# STUDIES OF THE EFFECT OF POLYMERIZING TEMPERATURES ON THE NUTRITIVE VALUE OF HERRING AND LINSEED OILS

by

Barbara Elizabeth Clark

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## ABSTRACT

Diets containing unheated and heat polymerized herring and linseed oils were fed to male white rats. The oils were incorporated in the diets at 20% (herring and linseed) and 10% (linseed) levels. Linseed oil, segregated by propanol or with additions of antioxidants were tested. Supplements of sheep feces and dried brewers yeast were fed with linseed oil diets to determine their prophylactic and curative properties.

Heat polymerization decreased the nutritive value of the oils as assessed by the growth rates, feed consumption, and the physical condition of the animals. Increasing the heated oil content of the diet from 10 to 20% resulted in lower growth rates. Propanol segregation or the additions of antioxidants to the heated linseed oil showed no effect on the nutritive value. Yeast was found to have slight prophylactic and curative properties when fed with heated oil diets.

# INTRODUCTION

The most widely used vegetable oil, for edible purposes, is olive oil, which is produced in abundance in the Mediterranean countries and Southern California. During 1939-45, a shortage of this oil persisted in many countries throughout the world, which necessitated attention to be focused on oils which could be produced domestically, for human consumption.

In Norway, a herring oil, following a deodorization-polymerization process, was used for canning purposes and to some extent as a cooking oil, salad oil, and table oil. Rapeseed oil was used extensively for the manufacture of wartime margarines in Germany, whereas other erucic acid oils have been used for a number of years in China and India.

Prior to 1939, Canada imported all the oil she required to satisfy the demand of human consumption, but in the years that followed she turned her attention to her oil-bearing seed crops, namely linseed, rapeseed, sunflower seed and soybean, in an attempt to meet her demand for oil. Linseed was used in a limited way in shortening during the war, but was found unsuitable because of the extent of "flavour reversion". Soybean and rapeseed oils showed the same type of deterioration but to a lesser extent.

In Switzerland, the use of twenty per cent linseed oil in a mixture of edible fats was not objectionable, whereas pure linseed oil was unsuitable from the standpoint of odour and flavour unstability.

This type of deterioration, referred to as "flavour reversion", is characteristic of all relatively unsaturated vegetable oils. Theories as to the cause of "flavour reversion" are many and controversial, as will be discussed later.

The production of edible shortenings of high nutritional value from vegetable oils, which exhibit this characteristic of reverted flavours, has provided a research problem of great interest, which when solved, would ensure many countries against a further world shortage of edible oil.

## REVIEW OF LITERATURE

Fat is an important human dietary component both because of its high fuel value and the palatability that it imparts to food. In recent years, the inclusion of fat in the diet of many species has been studied extensively and has been shown to have beneficial caloric and non-caloric functions.

Deuel et al (1947) obtained better growth, greater physical capacity and improved reproduction and lactation performance, when rats received diets containing from 5 to 50 per cent fat than on fat free diets. Optimum growth was attained with diets containing 20 to 40 per cent fat. Weanling and young adult rats, subjected to severe caloric restriction lost weight less rapidly, with lower mortality on diets containing liberal amounts of fat than those animals receiving 5 per cent fat or fat free diets of equal caloric value (Scheer et al 1947) (Scheer, Codie and Deuel 1947). Increasing the fat content of a ration from 0.45 to 7.0 per cent increased the milk production and milk fat yields of a lactating goat (Bender and Maynard 1932).

The rigid exclusion of fat from the diet of rats, produces a deficiency syndrome (Burr and Burr 1929), due to the inability of the animals to synthesize fatty acids with a double bond at $C_{12:13}$ position (Baldwin 1948). This condition, known as acrodynia, is characterized by a scaly condition of the skin, caudal necrosis

and a cessation of growth (Burr and Burr 1929) (Evans and Lepkovsky 1932). Linoleic and arachidonic acids are effective in preventing and curing this condition, while controversial evidence exists as to the effectiveness of linolenic acid (Burr and Burr 1930-1932) (Evans and Lepkovsky 1932) (Quackenbush, Kummerow and Steenbock 1942) (Burr and Barnes 1943) (Turpeinen 1938) (Martin 1939).

Evans and Lepkovsky (1928:1931:1932a) state that fat has a sparing action on thiamine. They found that low levels of thiamine which caused death to rats on a fat free diet in one month, would sustain life for four months when 10 per cent fat was fed.

The first essential for a food to be considered of high nutritive value, as stated by Deuel (1946) is that it be relatively completely digested and absorbed. In general fats as a class are well digested, the majority being more than 90 per cent assimilated. Practically all animal and vegetable fats were found to be digested by humans to the extent of 95 per cent or better; the high digestibility of butter and lard being matched by almost complete digestibility of such vegetable oils as cottonseed, corn, peanut and soybean (Langworthy 1923) (Deuel 1946a). Hoagland and Snider (1943) reported similar results with rats. The only exceptions were fats with melting points of 55°C. or above and these showed a marked decrease in digestibility (Holmes and Deuel 1921) (Crockett and Deuel 1947). Mattil (1946) states that the general limiting factor

of digestibility of a fat is the amount of saturated fatty acids present, particularly saturated acids of 18 or more carbons. Rapeseed oil was found to have the lowest coefficient of digestibility of any fat, liquid at ordinary temperatures,which has been investigated on rats. This is believed to be related to the poor absorbability of the erucic acid fraction (Deuel, Cheng and Morehouse 1948).

Studies on the rates of absorption of animal butter fats, body fats, vegetable oils and certain vegetable fats hydrogenated to varying degrees,in fasted rats, showed vegetable oils to be absorbed more slowly than cow butter fat and that hydrogenation lowered the absorption of the vegetable oils to an extent depending on the extent of hydrogenation. In general, it was found that unsaturated acids, namely linoleic and oleic, were more readily digested and that the short chain fatty acids were more readily absorbed than the long chain fatty acids (Bhalerao, Venkatappiah, and Anantakrishnan 1947). Crockett and Deuel (1947) state that fats melting at 55°C. or above, exhibit a decrease in the rate of absorption which is proportional to the decrease in digestibility. Steenbock, Irwin and Weber (1936) studied the percentage of fat absorbed from the alimentary canal of rats at 2, 4, 6, 8 and 12 hours after feeding definite quantities of fat. It was found that: -

(a) partially hydrogenated vegetable oils, as sold commercially for home use and bakers' use, were absorbed as rapidly as lard or corn oil, and

(b) butter oil, halibut liver oil and cod liver oil were absorbed uniformly at a more rapid rate than lard, corn oil and the partially hydrogenated fats.

Animal and vegetable fats, from the standpoint of digestion and absorption are largely interchangeable in nutrition, but their content of fat-soluble vitamins differ. While animal and marine fats contain vitamins A and D in variable amounts, vegetable oils contain vitamin E, a natural occurring antioxidant which gives protection against the onset of rancidity. Provided the fat soluble vitamins were supplied, vegetable oils maintain growth rates equal to those obtained with animal fats (Deuel and Movitt 1945).

Schantz, Elvehjem and Hart (1940) obtained good growth in rats on a diet of 4 per cent butter fat, corn oil, coconut oil, cottonseed oil or soybean oil homogenized into mineralized skimmed milk. However, more efficient gains were noted, during the first few weeks, in rats receiving butter fat. This growth stimulating effect was, in later work, attributed to the saturated fatty acid fraction of the butter fat (Schantz et al 1940). Boer, Jansen and Kentie (1947) also claimed that butter had a growth-promoting factor, which was later identified as vaccenic acid (Boer et al 1947).

Evidence in the literature, fails to support the theories that butter or vaccenic acid possess a growth stimulating value (Deuel, Movitt and Hallman 1943) (Deuel et al 1944) (Nath et al 1948a)

(Deuel et al 1948). Nath et al (1948) did find that butter fat promoted better growth than corn oil when lactose was the sole source of carbohydrate in the diet, but not when other carbohydrates were used, as had previously been reported by Boutwell et al (1943).

Vegetable oils, such as linseed, soybean and rapeseed, for use in human consumption received little attention before 1939, probably because their use as a food is adversely affected by flavour and odour unstability (Schar 1946) (Grace and Lemon 1949). Certain relatively unsaturated vegetable oils, as those already mentioned above, and marine oils, develop flavours after deodorization. This type of deterioration is termed "flavour reversion", implying that a bland, odourless oil regains the original characteristic flavour or odour, and in some cases, flavours and odours quite different from the original one.

Armstrong and McFarlane (1944) attributed the cause of flavour reversion in linseed oil to isolinolenic acid, a derivative of linolenic acid formed during hydrogenation. Bailey (1946) associated flavour reversion with the unsaturated fatty acid linolenic, since flavour stable oils do not contain it. This was also noted by Golumbic and Daubert (1947). Off-flavours present in reverted soybean oil were removed readily, by steam deodorization at 200°C. and 1-3 mm. pressure (Golumbic, Martin and Daubert 1946). One of the volatile products was later isolated and identified as $\alpha$-heptenal (Martin, Schepartz and Daubert 1948). Results,

from a later experiment, using a simulated soybean oil, indicated that the polyunsaturated fatty acids were not wholly responsible for flavour characteristics of the reverted oil (Golumbic, Schepartz and Daubert 1946). Davies and Gill (1936) investigated fishy flavours in oils and concluded that the fatty acids of linseed, and that the entry of N into organic combination, were involved. Supported by the theory that straw-like and fishy flavours of strongly reverted oils must evolve from nitrogen containing compounds, Mattil (1947) thought the non-saponifiable fraction to be related to flavour reversion.

The German process to increase flavour stability in vegetable oils, was by eliminating the phospholipids, which were assumed to be the causal agents of reversion. The methods employed were water washing followed by the addition of minute quantities of citric acid during deodorization, or treatment by live steam and then the fatty ethyl esters added to margarine (Goss 1946:1947).

In 1945, Privett, Pringle and McFarlane reported optimum conditions for the production of a "non-reverting" edible shortening from linseed oil by high temperature polymerization and solvent segregation. This process was based on the principle of converting linolenic acid into a conjugated isomer so that it could no longer act as a precursor for flavour reversion. The best oil was obtained by heating at 270° to 275°C. for 12 to 15 hours, while $CO_2$ was

continuously passed through. The polymerized oil was found to yield 60-65 per cent of acetone soluble oil, with an acid value of less than one per cent, calculated as oleic acid. Further studies indicated that polymerization proceeds according to the level of unsaturation in the triglyceride molecule, i.e., the most highly unsaturated are polymerized first. Solvent segregation yields an acetone soluble, non-polymer fraction (Privett, McFarlane and Gass 1947). A similar heat-polymerization process was used in Norway during the war, in which caustic-soda refined herring oil was bleached and then deodorized at a temperature of 280° to 300°C. under vacuum of 10-15 mm. Hg. for 8 to 12 hours. This oil, found to be more stable as regards to reversion of flavour and odour, was used for canning purposes and to some extent as a cooking oil, salad oil and table oil. However, this process resulted in a lowering of the nutritional value of the oil (Schwitzer 1948).

Linseed Oil, prepared by the method of Privett, Pringle and McFarlane (1945), was found in this laboratory to be toxic when fed at 20% level in the diet of young rats. The toxicity syndrome was described as severe diarrhea followed by death within 14 days. The nutritive value of corn, peanut, soybean and rapeseed oils was not affected by 15 hours heating, but was reduced when treated for 30 hours (Langerman 1949). Harris (1947) showed retarded growth, severe diarrhea and general poor condition in rats fed heat polymerized fish oils. Morris, Larsen and Lippincott (1943) studied groups of

rats fed diets containing 50 per cent lard. In some diets, part or all of the lard was heated at 300° to 350°C. for 30 minutes or 300°C. for 120 minutes. In the latter case, the animals either failed to grow or lost weight and some developed a progressive type of paralysis resembling that occurring in vitamin E deficiency. Little information is contained in the literature, on the subject of the effect of heated fats and oils in nutrition.

Langerman (1949) reported that acetone segregation was effective in partially concentrating the toxic factor, of heated linseed oil, in the soluble fraction and suggested heat degradation products or polymers soluble in acetone as possible toxic agents.

Kass (1947) stated that polymer formation may cause a decrease in the nutritive value of an oil through the inability of the body to metabolize such compounds. A polymer, soluble in acetone, could be a possible explanation for the linseed oil toxicity, since, Bernstein (1948) claimed that acetone was capable of dissolving, essentially, only the monomers, whereas the use of normal monohydric alcohols for fractionation, enabled the separation of the monomers, and in addition separated the polymer portion into a large number of fractions of varying polymeric sizes.

The heated linseed oils appeared to exert their effect by producing a deficiency of some essential nutrient, probably through the inhibition of biosynthesis (Langerman 1949). This was supported by the fact that supplements of rat feces (obtained from healthy animals) to the diets, tended to counteract the effect of heating,

which suggested that a member of the vitamin B family might be involved (Unpub. data 1949). Richter and Rice (1945) reported that feces were capable of replacing dried brewers yeast or liver powder, as the source of B-vitamins in a diet, in maintaining normal growth in the rat.

Fats, under other circumstances, have been shown to produce undesirable effects. For example, rat growth was inhibited by branched fatty acids due to the fact that they are not metabolized and are excreted in the urine (Thomas and Weitzel 1946). Olive oil and animal fats oxidized by heating and then fed orally to rats, all had a carcinogenic effect, although often the effect did not appear for a period of two years (Roffo 1939). The addition of oxidized fat or oxidized corn oil to the diet, effected decreased growth rates and low fertility in rats. The same oil fed by dropper produced no such effects. It was concluded that oxidized fat destroys some essential component of the non-lipid portion of the diet rather than exerting a direct toxic action (Kennelly and Quackenbush 1946).

Holman and Burr (1945) showed that linoleic, ethyl linoleate, linolenic, ethyl linolenate and methyl arachidonate were substrates for lipoxidase. Whereas studies on the rates of autoxidation of unsaturated fatty acids, containing 18 carbon atoms, and their esters, showed that the increase in the number of double bonds by one increased the rate of oxidation by at least a factor of two, and that the acids oxidized more rapidly than their esters (Holman and Elmer 1947).

Therefore, the essential fatty acid, linoleic is most readily oxidized. Peroxides of this acid are known to destroy alpha-tocopherol and have been reported by Pavcek and Shull (1942) to destroy biotin. With suboptimal quantities of linolate, alpha-tocopherol extends the effectiveness in preventing or curing the essential fat acid deficiency syndrome in the rat. When tocopherol, but no essential fatty acid, was fed to fat-deficient rats, the deficiency symptoms were aggravated (Hove and Harris 1946). The addition of small amounts of alpha-tocopherol to animal and vegetable fats does not markedly delay the onset of oxidation unless the fats were previously freed from their natural antioxidants (Thompson and Steenbock 1944).

A study of antioxidant effectiveness of several compounds on vegetable fats and oils was made by Mattil, Filer and Longenecker (1944). The most effective single compound was gallic acid, although nordihydroguairetic acid (N.D.G.A.), ascorbic acid and ascorbyl palmitate each about doubled the keeping time of any given vegetable fat or oil. The protective effect of N.D.G.A. was improved with either citric or phosphoric acid. N.D.G.A. was also found to be an effective antioxidant with lard (Higgins and Black 1944) (Stirton, Turer and Rieimenschneider 1945).

A review of the literature suggests many possible factors which singly, or in combination with one another, might prove to be the explanation of the cause of the heated linseed oil toxicity syndrome.

## OBJECT OF RESEARCH

In view of the results of recent experiments carried out in this laboratory in which heat polymerization decreased the nutritive value of some vegetable oils and rendered linseed oil toxic (Langerman 1949), this study was designed to observe the effect of polymerization on herring oil. Studies were also carried out in a further attempt to concentrate the toxic factor in heated linseed oil and to determine the cause of the toxicity syndrome.

## EXPERIMENTAL PROCEDURE

This experiment involved three feeding trials in which young, growing rats were fed a basal diet, in which the oils to be tested were incorporated at 10 and at 20 percent by weight. The oils studied were herring and linseed, heated for varying lengths of time at temperatures of 280°C. and 275°C., to correspond closely to the Norwegian and Privett's deodorization-polymerization processes, respectively.

(a) Animals.

Each feeding trial was based on a simple randomization block design and was conducted for either one or two periods of twenty eight days each. One hundred and ninety four male, white rats, approximately twenty eight days of age at the start of the test, were involved. They were allotted at random to individual wire-bottomed cages and grouped into lots of ten or sixteen animals, depending on the test. To determine the curative properties of the supplements, lots of ten rats were further sub-divided into lots of five animals each. All animals received feed and water ad libitum.

Live weight changes and feed consumption were recorded weekly. During the third or fourth week of the test period, where feasible, feces collections were made to permit the calculation of the digestibility coefficients.

(b) Diets.

Because the oils tested were eventually intended for human consumption, the basal diet consisted of ingredients which could be used for a table biscuit. Two basal diets, which differed primarily in level of fat, were used in this study. Increase in fat was made at the expense of the white flour, thus maintaining an approximately constant protein level by weight (27%).

Table I - Percentage composition of the basal diets

| Ingredients | 10% fat | 20% fat |
|---|---|---|
| White flour | 57.0 | 47.0 |
| Milk powder | 19.0 | 19.0 |
| Casein | 11.5 | 11.5 |
| Oil | 10.0 | 20.0 |
| Bonemeal | 2.0 | 2.0 |
| Salt | 0.5 | 0.5 |

The dry ingredients were mixed, the oil added and the whole was mixed thoroughly. The feed was then baked at 375°F. for approximately twenty minutes. The diets were granulated, air-dried, and stored in the refrigerator until required.

(i) Vitamins.

B-vitamins were added, prior to feeding, to the diets not otherwise supplemented. The amounts added were thiamine 10; riboflavin 100; and niacin 5 parts per million.

Vitamins A and D in oil, were administered directly to all animals, by means of a syringe, once weekly to provide 25 i.u. of vitamin A and 5 i.u. of vitamin D per day.

(ii) Chemical Preparation of the Oils.

(x) Herring oils*

The herring oils were caustic-soda refined, bleached and deodorized at a temperature of 280°C. for 10 hours or for 20 hours. The acetone segregation of the oil heated for 10 hours was similar to that used for the linseed oil as described below.

This deodorization-polymerization process corresponded to the Norwegian method, in which the heat treatment was for a period of 8 to 12 hours.

(y) Linseed oil**

Both crude and refined linseed oils were subjected to Privett's method of heat polymerization and solvent segregation for the production of edible oils. This method consisted of heating the oils at 275°C. for 12 hours, with a continuous passage of $CO_2$ through them. Solvent segregation involved a thorough mechanical stirring of seven volumes of acetone with one volume of oil and then allowing the mixture to stand overnight at 5°C. The supernatant (acetone soluble fraction) was removed and the acetone distilled off. The oil was kept under $CO_2$ until required for the diets.

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* The preparation of these oils by the National Research Council, Ottawa, was much appreciated.

** The author is indebted to Mr.L.Wiseblatt for the preparation of the linseed oils.

The procedure used for propanol segregation was the same as described for acetone. Both soluble and insoluble fractions were fed in the diets.

(z) Antioxidants

The antioxidants added to the linseed oil consisted of a combination of 0.025 per cent of nordihydroguairetic (N.D.G.A.) and 0.025 per cent of ascorbic acids.

The scheme of preparation of the oils to which additions of antioxidants were made or propanol segregation was employed, appears in the Appendix (Table 1).

(iii) Special Supplements.

It was desired further to test both prophylactic and curative effects which sheep feces or dried brewers yeast might exhibit when fed with the heated oil diets. The allotment plan showing the way in which this scheme was carried out appears in the Appendix (Table 2).

(x) Sheep feces

The sheep feces were obtained from sheep fed an all-timothy hay diet in metabolism studies. They were air-dried and ground, and then added to the diets on the basis of one part feces to 4 parts basal diet.

(y) Dried brewers yeast

Non-irradiated dried brewers yeast was mixed into the diets prior to feeding on the basis of one part yeast to 19 parts basal diet.

A description of the diets as fed in this study, together with information as to kind and level of oil, special supplements, the number of animals receiving each diet, and the number of test periods, is given in Table II.

TABLE II - Description of diets

| Diet No. | Oil | Heat treatment | Fraction of oil fed in diets | Oil level % | Supplement | No. animal male | Test period (28 day) |
|---|---|---|---|---|---|---|---|
| 1 | Herring R. | - | Whole oil | 20 | - | 16 | 1 |
| 2 | Herring R. | 10 hr. 280°C. | Whole oil | 20 | - | 16 | 1 |
| 3 | Herring R. | 20 hr. 280°C. | Whole oil | 20 | - | 16 | 1 |
| 4 | Herring R. | 10 hr. 280°C. | Acetone soluble | 20 | - | 16 | 1 |
| 5 | Linseed R. | - | Acetone soluble (whole oil) | 20 | - | 10 | 1 |
| 6 | Linseed R. | - | Acetone soluble (whole oil)* | 20 | - | 10 | 1 |
| 7 | Linseed R. | 12 hr. 275°C. | Acetone soluble | 20 | - | 10 | 1 |
| 8 | Linseed R. | 12 hr. 275°C. | Acetone soluble | 10 | - | 10 | 1 |
| 9 | Linseed R. | 12 hr. 275°C. | Acetone soluble* | 20 | - | 10 | 1 |
| 10 | Linseed R. | 12 hr. 275°C. | Propanol soluble | 20 | - | 10 | 1 |
| 11 | Linseed R. | 12 hr. 275°C. | Propanol insoluble | 20 | - | 10 | 1 |
| 12 | Linseed C. | - | Whole oil | 10 | - | 10 | 2 |
| 13 | Linseed C. | - | Whole oil | 10 | Sheep feces | 10 | 2 |
| 14 | Linseed C. | - | Whole oil | 10 | Brewers yeast | 10 | 2 |
| 15 | Linseed C. | 12 hr. 275°C. | Acetone soluble | 10 | - | 10 | 2 |
| 16 | Linseed C. | 12 hr. 275°C. | Acetone soluble | 10 | Sheep feces | 10 | 2 |
| 17 | Linseed C. | 12 hr. 275°C. | Acetone soluble | 10 | Brewers yeast | 10 | 2 |

R - denotes refined oils
C - denotes crude oils
* - antioxidants added.

(c) Criteria.

Criteria used to determine the nutritive value of the oils were, liveweight changes, feed consumption, general conditions of the animals during the test, and the results of post mortem examination of the viscera at the completion of the experiment.

## OBSERVATIONS

Seven of the one hundred and ninety-four animals placed on test, failed to survive their respective experiments. Post mortem examinations revealed that the animals were suffering from necrobacillosis, which was believed to be the cause of death.

The rats receiving diet number 11, containing the propanol insoluble portion of the heated linseed oil, developed scruffy, yellowish hair coats and produced dark, sticky feces. The remaining animals were in a healthy condition and no pathological disorders were evident during post mortem examinations at the completion of the experiment.

## EXPERIMENTAL RESULTS

### Heat Polymerization of Herring Oil.

The presence of heat polymerized herring oil in the diets, resulted in a lower feed consumption and hence lower liveweight gains in the animals, as compared to those receiving unheated oil. However, the lower gains were not due entirely to the decreased feed intakes but to a decrease in feed efficiency (Table III).

Table III - Mean gains, feed intakes and gain per 100 grams of feed of the herring oil diets

| Diet No. | Treatment of herring oil | Av.gain gms. | Av.feed gms. | Gain per 100 gm. feed |
|---|---|---|---|---|
| 1 | Unheated | 73.4 | 226.9 | 32.3 |
| 2 | Heated 10 hr. | 25.0 | 177.1 | 14.1 |
| 3 | Heated 20 hr. | 26.8 | 184.3 | 14.5 |
| 4 | Heated 10 hr.(acetone soluble) | 13.5 | 170.6 | 7.9 |

A comparison of the diets containing oil heated for 10 hours or 20 hours to the one containing unheated oil, showed that while there was approximately 22% decrease in the feed consumed, there was a 56% decrease in feed efficiency. With no appreciable change in feed consumption, a 44% decrease in feed efficiency was evident with the acetone soluble fraction of the oil heated for 10 hours, as compared to the other heated oil diets.

There was evidence to suggest that the effects of heating were not traceable to a decline in digestibility (Table IV).

Table IV - Digestibility of the dry matter

| Diet No. | % digested |
|---|---|
| 1 | 86.4 |
| 2 | 87.8 |
| 3 | 79.9 |
| 4 | 83.7 |

The Effect of the Level of Heated Linseed Oil in the Diet.

Previous experiments in this laboratory with heated linseed oil, indicated that the level of oil in the diet influenced the degree to which the toxic symptoms appeared, but no direct comparison had been made.

The liveweight gain attained on the diet containing 10 per cent level of heated oil was inferior to that produced with the 20 per cent level of unheated oil, whereas with 20 per cent heated oil in the diet the animals failed to increase their weight (Table V).

Table V - Mean gains, feed intakes and gains adjusted to equal feed according to oil and level fed

| Diet No. | Linseed oil | Oil level % | Av. gain gms. | Av. feed gms. | Adj. gain* |
|---|---|---|---|---|---|
| 5 | Unheated, acetone soluble | 20 | 105 | 282 | 98 |
| 7 | Heated, acetone soluble | 20 | -4 | 169 | 1 |
| 8 | Heated, acetone soluble | 10 | 64 | 231 | 62 |

* b = 0.1. Differences between averages exceeding 12 gms. are statistically significant at a probability of 5%.

The Effect of Propanol Segregation.

The data obtained from a comparison of diets containing propanol soluble, propanol insoluble or acetone soluble fractions of heat-polymerized linseed oil, are presented in Table VI. The latter fraction was used as a control, as the treatment of this oil had been found to be effective in partially concentrating the "toxic" factor (Langerman 1949).

Table VI - Mean gains, feed intakes and gains adjusted to equal feed for fractions of linseed oil

| Diet No. | Fraction of heated linseed oil | Av. gain gms. | Av. feed gms. | Adj. gain* |
|---|---|---|---|---|
| 7 | Acetone soluble | -4 | 169 | 1 |
| 10 | Propanol soluble | 6 | 153 | 12 |
| 11 | Propanol insoluble | 5 | 218 | 4 |

* b = 0.1. Difference between averages exceeding 12 gms. are statistically significant at a probability of 5%.

The results showed that the three fractions of oil were low in nutritive value and that propanol segregation was ineffective as a means of concentrating the "toxic" material.

The scruffy, yellowish appearance of the animals which received diet number 11, and the production of dark, sticky feces was attributed to the polymers contained in the oil.

The Effect of Antioxidants.

The addition of antioxidants to unheated and heat-polymerized linseed oil was effective in maintaining low peroxide values, whereas in the unstabilized oils peroxide values rose considerably, over the 28 day test period (see Appendix Table 3). However, the antioxidants failed to increase the nutritive value of the oils as was shown by the liveweight gains (Table VII).

Table VII - Mean gains, feed intakes and gains adjusted to equal feed for diets containing antioxidants

| Diet No. | Treatment of linseed oil | Av. gain gms. | Av.feed gms. | Adj. gain* |
|---|---|---|---|---|
| 5 | Unheated, acetone sol. | 105 | 282 | 98 |
| 6 | Unheated, acetone sol. + antioxidants | 107 | 268 | 102 |
| 7 | Heated, acetone sol. | -4 | 169 | 1 |
| 9 | Heated, acetone sol. + antioxidants | -4 | 163 | 1 |

* b = 0.1. Differences between averages exceeding 12 gms. are statistically significant at a probability of 5%.

Prophylactic and Curative Effects of the Special Supplements

To determine both the prophylactic and the curative properties of the supplements, a different trial design was necessary.

In this test, sixty animals were allotted and divided into twelve groups. During period I, two groups received the same diet and supplemental treatment, i.e. either unheated or heated oil diet with nil, sheep feces, or dried brewers yeast supplement. At the beginning of period II, the two identical groups were separated and each was fed the same oil diet as before, but with one or other of the supplements not previously received.

The oil in the basal diets was kept at 10 per cent level by weight, so that if any toxic effects were found they would not be severe enough to cause the death of the animals.

The average gain attained on the acetone soluble portion of heated linseed oil was far below that attained on the unheated oil, regardless of the supplementary treatments (Table VIII). This was partly accounted for by the difference in feed consumption since the heated oil diet was eaten in smaller amounts (Table IX).

The gains of the sub-groups could not be compared directly, because of the addition of 20 per cent sheep feces, presumably indigestible material, to some diets. Therefore the feed intakes were converted to digested calories consumed (Table IX). The factors used in the conversion are presented in the Appendix Table 4. The differences between the gains of the sub-groups in

period II, were due in part to the difference in initial weights of the animals at the beginning of this period. It was therefore necessary to modify the analysis of the data. In period I, the gains were adjusted to equal digested calories and in period II, partial regression was employed to adjust gains to equal digested calories and to equal initial weights (Table X).

TABLE VIII - Mean gains for the supplemental diets

| Linseed Oil | PERIOD I | | | PERIOD II | | |
|---|---|---|---|---|---|---|
| | Av. gain (all sub-groups) | Supple-ment | Av. gain | Av. gain (all sub-groups) | Supple-ment | Av. gain |
| Unheated | 97 | Nil | 89 | 74 | Feces | 52 |
| | | | | | Yeast | 92 |
| | | Feces | 98 | | Nil | 40 |
| | | | | | Yeast | 93 |
| | | Yeast | 105 | | Feces | 61 |
| | | | | | Nil | 70 |
| Heated, acetone soluble | 52 | Nil | 42 | 52 | Feces | 53 |
| | | | | | Yeast | 85 |
| | | Feces | 50 | | Nil | 26 |
| | | | | | Yeast | 72 |
| | | Yeast | 64 | | Feces | 47 |
| | | | | | Nil | 29 |

TABLE IX - Mean feed intakes and the digested calories consumed

| Linseed oil | PERIOD I | | | PERIOD II | | |
|---|---|---|---|---|---|---|
| | Av.feed and dig. calories (all sub-groups) | Supple-ment | Av.feed and dig. calories | Av.feed and dig. calories (all sub-groups) | Supple-ment | Av.feed and dig. calories |
| Unheated | (306)1271 | Nil | (255)*1165 | (393)1654 | Feces | (365)1288 |
| | | | | | Yeast | (402)1843 |
| | | Feces | (358) 1263 | | Nil | (318)1450 |
| | | | | | Yeast | (466)2137 |
| | | Yeast | (300) 1375 | | Feces | (429)1515 |
| | | | | | Nil | (379)1728 |
| Heated, acetone soluble | (258)1057 | Nil | (233) 1035 | (329)1342 | Feces | (312)1133 |
| | | | | | Yeast | (364)1551 |
| | | Feces | (280) 1015 | | Nil | (264)1177 |
| | | | | | Yeast | (351)1495 |
| | | Yeast | (263) 1121 | | Feces | (411)1492 |
| | | | | | Nil | (271)1205 |

* Figures in parentheses are grams of air-dry feed eaten in 28 days.

TABLE X - Average adjusted gains for the supplemented diets

| Linseed oil | PERIOD I | | | PERIOD II | | |
|---|---|---|---|---|---|---|
| | Adj.gain[1] (all sub-groups) | Supple-ment | Adj. gain[1] | Adj.gain[2] (all sub-groups) | Supple-ment | Adj. gain[2] |
| Unheated | 88 | Nil | 88 | 73 | Feces | 38 |
| | | | | | Yeast | 112 |
| | | Feces | 89 | | Nil | 34 |
| | | | | | Yeast | 128 |
| | | Yeast | 85 | | Feces | 57 |
| | | | | | Nil | 78 |
| Heated, acetone soluble | 62 | Nil | 54 | 46 | Feces | 36 |
| | | | | | Yeast | 94 |
| | | Feces | 64 | | Nil | 10 |
| | | | | | Yeast | 78 |
| | | Yeast | 68 | | Feces | 47 |
| | | | | | Nil | 14 |
| Nec.diff. | 5.7 | | 9.9 | 7.2 | | 17.7 |

[1] Gain adjusted to equal digested calories

[2] Gain adjusted to equal initial weights and equal digested calories.

The acetone soluble fraction of the heated oil was much less nutritious than the unheated oil. This was shown by the difference between the gains of these two groups, which was consistent throughout both periods, regardless of the supplemental treatments. (Table X).

In period I, no increase in gain was observed on the supplemented unheated oil diets, whereas a significant improvement in the nutritive value of the heated diets, containing the feces and the yeast, was present. However, it should be noted that an increase in gain such as was shown with the feces, over the nil treatment, would occur by random chance once in twenty trials. It was therefore assumed that some factor present in yeast, and possibly in feces, improved the nutritive value of the heated oil diets.

In period II, it was found that when the rats which had received the unheated oil with either no supplement or feces, were changed to feces or nil supplement respectively, they failed to increase their feed consumption sufficiently for their size and hence their gains were below average. Those groups which changed from nil or feces supplements to yeast increased their feed intakes remarkably and their gains were higher than average.

With the heated oil diets, the diet changes in period II were followed by similar gain responses as with the unheated oils, except for animals which previously received yeast. In this case,

those rats which changed from unheated oil with yeast to feces or nil, and from heated oil with yeast to feces, ate sufficient feed to compensate for the lower energy content, but changing from "yeast to nil" in the heated oil group resulted in a low feed and digestible caloric intake and hence a drop in feed efficiency.

From these results, it appeared that the presence of heated oil in diet increased the need for some factor contained in yeast.

## DISCUSSION

It was clear from a review of the results of these feeding trials, that heat polymerization had a detrimental effect upon the nutritive value of the oils. However, the heated linseed oil toxicity syndrome, namely severe diarrhea followed by death within fourteen days, as described by Langerman (1949) was not encountered.

Terrill (1946) stated that the ability of an oil to polymerize was related to its unsaturation, since it was the double bonds which were the reactive centres, about which polymerization took place. This was confirmed by Privett, McFarlane and Gass (1947) who found that the most highly unsaturated fatty acids were polymerized first. Therefore, the essential fatty acid, linoleic would be expected to be destroyed or built into a complex polymer by heat treatment. In this case, the destruction of linoleic acid might account for the lower gains attained by the animals on the heated oil diets, and no improvement would be expected by either feeding the propanol fractions or adding antioxidants to the oil. Increasing the level of heated oil in the diet, from 10 to 20 per cent, might aggravate the effect of the linoleic deficiency in a similar manner as was reported by Hove and Harris (1946), when small amounts of alpha-tocopherol were fed with fat-free diets.

In studies on the relation of B-vitamins to rat acrodynia, Jürgens, Pfaltz and Reinert (1945) found weight increase slackened in weanling rats on fat-free diets. With daily supplements of pyridoxin, pantothenic acid and nicotinamide, weight increase began but later

ceased. Kummerow, Chu and Randolph (1948) reported that when pyridoxin and Ca pantothenate were fed in addition to oxidized 10:12 ethyl linoleate, acrodynia was effectively cured whereas the oxidized fatty acid alone, was ineffective. It might, therefore, be postulated that the increase in gains shown by supplementing the heated oil diets with yeast, was due to the content of these vitamins in the yeast.

## CONCLUSIONS

1. Heat polymerization decreases the nutritive value of herring and of linseed oil.

2. Increasing the level of heated linseed oil in the diet, from 10 to 20 per cent, decreases the liveweight gains.

3. Propanol segregation is ineffective in concentrating the factor or material responsible for the lower nutritive value of heated linseed oil.

4. Addition of antioxidants to the heated oil fails to prevent the decrease in the nutritive value.

5. A stimulation of growth is obtained by supplementing the heated oil diets with yeast.

## LITERATURE CITED


Armstrong, J.C. and W.D.McFarlane
1944 — Flavor reversion in linseed shortening. Oil and Soap Vol.21,p.322.

Bailey, A.E.
1946 — Flavor reversion in edible fats. Oil and Soap Vol.23, p.55.

Baldwin, E.
1948 — Dynamic aspects of Biochemistry. Published by The MacMillan Co.N.Y. p.392.

Bender, R.C. and L.A.Maynard
1932 — Fat metabolism in the lactating goat. J. Dairy Sci. Vol.15, p.242.

Bernstein, I.M.
1948 — Polymer fractionation of heat-polymerized non-conjugated vegetable oils. J. Physical and Colloid.Chem. Vol.52, p.613.

Bhalerao, V.R., D.Venkatappiah and C.P. Anantakrishnan
1947 — The relative rate of absorption of different oils and fats. Indian J. Vet. Sci. Vol.17,p.221. Nutr.Abs. and Rev. Vol.18,p.823,1949.

Boer, J., B.C.P.Jansen and A.Kentie
1947 — On the growth-promoting factor for rats present in summer butter. J. of Nutr. Vol.33, p.339.

Boer, J., B.C.P.Jansen, A.Kentie and H.W.Knol
1947 — The growth-promoting action of vaccenic acid. J. of Nutr. Vol.33,p.359.

Boutwell, R.K., R.P.Geyer, C.A.Elvehjem and E.B.Hart
1943 — Further studies on the growth-promoting value of butter fat. J. Dairy Sci. Vol.26, p.429.

Burr, G.O. and M.M.Burr
1929 — A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem. Vol.82, p.345.

Burr, G.O. and M.M.Burr
1930
On the nature and role of the fatty acids essential in nutrition.
J. Biol. Chem. Vol.86, p.587.

Burr, G.O., M.M.Burr and E.S.Millar
1932
On fatty acids essential in nutrition.
J. Biol. Chem. Vol.97, p.1.

Burr, G.O. and R.H.Barnes
1943
Non-caloric functions of dietary fats.
Physiol. Revs. Vol.23, p.256.

Crockett, M.E. and H.J.Deuel, Jr.
1947
A comparison of the coefficient of digestibility and the rate of absorption of several natural and artificial fats as influenced by melting point.
J. of Nutr. Vol.33, p.187.

Davies, W.L., and E.Gill
1936
Investigations on fishy flavors.
J. Soc. Chem. Ind. Vol.55, p.141t.

Deuel, H.J.Jr., E.Movitt and L.F.Hallman
1943
The comparative nutritive value of butter and some vegetable fats.
Science Vol.98, p.139.

Deuel, H.J.Jr., E.Movitt, L.F.Hallman and F.Mattson
1944
Studies of the comparative nutritive value of fats. I. Growth rate and efficiency of conversion of various diets to tissue.
J. of Nutr. Vol.27, p.107.

Deuel, H.J.Jr., and E.Movitt
1945
Studies on the comparative nutritive value of fats. V. The growth rate and efficiency of conversion of various diets to tissue in rats weaned at 14 days.
J. of Nutr. Vol.29, p.237.

Deuel, H.J. Jr.
1946
Studies on the comparative nutritive value of fats. IX. The digestibility of margarine fat in human subjects.
J. of Nutr. Vol.32, p.69.

Deuel, H.J.Jr.
1946a
The role of fat in human nutrition.
Oil and Soap. Vol.23, p.209.

Deuel, H.J.Jr., E.R.Meserve, E. Straub, C. Hendrick and B.T.Scheer
1947
The effect of fat level of the diet on general nutrition.I.
J. of Nutr. Vol.33, p.569.

Deuel, H.J., A.L.S. Cheng and M.G.Morehouse
1948 Digestibility of rapeseed oil in the rat.
J. of Nutr. Vol. 35, p.295.

Deuel, H.J.Jr., S.M.Greenberg, E. Straub, D. Jue, C.M.Gooding and C.F.Brown
1948 Studies on the comparative nutritive value of fats. X. On the reputed growth-promoting activity of vaccenic acid.
J. of Nutr. Vol.35, p.301.

Evans, H.M., and S.Lepkovsky
1928 Sparing action of fat on the anti-neuritic vitamin.
Science, Vol.68, p.298.

Evans, H.M. and S.Lepkovsky
1931 Beneficial effects of fats in high sucrose diets when the requirements for the antineuritic vitamin B and the fat-soluble vitamins are fully satisfied.
J. Biol. Chem. Vol.92, p.615.

Evans, H.M. and S. Lepkovsky
1932 Vital need of the body for certain unsaturated fatty acids. I and II.
J. Biol. Chem. Vol.96, p.143 and 157.

Evans, H.M. and S. Lepkovsky
1932a The sparing action of fat on vitamin B. II and III.
J. Biol. Chem. Vol.96, p.165 and 179.

Golumbic, C., C.J.Martin and B.F.Daubert
1946 Flavor reversion in soybean oil. I. Preliminary spectral absorption and isolation studies.
Oil and Soap, Vol.23, p.187.

Golumbic, C., A.I. Schepartz and B.F.Daubert
1946 Flavor reversion in soybean oil. II The preparation and flavor characteristics of a simulated soybean oil.
Oil and Soap. Vol.23, p.380.

Golumbic, C. and B.F.Daubert
1947 Flavor reversion in soybean oil.
Food Indust. Vol.29, p.1075.

Goss, W.H.
1946 Processing oils and oils in Germany.
Oil and Soap, Vol.23, p.241.

Goss, W.H.
1947 Edible oil industry in Germany.
Food Indust. Vol.19, p.184 and 320.

Grace, N.H. and H.W.Lemon
1949
The Canadian oils industry.
Agr. Instit. Rev.Vol.4,p.341 and 420.

Harris, P.
1947
Cited in correspondence received from author.

Higgins, J.W. and H.C.Black
1944
A preliminary comparison of the stabilizing effect of several recently proposed antioxidants for edible fats and oils.
Oil and Soap, Vol.21, p.277.

Hoagland, R. and G.G.Snider
1943
Digestion of some animal and vegetable oils.
J. of Nutr. Vol.25, p.295.

Holman, R.T. and G.O.Burr
1945
Spectrophotometric studies of the oxidation of fats. IV. Ultra violet absorption spectra of lipoxidase-oxidized fats.
Archives of Biochem. Vol.7, p.47.

Holman, R.T. and O.C.Elmer
1947
The rates of oxidation of unsaturated fatty acids and esters.
J. A.O.C. Vol.24, p.127.

Holmes, A.D. and H.J.Deuel, Jr.
1921
Digestibility of some hydrogenated oils.
Am. J. Physiol. Vol.54, p.479.

Hove, E.L. and P.L.Harris
1946
Co-vitamin studies.V. The interrelation of alpha-tocopherol and the essential unsaturated fat acids.
J. of Nutr. Vol.31, p.699.

Jürgens, R., H.Pfaltz and M. Reinert
1945
Relationship of B-complex vitamins to the unsaturated fatty acids.
Helv. physiol. Acta. Vol.3,p.41
Nutr. Abs. and Revs. Vol.17,p.104,1947.

Kass, J.P.
1947
Polymerization of the unsaturated fatty acids.
Biol. Antioxid. Trans. 2nd. Conf. N.Y.
Pub. by Josiah Macy Jr.Foundation,p.27.

Kennelly, B. and F.W.Quackenbush
1946
Effects of oxidized fats in the diet of the rat.
Biol. Antioxid. Trans.1st. Conf. p.56.
Chem. Abst. Vol.42, p.1640,1946.
J.A.O.C. Soc. Vol.25,p.190,1948.

Kummerow, F.A., T.K.Chu and P.Randolph
1948 Effects of antoxidation on anti-acrodynic potency of fats and linoleic esters.
J. of Nutr. Vol.36, p.523.

Langerman, H.L.
1949 The effect of heat on the nutritive value of some vegetable oils.
M. Sc. Thesis, McGill University.

Langworthy, C.F.
1923 Digestibility of fats.
Ind. Eng. Chem. Vol.15, p.276.

Martin, G.J.
1939 Studies of fat-free diets.
J. of Nutr. Vol.17, p.127.

Martin, C.J., A.I. Schepartz and B.F.Daubert
1948 Flavor reversion in soybean oil.
IV. Isolation of reversion compounds in soybean oil.
J.A.O.C. Soc. Vol.25, p.113.

Mattil, K.F., L.J. Filer, Jr. and H.E.Longenecker
1944 A study of the antioxidant effectiveness of several compounds on vegetable fats and oils.
Oil and Soap, Vol.21, p.160.

Mattil, K.F.
1946 The digestibility of fats - A correlation of experimental data.
Oil and Soap. Vol.23, p.244.

Mattil, K.F.
1947 Flavor stability of soybean oil.
I. The role of the non-saponifiable portion.
J.A.O.C. Soc. Vol.24, p.243.

Morris, H.P., C.D. Larsen and J.W.Lippincott
1943 Effects of feeding heated lard to rats.
Histological description of lesions produced.
J. Nat. Cancer Inst. Vol.4, p.285.
Nutr. Abst. and Revs. Vol.15,p.718,1946.

Nath, H.,V.H.Barki, C.A. Elvehjem and E.B.Hart
1948 Further studies of the nutritive value of butterfat fractions.
J. Dairy Sci. Vol.31, p.793.

Nath, H., V.H.Barki, C.A.Elvehjem and E.B.Hart
1948a
Studies of the alleged growth promoting property of vaccenic acid.
J. of Nutr. Vol.36, p.761.

Pavcek, P.L. and G.M.Shull
1942
Inactivation of biotin by rancid fats.
J. Biol. Chem. Vol.146, p.351.

Privett, O.S., R.B.Pringle and W.D.McFarlane
1945
The elimination of flavor reversion in linseed shortening by heat polymerization and solvent segregation of the oil.
Oil and Soap, Vol.22, p.287.

Privett, O.S., W.D.McFarlane and J.H.Gass
1947
Studies on the heat polymerization and solvent segregation of vegetable oils.
J.A.O.C. Soc. Vol.24, p.204.

Quackenbush, F.W., F.A.Kummerow and H. Steenbock
1942
The effectiveness of linoleic, linolenic and arachidonic acids in reproduction and lactation.
J. of Nutr. Vol.24, p.213.

Richter, C.P. and K.K.Rice
1945
Self-selection studies on coprophagy as a source of vitamin B complex.
Am. J. Physiol. Vol.143,p.344.
Nutr.Abs. and Revs. Vol.15,p.266,1945.

Roffo, A.H.
1939
The development of malignant tumors in the digestive apparatus after ingestion of fat oxidized by heating.
Prensa med. argent. Vol.26, p.619.
Chem. Abs. Vol.37,p.2065,1943.

Schantz, E.J., C.A.Elvehjem and E.B.Hart
1940
The comparative nutritive value of butterfat and certain vegetable oils.
J. Dairy Sci. Vol.23, p.181.

Schantz, E.J., R.K.Boutwell, C.A.Elvehjem and E.B.Hart
1940
The nutritive value of the fatty acid fraction of butterfat.
J. Dairy Sci. Vol.23, p.1205.

Schar, A.
1946
Linseed oil for human consumption.
Mitt. Lebensm. Hyg. Vol.37,p.363.
J.A.O.C. Soc. Vol.24, p.284, 1947.

Scheer, B.T., D.F.Soule, M.Fields and H.J.Deuel,Jr.
1947 The effect of fat level of the diet on general nutrition. II. Growth, mortality and recovery in weanling rats maintained on restricted calories.
J. of Nutr. Vol.33, p.583.

Scheer, B.T., J.F. Codie and H.J.Deuel,Jr.
1947 The effect of fat level of the diet on general nutrition. III Weight loss, mortality and recovery in young adult rats maintained on restricted calories.
J. of Nutr. Vol.33, p.641.

Schwitzer, M.K.
1948 The herring industry in the United Kingdom.
Oleagineau Vol.3,p.243.
Cited in correspondence from H.J.Lips, N.R.C. Ottawa.

Steenbock, H., M.H.Irwin and J.Weber
1936 The comparative rate of absorption of different fats.
J. of Nutr. Vol.12, p.103.

Stirton, A.S., J. Turer and R.W.Rieimenschneider
1945 Oxygen absorption of methyl esters of fat acids and the effect of antioxidants.
Oil and Soap, Vol.22, p.81.

Terril, R.L.
1946 Polymer formation from natural and synthetic drying oils.
Oil and Soap, Vol.23, p.339.

Thomas, K. and G. Weitzel
1946 Deutsch Med. Wochenzeitschrift Vol.71, p.18.
Cited in F.A.O.Nutritional Studies No.2, 1949.

Thompson, C.R. and H. Steenbock
1944 The effect of $\alpha$-tocopherol and $\beta$-carotene in the oxidation of plant and animal fats.
Arch. Biochem. Vol.4,p.15.
Nutr.Abs. and Revs. Vol.14,p.265,1944.

Turpeinen, O.
1938

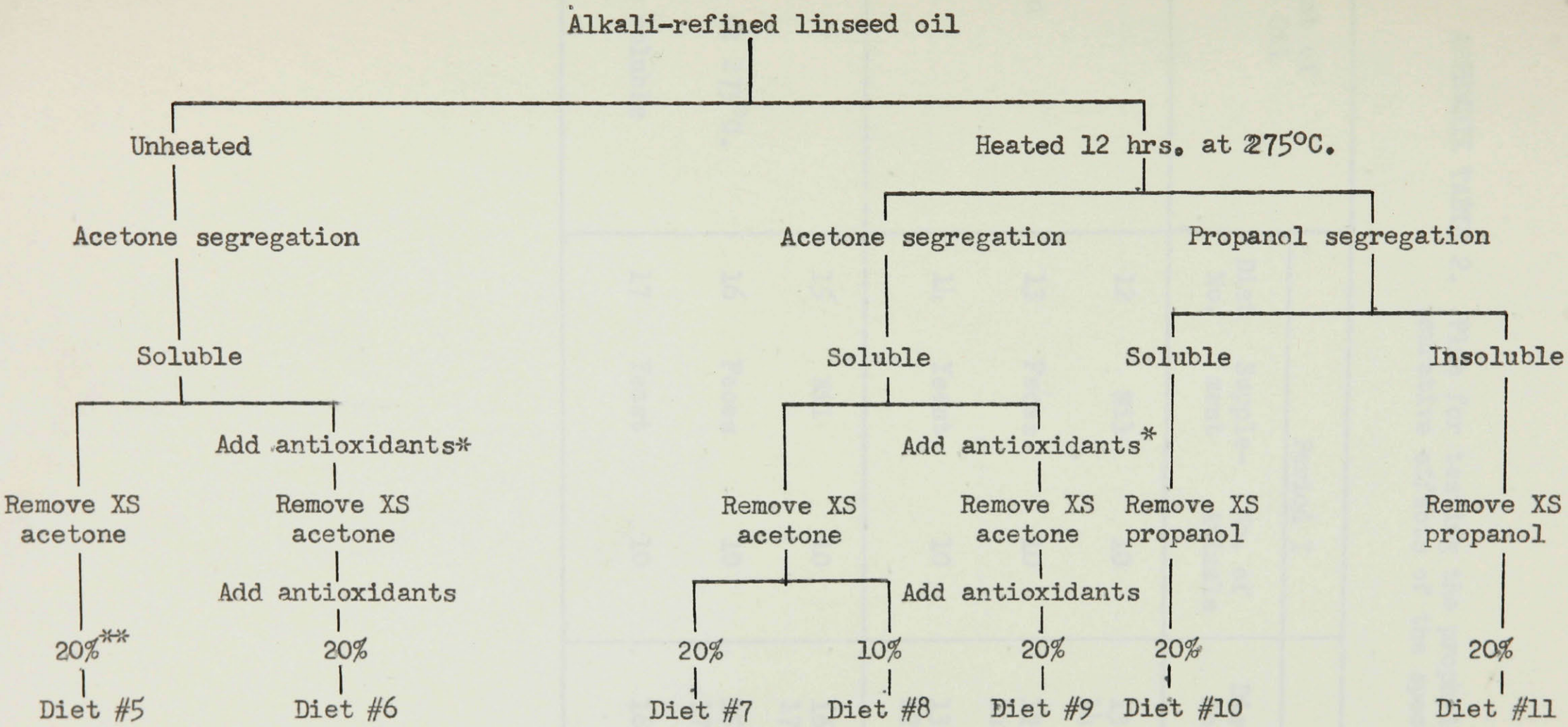
Further studies on the unsaturated fatty acids essential in nutrition.
J. of Nutr. Vol.15, p.351.

Unpublished
1949

Experiment 201
Nutrition Laboratory, Macdonald College, P.Q.

# APPENDIX

APPENDIX TABLE I - Scheme of preparation for oils used in diets 5 to 11




*Antioxidants: 0.025% N.D.G.A. )
0.025% Citric Acid) added at each addition

**Level (%) of oil in total diet fed.

APPENDIX TABLE 2. Plan for testing the prophylactic and curative effects of the special supplements

| Treatment of Linseed Oil | Period I | | | Period II | | |
|---|---|---|---|---|---|---|
| | Diet No. | Supplement | No. of Animals | Diet No. | Supplement | No. of Animals |
| Unheated | 12 | Nil | 10 | 13 | Feces | 5 |
| | | | | 14 | Yeast | 5 |
| | 13 | Feces | 10 | 12 | Nil | 5 |
| | | | | 14 | Yeast | 5 |
| | 14 | Yeast | 10 | 13 | Feces | 5 |
| | | | | 12 | Nil | 5 |
| Heated 12 hrs. at 275°C. | 15 | Nil | 10 | 16 | Feces | 5 |
| | | | | 17 | Yeast | 5 |
| | 16 | Feces | 10 | 15 | Nil | 5 |
| | | | | 17 | Yeast | 5 |
| Acetone soluble | 17 | Yeast | 10 | 16 | Feces | 5 |
| | | | | | Nil | 5 |

APPENDIX TABLE 3 - Peroxide values of the stabilized and unstabilized diets

| Diet No. | Treatment of linseed oil | Peroxide value | |
|---|---|---|---|
| | | 1st.day | 28th day |
| 5 | Unheated, acetone sol. | 99 | 276 |
| 6 | Unheated, acetone sol. + antioxidants | 26 | 55 |
| 7 | Heated, acetone sol. | 116 | 333 |
| 9 | Heated, acetone sol. + antioxidants | 25 | 38 |

APPENDIX TABLE 4. Total air-dry feed eaten and factors for conversion to digested calories

| Linseed oil | Period I | | | Factors for Conversion* | | | Period II | | |
|---|---|---|---|---|---|---|---|---|---|
| | Supplement | No. Animals | Total feed | % D.M. | Cal./gm. | % Cal.Dig. | Supplement | No. Animals | Total Feed |
| Unheated | Nil | 9 | 2299 | 94.07 | 5.04 | 96.16 | Feces<br>Yeast | 5<br>4 | 1824<br>1606 |
| | Feces | 10 | 3579 | 93.44 | 4.95 | 76.24 | Nil<br>Yeast | 5<br>5 | 1590<br>2328 |
| | Yeast | 10 | 2996 | 93.50 | 5.12 | 95.71 | Feces<br>Nil | 5<br>5 | 2146<br>1895 |
| Heated acetone soluble | Nil | 10 | 2325 | 93.29 | 5.09 | 93.73 | Feces<br>Yeast | 5<br>5 | 1560<br>1821 |
| | Feces | 10 | 2795 | 93.33 | 5.05 | 76.81 | Nil<br>Yeast | 5<br>5 | 1322<br>1755 |
| | Yeast | 10 | 2632 | 93.01 | 4.99 | 91.84 | Feces<br>Nil | 5<br>5 | 2055<br>1354 |

* These factors used to convert air-dry feed of corresponding diets in Period II to digested calories.