl esters were identified by gas liquid chromatography. The diene (diunsaturated) fraction was

rechromatographed (TLC) to remove traces of materials with different degrees of unsaturation than the major fraction.

(b) Oxidative Cleavage of the Double Bond in the Diene Fraction

The oxidation procedure used was essentially that described by Von Rudloff (1956). The oxidant solution was 21 g of sodium periodate plus 25 ml of 0.1 M potassium permanganate made up to one liter with water; 25 mg of the diene fraction were dissolved in 2.5 ml of pyridine and added to a mixture of 6.7 ml of the oxidant solution, 8.3 mg of potassium carbonate, and 7.5 ml of pyridine. The reaction mixture was refluxed for 1 hr. After refluxing, the excess oxidant was destroyed by the addition, with shaking, of sodium bicarbonate 0.1 g followed by 1.7 ml of 0.1 N sodium arsenite solution. The pyridine was removed by evaporation and the resulting aqueous solution was acidified by the addition of 0.35 ml of 10 per cent sulfuric acid. Three extractions of the acidified aqueous phase with ethyl ether served to extract the oxidized lipids.

The methyl esters of the degradation products were simultaneously analyzed by temperature programmed gas chromatography (over the range 120-200 C). The sample was first injected onto the column at 120 C and the column was operated isothermally for 10 minutes to obtain separation of the short chain monocarboxylic esters. Column temperature was then adjusted to 200 C

to elute dicarboxylic esters. Standard methyl linoleic acid was also oxidized by the same method for comparative purposes.

F. Measurements of Fat Oxidation

Techniques for measuring storage life of a fat or fatty food are based on subjecting the product to normal or accelerated storage conditions and measuring the progress of oxidation. The exact moment of rancidity is usually arbitrary since it must be based, in the final analysis, on human judgment as to when a product no longer becomes marketable or acceptable for a given use.

The following summarizes the methods employed in these studies:

(1) The oxygen absorption method:- Oxidative rancidity at 60 C was measured using a Barcroft-Warburg constant volume manometric apparatus. This apparatus was described by Johnston and Frey (1941). The Barcroft-Warburg apparatus is a closed system in which a sample is held, usually under oxygen, and oxidation is followed manometrically by measurement of oxygen uptake. Readings of the manometer were made at intervals of 1,3,6,9,12,18 and 24 hr and results were expressed in microliter ($\mu$l) of oxygen uptake per mg of the fat per hr.

(2) The active oxygen method (AOM):- The stability test described by Swift, Rose and Jamieson (1941) based on

peroxide content of the fat whose oxidation is accelerated by heat and aeration was also employed. A liquid sample in an aeration tube was inserted in an oil bath. A carefully controlled flow of dry oxygen was bubbled through the sample while the entire bath and sample were held at a temperature of 60 C. Fat samples were withdrawn periodically and analyzed for peroxide content by reactions using a starch-iodide indicator (AOCS, col-8, 1953).

(3) The 2- thiobarbituric acid method (TBA):- Various workers have used this chemical test based on the reaction of 2-thiobarbituric acid with the oxidation products of fats and oils to form red colour (Bernheim, Bernheim and Wilbur, 1947; Tarladgis, Pearson and Dugan, 1964). The formation of a red colour which can be measured spectrophotometrically indicates the degree of oxidation in a given sample.

In this study, one g of fat was emulsified with 0.2 g of Tween 20 in 20 ml of distilled water. Two ml of the emulsion was pipetted into a beaker and 3 ml of a 10 per cent ammonium sulfate solution in distilled water was added. The procedure was completed according to Tarladgis et al. (1964) and the mixture was set aside for 15 hr at 37 C and absorbancy at 533 mμ was read thereafter using a Spectronic 20 (Bausch and Lomb Ltd.).

A standard curve was prepared from 1, 1, 3, 3- tetra-ethoxypropane (TEP) according to the method proposed by Sinnhuber and Yu (1958) and is shown in Figure 4.

It is important to indicate that all glassware used in the three methods was cleaned as follows to prevent contamination:

a) degreasing with carbontetrachloride;

b) soaking in boiling hot alkaline detergent solution and rinsing;

c) soaking in chromic-sulfuric acid cleaning solution overnight and;

d) rinsing three times with tap water, twice with distilled water and drying in a vacuum oven.

G. Statistical Analysis

Data were analyzed by the analysis of variance with the aid of an IBM Model 1620 Computer, Macdonald College (1971). Differences between three or more treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1960).

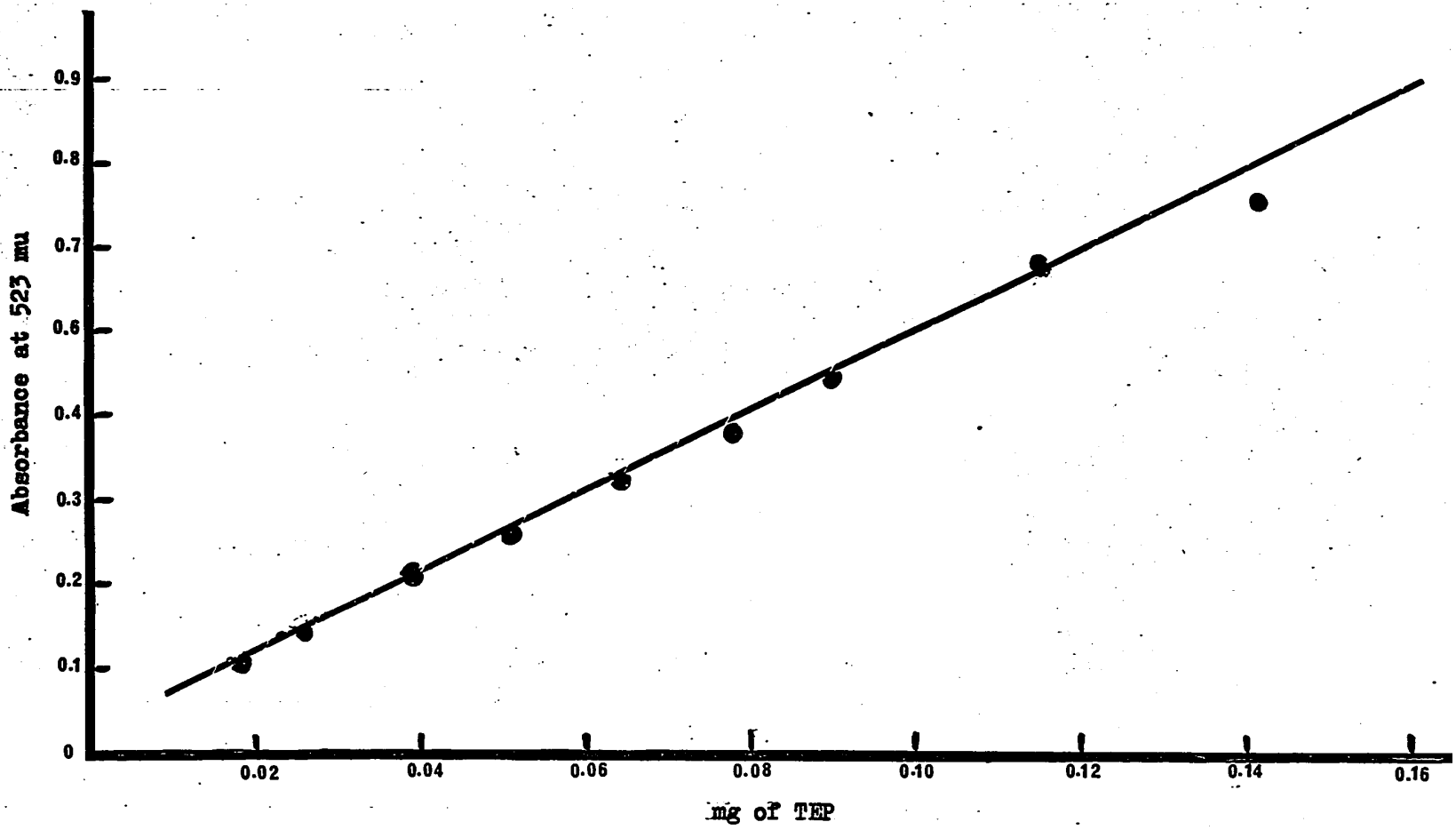



Figure 4. Standard curve for the determination of TBA reactive substances.

## EXPERIMENT I.

### A. Introduction

Swine, like many other mammals and certain amphibians, have some ability to alter the fatty acid composition of their depot fat. The composition and consistency of pork fat vary greatly according to the diet of the pig (Swern, 1964). It is common knowledge that pigs fed a fat rich in unsaturated fatty acids develop a soft fat, whereas, starch-fed pigs produce a hard fat (Hilditch, 1964).

Recent evidence has indicated that the level of copper in the diet affects the proportions of saturated and unsaturated fatty acids in adipose tissue of the growing-finishing pig. Elliot and Bowland (1968;1970) have indicated that feeding of diets high in copper (250 ppm) compared with diets containing the metabolic requirement (6-10 ppm) resulted in the deposition of a soft fat as indicated by a significant ($P < 0.01$) reduction in melting point. Soft fat resulting from copper ingestion was attributed to an increase in the monoenoic acids (16:1 and 18:1) and the dienoic acid (18:2) and a corresponding decrease in the saturated fatty acids (16:0 and 18:0). Their results also indicated that the effect of copper on fat composition was greater in diets containing animal protein supplements (fishmeal and meat meal) than in those containing exclusively plant protein supplements

(soybean and rapeseed meal). Moore et al. (1968) and Christie and Moore (1970) on the other hand, have emphasized that the significant changes in fat consistency could be related to alterations in the concentration of various triglyceride species present in the fat rather than to changes in the fatty acid composition per se. It has been well established that the physical properties of animal fats are greatly influenced by the component fatty acids and the way in which these are positioned in the glyceride molecule (Bailey, 1957).

The objective of Experiment I was to investigate: (a) the effect of feeding a diet, containing 250 ppm of supplemental copper, continuously throughout the growing and finishing periods on the melting point, fatty acid composition and triglyceride structure of the depot fat and (b) the effect of removal of supplemental copper from the diet after various periods of supplementation on the above mentioned characteristics in the backfat of the pig at market weight (92 kg).

B. Experimental

Animals

Forty-four gilts averaging 13.5 kg bodyweight were alloted to 11 treatments, 4 pigs in each treatment, as indicated in Table 1. One level (250 ppm) of supplemental copper was added to the basal diet and fed either throughout the growing period from

Table 1. Allotment of Pigs in Experiment I

| Group[1] | Copper[2] | | | Copper Removed at kg | Weight at Slaughter (kg) |
|---|---|---|---|---|---|
| | 0 | + | ± | | |
| 1 | x | | | | |
| 2 | | x | | | 23 |
| 3 | x | | | | |
| 4 | | x | | | 46 |
| 5 | x | | | | |
| 6 | | x | | | 69 |
| 7 | x | | | | |
| 8 | | x | | | 92 |
| 9 | | | x | 23 | |
| 10 | | | x | 46 | |
| 11 | | | x | 69 | 92 |

[1] 4 animals in each group

[2] Supplemental copper (250 ppm)

13.5 kg to slaughter at 23, 46, 69 or 92 kg liveweight or up to 23, 46 or 69 kg liveweight followed by the basal diet until slaughter at 92 kg. The basal diet used in this experiment had the composition indicated in Table 2. Feed and water were available to the pigs at all times.

The pigs were weighed weekly throughout the experiment and were slaughtered individually when their liveweight at the weekly weighing reached that set down in the experimental design.

Liver and backfat samples were collected, handled and analyzed as previously described under General Experimental B-1,2; C-1,2 (a-d); D-1,2 (a-f).

C. Results and Discussion

(a) Average Daily Gain and Liver Copper Concentration

No significant differences were observed among the treatment groups for ADG when 250 ppm of supplemental dietary copper was fed to pigs continuously throughout the growing-finishing period (Table 3). In the early stages of growth, the improvements in ADG associated with supplemental copper were +2.6, +1.8 and +1.6 per cent for pigs slaughtered at 23, 46 and 69 kg liveweight, respectively. It has been suggested that the action of supplementary copper in improving daily gain could be a result of its effect on the gut flora (Hawbaker et al., 1961). This mechanism presumably would be operative only to the early

Table 2. Composition of Diets for Experiments I and II

| Ingredients | Basal % | Basal plus Copper % |
|---|---|---|
| Barley | 32.39 | 32.39 |
| Wheat | 31.90 | 31.90 |
| Feather meal | 0.75 | 0.75 |
| Meat and bone meal | 2.49 | 2.49 |
| Wheat shorts | 29.91 | 29.91 |
| Dehydrated alfalfa | 0.50 | 0.50 |
| Limestone | 1.25 | 1.25 |
| Salt | 0.50 | 0.50 |
| Trace mineral mix[1] | 0.05 | 0.05 |
| Vitamin premix[2] | 0.12 | 0.12 |
| Bacitracin 25 | 0.02 | 0.02 |
| Arsanilic acid 20% | 0.05 | 0.05 |
| Methionine | 0.06 | 0.06 |
| Copper sulfate [3,4] | - | + |
| Composition by analysis | | |
| Crude protein % | 15.00 | 15.00 |
| Gross energy, Kcal/g | 4.50 | 4.50 |
| Fat % | 2.50 | 2.50 |
| Copper ppm | 12.00 | 247.90 |

[1] Trace mineral mix to supply: 60 ppm Mn; 60 ppm Zn; 25 ppm Fe; 10 ppm Cu; 1.25 ppm I and 1 ppm Co.

[2] From the vitamin premix: 1763.7 I.U./kg vitamin A; 268.9 I.U./kg vitamin D; 2.2 I.U./kg Riboflavin; 11.0 mg/kg Niacin; 3.5 mg/kg calcium pantotnenate and 11.0 mcg/kg vitamin $B_{12}$.

[3] To supply: 0.10% $CuSO_4.5H_2O$ in Experiment I.

[4] To supply: 0.10, 0.09, 0.08, 0.07, 0.06 and 0.05% $CuSO_4.5H_2O$ in Experiment II.

Table 3. Effect of Continuous Addition and Intermittent Removal of 250 ppm Supplemental Copper to a Practical Swine Diet on ADG and Liver Copper Content.

| Group[1] | Supplemental Copper | | | Weight at Time of Cu Removal | Weight at Slaughter | ADG(g) | Liver Cu (ppm) |
|---|---|---|---|---|---|---|---|
| | 0 | + | ± | | | | |
| 1 | x | | | | 23 kg | 340 | 18.8 |
| 2 | | x | | | | 349 | 268.1** |
| 3 | x | | | | | 476 | 49.1 |
| 4 | | x | | | 46 kg | 485 | 141.0** |
| 5 | x | | | | | 535 | 39.9 |
| 6 | | x | | | 69 kg | 544 | 249.9** |
| 7 | x | | | | | 599 | 51.8 |
| 8 | | x | | | 92 kg | 558 | 452.2** |
| 9 | | | x | 23 kg | | 549 | 51.4 |
| 10 | | | x | 46 kg | 92 kg | 581 | 48.4 |
| 11 | | | x | 69 kg | | 535 | 281.1** |

[1] 4 animals in each group

0 basal, + basal + copper continuous, ± basal + copper intermittent

** Significantly ($P < 0.01$) greater than control group

part of the growing period according to the data reported herein. When pigs continued to receive 250 ppm supplemental copper to market weight (92 kg liveweight), a/non-significant depression in ADG of -6.8 per cent was observed in comparison with control animals. Several research workers have reported a similar trend (Bowland, 1954; Castell and Bowland, 1968a; Hawbaker et al., 1961; Wallace et al., 1966). However, the inclusion of 250 ppm of supplemental copper in the swine diet has, in the majority of experiments, resulted in significant improvements in overall ADG (Braude, 1965).

Removal of copper from the diet at 23, 46 or 69 kg liveweight followed by feeding the basal diet until slaughter at 92 kg liveweight (Table 3), resulted in -8.3, -3.0 and -10.0 per cent depression in ADG respectively in comparison with control pigs receiving the control diet to 92 kg liveweight. This observation is in agreement with Teague and Grifo (1966) who reported that elimination of supplemental copper from the diet during the period from 46 kg liveweight to slaughter resulted in a slower rate of growth and poorer efficiency of feed utilization in comparison with control animals. The reason for the observed decrease in ADG resulting from the removal of supplemental copper from the diet is not clear. Research on this aspect of the problem is limited.

The data clearly illustrate the trend towards favourable response in the young pig to high level copper supplementation of the diet although the increase in ADG was not statistically significant. Certain factors affect the response to copper supplementation of the diet. The positive effects of copper on ADG compared to controls in a cold environment (9.2 C) were about twice as great as when the pigs were raised in a warm environment (18.3 C) (King, 1960). Bunch et al. (1963) observed that baby pigs failed to respond to high level copper supplementation when supplemental Zn was not added to the diet. Wallace et al. (1967) noted that the addition of 100 ppm of zinc to a diet containing 250 ppm copper prevented copper toxicosis and resulted in improved ADG. They recommended that this aspect of mineral fortification of the diet must be emphasized when 250 ppm copper is added to the diet. However, Barber et al. (1960) reported responses to copper in the absence of supplemental zinc in the diet. The diets employed herein contained 60 ppm supplemental zinc (Table 2).

Values for liver copper concentration are given in Table 3. The addition of 250 ppm copper to the diet of growing-finishing swine significantly ($P < 0.01$) increased the copper content of the liver between 7 and 15-fold. Highest liver copper concentrations were found in the group receiving

250 ppm of supplemental copper throughout the growing-finishing period to slaughter at 92 kg liveweight. The large increases in the concentration of copper in the livers of pigs fed copper supplemented diets in this experiment were within the range reported by numerous other workers (Bass et al., 1956; Ullrey et al., 1960; Bunch et al., 1961; Barber et al., 1961; Wallace, 1967; Castell and Bowland, 1968b).

Withdrawal of copper from the diet at 23 or 46 kg liveweight resulted in a liver copper concentration at slaughter (92 kg) which approximated that found in control pigs at the same weight. When the withdrawal of copper from the diet occurred at 69 kg, liver levels of copper were only reduced by about 50 per cent. The data indicates that the large amounts of copper stored in the liver when a diet containing a supplement of 250 ppm copper is being fed, are significantly reduced when the supplemental copper is removed from the diet. Lucas and Calder, (1957) similarly reported a large reduction in liver Cu stores when the supplemental Cu was removed from the diet for the period from 46 kg to 92 kg liveweight.

(b) Melting Point and Fatty Acid Composition

The saturated fatty acids (8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0 and 22:0), monounsaturated acids (14:1, 16:1 and 18:1) and polyunsaturated acids (18:2, 18:3 and 20:4) were

found to be present in the swine depot fat. Fatty acids with an odd number of carbon atoms (15 and 17) and several unknown peaks were found in trace amounts. An example of the fatty acid composition of triglycerides extracted from the backfat of pigs receiving the control diet is shown in Table 4. The order in which these acids appear is the order of elution from the DEGS column used in the study. The data represent an average of the values from four control animals at 92 kg.

To facilitate presentation of the data in this experiment, only the saturated fatty acids (14:0, 16:0 and 18:0) and the unsaturated fatty acids (16:1, 18:1 and 18:2) are discussed since only trace concentrations of the other fatty acids were detected. The composition of the depot fat in this study with respect to these acids compares favourably with that reported by others (Sink et al., 1964; Elliot and Bowland, 1968; Jurgens et al., 1970).

In this experiment, the addition of 250 ppm of supplemental Cu to the diet resulted in a significant ($P < 0.01$) decrease in the melting point of the backfat as reported in Table 5. This effect is largely accounted for by the significant ($P < 0.01$) increase in the proportions of unsaturated acids (16:1 + 18:1 + 18:2) at all slaughter weights. Control pigs (Table 5) displayed a significantly ($P < 0.01$) higher amount

Table 4. Weight % Fatty Acid Composition of the Backfat of Basal-Fed Pigs

| Fatty Acid | Weight % |
|---|---|
| 8.0 | tr.? |
| 10.0 | tr.? |
| 12:0 | tr.? |
| 14:0 | 1.3 |
| 14:1 | tr.? |
| 15:0 | tr.? |
| 16:0 | 25.0 |
| 16:1 | 6.8 |
| 17:0 | tr.? |
| 18:0 | 8.9 |
| 18:1 | 46.0 |
| 18:2 | 10.1 |
| 18:3 | 0.5 |
| 20:0 | 0.5 |
| 22:0 | tr.? |
| 20:4 | 0.4 |
| X * | 0.2 |
| 24:1 | tr.? |
| Y * | 0.2 |
| Z * | 0.1 |

* X, Y and Z peaks are the unknown components shown on the chromatogram

? These fatty acids were tentatively identified using the standard curve given in Figure 3, Page 65.

Table 5. Fatty Acid Composition (Weight % of the Total) and Melting Point of Backfat from Control and Continuous Copper-fed Pigs.

| Diet[1] | Weight at Slaughter (kg) | Fatty Acid Composition 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | Sum SFA | Sum UFA | Melting Point(C) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 23 | 1.95 | 25.92 | 4.52 | 9.97 | 46.77 | 10.27 | 38.44** | 61.56 | 26.80 |
| Copper | 23 | 1.85 | 22.72 | 6.77 | 7.00 | 48.72 | 12.12 | 32.39 | 67.61** | 23.50** |
| Control | 46 | 1.57 | 24.42 | 5.80 | 10.25 | 47.24 | 10.92 | 36.04** | 63.96 | 25.90 |
| Copper | 46 | 2.12 | 20.87 | 7.22 | 5.80 | 48.20 | 15.25 | 29.33 | 70.67** | 20.40** |
| Control | 69 | 1.47 | 26.77 | 5.52 | 10.75 | 44.20 | 10.97 | 39.31** | 60.69 | 28.00 |
| Copper | 69 | 1.90 | 17.57 | 8.65 | 7.42 | 50.55 | 12.95 | 27.85 | 72.15** | 19.10** |
| Control | 92 | 1.45 | 25.00 | 4.92 | 10.92 | 46.40 | 10.42 | 38.26** | 61.74 | 27.80 |
| Copper | 92 | 1.65 | 23.75 | 6.80 | 7.00 | 48.85 | 11.95 | 32.40 | 67.60** | 24.60** |

[1] 4 animals in each group

** Significant ($P < 0.01$) within weight groups and parameters

of the saturated acids (16:0 + 18:0) at all slaughter weights. The correlation of percent unsaturated fatty acids (UFA) with melting point was calculated using all sample values. A significant ($P<0.01$) negative correlation ($r = -0.924$) existed between the melting point and the total percentage UFA. This indicated that as melting point of a sample decreased, the percentage UFA in the sample increased and that 84.5 per cent ($r^2 \times 100$) of the difference in melting point could be explained by changes in the sum of the UFA in the sample. The regression coefficient ($b = -0.77$) indicated that for each decrease of 0.77 C in melting point, one could expect a one per cent increase in percentage UFA present in the sample (Figure 5). This indicates that the decrease in melting point is in a large part a consequence of the increase in the proportion of UFA. A similar negative correlation ($r = -0.814$) between these two parameters was reported by Elliot and Bowland (1969).

In the present experiment, there was a significantly ($P<0.01$) higher proportion of UFA in the backfat of pigs receiving Cu at all slaughter weights. The highest increase in UFA occurred amongst copper-fed pigs slaughtered at 46 or 69 kg liveweight. Similar results were described by Elliot and Bowland (1968). Measurements of the "consistency" of the porcine backfat by Taylor and Thomke (1964) also indicated that the depot fat of copper-fed animals was much softer than that of the

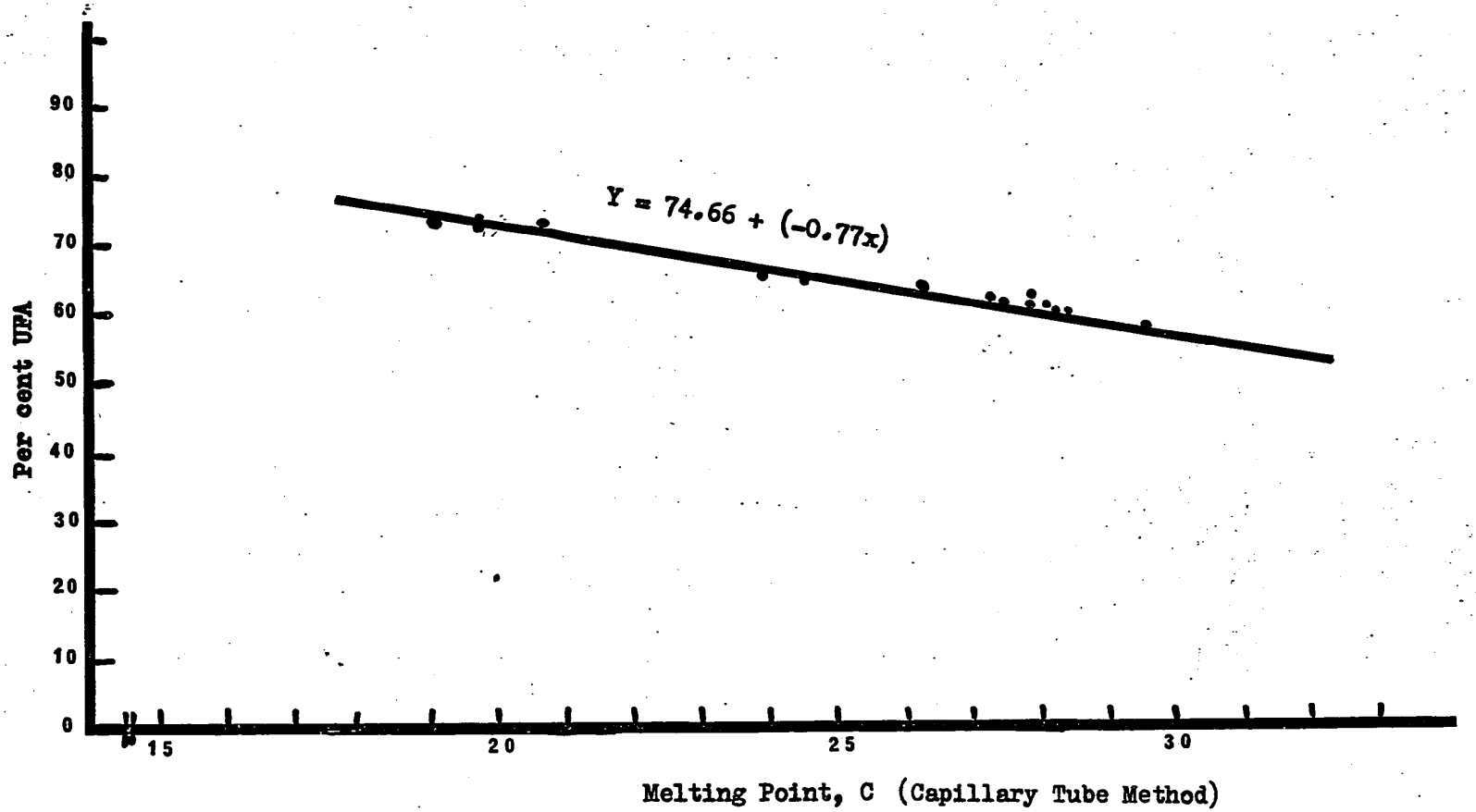



Figure 5. Regression of total melting point and total unsaturated fatty acids.

control animals due to the presence of higher UFA as determined by the iodine value of the backfat.

Removal of supplemental copper from the diet at 23, 46 or 69 kg liveweight resulted in a backfat at 92 kg which was not significantly different in melting point or per cent UFA from that of animals receiving the control diet throughout the experimental period (Table 6). The data showed conclusively that in order to obtain changes in the physical and chemical characteristics of porcine backfat, the copper supplement should be included in the diet for the entire period. This observation does not appear to have been previously reported.

(c) Neutral and Phospholipids in the Backfat of Normal and Copper-Fed Pigs

Average recovery of backfat lipid fractions from a silicic acid column was 99.5 $\pm$ 1.0 per cent. Thin-layer chromatography of the fractions indicated that the separation procedure gave excellent resolution of neutral and phospholipids. Neutral and phospholipids concentrations are given in Table 7 and Figure 6. The phospholipid concentration in backfat samples from copper-fed pigs were significantly ($P<0.01$) higher at all slaughter weights than the concentrations found in corresponding control samples.

Only one report (Gallagher et al., 1956) has suggested a role for copper in phosphatidic acid synthesis. Everson et al.

Table 6. Effect of Periodic Removal of 250 ppm Copper from Swine Diets on Fatty Acid Composition (Weight % of Total) and Melting Point of Backfat at 92 kg

| Diet* | Weight at Removal (kg) | Fatty Acid Composition | | | | | | Sum SFA | Sum UFA | Melting Point (C) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | | | |
| Control | | 1.45 | 25.00 | 4.92 | 10.92 | 46.40 | 10.42 | 38.26 | 61.74 | 27.80 |
| Copper | 23 | 1.03 | 25.60 | 5.95 | 9.83 | 45.55 | 10.63 | 37.87 | 62.13 | 27.10 |
| Copper | 46 | 1.60 | 27.28 | 6.78 | 8.55 | 44.18 | 10.53 | 38.51 | 61.49 | 25.50 |
| Copper | 69 | 2.48 | 24.30 | 5.08 | 10.65 | 45.80 | 10.23 | 38.89 | 61.11 | 27.80 |

* 4 animals in each group

Table 7. Percent of Neutral Lipids and Phospholipids in Backfat of Pigs Fed a Basal or a Copper-Supplemented Diet

| Diet | Weight at Slaughter (kg) | Neutral Lipid % of Fat | Phospholipid % of Fat |
|---|---|---|---|
| Control | 23 | 98.19 | 1.81 |
| Copper | 23 | 97.15 | 2.85** |
| Control | 46 | 98.10 | 1.90 |
| Copper | 46 | 97.25 | 2.75** |
| Control | 69 | 98.35 | 1.65 |
| Copper | 69 | 97.20 | 2.80** |
| Control | 92 | 98.57 | 1.43 |
| Copper | 92 | 97.21 | 2.79** |

** Significant ($P < 0.01$)

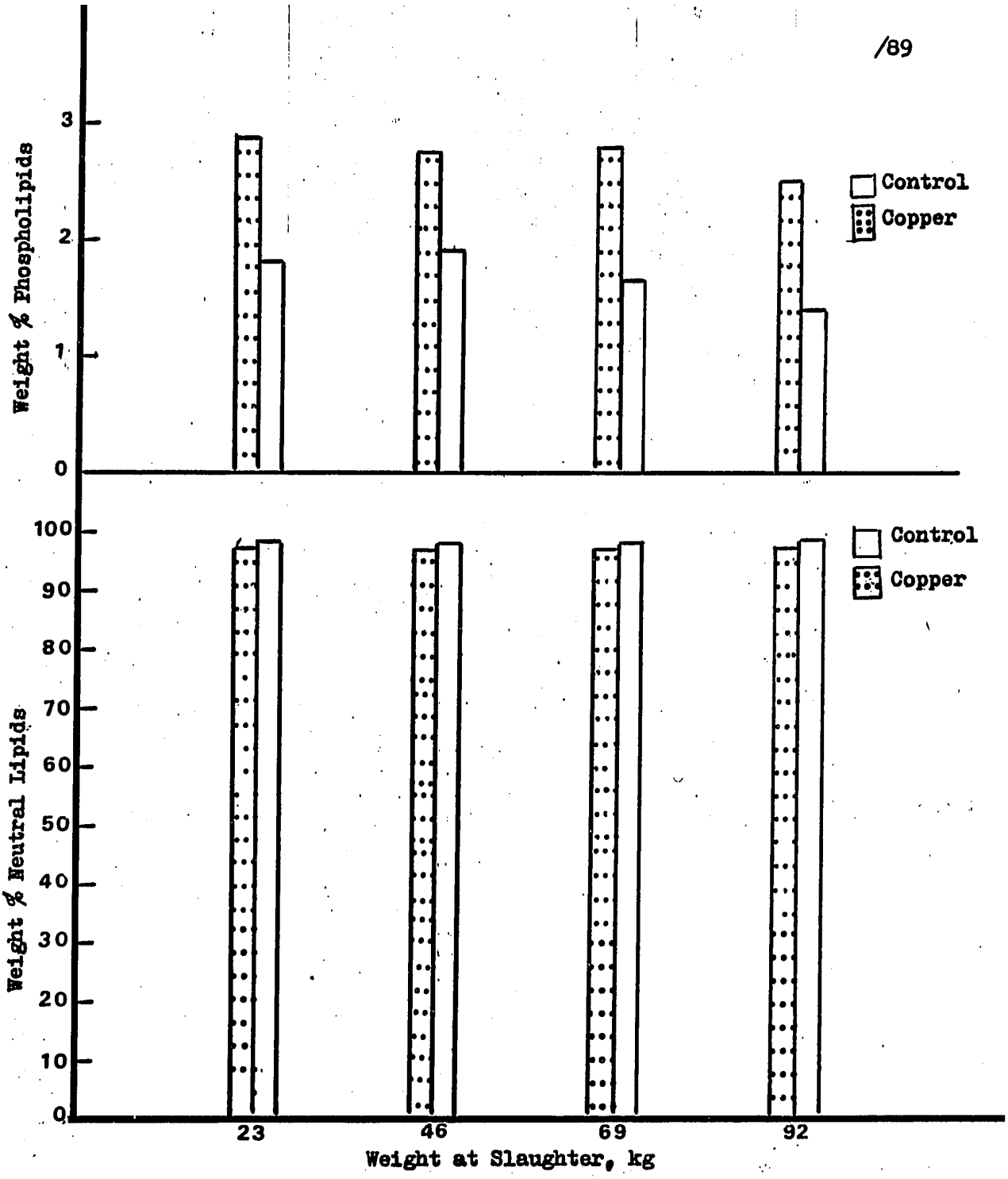



Figure 6. A comparison of weight percentage of neutral and phospholipids present in the backfat of control pigs and pigs fed 250 ppm supplemental copper in the diet.

(1968) showed a marked underdevelopment of myelin through the brain of copper-deficient guinea pigs. Copper deficiency in lambs has resulted in the degeneration of the myelin sheath structure which invests the nerve axon circumferentially (Underwood, 1966). In copper deficiency, a defect in phospholipid synthesis (via the phosphatidic acid pathway) could result in demyelination since myelin is rich in phospholipids as reported by Everson et al. (1968). On the basis of the foregoing discussion and the results reported herein, it is possible that copper plays an important role in phospholipid biosynthesis.

Based on the data, it appears that the effect of supplemental dietary copper on the composition of tissue lipids in the pig, is significant. Fatty acid metabolism, biosynthesis and desaturation appear to be affected by the intake of dietary copper. Taylor and Thomke (1964) have speculated that dietary high level copper might affect fat absorption or transport, or that the high level of copper found in the liver of animals on copper-supplemented diets might affect the normal fat metabolism of this tissue. However, O'Hea and Leveille (1968) have established that fatty acid biosynthesis takes place mainly in the adipose tissue (backfat) in the pig.

(d) Glyceride Distribution

Since pancreatic lipase (PL) preferentially hydrolyzes the ester bonds at the external positions of the glyceride

molecule (Mattson and Beck, 1956), fatty acid analysis of the monoglycerides formed during hydrolysis of a mixture of triglycerides will reveal the proportions of fatty acids esterified at the internal position of the glycerol molecule. The other acids present in the fat, are assumed to be preferentially esterified at the external positions. No differentiation is made between the two external positions of the glycerol molecule (1 and 3) in the present investigation. If one assumes that acids esterified at the 1- and 3- positions are randomly distributed, the pancreatic lipase technique permits the most detailed analysis of natural fats so far reported, an important point in this connection being the fact that isomeric triglycerides are distinguished by this method.

Comparison of the results obtained between the basal-fed and the copper-fed pigs for backfat triglycerides are given in Tables 8 and 9. For each group (Table 8), the first line gives the proportions of the fatty acids in the original fat, the second line the proportions of fatty acids in the 2- monoglyceride and the third line the percentage of each fatty acid that is in the 2- position. A study of the data reveals considerable difference between the fat samples from control animals and those receiving 250 ppm of copper in the diet. The backfat of copper-fed pigs contained significantly ($P < 0.01$) less 16:0 in the 2- position of their glycerides than the backfat of control

Table 8. Fatty Acid Composition (Weight %) of Control and Continuous Copper-Fed Pigs' Backfat Before Hydrolysis and of the Purified Monoglycerides Obtained After 15% Hydrolysis with Pancreatic Lipase

| Treatment | Analysis | Fatty Acid 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 |
|---|---|---|---|---|---|---|---|
| Control | Original | 1.95 | 25.92 | 4.52 | 9.97 | 46.77 | 10.27 |
| | MG | 5.20 | 67.70 | 7.50 | 2.10 | 14.80 | 2.70 |
| | % in 2-position | 88.80 | 87.10 | 55.30 | 7.00 | 10.50 | 8.80 |
| Copper to 23 kg | Original | 1.85 | 22.72 | 6.77 | 7.00 | 48.72 | 12.12 |
| | MG | 4.80 | 57.40** | 10.50** | 3.20 | 19.20** | 5.00** |
| | % in 2-position | 87.30 | 84.90 | 51.70 | 15.20 | 13.10 | 13.80 |
| Control to 46 kg | Original | 1.57 | 24.42 | 5.80 | 10.25 | 47.24 | 10.92 |
| | MG | 4.10 | 64.40 | 9.40 | 3.10 | 15.50 | 3.70 |
| | % in 2-position | 87.00 | 87.90 | 54.00 | 10.10 | 10.90 | 11.30 |
| Copper to 46 kg | Original | 2.12 | 20.87 | 7.22 | 5.80 | 48.20 | 15.25 |
| | MG | 5.60 | 53.40** | 12.20** | 2.60 | 20.20** | 6.10** |
| | % in 2-position | 88.10 | 85.30 | 56.30 | 14.90 | 14.00 | 13.30 |
| Control to 69 kg | Original | 1.47 | 26.77 | 5.52 | 10.75 | 44.20 | 10.97 |
| | MG | 3.80 | 67.80 | 9.00 | 3.30 | 13.00 | 3.40 |
| | % in 2-position | 86.10 | 84.40 | 54.30 | 10.20 | 9.80 | 10.30 |
| Copper to 69 kg | Original | 1.90 | 17.57 | 8.65 | 7.42 | 50.55 | 12.95 |
| | MG | 4.70 | 42.70** | 15.90** | 3.40 | 22.10** | 6.10** |
| | % in 2-position | 82.50 | 81.00 | 61.30 | 15.30 | 14.60 | 15.70 |
| Control to 92 kg | Original | 1.45 | 25.00 | 4.92 | 10.92 | 46.40 | 10.42 |
| | MG | 3.80 | 66.10 | 7.30 | 3.50 | 16.00 | 3.30 |
| | % in 2-position | 87.40 | 88.10 | 49.50 | 10.70 | 11.50 | 10.60 |
| Copper to 92 kg | Original | 1.65 | 23.75 | 7.80 | 7.00 | 48.85 | 11.95 |
| | MG | 4.00 | 60.60** | 9.90** | 2.60 | 17.20** | 3.90** |
| | % in 2-position | 80.80 | 85.10 | 42.30 | 12.30 | 11.70 | 10.90 |

** Significant ($P < 0.01$)

Table 9. Glyceride[1] Distribution in Backfat Samples from Control and Copper-Fed Pigs

| Groups | Glyceride Type | | | | Isomeric Forms | | | |
|---|---|---|---|---|---|---|---|---|
| | $S_3$ % | $S_2U$ % | $SU_2$ % | $U_3$ % | SUS % | SSU % | USU % | SUU % |
| Control to 23 kg | 2.78** | 12.60** | 52.86 | 16.30 | 0.93 | 11.67 | 48.97 | 3.89 |
| Copper to 23 kg | 1.41 | 8.93 | 51.97 | 25.20** | 0.74 | 8.19** | 47.64 | 4.33 |
| Control to 46 kg | 2.47** | 11.80** | 51.77 | 18.84 | 0.98 | 10.82 | 47.48 | 4.29 |
| Copper to 46 kg | 0.95 | 7.28 | 51.46 | 29.48** | 0.59 | 6.69** | 47.29 | 4.17 |
| Control to 69 kg | 3.32** | 13.56** | 50.88 | 15.65 | 1.11 | 12.45 | 46.71 | 4.17 |
| Copper to 69 kg | 1.13 | 7.56 | 43.02** | 35.60** | 1.10 | 6.46** | 36.77** | 6.25 |
| Control to 92 kg | 2.75** | 12.46** | 51.89 | 17.30 | 1.00 | 11.46 | 47.74 | 4.15 |
| Copper to 92 kg | 1.53 | 9.37 | 52.65 | 23.64** | 0.75 | 8.62 | 48.44 | 4.21 |

[1] From values found in 2- position acids (r).

** Significant ($P < 0.01$)

animals. Level of 14:0 and 18:0 tended to be decreased and increased respectively at the 2- position in fat samples from pigs receiving supplemental copper in the diet in comparison with pigs fed the basal diet. The 14:0 increased in all copper-fed groups except the group slaughtered at 23 kg, while the 18:0 increased in groups slaughtered at 23 and 69 kg and decreased in groups slaughtered at 46 and 92 kg liveweight. These differences between samples from copper-fed and control pigs were not statistically significant. Dietary 250 ppm of supplemental copper caused a significant ($P < 0.01$) increase in the amounts of 16:1, 18:1 and 18:2 in the 2- position of the glyceride molecule. Because of these significant differences in 2- esterified 16:0, 16:1, 18:1 and 18:2 between basal and copper-fed pigs, it is reasonable to conclude that high levels of dietary copper in some way altered the positional distribution of fatty acids in the triglyceride molecule. However, the general characteristics of porcine glyceride structure (predominant esterification of 16:0 at the 2- position, and 18:1 and 18:2 at the 1- and 3- positions, established for pork fat and lard, Hilditch and William, 1964) are still evident in samples from copper-fed pigs. Smith et al. (1965) stated that a value of 33.3 per cent of a fatty acid esterified at a given position would indicate no preferential esterification of the acid at either the internal or the external positions. It has been suggested that less than 28 per cent of a particular fatty acid esterified at the 2- position indicates

that the fatty acid is positioned preferentially at the other positions (1- and 3-), conversely, more than 38 per cent at the 2- position indicates preferential attachment of the acid at this position. The range 28-38 per cent is admittedly arbitrary, but it would allow for a relative standard deviation of 7 per cent in fatty acid analysis of the triglycerides and monoglycerides.

The glyceride type distribution as calculated from the pancreatic lipase hydrolysis data by the method of Vander Wal (1960) is given in Table 9. Definite variations in the concentration of the various glyceride types and their isomers amongst groups were observed. Backfat from control animals contained significantly ($P < 0.01$) higher proportions of $S_3$ and $S_2U$ than backfat from copper-fed pigs and tended to display greater amounts of the USU isomer than in the copper-fed group slaughtered at 69 kg liveweight. Backfat from copper-fed pigs contained a significantly ($P < 0.01$) higher proportion of $U_3$ in all groups. Only small differences were noticed amongst groups for the isomeric forms USU and SUU. The $S_2U$ (SUS and SSU isomers) and the $U_3$ glycerides serve well for comparative purposes (also $SU_2$ in the group slaughtered at 69 kg) since they are the second most abundant groups amongst the triglyceride species. The changes in the proportions of the various triglycerides species present in adipose tissue of

pigs fed copper appears to provide a further insight into the effects of dietary copper on the physical properties of pig adipose tissue. Variations in the melting points, ranging from a very soft backfat (group fed 250 ppm Cu and slaughtered when they reached 69 kg liveweight, melting point = 19.1 C) to a normal fat (control pigs, melting point = 28.0 C) were accompanied by variation in the concentration of the glyceride types ($S_3$, $S_2U$, $SU_2$, at 69 kg and $U_3$).

A significant ($P < 0.01$) negative correlation existed between the melting point and per cent $U_3$ triglyceride species in the sample. The correlation coefficient, ($r = -0.880$) indicates that as melting point of a sample decreased, the percentage of the $U_3$ triglyceride species in the sample increased and that 77.4 per cent ($r^2 \times 100$) of the difference in melting point could be explained by differences in the percentage $U_3$ species in the sample. The regression coefficient ($b = -0.45$) indicated that for each decrease of 0.45 C in melting point, one could expect a one per cent increase in the $U_3$ triglyceride species present in the sample.

Also, two positive significant ($P < 0.01$) correlations existed between melting point of a sample and the per cent $S_3$ or $S_2U$ triglyceride species in the sample. The significant ($P < 0.01$) correlation coefficients, ($r = 0.872$ for $S_3$ and $r =$

0.919 for $S_2U$) indicate that as melting point of a sample decreased, the percentage of the $S_3$ and $S_2U$ decreased and that 76.0 and 84.5 per cent ($r^2$ x 100) of the difference in melting point could be explained by differences in the percentage $S_3$ and $S_2U$ species in the sample, respectively.

Thus, the percentage of $S_3$, $S_2U$ and $U_3$ triglycerides species, as well as percentage UFA (discussed previously), can apparently both be used as reliable indicators of melting point (physical properties) and hence fat softness or hardness in these studies. This would indicate that the change in melting point of porcine backfat in response to supplemental dietary copper is related to both changes in fatty acid composition (increase in the proportions of UFA) as reported by Elliot and Bowland (1969) and to changes in triglyceride structure as reported by Christie and Moore (1970).

It became of interest, therefore, to attempt to determine the relative contributions of changes in percent UFA and percent $U_3$ to changes in melting point. A significant ($P < 0.01$) multiple correlation existed between melting point (dependent variable) and UFA and $U_3$ (independent variables). The multiple correlation coefficient ($RY.X_1X_2 = 0.930$), in addition to the information obtained from the two simple linear correlations between each of the two independent variables and the dependent variable, indicated that as melting point

of a sample decreased, the percentage UFA and percentage $U_3$ increased. The multiple regression equation was found to be:

$$Y = 69.91 - 0.68\ X_1 - 0.06\ X_2$$

The regression coefficients ($b_1 = -0.68$, $b_2 = -0.06$) indicate that for each one per cent increase in UFA, one could expect 0.68 C decrease in melting point and for each one per cent increase in $U_3$, one could expect 0.06 C decrease in melting point of the backfat of the pig.

Analysis of variance further indicated that the relative contribution of changes in percentage UFA to changes in melting point was significant ($P < 0.01$) while the relative contribution of percentage $U_3$ was not.

The partial correlation coefficients were calculated to investigate the relationship between melting point and each of the UFA and $U_3$. In comparing the partial correlation coefficients, it was found that:

$$rY_{1 \cdot 2} = rYX_{1 \cdot X_2} = -0.61^{**} \ (P < 0.01) \text{ and,}$$

$$rY_{2 \cdot 1} = rYX_{2 \cdot X_1} = -0.11$$

This indicates that percent UFA was the chief factor affecting the significant reduction in melting point when supplemental coper was added to swine diets. Thus, percent UFA can be used as a reliable predictor of melting point and hence of fat softness in fat analysis.

In the present experiment, there was a significantly ($P < 0.01$) higher proportion of $U_3$ glyceride species at all slaughter weights among copper-fed pigs than among control pigs, and significant ($P < 0.01$) reduction in melting point was observed. The failure of $U_3$ to show significant effects on melting point as described by the multiple regression coefficient and the partial correlation coefficients is in conflict with the evidence of others. Christie and Moore (1970) reported a significant increase in the most unsaturated glyceride species and a significant reduction in melting point of the fat samples from pigs fed a copper supplemented diet (250 ppm). These authors have claimed that alteration in triglyceride structure and not changes in fatty acid composition of the depot fat from copper-fed pigs is the factor mainly responsible for the changes in melting point of the fat.

It is generally assumed that the concentrations of solid glycerides ($S_3$ and $S_2U$) largely govern the consistency, in particular the melting point, of the fat. Bailey (1957) stated that crystals composed in large part of high melting glycerides, are more rigid and hence have greater stiffening power than crystals of lower melting point triglycerides. It is possible, then, that the observed increases in the percentage of 16:1, 18:1 and 18:2 fatty acids in the backfat in response to supplemental copper resulted in significant increases in the concentrations of the $SU_2$ (in the case of 69 kg copper group) and $U_3$ (in all copper-fed

groups) and significant reductions in $S_3$ and $S_2U$ triglyceride species present in the backfat. Moore et al. (1968) indicated that even a small change in the fatty acid composition of original fat can have an apparently disproportinate effect on the above mentioned glyceride species and consequently, a pronounced change in the melting point of the fat.

Data presented herein would indicate that relatively large changes in fatty acid composition in response to supplemental dietary copper result in changes in glyceride structure and that these changes are reflected in a decreased melting point.

Fatty acid distribution in the 2- monoglyceride fraction when copper was removed from the diet at 23, 46 or 69 kg liveweight (Table 10) indicated slight differences in the 2- esterified fatty acids. The observed differences in the level of all the fatty acids studied were not, however, statistically significant with the exception of 18:1 which showed significant ($P < 0.01$) decrease in the 2- monoglyceride molecule when copper was removed at 23, 46 or 69 kg liveweight. The saturated 16:0 acid tended to be preferentially esterified at the 2- position of the triglyceride molecule rather than at the 1- and 3- positions. The unsaturated 18:1 and 18:2 acids accumulated preferentially in positions 1 and 3 when copper was removed from the diet at 23, 46 or 69 kg liveweight. It seems likely that copper is necessary throughout the growing period of the pig to bring about the significant changes observed in Tables 8 and 9.

Table 10. Effect of Intermittent Addition of Copper to Swine Diet on Fatty Acid Composition of Backfat Before Hydrolysis and of Purified Monoglycerides Obtained After 15% Hydrolysis with Pancreatic Lipase

| Diet | Weight at Removal kg[1] | Analysis | Fatty Acid 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 |
|---|---|---|---|---|---|---|---|---|
| Control | | Original | 1.45 | 25.0 | 4.92 | 10.92 | 46.40 | 10.42 |
| | | MG | 3.80 | 66.1 | 7.30 | 3.50 | 16.00 | 3.30 |
| | | % in 2- position | 87.40 | 88.20 | 50.10 | 10.50 | 11.50 | 10.70 |
| Copper | 23 | Original | 1.03 | 15.60 | 5.45 | 9.83 | 45.55 | 10.63 |
| | | MG | 4.18 | 68.10 | 8.18 | 2.83 | 9.80** | 2.93 |
| | | % in 2- position | 85.98 | 88.60 | 48.33 | 9.53 | 7.13 | 9.78 |
| Copper | 46 | Original | 1.60 | 27.28 | 6.78 | 8.55 | 44.18 | 10.53 |
| | | MG | 3.50 | 71.80 | 8.43 | 2.70 | 10.73** | 2.88 |
| | | % in 2- position | 75.65 | 87.83 | 44.05 | 10.63 | 8.10 | 9.08 |
| Copper | 69 | Original | 2.48 | 24.30 | 5.08 | 10.65 | 45.80 | 10.23 |
| | | MG | 6.68 | 65.08 | 9.48 | 3.38 | 13.08** | 2.33 |
| | | % in 2- position | 90.35 | 89.28 | 60.80 | 10.45 | 9.48 | 7.80 |

[1] Slaughter was at 92 kg liveweight in all cases.

** Significant ($P < 0.01$)

In fact, the fatty acid distribution in the 2- monoglyceride fraction at 92 kg when Cu was removed from the diet at 23, 46 or 69 kg is to be expected since the gross fatty acid composition (in the reversed groups) did not differ significantly from the control animals. Furthermore, removal of copper from the diet at 23, 46 or 69 kg resulted in a significant decrease in liver copper concentration measured at 92 kg.

The glyceride type distribution as calculated from the pancreatic lipase hydrolysis is given in Table 11. No significant differences in the concentration of the various glyceride types and their isomers were observed at 92 kg when supplemental copper was removed from the diet at 23, 46 or 69 kg liveweight. This observation has not been reported before in the literature.

In copper-fed pigs (Table 8), increases in proportions of UFA appear to direct these fatty acids to position 2 of the glycerol molecule. This effect is most pronounced in the two groups slaughtered at 46 and 69 kg liveweight where 18:1 and 18:2 were higher in the original fat and the 2- monoglyceride fraction. In the reversed groups, the effect is eliminated by omitting copper from the diet.

The results obtained in this experiment provide useful information on how the supply of fatty acids (as influenced by the presence or absence of supplemented copper in the diet) for

Table 11. Effect of Removal of Copper from Swine Diet on Glyceride Distribution at 92 kg

| Diet | Weight at Removal kg | Glyceride Type | | | | Isomeric Forms | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $S_3$ % | $S_2U$ % | $SU_2$ % | $U_3$ % | SUS % | SSU % | USU % | SUU % |
| Control | | 2.80 | 12.46 | 51.71 | 17.41 | 0.99 | 11.47 | 47.58 | 4.13 |
| Copper | 23 | 2.41 | 11.83 | 54.08 | 16.77 | 0.80 | 11.03 | 50.44 | 3.64 |
| Copper | 46 | 2.36 | 11.71 | 56.62 | 15.20 | 0.67 | 11.04 | 53.53 | 3.09 |
| Copper | 69 | 2.60 | 12.20 | 53.71 | 16.45 | 0.86 | 11.34 | 49.98 | 3.73 |

acylating the three positions of the glycerol moiety in triglyceride synthesis in the pig adipose tissue influenced the triglyceride structure and composition. The increased esterification of 18:1 and 18:2 at the 2- position of the triglyceride molecule could indicate a change in the common biosynthetic pathway of the deposited triglyceride molecule. Triglycerides are probably synthesized in the pig adipose tissue by the glycerophosphate pathway (Weiss et al., 1960), i.e., L -X- glycerophosphate is esterified in positions 1 and 2 with fatty acids to form phosphatidic acid and copper may be involved in the formation of phosphatidic acid as described by Gallagher et al. (1956). Phosphatidic acid is dephosphorylated and in return, acylated in position 3 to triglyceride. It has yet to be established whether position 1 or 2 of L-X-glycerophosphate is esterified first or whether both positions are esterified simultaneously. It is now established by O'Hea and Leveille (1968) that adipose tissue of the pig is the major site for de novo synthesis of fatty acids, but how such fatty acids are positioned in a glycerol to form glycerides and how copper affects this positioning is a subject for further research.

## SUMMARY

The inclusion of 250 ppm of supplemental copper (0.1% Cu $SO_4 \cdot 5H_2O$) in a practical swine ration did not significantly increase growth rate. Pigs receiving supplemental copper had a significantly ($P < 0.01$) higher level of copper stored in the liver.

Removal of copper from the ration at either 23, 46 or 69 kg liveweight, caused a reduction in growth rate and markedly decreased liver copper concentration, measured at slaughter (92 kg) to a level approximating that present in the livers of the control animals.

Copper supplementation of the diet altered the consistency of the depot fat as indicated by a decrease in melting point and an increase in the proportions of unsaturated fatty acids and the $U_3$ triglyceride species.

Elimination of copper from the diet at 23, 46 or 69 kg liveweight resulted in a depot fat, at 92 kg, which was not markedly different from that of animals receiving the basal ration.

# EXPERIMENT II.

## A. Introduction

Supplemental copper at a level of 250 ppm did not significantly improve performance of gilts in Experiment I when included continuously in the diet from 13.5 to 92 kg liveweight. Bunch et al (1961) have reported that 125 to 250 ppm of copper added to the diet of 2 to 3-week old pigs for 4 to 6 weeks significantly increased ADG and FC. Barber, Braude and Mitchell (1955b) could find no effect on ADG and FC when approximately 150 ppm copper was included in a swine diet.

The relationship between the softness of the fat and the presence of 250 ppm of supplemental copper in the diet has been well established in Experiment I as well as in other studies (Elliot and Bowland, 1968,1970; Moore et al., 1968; Christie and Moore, 1970). The pig deposits unsaturated fat in preference to saturated fat in its adipose tissue when there is 250 ppm of supplemental copper in the diet. However, there are no reports in the literature concerning the effect of level of supplemental copper on changes in depot fat composition.

Experiment II was conducted, therefore, to study the effect of feeding supplemental copper at levels of 125, 150, 175, 200, 225 and 250 ppm on the performance and backfat characteristics of the pig.

B. Experimental

Fifty-six pigs, divided on the basis of liveweight and sex, into seven groups of eight (four males and four females/group) were used in this study. The basal diet was formulated as described in Experiment I (Table 2) and supplemented with 0, 125, 150, 175, 200, 225 and 250 ppm copper. Diets were fed ad libitum. Pigs were weighed weekly and feed consumption recorded. Individual pigs were removed from the experiment and slaughtered as they reached approximately 92 kg liveweight.

Samples of the backfat and liver were obtained at the time of slaughter as described under General Experimental, Section B-2 and B-3. The samples were prepared for anlaysis, analyzed and results calculated as described under General Experimental, Sections C-1, C-2 (a), (b); D-2 (a), (d), (e).

C. Results and Discussion

(a) Average Daily Gain, Feed Conversion and Liver Copper

There were no significant differences in ADG among the groups of pigs fed the seven levels of supplemental copper (Table 12 and Figure 7). The results showed that levels of supplemental copper below 150 ppm or above 200 ppm slightly depressed the overall (male + female) ADG. Dietary 175 or 200 ppm of supplemental copper slightly stimulated growth by +1.8 per cent. Male pigs fed diets supplemented with 150, 175 or 200 ppm of copper gained markedly faster (+3.9, +5.4 and +1.4%) as compared with

Table 12. Effect of Various Levels of Supplemental Dietary Copper on Average Daily Gain, Feed Conversion and Liver Copper Concentration

| Level of Cu, ppm ⟶ | | 0 | 125 | 150 | 175 | 200 | 225 | 250 |
|---|---|---|---|---|---|---|---|---|
| No. of pigs/pen | | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| ADG, gm | (a) | 719* | 692* | 747* | 758* | 729* | 713* | 602 |
| | (b) | 688 | 657 | 661 | 677 | 703 | 632 | 662* |
| | (Avg) | 704 | 674 | 704 | 717 | 717 | 678 | 632 |
| FC | | 3.18 | 2.89 | 3.17 | 3.12 | 2.89 | 3.12 | 3.14 |
| Liver Cu, ppm | (a) | 22 | 70 | 101 | 150 | 241 | 289 | 301 |
| | (b) | 15 | 61 | 98 | 138 | 231 | 270 | 289 |
| | (Avg) | 18 | 65** | 99** | 144** | 236** | 279** | 295** |

(a) Barrows
(b) Gilts

* Significant ($P < 0.05$)
** Significant ($P < 0.01$)

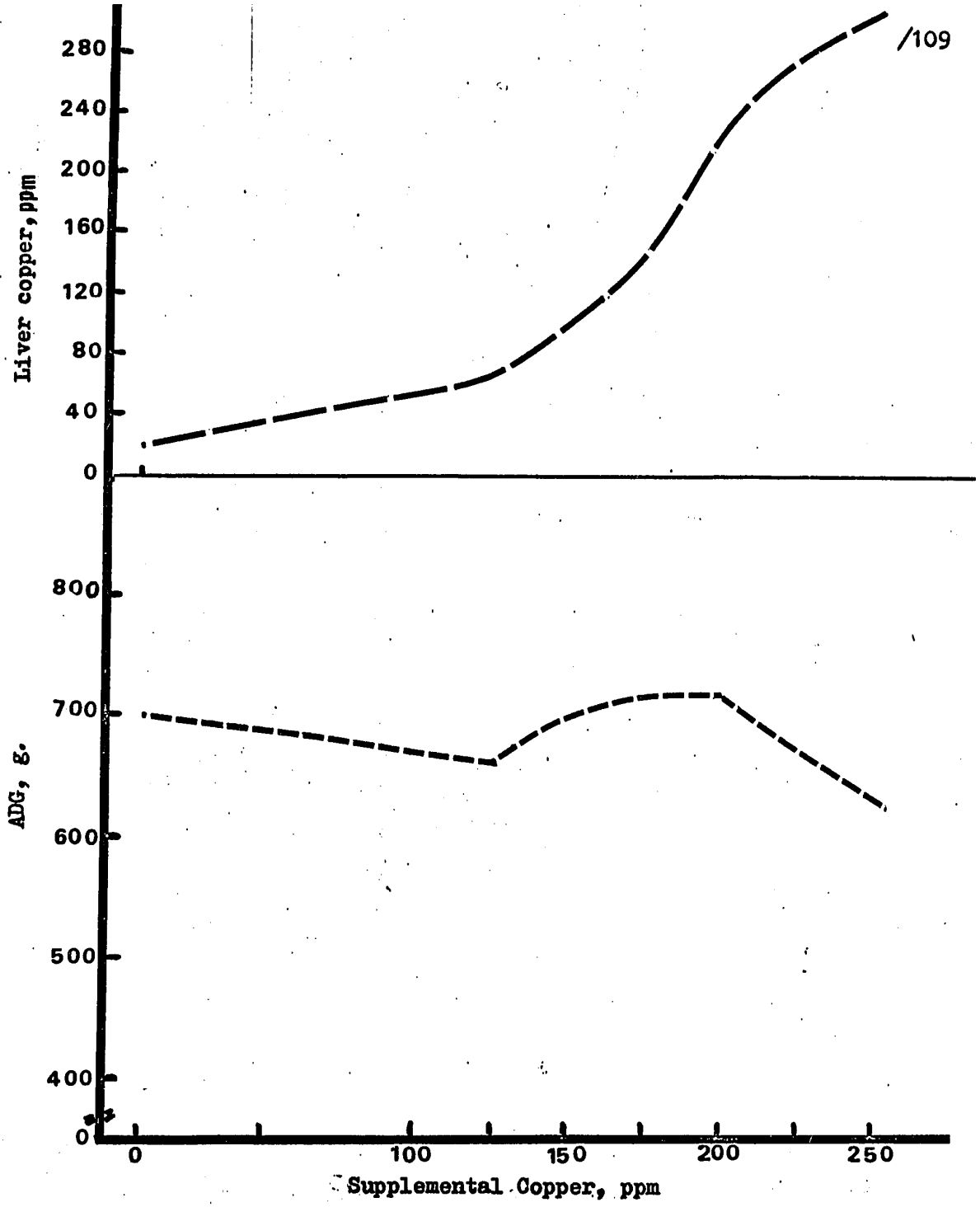



Figure 7. Effect of various levels of supplemental copper on average daily gain and liver copper content.

male pigs fed similar diets without supplemental copper. All but the 200 ppm copper level in the female diets produced marked depressions in ADG as compared with female pigs on the unsupplemented diet. The lack of gain response indicates that level of copper below 175 ppm or above 200 ppm is not adequate enough to elicit an increase in ADG. At all levels below 250 ppm, barrows grew significantly ($P < 0.05$) faster than gilts. At the 250 ppm level of supplemental copper, the reverse was evident.

These results are in general agreement with those of Wallace et al. (1967) who found no marked or consistent improvement in growth rate and feed efficiency at any level of copper supplementation up to 250 ppm. Both sets of data show no clear relation between level of supplemental copper and pig performance. Lucas and Calder (1957b) at first indicated that 125 ppm copper was as effective as 250 ppm, but later work (Lucas et al., 1961) indicated that 250 ppm was more effective. Dammers et al. (1959) found that 187½ ppm of supplemental copper was as effective as 250 ppm but significantly better than 125 ppm. Bellis (1961) reported an improvement in ADG when dietary copper levels of less than 250 ppm were fed. Braude et al. (1970) reported a significant increase in ADG when 170, 210 or 250 ppm of supplemental copper was included in the swine diet. Their results showed no significant differences in performance of pigs receiving copper at the different levels (170, 210 or 250 ppm copper).

The mechanism by which the addition of copper to diets improves or regresses performance of growing pigs is poorly understood although it has been suggested that the action is bactericidal or perhaps anthelmintic in nature (Hawbaker et al., 1961). The growth response to the addition of copper to pig diet, which has been firmly established by many experiments (Braude, 1965), could be due either to a systemic effect resulting from the absorbed copper or to the effect of soluble copper acting within the digestive tract or to both. The slight improvements noted in the experiment at the 175 and 200 ppm levels do not indicate in which of these ways the action takes place. No conclusions are possible from these observations since the response in ADG of pigs given high levels of copper does not appear to be related to the amount of copper included in the diet.

The influence of supplemental copper on feed conversion was not consistent. The amount of feed required per unit of gain was considerably improved by copper supplementation at 125 and 200 ppm (-9.12% vs. the control). This trend was less pronounced at the other levels of supplemental copper. Barber et al. (1957) reported that copper supplementation of diets fed to fattening pigs increased feed consumption and ADG significantly with a small, non-significant effect on FC. When Wallace et al. (1966) offered pigs simultaneous access to meal mixtures containing 0, 125, 250, 500 and 1,000 ppm of supplemental copper,

the pigs ate more of the meal mixture with no supplemental copper and ate progressively less of the various mixtures as copper level increased, an observation which contradicts that made by Mitchell (1953). Braude et al. (1970) also indicated that pigs fed supplemental copper (170, 210 or 250 ppm) consumed significantly less feed per unit of gain.

Pigs receiving supplemental copper stored significantly ($P < 0.01$) higher levels of copper in their livers. The level of copper accumulation in the liver followed closely the level of copper in the diet (Figure 7). No significant differences were noted with respect to sex, gilts accumulated slightly greater amounts of copper in their livers than barrows. Several workers (Barber et al., 1961; Suttle and Mills, 1964; Braude, 1970) have reported that the amount of copper stored in the liver can be greatly influenced by the amount of copper in the diet as well as by the level of other metals in the diet specifically zinc and iron. In this connection, the diets used in this experiment were supplemented with both zinc and iron at levels of 60 and 25 ppm, respectively.

(b) Fatty Acid Composition and Melting Point of Backfat

Gas chromatographic analysis of the methyl esters of fatty acids extracted from pigs' backfat are summarized in Tables 13 and 14. Although more than 24 different peaks were

Table 13. Weight % of the Saturated Fatty Acids in the Backfat of Pigs fed Basal and Several Copper Supplemented Diets at 92 kg.

| Treatment | Melting Point C | Wt. % Saturated Fatty Acids 14:0 | 16:0 | 18:0 | Total |
|---|---|---|---|---|---|
| Control | 28.1 | 1.47 | 25.38 | 11.82 | 38.67 |
| 125 ppm | 22.7** | 1.50 | 19.57** | 9.00 | 30.07** |
| 150 ppm | 21.8** | 1.76 | 20.84** | 9.14 | 31.74** |
| 175 ppm | 20.3** | 1.63 | 20.02** | 8.59 | 30.24** |
| 200 ppm | 19.7** | 1.56 | 19.84** | 8.92 | 30.32** |
| 225 ppm | 23.2** | 1.57 | 21.53** | 8.82 | 31.92** |
| 250 ppm | 25.1** | 1.45 | 23.84** | 9.53 | 34.82** |

** Significant ($P < 0.01$).

Table 14. Weight % of the Unsaturated Fatty Acids in the Backfat of Pigs Fed Basal and Several Copper Supplemental Diets at 92 kg.

| Treatment | Weight % Unsaturated Fatty Acids | | | | | |
|---|---|---|---|---|---|---|
| | 16:1 | 18:1 | 18:2 | 18:3 | 20:4 | Total |
| Control | 5.36 | 45.95 | 9.30 | 0.38 | 0.37 | 61.34 |
| 125 ppm | 6.24** | 50.92** | 10.97** | 0.33** | 0.32** | 68.78** |
| 150 ppm | 7.47** | 48.30** | 11.54** | 0.49** | 0.47** | 68.24** |
| 175 ppm | 6.69** | 49.70** | 12.53** | 0.34** | 0.39** | 69.72** |
| 200 ppm | 6.54** | 49.40** | 12.14** | 0.52** | 0.50** | 69.59** |
| 225 ppm | 6.72** | 49.67** | 10.77** | 0.45** | 0.45** | 68.05** |
| 250 ppm | 6.41** | 48.02** | 10.00** | 0.38** | 0.37** | 65.17** |

** Significant ($P < 0.01$).

observed, three saturated (14:0, 16:0 and 18:0) and five unsaturated (16:1, 18:1, 18:2, 18:3 and 20:4) acids were found to account for more than 98 per cent of the total based on peak areas. This is in general agreement with Elliot and Bowland (1968;1970) and Jurgens et al. (1970).

Supplemental copper (125, 150, 175, 200, 225 or 250 ppm) to the swine diet caused a significant ($P < 0.01$) fall in the 16:0 and 18:0 acid content accompanied by concomitant significant ($P < 0.01$) increases in 16:1, 18:1, 18:2, and 18:3 acid content, the changes being less pronounced with the diet containing 250 ppm of supplemental copper. However, the deposition of SFA and UFA in the fat of this group of pigs did not differ significantly from the other copper-fed pigs (125 to 225 ppm groups).

The broad generalization to be made from these results is that the presence of supplemental (125 to 250 ppm) copper in the diet results in a higher percentage of UFA in the backfat of pigs as compared with the essential dietary level (6-10 ppm) established for the pig. There is no direct evidence to cite in support of the above findings. From the point of view of the major objectives of this research, attention is called to the clear relationship between the composition of the backfat and supplemental copper to the swine diet. The data emphasize the conclusion drawn in the previous experiment (Experiment I)

as well as by other investigators (Elliot and Bowland, 1968;1970) that significant changes in percentage composition of swine backfat could be brought about by dietary 250 ppm of supplemental copper. The fall in percentage of 16:0 and 18:0 acids may reflect a slower rate of deposition than the deposition of other fatty acids, mainly 16:1, 18:1, 18:2, 18:3 and 20:4. It would not be surprising if SFA and UFA competed with each other under these conditions of copper supplementation in the formation of the backfat.

No significant differences attributable to sex were observed in control and copper-fed pigs. It appeared, however, that barrows deposited slightly higher linoleic acid than gilts. This is in contradiction to the report of Friend and Cunningham (1967) and Elliot and Bowland (1970) who reported a significant copper x sex interaction resulting from a greater response to copper supplementation of the diet by gilts as compared with barrows, i.e., the increase in the proportion of UFA and the decrease in the proportion of SFA were more pronounced in the backfat of copper-supplemented gilts than in copper-supplemented barrows. The data reported herein is in agreement, however, with that of Babatunde et al. (1967) who found no significant differences in fatty acid composition of backfat samples from barrows and gilts.

In this experiment, the rate of growth appeared to have an effect on the nature of the fat deposited by the pig. Fat

produced by slow-growing pigs (groups receiving 125, 225 or 250 ppm copper) was significantly ($P < 0.01$) less saturated than fat produced by control pigs. However, when one compares relatively fast growing pigs (groups receiving 175 or 200 ppm copper) the fat was even less saturated than amongst slow-growing pigs. This finding is not consistent with the report of Callow (1935) who concluded that a slowly growing pig usually has a slower rate of fat synthesis and deposition, which, in turn, is related to a softer backfat. Fat produced by slow-growing pigs in Callow's experiments was more saturated than fat produced by fast-growing pigs.

The melting point of the backfat of the control pigs was 5-10 C higher than that of the fat from copper-fed pigs. From the significant inverse relationship existing between melting point and percentage UFA (Experiment I, Elliot and Bowland, 1969), it seemed that such large differences in melting point could be explained solely on the basis of the observed differences in fatty acid composition. The marked differences in melting point were accounted for by significant ($P < 0.01$) differences in the positional distribution of fatty acids in backfat triglycerides (Experiment I, Christie and Moore, 1970). However, as discussed in Experiment I, the significant increase in UFA was more effective in producing significant differences in melting point than the triglyceride structure.

The fact that there appears to be a definite relationship between dietary copper and fatty acid composition of the backfat, may imply that significant changes in fatty acid biosynthesis had occurred. This suggests that a significant amount of 16:1, 18:1 and 18:2 had been formed from 16:0 and 18:0 acids. Possibly increased synthesis of the low melting point fatty acids may explain the apparent increased unsaturation and softness of swine backfat. To date, the role of copper in fatty acid biosynthesis or desaturation has not been reported in any biochemical studies with the exception of its involvement in the synthesis of phosphatidic acid. The effect of copper ions on lipogenesis may be direct; the possibility exists that copper could influence fatty acid synthesis indirectly by its effect on carbohydrate metabolism (Weissberger and Luvalle, 1944).

There remained the question of the site of fatty acid desaturation. It has been recently suggested (O'Hea and Leveille, 1968) that the adipose tissue of the pig carries out four major functions: conversion of glucose carbon to fatty acids and $\alpha$-glycerophosphate; uptake of the fatty acids from extracellular triglycerides; esterification of fatty acids with $\alpha$-glycerophosphate; mobilization of stored triglyceride fatty acids as free fatty acids (FFA), although thus far, no observations have been reported to indicate that the adipose tissue is a metabolically active site for fatty acid desaturation.

(c) Identification of Linoleic Acid

In previous experiments (Elliot and Bowland, 1970), as well as in Experiments I and II, an increase in the level of linoleic acid in the backfat of pigs fed copper supplemented diets was noted. According to the results in Experiment II, the significant ($P<0.01$) increase in the linoleic acid closely followed the significant changes in melting point of swine backfat (Figure 8).

Linoleic acid (18:2W6,9) is an essential fatty acid generally considered not to be synthesized by mammals and, therefore, required in the diet (Hilditch, 1939). However, Babatunde et al. (1968a) have demonstrated that a fat supplying almost entirely saturated fatty acids is capable of preventing skin lesions in the pig as contrasted to aggrevating them as in the rat (Evans and Lepkovsky, 1932). They presented evidence indicating a considerable net increase in linoleic acid (18:2W6,9) in porcine adipose tissue after 21 weeks on a fat-free diet. Accordingly, they claimed that linoleic acid does not appear to be a dietary requirement for young pigs. Babatunde et al. (1968b) have reported similar results.

Elliot and Bowland (1970) have suggested that the observed increase in the relative incorporation of linoleic acid into the depot triglycerides from pigs fed supplemental copper, may, in fact, be the result of an increased synthesis of a

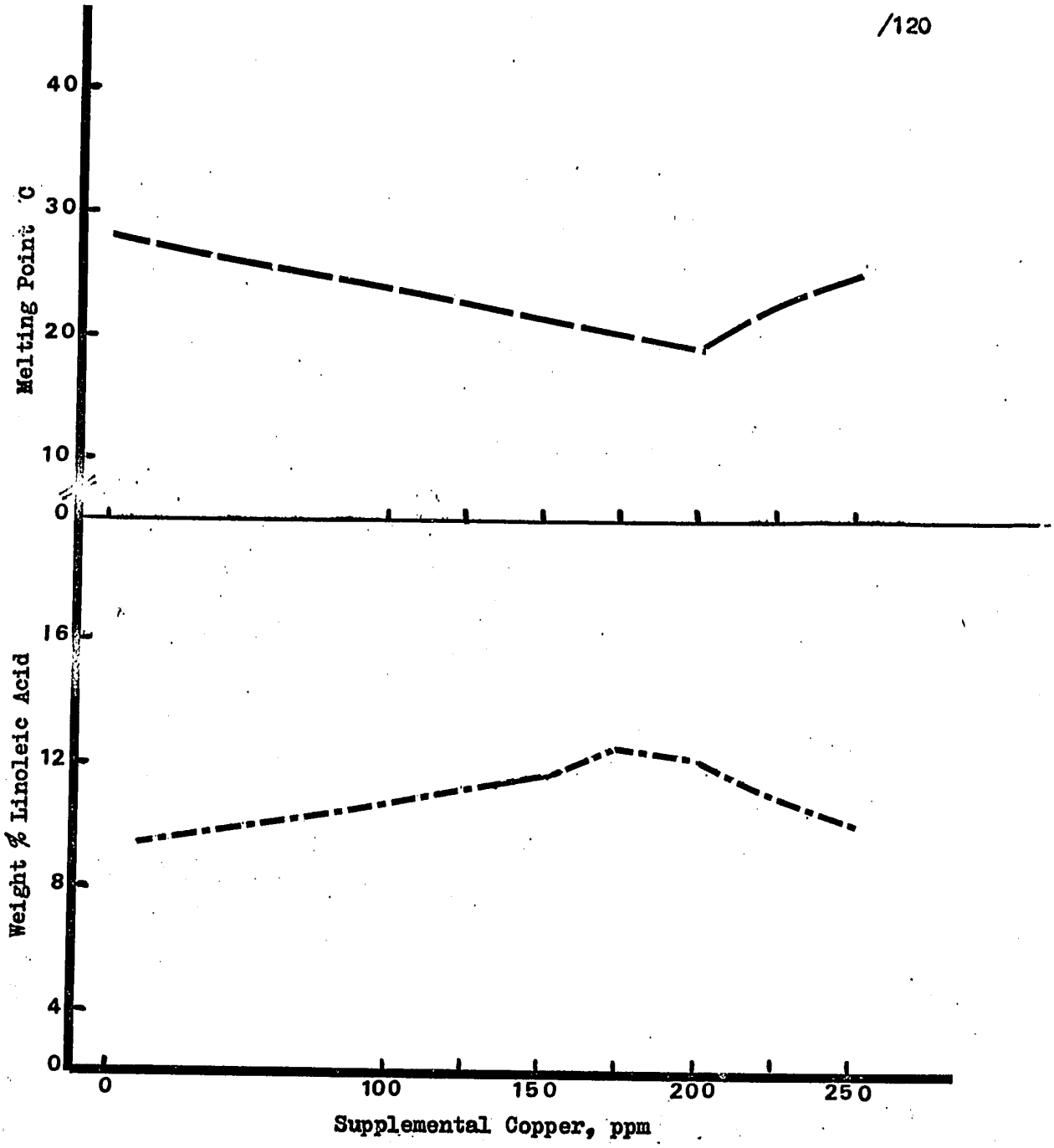



Figure 8. A comparison of melting point and level of linoleic acid present in the backfat of pigs fed various levels of copper.

di-unsaturated 18 carbon acid with its double bonds located at the 9 and 12 carbons from the methyl end of the fatty acid molecule (18:2W9,12) which the mammals can synthesize. Holloway et al. (1963) demonstrated the synthesis of this acid (18:2W9,12) by desaturation of 18:1 in rat liver cells. This acid has been separated by gas liquid chromatography as a shoulder on the 18:2W6,9 peak (Holloway et al., 1963; Walker, 1966).

This part of the investigation was, therefore, undertaken to study the relation between copper supplementation of the diet and the apparent synthesis of linoleic acid to determine if this acid, chromatographically identified as 18:2W6,9 by comparison of its retention time with that of pure methyl 18:2W6,9; might not be the positional isomer 18:2W9,12. Various chromatographic techniques were used to gain some information on the structure of the di-unsaturated 18 carbon acid present in samples from copper-fed and control pigs.

The diene fractions were isolated from the total methyl esters derived from selected samples (samples containing increased levels of linoleic acids, from the group of pigs receiving supplemental Cu continuously in the diet and slaughtered at 46 or 69 kg live-weight) by thin-layer chromatography using the $AgNO_3$-silica gel system described under General Experimental, Section E-(1). The band corresponding to the di-unsaturated fatty acid (Figure 9) were scraped from the thin-layer plates and extracted with diethyl


A
B
C

ether. In each case, the diene fraction obtained was re-chromatogrammed on $AgNO_3$-silica gel plates using benzene as a solvent to remove impurities with different degrees of unsaturation than the major fraction.

The purified diene methyl ester fraction and standard methyl 18:2W6,9 were chromatogrammed on a 15 per cent DEGS column operated isothermally at 190 C. Typical chromatograms of the diene fraction and standard 18:2W6,9 are shown in Figure 10. The retention times were identical in both cases, indicating, therefore, that the di-unsaturated 18 carbon acid in porcine depot fat whose relative concentration increased in response to copper supplementation of the diet, was, in fact, 18:2W6,9. Identification of peaks (Figure 10) was made by comparison of retention times with that of the standard, 18:2W6,9 methyl ester, and by determining separation factors as proposed by Ackman (1967) and reported in Table 15. The linoleic acid methyl esters from control or copper-fed pigs were eluted from the gas chromatograph in the same position as the standard methyl ester having identical retention times and separation factors.

Further independent evidence for the conclusion that the di-unsaturated fatty acid found in copper-fed pigs is the essential 18:2W6,9 linoleic acid was achieved by re-chromatogramming the isolated pure diene fractions as well as the standard 18:2W6,9 methyl ester on silver nitrate-silica gel plates and using a more

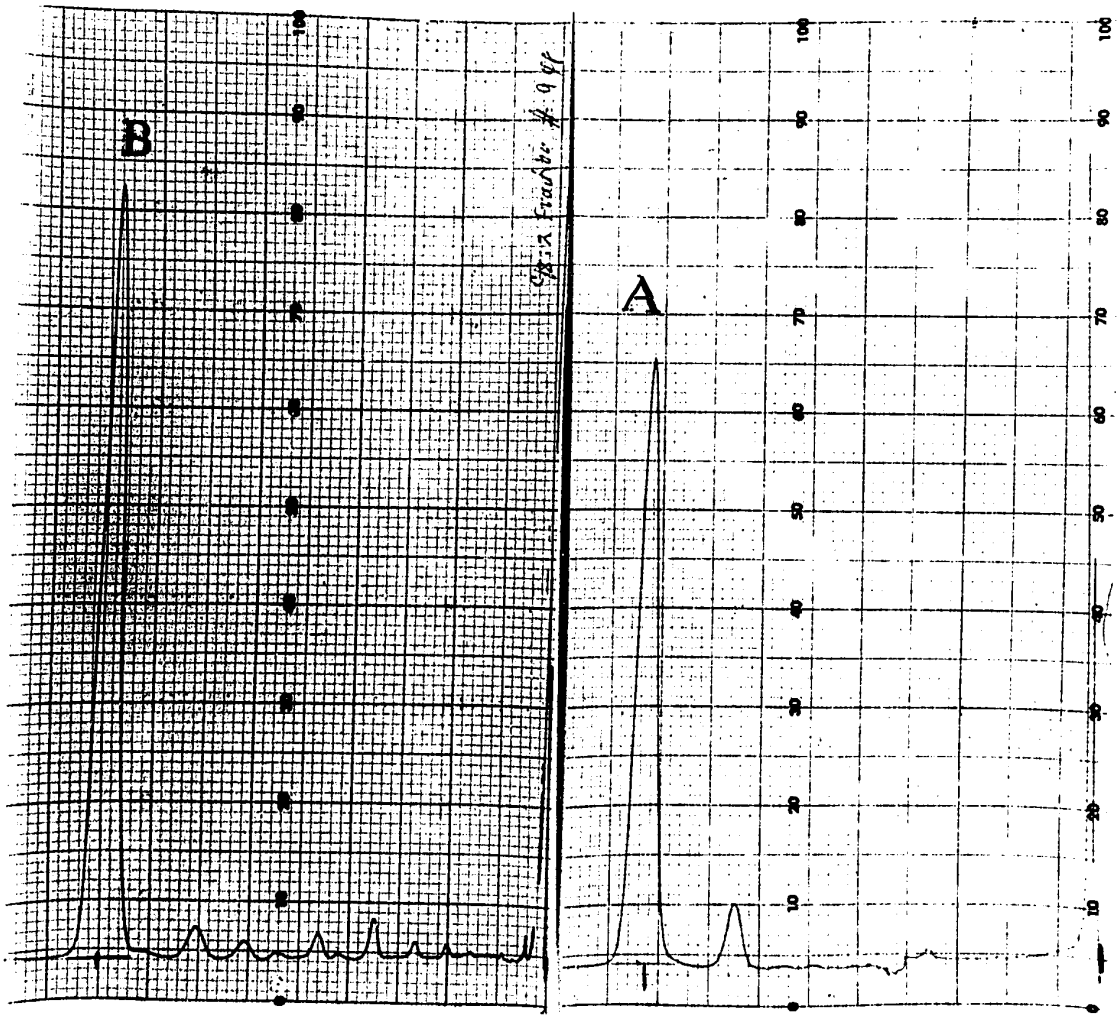



Figure 10. Typical gas chromatogram of the di-unsaturated fatty acid methyl esters:
A - Standard 18:2W6,9 methyl ester;
B - Di-unsaturated methyl ester isolated from the backfat of pigs fed 250 ppm Cu.

Table 15. Separation Factors for the Diene Fractions Isolated from Control and Copper-Fed Pigs Methyl Esters and the Standard 18:2W6,9 Methyl Ester

| Methyl Ester | RT, min. | r* 18 | r** | r*** |
|---|---|---|---|---|
| Diene from control pigs | 4.833 | 1.288 | 1.4673 | 1.0403 |
| Diene from copper-fed pigs | 4.833 | 1.288 | 1.4673 | 1.0403 |
| Standard 18:2W6,9 | 4.833 | 1.288 | 1.4673 | 1.0403 |

* $r_{18} = \frac{\text{RT of diene}}{\text{RT of 18:0}}$

** r = Separation factor relative to the 18:3W6,9,12 standard which has a $r_{18}$ value of $\frac{6.833}{3.616} = 1.890$.

*** r = Separation factor relative to the 18:2W9,12, its $r_{18}$ value as reported in the literature = 1.340.

The value 1.0403 is reported by Ackman (1964) as a separation factor for $\frac{\text{18:2W9,12}}{\text{18:2W6,9}}$ isomers.

polar developing solvent than that used in isolating the methyl esters (Morris et al., 1967). Only one distinct spot was obtained for each diene fraction having the same $R_f$ value for control, copper-fed pigs and standard 18:2W6,9 unsaturated fatty acid. The $R_f$ value was equal to 70 relative to methyl oleate (100).

In order to confirm these observations, samples of the purified diene fraction and 18:2W6,9 methyl esters were subjected to $KMnO_4$-$NaIO_4$ oxidation. The recovered oxidation products were separated on GLC using 15 per cent diethylene glycol succinate column programmed from 120 to 200 C. Identical chromatograms were obtained from the oxidation products of the diene fraction and standard 18:2W6,9. Typical chromatograms of these are shown in Figures 11 and 12, respectively.

The results, as presented, do not permit any qualitative identification of the various peaks obtained by oxidation of the 18 carbon diene fraction, however, it provides a strong evidence that the di-unsaturated fatty acid isolated from the methyl esters of fatty acids from copper-fed pigs is, in fact, 18:2W6,9.

According to these interesting observations supported by chromatographic and classical chemical analytical techniques, it is suggested that the essential fatty acid 18:2W6,9 is synthesized by the pig when supplemental copper is included in the diet. Babatunde et al. (1968a,b) have suggested that

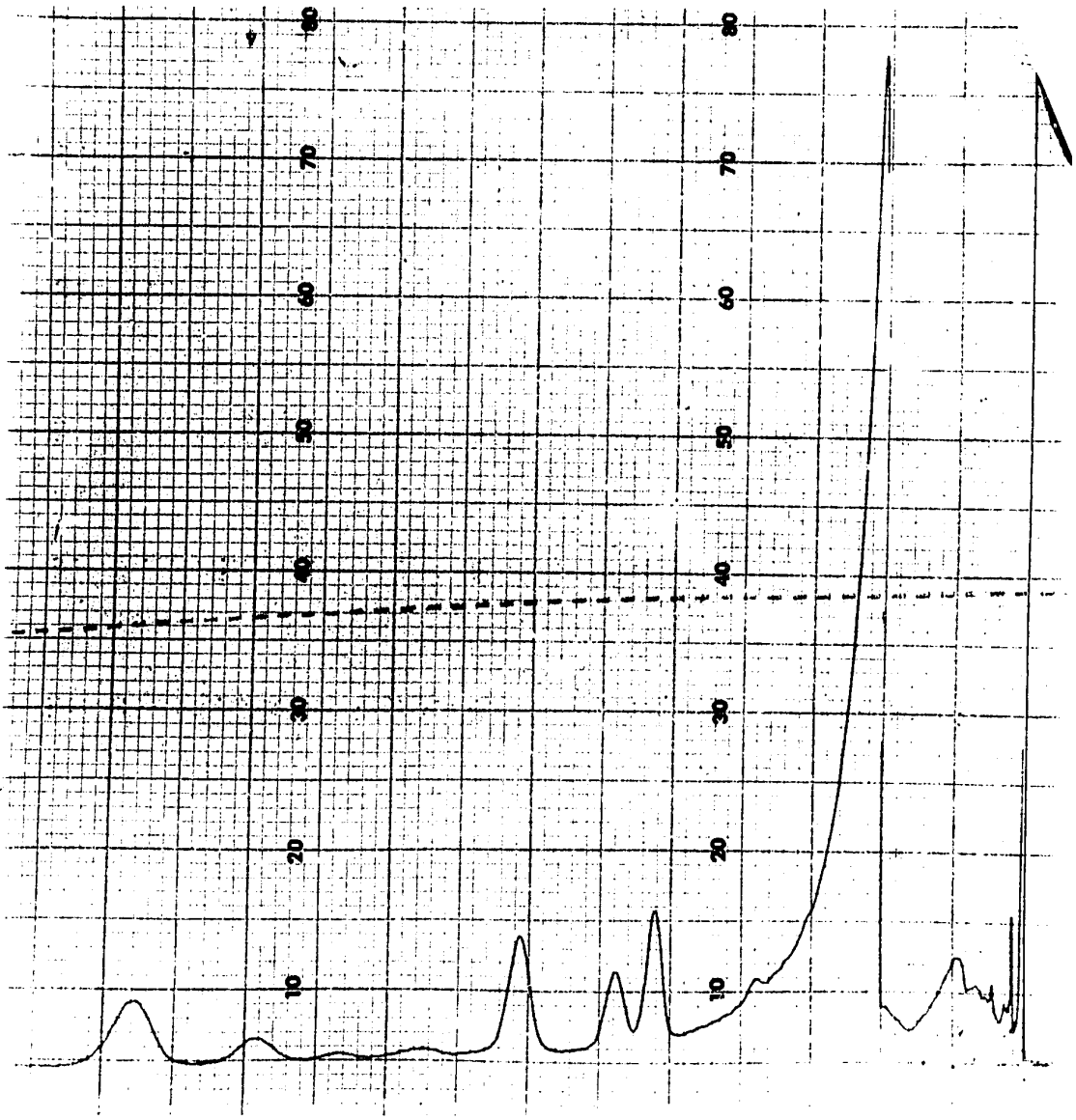
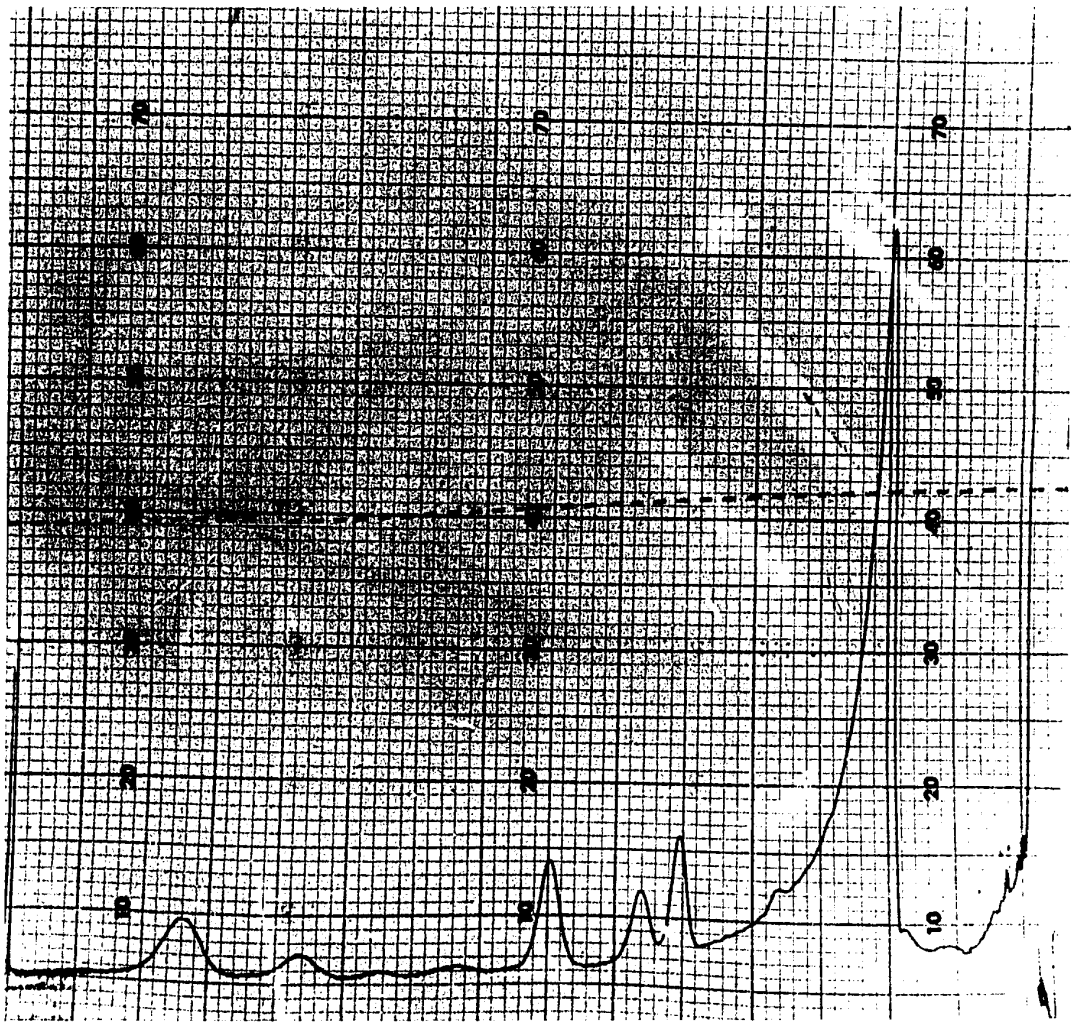



Figure 11. Typical gas chromatogram of the oxidation products obtained by the permanganate-periodate oxidation of the diene fraction isolated from the backfat methyl esters of a pig fed 250 ppm Cu.



pigs can synthesize linoleic acid. Klenk (1965) have noted that the rat is able to convert hexadeca 7,10- dienoate (16:2W6,9) to linoleic acid and longer chain polyunsaturated fatty acids of the W-6 family.

It appears, therefore, that the hypothesis of Elliot and Bowland (1970) is not valid under the present experimental conditions. The data herein is suggestive of a role for copper in the biosynthesis of unsaturated fatty acids but this aspect requires further experimentation.

## SUMMARY

No significant differences were observed in average daily gain of pigs fed 7 levels of supplemental copper (0, 125, 150, 175, 200, 225 and 250 ppm). The results showed that levels of copper below 150 ppm or above 200 ppm slightly depress the overall (male + female) average daily gain.

The amount of feed required per unit of gain was decreased by copper supplementation of the diet at 125 and 200 ppm (-9.12% vs. the control). This improvement in FC was less pronounced at the other copper levels studied.

Pigs receiving supplemental copper had a significantly higher level of copper stored in the liver. There was a relationship between dietary copper level and liver accumulation. No significant differences ($P < 0.05$) were found with respect to sex with gilts accumulating a higher level of copper in their livers than barrows.

Statistical analysis of the data for the individual saturated and unsaturated fatty acids of the backfat showed significant difference due to the level of copper in the diet. No significant differences attributable to sex were observed in control and copper-fed pigs. It appeared, however, that barrows deposited slightly more linoleic acid than gilts. The significant

increase in unsaturation of the backfat as a result of copper supplementation of the diet resulted in a significant ($P < 0.01$) reduction in melting point.

Evidence regarding the synthesis of linoleic acid (18:2W6,9) by pigs is provided.

## EXPERIMENT III.

### A. Introduction

There are two recognized factors governing the resistance of natural fats to rancidification; content of various unsaturated fatty acids and content of natural antioxidants.

In Experiments I and II, it has been established that the backfat of pigs fed diets supplemented with various levels of copper was more unsaturated than the backfat of control animals. Under normal conditions, the stability of pork fat relative to other animal fats is known to be poor (Bailey, 1964). This has been attributed, in part, to its relatively higher content of UFA and, in part, to its lower content of natural antioxidants. Any dietary treatment which further increases the degree of pork fat unsaturation could, therefore, result in further deterioration in stability.

Rancidity may be prevented in rendered lard by the incorporation of antioxidants after rendering (Lundberg et al., 1944), but such incorporation in pork that is to be preserved by curing, freezing, etc., is physically impractical. In such situations, antioxidants must be introduced by dietary means if possible. Tocopherols are apparently the only antioxidants of a large number investigated which are capable of being stored in animal fat (Hanson et al., 1944).

B. Preliminary Investigation

(a) Experimental

Backfat samples derived in Experiment I which had been stored under nitrogen at -20 C were used to determine the effect of supplemental dietary copper on the oxidative stability of pork fat.

The samples were extracted as described under General Experimental, Section C-2 (a) and subjected to the following analysis:

(i) Oxygen absorption by the absorption method described under General Experimental, Section F-1.

(ii) Peroxide value by the active oxygen method (AOM) as described under General Experimental, Section F-2.

(b) Results and Discussion

As discussed previously (Experiments I and II), feeding supplemental copper to swine resulted in a backfat containing increased proportions of the UFA (16:1, 18:1 and 18:2) and decreased proportions of the SFA (16:0 and 18:0) in comparison with the backfat of control pigs (Tables 5, 13 and 14). Fats containing relatively high proportions of UFA are subject to autoxidation, the end products of which result in undesirable odors and flavours in the fat. Accordingly, the present study was made to elucidate the relative importance of the change in fatty acid composition resulting from the feeding of supplemental copper on the oxidative susceptibility of the fat.

Stability may be defined as the resistance to auto-oxidation under prescribed conditions of aging and is measured in units of time required for the product to (a) acquire a state of oxidation which correlates with organoleptic detection of rancid odor and flavour, or (b) to reach the end of the induction period if oxygen absorption measurements or peroxide analysis are used. Under usual conditions of storage, this time is too long for practical evaluation, therefore, it is necessary to employ accelerated oxidation tests.

When the autoxidation of the fat from copper-fed and control pigs was studied, either by measuring the amount of oxygen absorbed (Table 16) or by determining the peroxide value (Table 17), it was found that the course of oxidation exhibited two distinct phases (Figures 13 and 14). During the initial phase, oxidation proceeded at a relatively slow rate, then, after a certain critical amount of oxidation had occurred, the reaction entered a second phase characterized by a rapidly accelerating rate of oxidation, and an eventual rate many times greater than that observed in the initial phase. The initial period of relatively slow oxidation of a fat is termed "induction period" (Lundberg, 1954).

Fats from copper-fed and control pigs differed significantly ($P < 0.01$) in the manner in which their oxidation proceeded. The more unsaturated fat (from copper-fed pigs) was

Table 16. Effect of Copper Supplementation of Swine Diets on Oxidative Stability of Pork Fat at 60 C

| Diet | kg Weight at Slaughter | µl $O_2$ Uptake Per mg of Fat After: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hr | 3 hr | 6 hr | 9 hr | 12 hr | 18 hr |
| Control | 23 | 0.006 | 0.009 | 0.016 | 0.027 | 0.046 | 0.061 |
| Copper | 23 | 0.015 | 0.024 | 0.038 | 0.049** | 0.081** | 0.108** |
| Control | 46 | 0.008 | 0.015 | 0.024 | 0.033 | 0.054 | 0.083 |
| Copper | 46 | 0.014 | 0.031 | 0.044 | 0.058** | 0.176** | 0.219** |
| Control | 69 | 0.007 | 0.014 | 0.018 | 0.023 | 0.044 | 0.054 |
| Copper | 69 | 0.018 | 0.030 | 0.054 | 0.074** | 0.130** | 0.252** |
| Control | 92 | 0.009 | 0.018 | 0.026 | 0.033 | 0.048 | 0.060 |
| Copper | 92 | 0.010 | 0.018 | 0.026 | 0.036** | 0.072** | 0.140** |

** Significant ($P < 0.01$)

Table 17. Effect of Copper Supplementation of Swine Diets on Oxidative Changes in the Backfat at 60 C

| Diet | kg Weight at Slaughter | Peroxide Value[1] After: 1 hr | 3 hr | 6 hr | 9 hr | 12 hr | 18 hr |
|---|---|---|---|---|---|---|---|
| Control | 23 | 1.07 | 1.85 | 3.10 | 5.30 | 9.25 | 12.25 |
| Copper | 23 | 3.15 | 4.90 | 7.70 | 11.85 | 16.35 | 21.40** |
| Control | 46 | 1.75 | 2.90 | 5.30 | 8.95 | 10.90 | 16.60 |
| Copper | 46 | 2.60 | 6.25 | 8.70 | 11.60 | 35.25 | 43.95** |
| Control | 69 | 1.45 | 2.85 | 3.55 | 4.65 | 8.85 | 10.85 |
| Copper | 69 | 3.70 | 5.50 | 10.45 | 14.95 | 26.05 | 51.20** |
| Control | 92 | 1.80 | 3.10 | 5.20 | 6.70 | 9.65 | 12.10 |
| Copper | 92 | 2.00 | 3.60 | 5.25 | 7.20 | 14.40 | 25.18** |

[1] meq/mg of fat.

** Significant ($P < 0.01$)

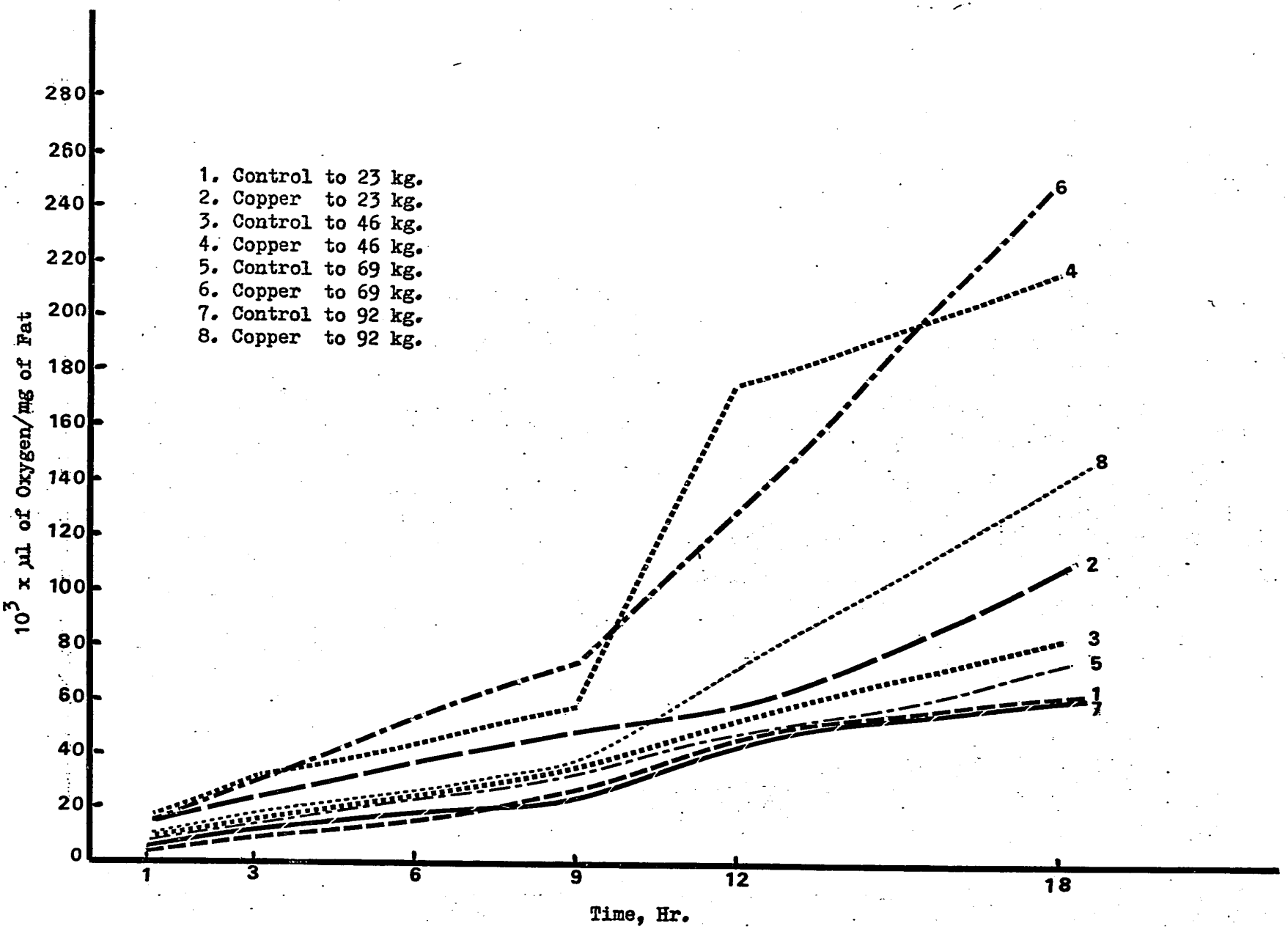



Figure 13. Effect of 250 ppm supplemental dietary copper on oxygen absorption by the porcine backfat.

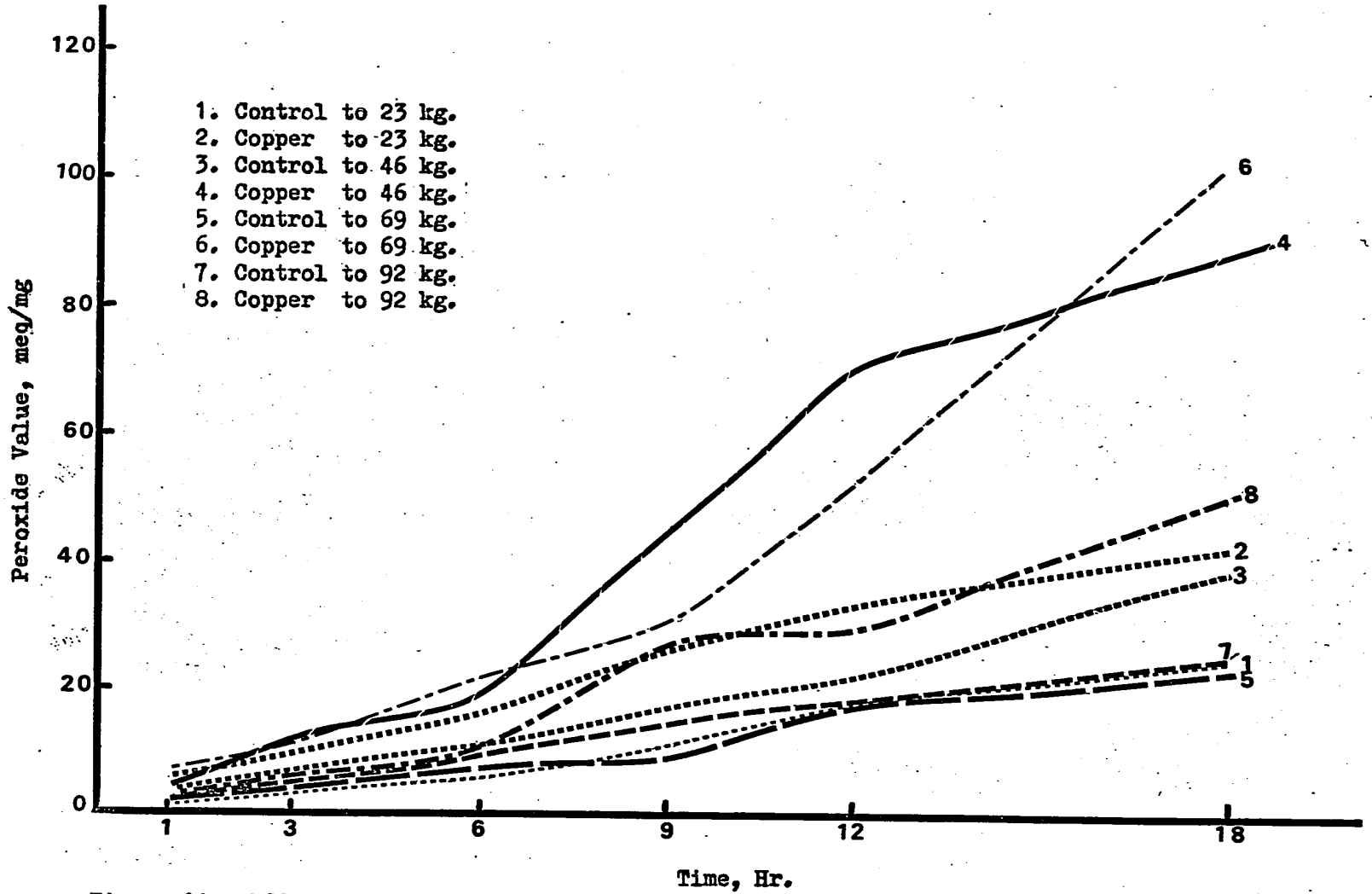



Figure 14. Effect of 250 ppm supplemental dietary copper on the peroxide value of the porcine backfat.

more rapidly oxidized than the less unsaturated fat (from control pigs). Under test conditions that accelerated rate of oxidation, the fat from copper-fed pigs, especially groups slaughtered at 46 or 69 kg liveweight, absorbed signifcantly ($P < 0.01$) more oxygen and developed significantly ($P < 0.01$) higher peroxide values than the fat from control pigs.

Fat high in linoleic acid (copper-fed pigs) became rancid after the absorption of less oxygen than fat low in linoleic acid (control pigs). The peroxide value of the fat tended to increase as the linoleic acid content of the fat increased. The stability of a fat was shown to be a function of the polyethenoid (methylene interrupted type) acid content rather than the iodine number (Bailey, 1964). The readily formed peroxides of linoleic acid catalyze the autoxidation of 18:1 and 16:1 acids, and thus contribute to poor stability in more highly unsaturated fat.

Samples from pigs receiving the supplemental copper up to 23, 46 or 69 kg, then continued on the basal diet and slaughtered at 92 kg liveweight, displayed a rate of oxidation similar to that of the control samples (Table 18). This is probably due to the decreased proportions of palmitoleic, oleic and linoleic acid in these samples as a result of removal of supplemental copper from the diet (Table 6 ). This decrease in the relative proportion of the UFA would decrease the susceptibility of the fat to oxidation. The data in Tables 16, 17 and 18 show that the samples

Table 18. Effect of Intermittent Addition of Copper to Swine Diets on Oxidative Stability of Pork Fat at 60 C

| Diet | Weight at Cu Removal (kg) | µl $O_2$ Uptake per mg of Fat After: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 hr | 3 hr | 6 hr | 9 hr | 12 hr | 18 hr |
| Control[1] | | .009 | .0184 | .0623 | .0330 | .0480 | .0605 |
| Copper | 23[1] | .0095 | .0165 | .0223 | .0290 | .0480 | .0635 |
| Copper | 46[1] | .0097 | .0184 | .0250 | .0442 | .0535 | .0715 |
| Copper | 69[1] | .0093 | .0145 | .0200 | .0270 | .0705 | .0784 |

[1] Samples obtained at slaughter, 92 kg

from pigs which received the supplemental copper for a limited period of time were significantly ($P < 0.01$) more resistant to oxidation than the samples from pigs fed copper continuously.

On the basis of these results, Experiment III was conducted to study the effect of supplemental dietary copper plus supplemental dl-$\alpha$-tocopherol (vitamin E) on the fatty acid composition and oxidative stability of porcine depot fat.

C. Experiment III Experimental

Animals

Forty crossbred pigs averaging 14.5 kg bodyweight at the commencement of the experiment were assigned at random to 5 dietary treatments with 8 pigs (4 males and 4 females) in each treatment. The composition of diets is given in Table 19. Since 200 ppm of supplemental copper in Experiment II was more effective in improving ADG and FC than other levels studied, this level was used in Experiment III. The treatments in this experiment consisted of: Treatment 1, basal; Treatment 2, basal plus 200 ppm copper; Treatment 3, basal plus 200 ppm copper plus 22 I.U./kg vitamin E; Treatment 4, basal plus 200 ppm copper plus 44 I.U./kg vitamin E and Treatment 5, basal plus 200 ppm copper plus 88 I.U./kg vitamin E. The basal diet contained 1.4 I.U./kg vitamin E.

Feed and water were allowed ad lib throughout the experimental period. The pigs were weighed biweekly until market

Table 19. Composition of Diets for Experiment III.

| Ingredients | Basal % | Basal plus Copper % | Basal plus Copper plus Vitamin E % |
|---|---|---|---|
| Barley | 46.16 | 46.16 | 46.16 |
| Wheat | 11.48 | 11.48 | 11.48 |
| Wheat shorts | 23.70 | 23.70 | 23.70 |
| Malt sprouts | 6.98 | 6.98 | 6.98 |
| Soybean meal 49% | 4.99 | 4.99 | 4.99 |
| Alfalfa dehydrated 17% | 0.50 | 0.50 | 0.50 |
| Animal fat | 0.50 | 0.50 | 0.50 |
| Molasses cane | 2.50 | 2.50 | 2.50 |
| Calcium carbonate | 2.05 | 2.05 | 2.05 |
| Calcium phosphate | 0.20 | 0.20 | 0.20 |
| Salt | 0.50 | 0.50 | 0.50 |
| Trace mineral mix[1] | 0.05 | 0.05 | 0.05 |
| Vitamin premix[2] | 0.25 | 0.25 | 0.25 |
| Methionine | 0.05 | 0.05 | 0.05 |
| Arsanilic acid 20% | 0.05 | 0.05 | 0.05 |
| Bacitracin 10 | 0.05 | 0.05 | 0.05 |
| Copper sulfate[3] | - | + | + |
| Vitamin E[4] | - | - | + |

[1] Trace mineral mix to supply: 60 ppm Mn; 60 ppm Zn; 25 ppm Fe; 10 ppm Cu; 125 ppm I and 1 ppm Co.

[2] From the vitamin premix: 2280.0 I.U./kg vitamin A; 570.0 I.U./kg vitamin $D_3$; 1.4 I.U./kg vitamin E; 2.1 mg/kg Roboflavin; 5.7 mg/kg Niacin; 5.13 mg/kg d-calcium pantoghenate; 17.10 mg/kg choline chloride and 11.4 mcg vitamin $B_{12}$

[3] To supply: 0.08% $CuSO_4 \cdot 5H_2O$

[4] To supply: 22, 44 or 88 I.U./kg vitamin E

weight was approached and weekly thereafter. Individual pigs were removed from the experiment and slaughtered as their weight on weigh-day reached approximately 92 kg.

Samples of the backfat and liver were obtained at the time of slaughter as described under General Experimental, Section B-2 and B-3. The samples were prepared for analysis, analyzed and results calculated as described in Section C-1, C-2 (a), (b); F-1 and F-3.

D. Results and Discussion

(a) Average Daily Gain, Feed Conversion and Liver Copper

The results for ADG, FC and liver copper concentration are presented in Table 20. Average daily gain was improved +5.6, +6.5 and +6.9 per cent when 200 ppm of supplemental copper or 200 ppm copper plus 22 I.U. or 44 I.U./kg of vitamin E was added to the basal diet. With the diet containing 200 ppm copper plus 88 I.U./kg of vitamin E, a reduction of -0.7 per cent in ADG relative to the control pigs was observed. None of the observed differences were statistically significant.

The beneficial effect of 200 ppm supplemental copper in the diet on ADG reported in this experiment was higher by approximately 4 per cent than the response reported in Experiment II.

Table 20. Effect of 200 ppm of Supplemental Dietary Copper and Three Levels of dl-α-Tocopherol on Average Daily Gain, Feed Conversion and Liver Copper Content

| Treatment | | Control | 200 ppm Cu | 200 ppm Cu + 22 I.U. Vit. E | 200 ppm Cu + 44 I.U. Vit. E | 200 ppm Cu + 88 I.U. Vit.E |
|---|---|---|---|---|---|---|
| No. of pigs[1] per pen | | 8 | 8 | 8 | 8 | 8 |
| ADG, gm | (a) | 700 | 749 | 768 | 747 | 676 |
| | (b) | 644 | 671 | 664 | 691 | 657 |
| Average | | 672 | 710 | 716 | 719 | 667 |
| FC | | 3.35 | 3.07 | 3.17 | 3.15 | 3.34 |
| Liver Cu, ppm | (a) | 11.88 | 138.48 | 136.40 | 128.63 | 137.73 |
| | (b) | 12.23 | 125.93 | 134.55 | 124.78 | 126.35 |
| Average | | 12.05 | 132.20** | 135.48** | 126.70** | 132.04** |

[1] 4 males and 4 females in each treatment

(a) male pigs

(b) female pigs

** Significant ($P < 0.01$)

Responses in ADG obtained with vitamin E in the presence of copper indicate that there may be a slight extra benefit when 22 and 44 I.U./kg of vitamin E are included with 200 ppm of supplemental copper in the swine diet. The magnitude of the effect was too small to be of great practical importance. The NCR - 42 Committee on Swine Nutrition (1970) reported that there was a growth response to copper but not to vitamin E and they concluded that copper, not vitamin E, functioned in stimulating appetite and rate but not efficiency of gain. Hays, Cromwell and Overfield (1971) studied the effect of supplemental copper plus vitamin E on response of pigs fed corn and wheat-base diets. In the first forty-two days, they found a significant ($P<0.05$) linear increase in the rate of gain with increasing copper level (0, 62, 125, 188 and 250 ppm) with or without 22 I.U./kg vitamin E. They concluded that vitamin E had no significant effect on ADG but did significantly improve feed conversion. For the entire period, neither copper nor vitamin E in their experiments had a significant effect on ADG, F/G or Hb levels. Hvidsten and Astrup (1963) obtained slight improvement in ADG by addition of supplemental vitamin E to swine diets either throughout the whole growing-finishing phase or during the finishing period only.

While the evidence would indicate that some improvement occurred when 200 ppm of supplemental copper was fed in combination with 22 or 44 I.U./kg of vitamin E, it should be pointed out that

the performance of pigs receiving diets supplemented with 200 ppm copper plus 88 I.U./kg of vitamin E was poorer (-0.7% reduction in ADG) as compared with control pigs. There is no evidence in the literature to support or to contradict this observation.

Pigs fed the diet supplemented with copper or copper plus vitamin E required less feed per unit of gain than pigs receiving the control diet (Table 20). Efficiency of feed utilization was improved by +8.4, 5.4, 6.0 and 0.3 per cent when 200 ppm copper or 200 ppm copper plus 22, 44 or 88 I.U./kg of vitamin E, respectively, were added to pig diets.

Liver copper concentrations of pigs fed the diet supplemented with copper or copper plus vitamin E were significantly ($P < 0.01$) different than liver copper concentrations of pigs receiving the basal diet. Barrows accumulated higher copper in their livers than did gilts although these differences were not statistically significant. This suggests that the addition of vitamin E to swine diets has no significant effect on the accumulation of copper in the liver.

(b) Fatty Acid Composition and Melting Point

Supplementation of the diet with 200 ppm copper or 200 ppm copper plus 22 or 44 I.U./kg of vitamin E of diet fed to swine from 14.5 kg until slaughter at 92 kg significantly ($P < 0.05$) increased the percentage UFA in swine depot fat and significantly

($P < 0.05$) decreased the percentage SFA (Table 21). These changes were most marked in the case of fat from pigs receiving 200 ppm of copper alone. In the case of the vitamin E supplemented animals, there was a marked decrease in the percentage UFA as the level of vitamin E in the diet increased. In fact, the highest level of vitamin E (88 I.U./kg) significantly ($P < 0.01$) lowered the percentage UFA in comparison with fat from control animals (Figure 15).

There was also a significant ($P < 0.05$) reduction in the melting point of the backfat of pigs fed supplemental copper or copper plus 22 or 44 I.U./kg of vitamin E as compared with control animals. Melting point of fat from pigs fed 200 ppm of supplemental copper plus 88 I.U./kg of vitamin E was significantly ($P < 0.01$) lower than the fat from control animals. As established in Experiment I, as well as by others (Elliot and Bowland, 1969), the sum of the percentage UFA can be used as a reliable indicator of fat softness.

With regard to sex, no significant differences were observed between percentage UFA of the backfat from barrows and gilts when supplemental copper or copper plus vitamin E were present in the diet. In the control group, gilts accumulated significantly higher ($P < 0.01$) UFA and significantly lower ($P < 0.01$) SFA values than barrows. This observation is in contradiction to results reported in Experiment II where barrows accumulated slightly higher UFA than gilts. Friend and Cunningham (1967) and Elliot and Bowland (1970) reported that gilts accumulated significantly ($P < 0.01$)

Table 21. Effect of Supplemental 200 ppm Copper and Three Levels of dl-α-Tocopherol to Swine Diets on Melting Point and Weight % of the Saturated and Unsaturated Fatty Acids

| Treatment | | Melting Point C | % Saturated Fatty Acids 14:0 | 16:0 | 18:0 | 20:0 | Total |
|---|---|---|---|---|---|---|---|
| Control | (a) | 31.0 | 1.23 | 26.64 | 12.31 | 0.64 | 40.82 |
| | (b) | 24.8 | 1.47 | 22.49 | 9.53 | 0.46 | 33.95** |
| | (Avg) | 27.9[a] | 1.35 | 24.57 | 10.92 | 0.55 | 37.39[a] |
| 200 ppm Cu | (a) | 19.8 | 1.52 | 19.52 | 8.30 | 0.60 | 29.94 |
| | (b) | 22.5 | 1.68 | 21.05 | 8.29 | 0.53 | 31.55 |
| | (Avg) | 21.1[b] | 1.60 | 20.29 | 8.29 | 0.57 | 30.75[b] |
| 200 ppm Cu + 22 I.U./kg Vit. E | (a) | 22.1 | 1.52 | 23.51 | 8.42 | 0.67 | 34.12 |
| | (b) | 23.1 | 1.68 | 22.40 | 9.43 | 0.61 | 34.12 |
| | (Avg) | 22.6[b] | 1.60 | 22.95 | 8.93 | 0.64 | 34.12[b] |
| 200 ppm Cu + 44 I.U./kg Vit. E | (a) | 23.2 | 1.27 | 23.11 | 10.56 | 0.52 | 35.46 |
| | (b) | 22.5 | 1.41 | 22.48 | 9.94 | 0.61 | 34.44 |
| | (Avg) | 22.8[b] | 1.34 | 22.78 | 10.26 | 0.57 | 34.95[b] |
| 200 ppm Cu + 88 I.U./kg Vit. E | (a) | 29.6 | 1.42 | 27.05 | 11.45 | 0.89 | 40.61 |
| | (b) | 30.6 | 1.23 | 27.57 | 12.92 | 0.71 | 42.43 |
| | (Avg) | 30.1[c] | 1.34 | 27.31 | 12.18 | 0.70 | 41.53[c] |

| Treatment | | % Unsaturated Fatty Acids 16:1 | 18:1 | 18:2 | 18:3 | 20:4 | Total |
|---|---|---|---|---|---|---|---|
| Control | (a) | 4.63 | 45.74 | 8.28 | 0.15 | 0.38 | 59.18 |
| | (b) | 4.87 | 50.04 | 10.64 | 0.23 | 0.27 | 66.05** |
| | (Avg) | 4.75 | 47.89 | 9.46 | 0.19 | 0.32 | 62.61[a] |
| 200 ppm Cu | (a) | 6.41 | 49.81 | 13.19 | 0.29 | 0.36 | 70.06 |
| | (b) | 7.14 | 50.41 | 10.34 | 0.23 | 0.33 | 68.45 |
| | (Avg) | 6.78 | 50.12 | 11.76 | 0.26 | 0.34 | 69.26[b] |
| 200 ppm Cu + 22 I.U./kg Vit. E | (a) | 6.02 | 48.27 | 11.08 | 0.21 | 0.30 | 65.88 |
| | (b) | 6.29 | 47.96 | 11.12 | 0.22 | 0.29 | 65.88 |
| | (Avg) | 6.16 | 48.12 | 11.10 | 0.21 | 0.30 | 65.88[b] |
| 200 ppm Cu + 44 I.U./kg Vit. E | (a) | 7.21 | 46.68 | 11.04 | 0.23 | 0.38 | 65.54 |
| | (b) | 5.68 | 47.54 | 11.84 | 0.21 | 0.29 | 65.56 |
| | (Avg) | 6.45 | 47.11 | 11.44 | 0.72 | 0.34 | 65.55[b] |
| 200 ppm Cu + 88 I.U./kg Vit. E | (a) | 4.72 | 45.55 | 8.71 | 0.14 | 0.27 | 59.39 |
| | (b) | 4.65 | 43.55 | 8.91 | 0.15 | 0.31 | 57.57 |
| | (Avg) | 4.68 | 44.57 | 8.12 | 0.15 | 0.29 | 58.81[c] |

(a) = male pigs, (b) = female pigs

a,b,c = means followed by the same superscript are not significantly ($P < 0.05$) different

** Significant ($P < 0.01$)

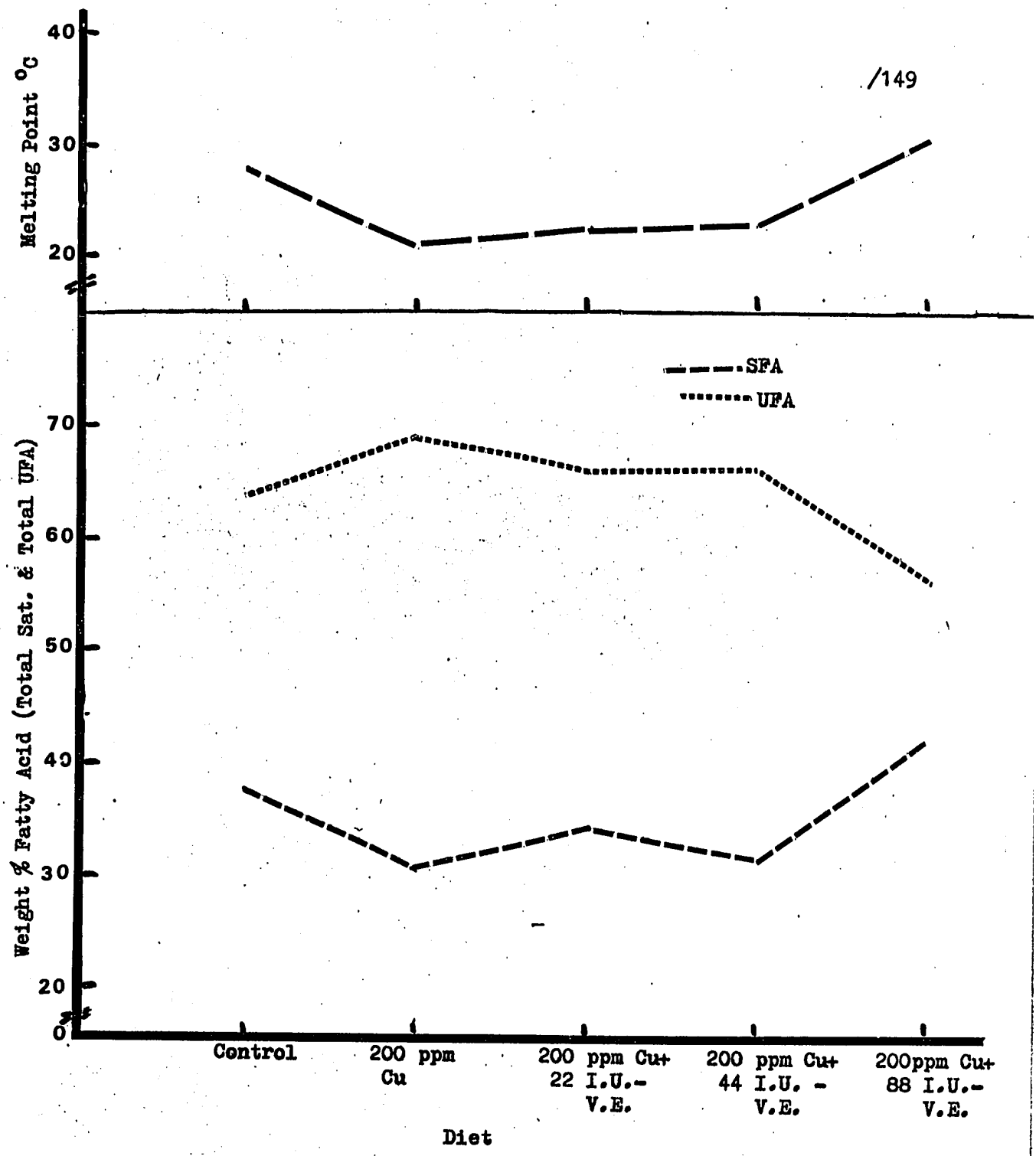



Figure 15. A comparison of melting point and levels of saturated and unsaturated fatty acids of backfat from pigs fed supplemental 200 ppm copper plus three levels of dl-$\alpha$-tocopherol.

higher UFA than barrows. Babatunde et al. (1967) found no significant differences in fatty acid composition of backfat samples from barrows and gilts.

In addition, the 18:2 values indicated a net synthesis of this acid in response to supplemental copper in the diet which would further indicate the ability of the pig to synthesize fatty acids containing more than one double bond when copper is supplemented in the diet. Fat from pigs fed the copper supplement plus the highest level of tocopherol had an average linoleic acid content of about 8.12 per cent which was significantly ($P < 0.01$) lower than linoleic acid content of fat from only copper-fed pigs. The results indicated that control fat contained a higher amount of linoleic acid than fat from pigs fed copper plus 88 I.U./kg of vitamin E.

Bratzler et al. (1950) reported that supplements of mixed tocopherols caused an increase in the unsaturation (iodine value) of the depot fat of pigs fed a low fat, tocopherol deficient ration. This conclusion, as presented in their work, is based on the average iodine and thiocyanogen values of 5 fats from different sites in the body of each animal, namely leaf (omental), ruffle (mesenteric), jowl (cheek), back and ham-facing fats. On this basis, no conclusion regarding the effect of dietary tocopherols on the unsaturation of fatty acids can be drawn.

Hove and Seibold (1955) found that vitamin E supplementation of the diet led to an increase in liver fat from 1.6 to 2.3 per cent, expressed on a wet-weight basis. Although the iodine values of the liver fats did not differ appreciably, the proportions of pentaethenoid and diethenoid acids were increased (mainly at the expense of the oleic and triethenoid acids) in the fat from vitamin E deficient pigs compared with the fat from livers of pigs that had been given the dietary supplement of vitamin E.

One might suggest, therefore, that tocopherols may be concerned in the synthesis of palmitoleic, oleic and linoleic acid although the studies reported here do not provide any insight into the mode of action of tocopherols in such syntheses.

(c) The Role of Vitamin E in the Comparitive Stability of the Fat

In this study, the stability of the backfat was shown to be a function of the UFA content and the vitamin E level in the swine diet (Tables 22 and 23). The progress of oxidative rancidity expressed as µl of oxygen ($O_2$) uptake per mg of fat is illustrated in Figure 16. In general, the amount of $O_2$ absorbed increased at different rates with increase in the period of test. In all cases, there was a period of low and relatively uniform $O_2$ uptake (induction period) which ended in a sudden and rapidly accelerated increase in the oxidation rate (Figure 16). The rise was sufficiently sharper in fat samples obtained from pigs receiving the 200 ppm of

Table 22. Oxygen Absorption by Fat Samples Taken from Pigs Which Received 200 ppm of Supplemental Copper and Three Levels of dl-∝-Tocopherol in Their Diet

| Treatment | | μl $O_2$ per mg of Fat After: 1 hr | 3 hr | 6 hr | 12 hr | 18 hr | 24 hr |
|---|---|---|---|---|---|---|---|
| Control | (a) | 0.003 | 0.005 | 0.014 | 0.027 | 0.037 | 0.043 |
| | (b) | 0.005 | 0.007 | 0.013 | 0.025 | 0.049 | 0.057 |
| Average | | 0.004 | 0.006 | 0.014 | 0.026 | 0.043 | 0.050[a] |
| 200 ppm Cu | (a) | 0.009 | 0.019 | 0.040 | 0.093 | 0.162 | 0.196 |
| | (b) | 0.006 | 0.013 | 0.025 | 0.036 | 0.057 | 0.077 |
| Average | | 0.007 | 0.016 | 0.032 | 0.064 | 0.109 | 0.136[b] |
| 200 ppm Cu+22 I.U.Vit.E | (a) | 0.003 | 0.045 | 0.025 | 0.065 | 0.086 | 0.145 |
| | (b) | 0.003 | 0.007 | 0.020 | 0.043 | 0.054 | 0.071 |
| Average | | 0.003 | 0.018 | 0.022 | 0.054 | 0.073 | 0.108[c] |
| 200 ppm Cu+44 I.U.Vit.E | (a) | 0.004 | 0.026 | 0.038 | 0.074 | 0.092 | 0.143 |
| | (b) | 0.005 | 0.010 | 0.028 | 0.058 | 0.080 | 0.098 |
| Average | | 0.005 | 0.018 | 0.033 | 0.066 | 0.086 | 0.121[c] |
| 200 ppm Cu+88 I.U.Vit.E | (a) | 0.002 | 0.005 | 0.008 | 0.027 | 0.041 | 0.068 |
| | (b) | 0.001 | 0.004 | 0.007 | 0.010 | 0.021 | 0.081 |
| Average | | 0.002 | 0.004 | 0.008 | 0.019 | 0.031 | 0.075[d] |

(a) = male pigs
(b) = female pigs
a,b,c,d = means followed by the same superscript are not significantly ($P < 0.05$) different

Table 23. TBA Values for Fat Samples Taken from Pigs which Received 200 ppm of Supplemental Copper and Three Levels of dl-α-Tocopherol in their Diet

| Treatment | | TBA Value at 37 C for 15 hrs | TBA Value at -20 C for 30 days |
|---|---|---|---|
| Control | (a) | 8.23 | 10.33 |
| | (b) | 9.15 | 11.43 |
| Average | | 8.69[a] | 10.88[a] |
| 200 ppm Cu | (a) | 15.73 | 21.13 |
| | (b) | 10.43 | 14.45 |
| Average | | 13.08[b] | 17.79[b] |
| 200 ppm Cu+22 I.U. Vit. E | (a) | 13.55 | 15.28 |
| | (b) | 9.58 | 12.48 |
| Average | | 11.56[c] | 13.88[c] |
| 200 ppm Cu+44 I.U. Vit. E | (a) | 11.90 | 13.40 |
| | (b) | 10.53 | 12.58 |
| Average | | 11.21[d] | 12.79[c] |
| 200 ppm Cu+88 I.U. Vit. E | (a) | 7.78 | 9.78 |
| | (b) | 9.75 | 11.65 |
| Average | | 8.76[a] | 10.71[a] |

(a) = male pigs
(b) = female pigs

a,b,c,d = means followed by the same superscript are not significantly ($P < 0.05$) different

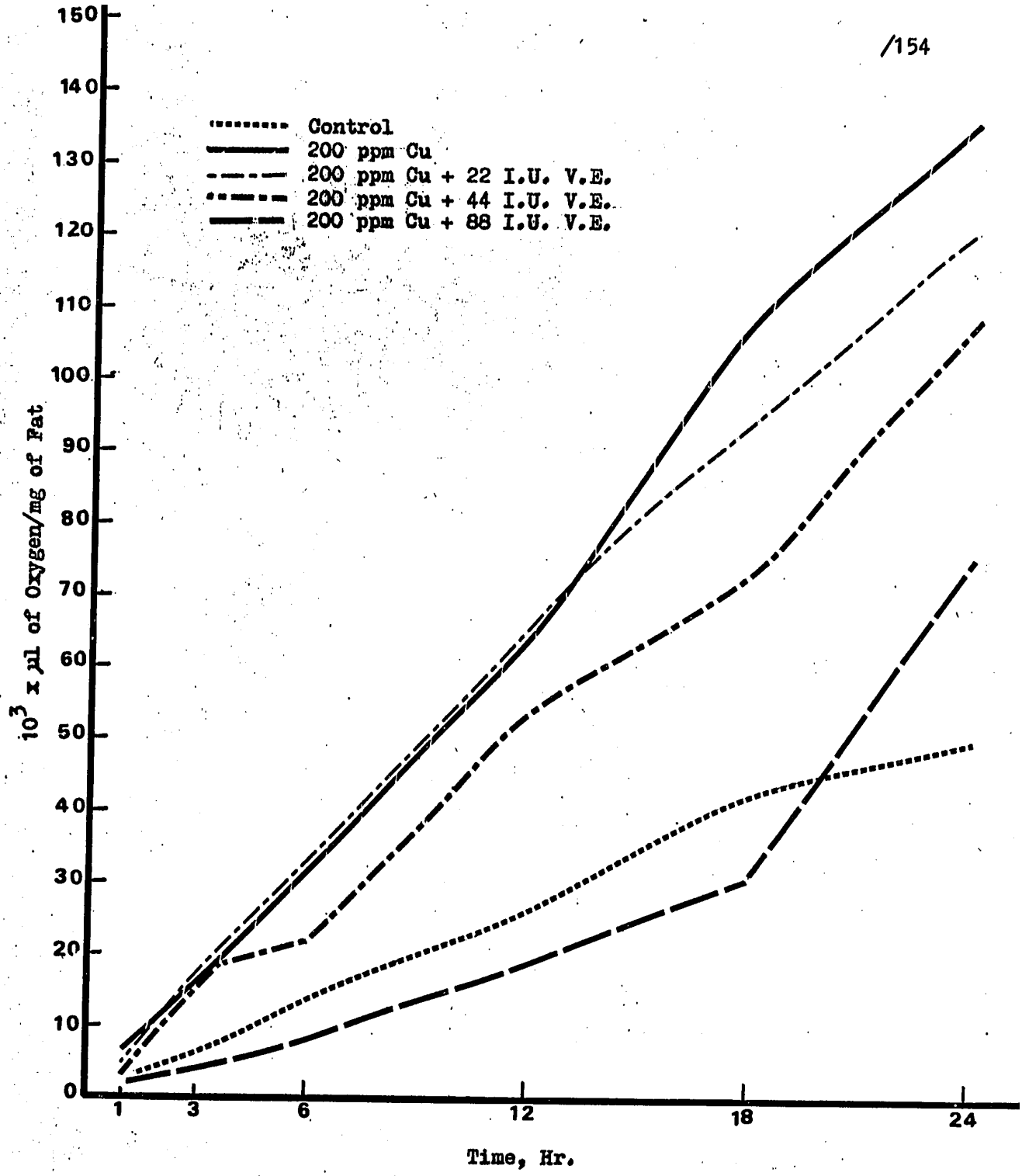



**Figure 16.** Effect of 200 ppm of supplemental dietary copper plus three levels of dl-∝-tocopherol on oxygen absorption by porcine backfat.

supplemental copper. This exhibited the highest rate of oxidation followed by fat obtained from pigs receiving 200 ppm of supplemental copper plus 22 or 44 I.U./kg of vitamin E. The control and the group receiving 200 ppm of supplemental copper plus 88 I.U./kg of vitamin E exhibited the slowest rate of oxidation amongst the 5 groups.

Results given in Tables 22 and 23 clearly indicate that addition of vitamin E to a swine diet in the presence of 200 ppm copper significantly ($P < 0.01$) reduced oxidative susceptibility of the resulting backfat ($\mu$l of $O_2$ or TBA value) in comparison with fat from pigs receiving only 200 ppm of copper. However, $O_2$ uptake by the fat from pigs receiving the three levels (22, 44 or 88 I.U./kg) of vitamin E was significantly ($P < 0.01$) greater than the fat from control pigs.

It has been pointed out previously that the oxidative characteristics of the UFA lead to a linear relationship between the keeping quality of a fat and the concentration of active methylene groups, as determined by the content of linoleic acid. In the present experiment, it appeared that this relationship is not valid when the 88 I.U./kg of vitamin E group was compared with the control group. The author is not certain as to the cause of the decreased concentrations of the UFA and increased oxygen absorption in the group receiving 88 I.U./kg of vitamin E.

In common with the observations made by numerous investigators regarding the antioxygenic behaviour of a α -tocopherol (and other phenolic antioxidants), it was found that a given increment of the antioxidant is less effective in lengthening the induction period as the antioxidant concentration increases. Dammers et al. (1958) drew the conclusion from their experiments that 40 mg of vitamin E per day in the pigs' diet was desirable to give pork a satisfactory keeping quality. Except in the control group, diets used in this experiment contained 200 ppm of supplemental copper and the quality of pork fat produced on these diets varied. These results show, however, that further improvement in fat stability results from supplementing the swine diet containing high levels of copper with vitamin E. There seems to be a tendency towards a better stability of the fat produced under conditions described in this experiment when 22 I.U./kg of vitamin E was supplemented in conjunction with 200 ppm of supplemental copper. The increased stability of the fat in this group may indicate a better utilization of the antioxidant at low (22 I.U./kg) rather than at high (44 or 88 I.U./kg) level. Some evidence has been presented (Hines and Mattill, 1943) indicating that when excessive amounts of tocopherols are fed, large fractions are excreted in the faeces. Mattill (1945) suggested that at high concentrations of dl-α-tocopherol, an appreciable portion of the antioxidant is consumed in side reactions, and thus does not serve

its function as a breaker of the main reaction chain. He concluded that tocopherols attain their effectiveness at comparatively low levels and above the optimum concentration, tocopherols function as pro-oxidants.

## SUMMARY

Experiment III deals with the keeping quality of pork fat as influenced by supplementing practical swine rations with 200 ppm copper and the effect of supplemental vitamin E in the presence of copper on stability from 14.5 kg to slaughter at 92 kg liveweight.

With a content of 22 and 44 I.U./kg of vitamin E in the feed supplemented with 200 ppm copper, an improved keeping quality of pork fat was obtained.

Feeding 88 I.U./kg of dl- $\alpha$ -tocopherol, markedly affected the fatty acid composition of the backfat by increasing the percentage of palmitic and stearic acids at the expense of palmitoleic, oleic and linoleic acids. However, the resistance of the backfat to oxidative rancidity was not significantly improved in comparison with fat from control animals.

## GENERAL SUMMARY AND CONCLUSION

### A. Average Daily Gain and Feed Conversion

Although there is now much evidence that supplementation of swine diets with copper results in increased rate of growth (Braude, 1965; Wallace, 1967; Braude et al.,1970), under conditions described in these experiments, copper supplementation of the diet at various levels (125 to 250 ppm) did not significantly improve ADG. Two reports (NCR - 42 Committee, 1970; Hays et al., 1971) are in accordance with results obtained herein, that for the entire period of growth neither copper nor vitamin E level had a significant effect on ADG. A level of 200 ppm of supplemental copper in the diet stimulated growth and resulted in +5.8 per cent increase in ADG under these experimental conditions. Supplemental copper at the above mentioned level markedly improved FC (approximately +9.1%). Copper supplementation of the swine diet has been reported to improve FC by others (Braude, 1965; Wallace, 1967).

### B. Liver Copper Accumulation

Liver copper concentration increased as the level of copper in the diet increased (Experiments II and III). The presence of supplemental vitamin E in the diet (Experiment III) did not appear to have any effect on liver copper accumulation. Wide variation in the extent of liver copper accumulation within the copper supplemented pigs was noted. Levels of liver copper

accumulation when various levels of copper were included in the diet are within the range of liver copper concentrations reported by others when similar levels of supplemental copper were fed (Wallace, 1967; Braude et al., 1970).

Removal of supplemental copper from the diet during the growing period (at 23 or 46 kg liveweight) resulted in liver copper concentration at slaughter (92 kg liveweight) which approximated that found in the control pigs at the same weight. When withdrawal of copper occurred at 69 kg, liver copper was only reduced by about 50 per cent. This is supported by the report of Lucas and Calder (1957).

It would appear, based on these results, that to obtain an improvement in ADG and/or FC, copper must be included in the pigs' diet continuously from weaning to market. Removal of copper from the diet prior to market weight to reduce the level of copper accumulation in the liver results in reduced ADG, below that observed in control animals not receiving supplemental copper.

C. Characteristics of the Backfat

The relationship between the softness of the fat and the presence of supplemental copper in the swine diet has been well established in these studies as well as in others (Moore et al., 1968; Elliot and Bowland, 1968;1970; Christie and Moore, 1970). It is evident that the pig tends to accumulate UFA in preference

to SFA when supplemental copper is included in the diet at high levels. This was accompanied by marked alteration in the consistency of the fat as indicated by a significant ($P < 0.01$) decrease in melting point. The data emphasize the conclusion drawn by Elliot and Bowland (1969) that the significant ($P < 0.01$) reduction in melting point which led to soft fat could be explained in large part on the basis of the significant ($P < 0.01$) increase in the UFA present in the fat.

The changes observed in the proportions of the various triglyceride species present in the backfat appeared to provide further insight into the effects of supplemental dietary copper on the fat consistency. However, as was evident from the calculated multiple regression and partial correlation coefficients, the percentage UFA seemed to be the factor largely responsible in introducing the significant changes in melting point rather than the changes in triglyceride structure or species. It is possible, however, that the observed significant ($P < 0.01$) increase in UFA in the backfat in response to supplemental copper in the diet can have disproportionate effects on the triglyceride species in the fat and consequently, on the changes in the melting point of the fat.

With the experiments of Hilditch (1939) in mind, which demonstrated that no synthesis of linoleic acid is likely to occur in the pig, it is evident from the data reported herein that appreciable amounts of linoleic acid were deposited in the backfat

of pigs fed diets containing supplemental copper. Possibly, based on these findings, the increase in linoleic acid noted in these studies, as well as by others (Elliot and Bowland, 1970), was the result of the effect of copper on the metabolic pathways concerned in fatty acid desaturation. It is unlikely that linoleic acid synthesis by gastrointestinal microflora could account for more than negligible amounts. The proof given in this work does not support the previous hypothesis (Elliot and Bowland, 1970) in that copper supplementation could stimulate the synthesis of the isomer 18:2W9,12 which was not separable from 18:2W6,9 in their studies.

Based on the proof provided herein, and supported by the suggestion of others (Babatunde et al., 1968a,b), a question regarding the essentiality of linoleic acid (18:2W6,9) for the pig, must be raised. Further research is urgently required to further fully elucidate this point.

Soft fat in pig carcasses is undesirable because it results in a poor product in the market place and results in extra losses during cooking. Moreover, soft fat is more liable to develop rancidity because it is more unsaturated than hard fat, and is, therefore, more reactive chemically. Unsaturated fats tend to take up oxygen from the atmosphere and form peroxides, which, in turn, undergo further chemical changes resulting in off flavours and odors in the product. The addition of 22 I.U./kg of vitamin E in the presence of 200 ppm of supplemental copper appeared to be useful in significantly improving fat stability.

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# APPENDIX

Appendix Table 1. Summary of the Analysis of Variance of Fatty Acids, Melting Point, Liver Copper Content and Average Daily Gain (Exp. I).

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 1. Fatty Acids | | |
| a. Myristic: | | |
| Cu | 1 | 0.577* |
| Slght. Wt. | 3 | 0.278* |
| Cu x Slght. Wt. | 3 | 0.089 |
| Error | 24 | 0.087 |
| b. Palmitic: | | |
| Cu | 1 | 124.820** |
| Slght. Wt. | 3 | 11.901* |
| Cu x Slght. Wt. | 3 | 20.983** |
| Error | 24 | 3.144 |
| c. Palmitoleic: | | |
| Cu | 1 | 68.152** |
| Slght. Wt. | 3 | 5.664 |
| Cu x Slght. Wt. | 3 | 2.165 |
| Error | 24 | 2.195 |
| d. Stearic: | | |
| Cu | 1 | 71.700** |
| Slght. Wt. | 3 | 4.705 |
| Cu x Slght. Wt. | 3 | 2.182 |
| Error | 24 | 1.909 |
| e. Oleic: | | |
| Cu | 1 | 44.650* |
| Slght. Wt. | 3 | 1.100 |
| Cu x Slght. Wt. | 3 | 3.138* |
| Error | 24 | 6.415 |
| f. Linoleic: | | |
| Cu | 1 | 14.445 |
| Slght. Wt. | 3 | 6.046 |
| Cu x Slght. Wt. | 3 | 1.737 |
| Error | 24 | 5.254 |
| 2. Melting Point | | |
| Cu | 1 | 219.975** |
| Slght. Wt. | 3 | 16.432* |
| Cu x Slght. Wt. | 3 | 14.364 |
| Error | 24 | 5.075 |

Continued

Appendix Table 1. (contd.)

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 3. Liver Copper | | |
| Cu | 1 | 453556.700** |
| Slght. Wt. | 3 | 35168.460 |
| Cu x Slght. Wt. | 3 | 32368.506 |
| Error | 24 | 26408.169 |
| 4. Average Daily Gain | | |
| Cu | 1 | 0.00451 |
| Slght. Wt. | 3 | 0.56674 |
| Cu x Slght. Wt. | 3 | 0.01295 |
| Error | 24 | 0.0128 |

* Significant ($P < 0.05$)
** Highly Significant ($P < 0.01$)

Appendix Table 2. Summary of the Analysis of Variance of % Fatty Acids Esterified in the 2- Position and Glyceride Species (Exp. I).

| Source of Variations | d.f | Mean Square |
|---|---|---|
| 1. Fatty Acids Esterified at 2- Position | | |
| a. Myristic: | | |
| Cu | 1 | 2.365 |
| Slght. Wt. | 3 | 2.058* |
| Cu x Slght. Wt. | 3 | 1.276 |
| Error | 24 | 0.650 |
| b. Palmitic: | | |
| Cu | 1 | 1399.200** |
| Slght. Wt. | 3 | 102.260** |
| Cu x Slght. Wt. | 3 | 133.123** |
| Error | 24 | 15.615 |
| c. Palmitoleic: | | |
| Cu | 1 | 115.520** |
| Slght. Wt. | 3 | 24.840* |
| Cu x Slght. Wt. | 3 | 8.188 |
| Error | 24 | 5.256 |
| d. Stearic: | | |
| Cu | 1 | 0.020 |
| Slght. Wt. | 3 | 0.582 |
| Cu x Slght. Wt. | 3 | 1.442 |
| Error | 24 | 0.899 |
| e. Oleic: | | |
| Cu | 1 | 187.211** |
| Slght. Wt. | 3 | 2.442 |
| Cu x Slght. Wt. | 3 | 20.658* |
| Error | 24 | 5.431 |
| f. Linoleic: | | |
| Cu | 1 | 32.200** |
| Slght. Wt. | 3 | 3.280 |
| Cu x Slght. Wt. | 3 | 1.838 |
| Error | 24 | 1.379 |
| 2. Triglycerides | | |
| a. SSS: | | |
| Cu | 1 | 16.603** |
| Slght. Wt. | 3 | 0.611 |
| Cu x Slght. Wt. | 3 | 0.611 |
| Error | 24 | 0.396 |
| b. SSU: | | |
| Cu | 1 | 121.173** |
| Slght. Wt. | 3 | 7.484 |
| Cu x Slght. Wt. | 3 | 8.773* |
| Error | 24 | 1.780 |

Continued

Appendix Table 2. (contd.)

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 2. Triglycerides | | |
| c. USS: | | |
| Cu | 1 | 120.318** |
| Slght. Wt. | 3 | 7.365* |
| Cu x Slght. Wt. | 3 | 8.758 |
| Error | 24 | 1.779 |
| d. USU: | | |
| Cu | 1 | 124.225** |
| Slght. Wt. | 3 | 17.940 |
| Cu x Slght. Wt. | 3 | 40.623 |
| Error | 24 | 17.601 |
| e. SUS: | | |
| Cu | 1 | 0.234 |
| Slght. Wt. | 3 | 0.076 |
| Cu x Slght. Wt. | 3 | 0.035 |
| Error | 24 | 0.100 |
| f. SUU: | | |
| Cu | 1 | 2.480 |
| Slght. Wt. | 3 | 0.171 |
| Cu x Slght. Wt. | 3 | 0.592 |
| Error | 24 | 0.947 |
| g. UUS: | | |
| Cu | 1 | 2.480 |
| Slght. Wt. | 3 | 0.171 |
| Cu x Slght. Wt. | 3 | 0.592 |
| Error | 24 | 0.947 |
| h. UUU: | | |
| Cu | 1 | 974.500** |
| Slght. Wt. | 3 | 57.484* |
| Cu x Slght. Wt. | 3 | 106.498** |
| Error | 24 | 12.010 |

* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)

Appendix Table 3. Summary of the Analysis of Variance of Oxygen Absorption and Peroxide Value of the Fat (Exp. I).

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 1. Oxygen Absorption after: | | |
| a. 9 hr | | |
| Cu | 1 | 0.005** |
| Slght. Wt. | 3 | 0.0003* |
| Cu x Slght. Wt. | 3 | 0.0006* |
| Error | 24 | 0.00006 |
| b. 12 hr | | |
| Cu | 1 | 0.035** |
| Slght. Wt. | 3 | 0.005** |
| Cu x Slght. Wt. | 3 | 0.004** |
| Error | 24 | 0.0003 |
| c. 18 hr | | |
| Cu | 1 | 0.099** |
| Slght. Wt. | 3 | 0.010** |
| Cu x Slght. Wt. | 3 | 0.009** |
| Error | 24 | 0.001 |
| 2. Peroxide Value after: | | |
| 18 hr | | |
| Cu | 1 | 4056.723** |
| Slght. Wt. | 3 | 447.246** |
| Cu x Slght. Wt. | 3 | 403.476** |
| Error | 24 | 51.642 |

* Significant ($P < 0.05$)

** Highly significant ($P < 0.01$)

Appendix Table 4. Summary of the Analysis of Variance of Fatty Acids, Melting Point, Liver Copper Content and Average Daily Gain (Exp. II).

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 1. Fatty Acids | | |
| a. Myristic: | | |
| Cu | 6 | 0.084 |
| Sex | 1 | 0.220* |
| Cu x Sex | 6 | 0.023 |
| Error | 42 | 0.051 |
| b. Palmitic: | | |
| Cu | 6 | 38.197* |
| Sex | 1 | 0.965 |
| Cu x Sex | 6 | 1.726 |
| Error | 42 | 5.858 |
| c. Palmitoleic: | | |
| Cu | 6 | 2.511* |
| Sex | 1 | 0.033 |
| Cu x Sex | 6 | 1.034 |
| Error | 42 | 1.876 |
| d. Stearic: | | |
| Cu | 6 | 9.473 |
| Sex | 1 | 0.007 |
| Cu x Sex | 6 | 1.015 |
| Error | 42 | 2.553 |
| e. Oleic: | | |
| Cu | 6 | 16.440** |
| Sex | 1 | 2.910 |
| Cu x Sex | 6 | 2.626 |
| Error | 42 | 4.218 |
| f. Linoleic: | | |
| Cu | 6 | 5.405** |
| Sex | 1 | 0.962 |
| Cu x Sex | 6 | 0.077 |
| Error | 42 | 1.828 |
| g. Linolenic: | | |
| Cu | 6 | 0.038** |
| Sex | 1 | 0.003 |
| Cu x Sex | 6 | 0.019 |
| Error | 42 | 0.011 |
| h. Arachidonic: | | |
| Cu | 6 | 0.039* |
| Sex | 1 | 0.002 |
| Cu x Sex | 6 | 0.005 |
| Error | 42 | 0.012 |

Continued

Appendix Table 4. (contd.)

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 2. Melting Point | | |
| Cu | 6 | 70.660** |
| Sex | 1 | 6.379 |
| Cu x Sex | 6 | 0.327 |
| Error | 42 | 8.600 |
| 3. Liver Copper Content | | |
| Cu | 6 | 91985.083** |
| Sex | 1 | 3090.300 |
| Cu x Sex | 6 | 129.200 |
| Error | 42 | 1069.523 |
| 4. Average Daily Gain | | |
| Cu | 6 | 0.006 |
| Sex | 1 | 0.022* |
| Cu x Sex | 6 | 0.004 |
| Error | 42 | 0.005 |

* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)

Appendix Table 5. Summary of the Analysis of Variance of Fatty Acids, Melting Point, Liver Copper Content and Average Daily Gain (Exp. III).

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 1. Fatty Acids | | |
| a. Myristic: | | |
| Treatment | 4 | 0.158* |
| Sex | 1 | 0.119 |
| Treatment x Sex | 4 | 0.050 |
| Error | 30 | 0.053 |
| b. Palmitic: | | |
| Treatment | 4 | 55.580* |
| Sex | 1 | 5.829 |
| Treatment x Sex | 4 | 11.592 |
| Error | 30 | 10.047 |
| c. Palmitoleic: | | |
| Treatment | 4 | 7.690** |
| Sex | 1 | 0.055 |
| Treatment x Sex | 4 | 1.492 |
| Error | 30 | 1.336 |
| d. Stearic: | | |
| Treatment | 4 | 16.640** |
| Sex | 1 | 2.371 |
| Treatment x Sex | 4 | 4.658 |
| Error | 30 | 2.091 |
| e. Arachidic: | | |
| Treatment | 4 | 0.031* |
| Sex | 1 | 0.017 |
| Treatment x Sex | 4 | 0.019 |
| Error | 30 | 0.009 |
| f. Oleic: | | |
| Treatment | 4 | 32.242* |
| Sex | 1 | 4.830 |
| Treatment x Sex | 4 | 10.583 |
| Error | 30 | 8.016 |
| g. Linoleic: | | |
| Treatment | 4 | 13.533** |
| Sex | 1 | 0.117 |
| Treatment x Sex | 4 | 7.138* |
| Error | 30 | 2.122 |
| h. Linolenic: | | |
| Treatment | 4 | 0.014** |
| Sex | 1 | 0.000 |
| Treatment x Sex | 4 | 0.005** |
| Error | 30 | 0.0007 |

Continued

Appendix Table 5. (contd.)

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| i. Arachidonic | | |
| Treatment | 4 | 0.004 |
| Sex | 1 | 0.017 |
| Treatment x Sex | 4 | 0.006 |
| Error | 30 | 0.010 |
| 2. Melting Point | | |
| Treatment | 4 | 119.192** |
| Sex | 1 | 1.640 |
| Treatment x Sex | 4 | 23.657 |
| Error | 30 | 15.895 |
| 3. Liver Copper Content | | |
| Treatment | 4 | 22947.865** |
| Sex | 1 | 342.810 |
| Treatment x Sex | 4 | 66.927 |
| Error | 30 | 892.794 |
| 4. Average Daily Gain | | |
| Treatment | 4 | 0.0052 |
| Sex | 1 | 0.0389** |
| Treatment x Sex | 4 | 0.0019 |
| Error | 30 | 0.0042 |

* Significant ($P < 0.05$)
** Significant ($P < 0.01$)

Appendix Table 6. Summary of the Analysis of Variance of Oxygen Absorption and TBA Value of the Fat (Exp. III)

| Source of Variations | d.f. | Mean Square |
|---|---|---|
| 1. Oxygen Absorption after: | | |
| a. 12 hr | | |
| Treatment | 4 | 0.003** |
| Sex | 1 | 0.005** |
| Treatment x Sex | 4 | 0.008 |
| Error | 30 | 0.006 |
| b. 18 hr | | |
| Treatment | 4 | 0.008** |
| Sex | 1 | 0.009** |
| Treatment x Sex | 4 | 0.003* |
| Error | 30 | 0.001 |
| c. 24 hr | | |
| Treatment | 4 | 0.009** |
| Sex | 1 | 0.017** |
| Treatment x Sex | 4 | 0.006** |
| Error | 30 | 0.001 |
| 2. TBA Value at: | | |
| a. 37 C | | |
| Treatment | 4 | 28.886** |
| Sex | 1 | 24.025** |
| Treatment x Sex | 4 | 19.262** |
| Error | 30 | 1.914 |
| b. -20 C | | |
| Treatment | 4 | 66.527** |
| Sex | 1 | 23.870** |
| Treatment x Sex | 4 | 23.343** |
| Error | 30 | 2.499 |

* Significant ($P < 0.05$)
** Highly significant ($P < 0.01$)