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Effects of dietary fatty acids, polyunsaturated/saturated ratios, and fat levels on growth and mineral deposition in young male rats

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EFFECTS OF DIETARY FATTY ACIDS, POLYUNSATURATED/
SATURATED RATIOS, AND FAT LEVELS ON GROWTH
AND MINERAL DEPOSITION IN YOUNG MALE RATS

by

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Approved by

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APPROVAL PAGE

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The purpose of this study was to investigate dietary effects of short-chain saturated (SCSFA), long-chain saturated (LCSFA), long-chain monounsaturated (LCMFA), and long-chain polyunsaturated (LCPFA) fatty acids on growth and mineral status in male weanling rats. Two experiments were used, and the length of each experiment was four weeks. Two levels (5% and 10%) of dietary fat were used in each experiment. In Experiment 1 butyric and caproic acids (SCSFA), stearic and palmitic acids (LCSFA), oleic acid (LCMFA) and linoleic and linolenic acids (LCPFA) were used to formulate four test diets. A corn oil reference diet was also included in Experiment 1. In Experiment 2 linoleic, linolenic, palmitic and stearic acids were used to formulate P/S ratios of 0.1, 0.4, 1.0, 4.0 and 8.0. Parameters used for evaluating animal responses included weight gain, hemoglobin, hematocrit, liver, kidney, spleen and testes concentrations of copper, iron, zinc and manganese and bone (femur and tibia) levels of calcium, phosphorus, magnesium and zinc.

The presence of saturated fatty acids and increases in dietary fat levels, regardless of the type of fatty acid, were associated with significant decreases in weight gains. Increases in P/S ratios were associated with increases in weight gains. Neither type of fatty acid, P/S ratio nor the level of dietary fat had any effect on hemoglobin and hematocrit levels.

Animals on LCSFA diets had higher liver, kidney and testes copper levels than animals on the other test diets. Increasing

level of fat from 5% to 10% resulted in decreased spleen copper deposition in animals on LCMFA and LCPFA diets. P/S ratios in the diet had no apparent effect on liver, kidney and spleen copper levels.

Saturated fatty acids significantly increased liver and kidney iron levels in this study. Spleen and testes iron levels tended to be higher in animals fed saturated fatty acids. Higher P/S ratios were associated with significantly lower liver and higher spleen iron deposition.

LCPFA diets significantly decreased kidney zinc levels regardless of level of fat, but no effects of LCPFA were observed on liver and spleen zinc values. In the testes, animals on LCSFA diet at both levels of fat had higher zinc content than animals on other experimental diets. P/S ratios had no apparent effect on liver and kidney zinc levels. Highest spleen zinc levels were observed in animals fed diets of P/S ratio of 1, and increases in dietary fat were associated with decreased spleen zinc levels.

Neither dietary fatty acids nor level of fat had an effect on kidney, spleen and testes manganese. In the liver higher manganese levels were found in animals on LCMFA and LCPFA diets. Neither P/S ratio nor level of fat had an effect on liver, kidney, testes and spleen manganese levels.

Dietary fatty acids, P/S ratios and fat levels were found to have no effects on the tibia and femur mineral levels. This could be due to the fact that dietary effects take longer periods of time to induce observable changes in the bone.

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This dissertation is dedicated to Dr. Aden C. Magee for his dedication and commitment to his students at the University of North Carolina at Greensboro. God bless you, Dr. Magee.

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CHAPTER I

INTRODUCTION

Recent evidence seems to indicate that the type of fat in the diet has an influence on the bioavailability of trace minerals (Johnson, Lukaski, & Bowman, 1987; Lukaski, Klevay, Bolonchuk, Mahalko, Milne, Johnson, & Sanstead, 1982). Dietary guidelines published by health-oriented professional agencies, like the American Heart Association (1985), as well as The Surgeon Generals Report (1988), have emphasized the need to reduce total dietary fat consumption, especially the intake of animal fats which are high in saturated fatty acids, to protect against the incidence of coronary heart disease. Associations like the American Academy of Pediatrics (1986) have also formulated dietary guidelines for infants and children in an attempt to reduce the early development of atherosclerosis. The importance of incorporating more polyunsaturated fats at the expense of saturated fats has been greatly emphasized and has resulted in more Americans favoring a vegan type of dietary regime. Animal protein and fats in the diet are being replaced by plant protein sources and vegetable oils and fats (Goor, 1985; Hegstead, 1985). It has been known that replacing animal protein with plant protein decreases the biovavailability of some trace minerals like iron, copper and zinc (Mahalko, Sanstead, Johnson, & Milne, 1983). Phytate, a compound found in many plant components--seeds, roots, and tubers--

has been reported to impair trace mineral absorption, especially zinc (Hambridge, Casey, & Krebs, 1986; Prasad, 1982). Reducing animal protein to reduce consumption of fat and cholesterol could result in unintentional consequences regarding trace mineral status. Magnesium deficiency has been reported to produce spasms of the coronary arteries that could contribute to ischemic heart disease (Turlapaty & Alturn, 1980). Zinc deficiency is thought to alter serum cholesterol levels (Hambridge, Casey, & Krebs, 1986). Optimal intakes of calcium and iron are thought necessary to keep cholesterol levels normal and prevent atherosclerosis (Mertz, 1982). While it may seem beneficial to modify dietary patterns to prevent the incidence of coronary heart disease, it may not be so advantageous to do so if the dietary regime precipitates other health hazards like trace mineral deficiencies. Although the effects of dietary sources of fat on some trace minerals have been reported, the exact nature of the relationship between individual types of dietary fatty acids and polyunsaturated/saturated fat ratios on trace mineral deposition is rather fragmentary and needs to be studied further due to its implications on health.

The purposes of this study were to investigate the dietary effects of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) and the effects of different polyunsaturated/saturated fatty acid (P/S) ratios on growth and tissue deposition of calcium, phosphorus, magnesium, copper, iron manganese, and zinc in weanling rats. Two fat levels, 5% and 10%, were selected in order to determine which dietary level might have the most prominent effect on mineral deposition in the tissues studied.

CHAPTER II

REVIEW OF LITERATURE

One of the earliest indications of a possible relationship between dietary fat and trace mineral bioavailability was indicated in a study conducted by Babatunde in 1972. Babatunde reported that the level of zinc required for optimum growth and feed utilization increased as the level of saturated fat in the form of lard was increased. In 1979, Bettger, Reeves, Moscatelli, Reynolds, and O'Dell also reported that rats fed a zinc deficient diet supplemented with corn oil (high in polyunsaturated fatty acids) grew more rapidly than rats fed a zinc deficient diet supplemented with hydrogenated coconut oil (high in saturated fatty acids). When the diet was adequate in zinc, similar growth rates were observed in animals fed either types of fat sources. In another study with rats, Bettger, Wong and Paterson (1986) reported that polyunsaturated fatty acids aggravated the signs of zinc deficiency and did not support optimal growth. However, in contrast to studies with rats, Bettger, Reeves, Moscatelli, Savage, and O'Dell (1980) found that chicks on a zinc deficient diet which was supplemented with hydrogenated coconut oil had higher growth rates than chicks fed a zinc deficient diet supplemented with corn oil. When zinc was adequately provided in the diet, growth rate was similar regardless of the type of fat in the diet. The investigators concluded that the differences in the zinc-fatty acid interactions noted in each of their studies could be due to differences in basal matabolic rates in the two animal species.

Amine and Hegstead (1975) reported that diets high in fat favored iron absorption and utilization, and iron absorption was greater in rats fed diets in which the fat was supplied as corn oil. These investigators also observed that more iron was absorbed when iron deficient rats were repleted with diets containing 30% fat than with diets containing 5% fat. Onderka and Kirksey (1975) reported higher iron levels in the liver, spleen and kidneys of rats fed diets containing coconut oil as compared to those fed safflower oil (high in PUFAs). Bowering, Masch, and Lewis (1977) found that increases in dietary fat level from 5% to 20% and incorporation of a more saturated fat source (from corn oil to lard) were associated with small but significant increases in iron absorption when compared to the control diet containing 5% corn oil. An increase in dietary fat and a change from corn oil to lard produced significant increases in liver iron accumulation. Monsen and Cook (1979) reported that fat as corn oil had little influence on the absorption of nonheme iron but when the basal diet was deleted of the fat (as corn oil), the absorption of nonheme iron was found to increase. Doubling the level of dietary fat (as corn oil) in the basal diet had no effect on the absorption of nonheme iron. These results seem to be contrary to studies which have reported enhancement of iron absorption with increase in fat intake irrespective of type of fat. Jones (1985) reported that liver iron deposition was low in rats fed corn oil when compared to those fed vegetable shortening as the fat source. In a recent study Johnson, Lukaski, and Bowman (1987) reported that a higher level of fat in the diet enhanced the absorption of heme and nonheme iron. At lower iron intakes, cocomut oil tended to enhance absorption of iron compared to safflower oil. The investigators reported significant effects of dietary fat level, type of fat, and dietary iron level on liver iron of both heme and nonheme groups. In general, high fat diets resulted in higher liver iron levels with coconut oil enhancing liver iron content as compared to safflower oil.

Sinthusek and Magee (1984) reported that rats fed corn oil diets had significantly lower liver copper levels than did the group of rats fed coconut oil, which led the above researchers to conclude that the availability of copper is enhanced if the diet contains a high percentage of saturated fats. Lin (1985), however, observed no significant differences between liver copper, iron and zinc levels in young rats fed coconut oil or lard. From their combined works, investigators Magee, Jones, Lin, Sinthusek, Frimpong, and Wu (1986) have concluded that the bioavailability of copper, iron and zinc in the rat may be dependent upon the type and level of fat in the diet.

Although knowledge about fatty acid and trace mineral bioavailability is very fragmentary and contradictory, a relationship seems to emerge indicating saturated fatty acids tend to enhance trace mineral bioavailability compared to polyunsaturated fatty acid sources. On the other hand, in response to early American dietary trends (Pennington, 1986; Welsh & Martson, 1982) many health professionals and nutritionists were concerned about the increasing consumption of animal fat from meat and dairy products (mainly saturated fat) and the decreasing use of complex carbohydrates, especially since these dietary trends were associated with major killer diseases like cardiovascular heart disease (CHD) (Grundy, 1986; Multiple Risk Factor Intervention Trial Research Group, 1982) and cancer (American Cancer Society Special Report, 1984; Committee on Diet, Nutrition and Cancer, National Research Council, 1982; Palmer, 1983). In 1988, the Surgeon General's Report on Nutrition and Health concluded that overconsumption of foods high in fat and underconsumption of foods high in complex carbohydrate were responsible for the high incidence of CHD and cancer in the country. The 1989 National Research Council's report, "Diet and Health," drew similar conclusions. Several health agencies of the government and the public sector (American Academy of Pediatrics, 1986; Food and Nutrition Board, 1989; Nutrition Committee, American Heart Association, 1988) have recommended that Americans reduce their intake of total fat, especially saturated fat and cholesterol and increase consumption of polyunsaturated fats, complex carbohydrates and dietary fiber. Dietary fiber and phytate, major plant components have also been reported to impair mineral absorption (Hambridge et al., 1986; Monsen, 1988; Oberleas & Harland, 1981; O'Dell, 1984; Turnland, 1988). Although mineral elements represent only a very small fraction of human body weight, they are known to play important functions in highly specific ways.

Calcium is the most abundant mineral in the human body and is mainly stored in the bones where it plays two important roles. It serves as a resevoir to prevent alteration of blood calcium concentration and to provide a rigid frame to hold the body upright. Calcium is also involved in normal muscle contraction and relaxation, proper nerve functioning, blood clotting, blood pressure and immune defenses. Hormones that promote growth, stomach acid, vitamin D and lactose are known to enhance calcium bioavailability. High fiber and protein diets, phytic and oxalic acids have been reported to impair calcium balance (Heaney, Recker, & Hinders, 1988; Heaney, Recker, & Hinders, 1989). Stunted growth in children and bone loss (osteoporosis) in adults are symptoms of calcium deficiency. Similar signs of deficiency have been observed in experimental animals (Allen, 1982).

Phosphorus is the mineral in the second largest quantity in the body and is found combined with calcium in the bones and teeth. Phorphorus is part of every cell, as part of deoxyribonucleic acid (DNA) and of ribonucleic acid (RNA). It is found in phospolipids of cell membranes and plays an important role in energy transfer and buffer systems in the body. Animal protein is the best source of phosphorus, and deficiencies of this mineral are yet unknown (Whitney, Hamilton, & Rolfes, 1990).

Most of the magnesium present in the body is stored in the bone. The mineral plays an important role in bone mineralization, building of protein, enzyme action, normal muscular contraction, transmission of nerve impulses and maintenance of teeth (Wester, 1987). Deficiency results in growth failure, weakness and convulsions in humans and experimental animals (Li & Vallee, 1980).

The trace mineral iron performs important roles as part of the protein hemoglobin which transports oxygen to various parts of the body and as part of the protein myoglobin in muscles which makes oxygen available for muscle contraction (Hallberg, 1984). Reserve or storage iron, ferritin and hemosiderrin occur in highest concentrations in the liver, spleen and bone marrow, and these tissues serve as useful index of body stores (Mertz, 1981). The reticuloendothelial cells of these tissues are involved in the removal of hemoglobin from the red blood cells, breakdown of the heme moiety, release of iron and the return of this iron to the plasma. Mobilization of iron from stores requires the copper containing enzyme of the plasma-cerulopasmin (ferroxidase I) (Morris, 1987). Iron absorption is decreased by increased levels of dietary calcium and phosphate. Increased dietary zinc, copper and manganese are reported to compete with iron for absorption binding sites (Monsen, 1988).

The major organ involved in zinc metabolism is the liver, and liver biopsy has been used in the assessment of zinc status (Keen, 1988). Zinc is an important cofactor for various enzymes, is associated with the functioning of the hormone insulin, involved in making genetic material and proteins, in taste perception, wound healing, spermatogenesis in the testes, normal growth and collagen synthesis. Dietary fiber, phytate, calcium, phosphate and iron are known to have inhibitory effects on zinc absorption. Zinc toxicity results in anemia due to reduced hemoglobin synthesis (Hambridge et al., 1986).

Copper performs several vital roles in the body--it serves as a cofactor for several enzymes, it is necessary for the absorption and use of iron in the formation of hemoglobin, manufacture of collagen and the healing of wounds. Tissues that contain relatively high concentration of copper include the liver and the spleen, both of which are very responsive to variations in dietary copper intakes (Davis & Mertz, 1987). Copper deficiency is rare but has been reported in children with iron deficiency anemia. It severely disturbs growth in children and experimental animals. Excess zinc interferes with copper absorption and can cause deficiency (Rosenbury & Solomons, 1982; Turnland, 1988).

Manganese occurs only in trace amount in human and animal tissues and is distributed widely throughout the body fluids and tissues. The mineral tends to be in higher concentrations in tissues rich in mitochondria. Bones, liver and kidney normally contain higher concentrations of manganese than other organs. Levels in the bones can be raised or lowered by substantially varying the manganese intakes of the experimental animal and may be used as a bioassay for dietary manganese bioavailability (Davis & Mertz, 1987). Manganese functions as a cofactor for various enzymes in the body and deficiency (in animals), results in poor growth, nervous system disorders and reproductive abnormalities (Whitney et al., 1990).

Thus, these minerals play important roles in the normal functioning of the body. If altering dietary fat does alter mineral bioavailability as studies mentioned earlier seem to indicate, it brings out the need to closely study the relationship between dietary fat and polyunsaturated to saturated (P/S) ratios on mineral bioavailability. The present study, using individual types of fatty acids and polyunsaturated to saturated (P/S) ratios, was conducted to examine the type of relationship existing between fatty acids and trace minerals. A search of the literature also indicated that little information was available on the effects of level and source of fats on the bioavailability of calcium, phosphorus, magnesium or manganese. Since there is a possibility that these minerals may be affected by the degree of saturation of a fat, this study attempts to focus on the effects of saturated and polyunsaturated fatty acids on the status of these minerals in the rat.

CHAPTER III

METHODS AND PROCEDURES

The purposes of this study were to investigate the effects of saturated and unsaturated fatty acids and the effects of different polyunsaturated fatty acid: saturated fatty acid (P/S) ratios on growth and tissue deposition of calcium, phosphorus, magnesium, copper, iron, manganese, and zinc in male weanling Sprague-Dawley[1] rats.

The study was conducted in two phases. In Experiment 1, the fatty acids used as fat sources in the diet included short-chain saturated fatty acids (SCSFA) (i.e., butyric and caproic acids), long-chain saturated fatty acids (LCSFA) (i.e., stearic and palmitic acids), long-chain monounsaturated fatty acid (LCMFA) (i.e., oleic acid) and long-chain polyunsaturated fatty acids (LCPFA) (i.e., linoleic and linolenic acids). Corn oil was also included as a fat source for one group of animals to provide a reference for judging the performance of animals on the test diets. Corn oil is the standard fat source used in animal studies of this nature. Each fat source was fed at two levels, 5% and 10%, of the diet. In Experiment 2, five different P/S ratios of fatty acids (0.1, 0.4, 1.0, 4.0, 8.0) were fed at two levels, 5% and 10%, of the diet. The polyunsaturated

[1]Purchased from Holtzman Company, Madison, WI.

fatty acids--linoleic and linolenic--and the saturated fatty acids--palmitic and stearic--were the fatty acids used to formulate diets with various P/S ratios.

The parameters used for evaluating the results from both experiments included food intake, weight gain, hemoglobin, hematocrit, concentrations of copper, iron, zinc and manganese in the liver, kidney, spleen and testes; and concentrations of calcium, phosphorus, magnesium, and zinc in the bone (both femur and tibia).

A 2X5 factorial randomized block design was used for both experiments. For Experiment 1, dietary factors included two levels of fat (i.e., 5%, 10%) and five sources of dietary fat (i.e., SCSFA, LCSFA, LCMFA, LCPFA, and a corn oil reference diet). In Experiment 2, two levels of fat (i.e., 5%, 10%) and five P/S fatty acid ratios (0.1, 0.4, 1.0, 4.0, 8.0) constituted the dietary variables.

Animals

Sixty male weanling Sprague-Dawley rats were used in each experiment. The rats used in both experiments were approximately 21-24 days old and averaged 54 grams (Experiment 1) and 55 grams (Experiment 2) in weight initially.

A randomized block design involving six replications was used for both experiments. The animals were housed in individual stainless steel, wire mesh cages and had free access to the test diets and water during the experiments. The experimental diets were randomly assigned to individual animals within a replication, and food consumption records were maintained daily during both experiments. Spillage of the diet was accounted for on a biweekly basis. The experimental period for each of the experiments was four weeks during which the animals were weighed weekly. Total weight gain while on the experimental diet was calculated.

At the end of the experimental periods, the animals were fasted for 12 hours, and tail blood was drawn for hemoglobin and hematocrit determinations. All the animals were sacrificed from Experiments 1 and 2, and the liver, kidneys, spleen, testes, femur and tibia of each animal were removed and weighed. The tissues were dried to constant weight in an oven at 60C, and dry weights were obtained on the samples. The dried tissues and bones were then ashed initially with hot concentrated nitrate acid to remove all organic matter. Final ashing was accomplished using hot percloric acid. The ash of each sample was dissolved in 3ml of 0.6N HCl. The volume was made up to 25ml with redistilled water for the tissue samples. The bone samples were diluted to 100ml. All copper, iron, zinc, manganese calcium, and magnesium concentrations were determined on the tissues and bones by atomic absorption spectrometry[2]. Inorganic phosphorus levels in tibias and femurs were determined colorimetrically by the method of Simonsen, Wertman, Westover, and Mehl (1946).

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[2]Model Video 12E, Thermo Jarell Ash Corporation, Waltham, MA.

Diets

Diets in Experiment 1 differed in the fatty acids used as fat sources. The test diets included short-chain saturated fatty acids[3] (butyric and caproic acids), long-chain saturated fatty acids[4] (stearic and palmitic acids), monounsaturated fatty acid[5] (oleic acid) and polyunsaturated fatty acids (linoleic[6] and linolenic[7] acids). The fatty acids were added in equal proportions (1:1) to each of the test diets. Corn oil[8], a mixture of LCSFA, LCMFA and LCPFA (60: 25: 10, v/v), served as the fat source in the reference diet. Experiment 2 diets mainly varied in the P/S fatty acid ratio. Polyunsaturated fatty acids--linoleic and linolenic acids--and saturated fatty acids--palmitic and stearic--were used in equal amounts to formulate diets with P/S ratios of 0.1, 0.4, 1.0, 4.0 and 8.0.

In each of the experiments the fat sources were fed at two levels, 5% and 10% of the diet including the corn oil control group in Experiment 1. In addition to the fat component, the other dietary ingredients of the test diets included 50% dextrose[9], 17-22%

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[3]ICN Biochemicals Inc., Cleveland OH.

[4]Eastman Kodak Co., Rochester, NY.

[5]Fisher Scientific Company, Fairlawn, NJ.

[6]Fisher Scientific Company, Fairlawn, NJ.

[7]Eastman Kodak Co., Rochester, NY.

[8]ICN Biomedicals Inc., Cleveland OH.

[9]Dextrose monohydrate, Teklad Test Diets, Madison, WI.

cornstarch[10], 15% egg white solids[11], 4% mineral mix[12], and 2% vitamin mix, 2% cellulose[13] and 2% vitamin A and D mix[14]. The composition of all diets are given in Appendix A, Tables A-1 through A-4.

The diets were ashed and analyzed for copper, iron, zinc, manganese, calcium, and magnesium using atomic absorption spectrophotometry. Inorganic phosphorus content of the diet was determined by the colorimetric method of Simonsen et al. (1946).

Analytical Methods

Hemoglobin was determined by the method of Shenk, Hall, and King (1934). Using a sharp razor blade, a small portion of the tip of the tail of the animal was removed, and blood was drawn to the first mark on a graduated diluting pipette. The pipette containing the blood was placed in a freshly prepared 0.1% sodium carbonate, and the pipette was immediately filled to the second mark with the solution. The resulting mixture was then drained freely into a test tube.

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[10]Cornstarch, Teklad Test Diets, Madison, WI.

[11]Egg white solids, Teklad est Diets, Madison, WI.

[12]Wessons modified Osborne-Mendel mineral mix, Teklad Test Diets, Madison, WI.

[13]Alphacel, ICN Nutritional Biochemicals, Cleveland, OH.

[14]The mix contained 2000 units of vitamin A palmitate/gram and 400 units of vitamin D/gram. The vitamins were purchased from Teklad Test Diets, Madison, WI.

The optical density of a sample was read against a water reference in a spectrophotometer[15] with the wavelength set at 542 nm. The grams of hemoglobin per 100 mls of blood was calculated using the equation: optical density (O.D.) x 28.575 = grams hemoglobin per 100ml blood.

For the hematocrit determinations, blood was drawn from the tail at the same time as the blood for the hemoglobin was drawn. The heparinized hematocrit capillary tubes were filled, plugged with critoseal and centrifuged in the hematocrit centrifuge. The hematocrit percentage was then read from the Lancer Critocap Manual reader and recorded.

After hemoglobin and hematocrit samples were obtained, the animals were sacrificed by decapitation. Liver, spleen, kidneys and testes along with the femur and tibia from one of the legs were removed, completely cleaned of tissue, weighed and dried at 60C in an oven. The dried tissues and bones were weighed again and ashed. As already noted the copper, iron, zinc and magnesium concentrations of the liver, spleen, kidneys and testes as well as the calcium, zinc and manganese concentrations of the femur and tibia were determined by atomic absorption spectrophotometry. The copper determinations were read at 324.8 nm, iron at 248.3 nm, zinc at 213.9 nm, manganese at 279.5 nm, magnesium at 285.2 nm, and calcium at 422.7 nm using an air acetylene flame.

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[15]Spectronic 20, Bausch and Lomb, Rochester, NY.

Phosphorous concentrations in the femur and tibia were determined colorimetrically by the molybdivanadate method (Simonsen et al., 1946). The principle of the method is based upon the yellow color formed when an excess of molybdate is added to an acidified solution of an orthophosphate.

Statistical Methods

Data collected throughout the experimental phase were analyzed using analysis of variance (ANOVA) procedures (SAS Institute Incorporated, 1985). Statistical results were considered significant if the probability of observing such a result under the null hypothesis was less than 1 chance in 20 and highly significant if the probability of observing a result was less than 1 chance in 100 (Snedecor & Cochran, 1980). Covariant analysis were done on weight gain data of the animals to determine the effect of diet on weight gain after adjusting for food consumption. A three-way ANOVA was used for the initial analysis of the data. For Experiment 1, sources of variation included replicates, fatty acid type and fat level. In Experiment 2 these sources of variation were replicates, P/S fat ratios and fat level.

In order to provide the reader with a convenient means of determining differences between mean responses to test diets (without having to resort to ANOVA appendix tables) least significant differences (LSD) were provided (Snedecor & Cochran, 1980) as an indicator of differences between individual means at the 0.05 and 0.01 levels of probability. Duncan's Multiple Rank test was used to compare group means associated with fatty acid sources, P/S ratios or fat levels at the 0.05 level of probability.

CHAPTER IV

RESULTS

The raw data obtained from both experiments in this study are presented in Appendix B. The results of the statistical analysis of the data are found in Appendix C.

Growth

The effects of different types of fatty acids and corn oil used in Experiment 1 on growth and food intakes are given in Table 1. Increases in the degree of saturation in the fatty acid in the diet were associated with significant ($p < 0.05$) decreases in growth of young rats (Table 1). Animals fed the corn oil reference diet had significantly higher weight gains than animals fed diets containing various types of fatty acids. Increasing the level of dietary fat was associated with significant decreases in weight gains of animals regardless of type of fat in the diet. Differences in weight gains of animals fed the various dietary regimens were still significant after weight gains were adjusted for food consumption using covariant analysis.

Weight gains of animals fed diets with different P/S fat ratios (Experiment 2) are also presented in Table 1. Statistical analysis of the data (Appendix C, Table C-2) indicated no significant effects of dietary P/S ratios or fat level on weight gains of the animals.

Table 1

Growth and Feed Intake of Young Male Rats Fed Different Sources or P/S Ratios at Two Levels of Fat for Experiments 1 and 2

	Fat Level (%)					
Source	5	10	Mean[2]	5	10	Mean[2]
<u>Experiment 1</u>	4 weeks weight gain (gm)[1]			4 weeks food intake (gm)[1]		
Corn oil	191 ± 12	185 ± 8	188 ± 7^{c}	417 ± 12	376 ± 16	397 ± 14
SCSFA	147 ± 3	106 ± 14	127 ± 9^{f}	334 ± 9	234 ± 23	284 ± 19
LCSFA	146 ± 6	131 ± 5	139 ± 4^{ef}	377 ± 13	329 ± 10	353 ± 11
LCMFA	148 ± 4	155 ± 3	152 ± 2^{de}	365 ± 11	341 ± 6	353 ± 7
LCPFA	159 ± 7	153 ± 6	156 ± 4^{d}	353 ± 9	336 ± 12	344 ± 8
Mean	158 ± 4^{a}	146 ± 6^{b}		369 ± 8	323 ± 11	
LSD[3] 0.05	19					
0.01	26					

Source	Fat Level (%) 5	10	Mean[2]	5	10	Mean[2]
Experiment 2	4 weeks weight gain (gm)[1]			4 weeks food intake (gm)[1]		
0.1	92 ± 5	102 ± 4	97 ± 3	355 ± 0	355 ± 0	355 ± 0
0.4	87 ± 2	100 ± 5	94 ± 3	358 ± 2	355 ± 0	357 ± 1
1.0	94 ± 5	103 ± 3	99 ± 3	355 ± 2	355 ± 0	355 ± 0
4.0	101 ± 3	103 ± 8	102 ± 4	357 ± 0	355 ± 0	356 ± 1
8.0	113 ± 3	92 ± 3	103 ± 4	355 ± 0	355 ± 0	355 ± 0
Mean[2]	97 ± 2	100 ± 2		356 ± 1	355 ± 0	
LSD[3]						
0.05	11					
0.01	15					

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same row or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of a superscript indicates no significant difference.

[3]Least significant differences at specified probability levels.

Hemoglobin and Hematocrit

Hemoglobin and hematocrit concentrations obtained from rats in Experiments 1 and 2 are shown in Table 2. Analysis of data (Appendix C, Table C-3) for Experiment 1 indicated that neither the types of fatty acids nor the level of fat in the diet had any effect on the hemoglobin and hematocrit levels of animals. Analysis of the data (Appendix C, Table C-4) for Experiment 2 indicated that neither the P/S ratios nor the level of fat in the diet had an effect on the hemoglobin and hematocrit levels of animals on the diets.

Tissue Copper

Tissue copper concentrations of animals from Experiment 1 are given in Table 3. The statistical analysis of the data for Experiment 1 are found in Appendix C, Table C-5. The livers of rats fed the LCSFA diet contained significantly more copper than the livers of rats fed the other four diets. Although lower liver copper concentrations were observed in rats fed 10% fat, these concentrations were not statistically different from liver copper levels of animals fed 5% fat. Kidney copper concentrations were significantly ($p < 0.05$) influenced by the type of dietary fat. Animals fed LCSFA had significantly ($p < 0.05$) greater kidney copper concentrations than those fed other sources of fatty acids. Lowest kidney copper levels were observed in animals fed corn oil reference diets. With regards to the spleen, significantly higher copper concentrations were observed in animals fed LCSFA as the dietary fat source than the other fatty

Table 2

Hemoglobin and Hematocrit Levels of Young Male Rats Fed Different Sources or P/S Ratios at Two Levels of Fat for Experiments 1 and 2

	Fat Level (%)					
Source	5	10	Mean[2]	5	10	Mean[2]
<u>Experiment 1</u>	Hemoglobin (gm/dl)[1]			Hematocrit (%)[1]		
Corn oil	12.9 ± 0.3	11.7 ± 0.5	12.3 ± 0 3	48 ± 3	48 ± 3	48 ± 1
SCSFA	11.9 ± 0.2	11.8 ± 0.2	11.8 ± 0.2	46 ± 2	47 ± 1	47 ± 1
LCSFA	11.8 ± 0.2	12.0 ± 0.4	11.9 ± 0.2	43 ± 1	43 ± 1	43 ± 1
LCMFA	12.2 ± 0.4	12.4 ± 0.5	12.3 ± 0.3	46 ± 1	45 ± 2	46 ± 1
LCPFA	11.8 ± 0.3	12.2 ± 0.4	12.0 ± 0.2	47 ± 1	48 ± 2	48 ± 1
Mean[2]	12.1 ± 0.2	12.1 ± 0.2		46 ± 1	46 ± 1	
LSD[3]			LSD[3]			
0.05	1.2			0.05	4	
0.01	1.6			0.01	5	

Source	Fat Level (%) 5	10	Mean[2]	5	10	Mean[2]
Experiment 2	Hemoglobin (gm/dl)[1]			Hematocrit (%)[1]		
0.1	15.0 ± 0.2	13.3 ± 0.6	14.2 ± 0.4	55 ± 3	51 ± 3	53 ± 2
0.4	14.7 ± 0.6	14.1 ± 0.5	14.4 ± 0.4	54 ± 2	55 ± 9	55 ± 5
1.0	14.3 ± 0.2	13.9 ± 0.5	14.1 ± 0.3	51 ± 3	53 ± 3	52 ± 2
4.0	15.1 ± 0.8	14.8 ± 0.9	15.0 ± 0.6	52 ± 1	54 ± 2	53 ± 1
8.0	13.1 ± 0.4	14.4 ± 0.4	14.0 ± 0.3	54 ± 2	56 ± 2	55 ± 2
Mean[2]	14.4 ± 0.2	14.1 ± 0.3		53 ± 1	54 ± 2	
LSD[3]			LSD[3]			
0.05	1.2		0.05	4		
0.01	1.6		0.01	5		

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 3

Tissue Copper Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source		Fat Level (%) 5	10	Mean[2]
Liver		ug/gram dry weight[1]		
Corn oil		8.9 ± 0.2	9.3 ± 0.6	9.1 ± 0.3[e]
SCSFA		10.6 ± 0.4	9.4 ± 0.4	10.0 ± 0.4[e]
LCSFA		11.3 ± 0.7	11.2 ± 0.9	11.3 ± 0.6[d]
LCMFA		10.4 ± 0.3	9.3 ± 0.7	9.9 ± 0.4[e]
LCPFA		9.5 ± 0.5	8.4 ± 0.5	9.0 ± 0.4[e]
Mean[2]		10.1 ± 0.3	9.5 ± 0.3	
LSD[3]				
0.05	1.6			
0.01	2.2			
Kidney				
Corn oil		25.8 ± 1.6	22.5 ± 1.9	24.1 ± 1.3[e]
SCSFA		31.0 ± 11.0	35.6 ± 11.7	33.3 ± 7.7[e]
LCSFA		55.4 ± 20.0	75.5 ± 8.4	65.5 ± 10.8[d]
LCMFA		37.2 ± 8.5	33.4 ± 12.4	35.4 ± 7.2[e]
LCPFA		31.6 ± 4.8	35.3 ± 10.8	33.5 ± 5.6[e]
Mean[2]		36.2 ± 5.0	40.5 ± 5.3	
LSD[3]				
0.05	29.6			
0.01	39.5			

Source	Fat Level (%) 5	10	Mean[2]
Spleen	ug/gram dry weight[1]		
Corn oil	4.8 ± 0.9	5.6 ± 0.3	5.2 ± 0.5^{d,e}
SCSFA	4.8 ± 0.5	4.8 ± 0.9	4.8 ± 0.5^{e}
LCSFA	5.0 ± 0.5	7.7 ± 0.8	6.4 ± 0.6^{d}
LCMFA	5.8 ± 0.4	3.7 ± 0.4	4.8 ± 0.4^{e}
LCPFA	5.9 ± 0.5	4.7 ± 0.4	5.3 ± 0.4^{d,e}
Mean[2]	5.3 ± 0.3	5.3 ± 0.4	
LSD[3]			
0.05 1.6			
0.01 2.2			
Testes			
Corn oil	13.3 ± 0.6	13.9 ± 0.7	13.6 ± 0.5^{e}
SCSFA	12.8 ± 0.7	12.1 ± 0.4	12.5 ± 0.4^{e}
LCSFA	17.2 ± 2.8	14.8 ± 0.4	16.0 ± 1.4^{d}
LCMFA	13.1 ± 0.5	11.5 ± 0.5	12.3 ± 0.4^{e}
LCPFA	12.9 ± 0.4	12.3 ± 0.5	12.6 ± 0.3^{e}
Mean[2]	13.9 ± 0.6	13.0 ± 0.3	
LSD[3]			
0.05 3.1			
0.01 4.1			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

acid types. Increases in dietary fat levels were not associated with marked changes in spleen copper levels for animals on various diets. Although not statistically significant, animals fed long-chain mono- and polyunsaturated fatty acids at the 5% level had higher spleen copper levels than animals on the other diets. As the level of fat in the diet increased, spleen copper levels significantly increased in animals fed LCSFAs. The presence of LCMFA and LCPFA in the diets were associated with decreases in spleen copper concentrations at the 10% fat level. Analysis of the data revealed that there was a significant effect of diet on testes copper deposition. The testes of rats fed the LCSFA diet contained significantly more copper than rats fed the other four diets. As the level of fat in the diet increased, testes copper levels tended to decrease regardless of fat source.

Tissue copper concentrations of animals fed diets containing different P/S ratios and levels of fat are given in Table 4. The statistical analysis of the data is presented in Appendix C, Table C-6. There were no significant differences in liver copper levels associated with the various P/S ratios and dietary fat levels. When the level of dietary fat was increased to 10%, liver copper contents significantly increased ($\underline{p} < 0.05$) in animals fed diets with P/S ratio of four. Significant dietary fat level effects were observed in relation to kidney copper levels. Animals fed diets containing 10% fat had higher kidney copper levels than those fed diets containing fat at the 5% level. Animals on diets with P/S ratios of 0.1 had significantly ($\underline{p} < 0.05$) higher kidney copper levels than animals

Table 4

Tissue Copper Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

	Fat Level (%)		
P/S Ratio	5	10	Mean[2]
Liver	ug/gram dry weight[1]		
0.1	7.9 ± 0.4	8.5 ± 0.5	8.2 ± 0.3
0.4	8.0 ± 0.3	7.6 ± 0.4	7.8 ± 0.2
1.0	7.8 ± 0.6	8.2 ± 0.3	8.0 ± 0.3
4.0	6.7 ± 0.3	8.4 ± 0.4	7.6 ± 0.4
8.0	7.5 ± 1.1	8.8 ± 0.3	8.2 ± 0.6
Mean[2]	7.6 ± 0.3	8.3 ± 0.2	
LSD[3]			
0.05 1.6			
0.01 2.2			
Kidney			
0.1	40.2 ± 4.0	53.6 ± 6.4	46.9 ± 4.1[c]
0.4	31.8 ± 1.7	39.8 ± 4.9	35.8 ± 2.7[d]
1.0	32.8 ± 2.4	44.0 ± 3.2	38.4 ± 2.5[d]
4.0	35.1 ± 3.1	41.5 ± 4.3	38.3 ± 2.7[d]
8.0	42.2 ± 5.4	32.5 ± 1.3	37.4 ± 3.0[d]
Mean[2]	36.4 ± 1.7[b]	42.3 ± 2.2[a]	
LSD[3]			
0.05 11.1			
0.01 15.0			

P/S Ratio			Fat Level (%) 5	10	Mean[2]
Spleen			ug/gram dry weight[1]		
0.1			7.8 ± 0.8	8.0 ± 0.9	7.9 ± 0.6
0.4			8.9 ± 0.4	6.6 ± 1.1	7.8 ± 0.7
1.0			7.7 ± 8.0	7.2 ± 1.5	7.5 ± 0.8
4.0			8.3 ± 1.2	7.5 ± 0.7	7.9 ± 0.7
8.0			9.5 ± 1.0	8.0 ± 1.4	8.8 ± 0.9
Mean[2]			8.4 ± 0.4	7.5 ± 0.5	
LSD[3]					
	0.05	2.8			
	0.01	3.8			
Testes					
0.1			13.5 ± 1.6	18.6 ± 3.0	16.1 ± 1.8
0.4			19.1 ± 4.1	15.5 ± 1.7	17.3 ± 2.2
1.0			17.2 ± 2.2	15.8 ± 1.8	16.5 ± 1.4
4.0			15.8 ± 1.6	15.8 ± 1.9	15.8 ± 1.2
8.0			16.2 ± 1.8	16.3 ± 1.9	16.3 ± 1.3
Mean[2]			16.4 ± 1.1	16.4 ± 0.9	
LSD[3]					
	0.05	4.3			
	0.01	5.8			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

receiving the other P/S ratios. Animals on diets with P/S ratios of 0.4 had the lowest levels of kidney copper. Copper levels of the spleen were not influenced by dietary P/S ratios nor by the level of fat in the diet. Animals fed the 5% fat diet with a P/S ratio of eight, however, tended to have higher copper levels than animals fed the other dietary combinations. Testes copper values did not change with increasing P/S ratios and were essentially the same with either level of dietary fat.

Tissue Iron

Tissue iron deposition in rats in Experiment 1 are given in Table 5, and the statistical analysis of the data are presented in Appendix C, Table C-7. Statistical analysis of liver iron levels revealed significant effects attributed to both type of dietary fat and level of fat in the diet. Animals fed LCSFA had the highest ($p < 0.05$) concentrations of liver iron while animals fed corn oil or LCPFA had the lowest ($p < 0.05$) levels of iron in the liver. Increasing the level of dietary fat from 5% to 10% was associated with significant increases in liver iron deposition. Animals fed LCSFA had liver iron levels 1.5 to 3 times higher than those found in animals fed the other dietary combinations. The type of diet was found to have a significant effect ($p < 0.05$) on kidney iron deposition. Diets containing LCSFA were associated with significant ($p < 0.05$) increases in kidney levels of iron in animals fed these fatty acids. Although not statistically significant, kidney iron

Table 5

Tissue Iron Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source		Fat Level (%) 5	10	Mean[2]
Liver		ug/gram dry weight[1]		
Corn oil		254 ± 18	268 ± 22	261 ± 14[e]
SCSFA		443 ± 51	479 ± 50	461 ± 34[d]
LCSFA		625 ± 64	973 ± 57	799 ± 66[c]
LCMFA		417 ± 41	373 ± 29	395 ± 66[d]
LCPFA		270 ± 17	278 ± 19	274 ± 12[e]
Mean[2]		402 ± 31[b]	474 ± 51[a]	
LSD[3]				
0.05	118			
0.01	158			
Kidney				
Corn oil		166 ± 6	162 ± 9	164 ± 5[d]
SCSFA		177 ± 14	183 ± 12	180 ± 9[cd]
LCSFA		180 ± 7	209 ± 10	195 ± 7[c]
LCMFA		158 ± 11	174 ± 9	166 ± 7[d]
LCPFA		170 ± 9	168 ± 11	169 ± 7[d]
Mean[2]		170 ± 4	179 ± 5	
LSD[3]				
0.05	28			
0.01	38			

Source			Fat Level (%) 5	10	Mean[2]
			ug/gram dry weight[1]		
Spleen					
Corn oil			658 ± 27	872 ± 123	765 ± 68[de]
SCSFA			925 ± 146	1058 ± 71	992 ± 80[d]
LCSFA			1584 ± 268	1728 ± 253	1656 ± 177[c]
LCMFA			1007 ± 224	876 ± 56	942 ± 112[de]
LCPFA			656 ± 45	630 ± 31	643 ± 26[f]
Mean[2]			966 ± 95	1033 ± 89	
LSD[3]					
	0.05	448			
	0.01	602			
Testes					
Corn oil			165 ± 19	140 ± 19	153 ± 13
SCSFA			158 ± 19	144 ± 23	151 ± 14
LCSFA			154 ± 20	163 ± 18	159 ± 13
LCMFA			142 ± 17	134 ± 12	138 ± 10
LCPFA			138 ± 20	109 ± 6	124 ± 11
Mean[2]			151 ± 8	138 ± 8	
LSD[3]					
	0.05	42			
	0.01	57			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

levels tended to be higher in animals on the SCSFA, LCSFA, and LCMFA fed at a 10% level. Dietary fat source had a significant effect on spleen iron concentartions. Spleen iron levels were highest ($p < 0.05$) in those animals fed LCSFA. As the degree of unsaturation of fatty acids in the diet increased, spleen iron levels decreased. Rats fed the LCSFA had the lowest ($p < 0.05$) levels of iron in their spleens. Increases in fat from 5% to 10% resulted in a nonsignificant increase in spleen iron concentrations in those animals fed SCSFA and LCSFA diets. No significant dietary effect was observed on testes iron concentrations in the rat, but animals on the SCSFA and LCSFA diets at either fat levels tended to have higher testes iron concentrations than those on the LCMFA or LCPFA diets. At higher levels of fat in the diet, iron concentrations in the testes tended to be lower.

Tissue iron levels of rats fed different P/S ratios are given in Table 6. The statistical analysis of the data from Experiment 2 is presented in Appendix C, Table C-8. Analysis of liver iron levels indicated significant ($p < 0.05$) diet and level effects. Animals on diets with P/S ratios of 0.1 had highest ($p < 0.05$) liver iron levels while animals on diets with P/S ratios of eight had the lowest ($p < 0.05$) tissue mineral levels. As the level of dietary fat increased, liver iron levels significantly ($p < 0.05$) decreased. Although P/S ratio and level of dietary fat had no significant effects on kidney iron deposition in animals in Experiment 2, animals fed diets with P/S ratios of 0.1 and 0.4 tended to have higher kidney iron levels than animals fed the other P/S ratio diets. Significant

Table 6

Tissue Iron Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

Source			Fat Level (%)		
			5	10	Mean[2]
Liver			ug/gram dry weight[1]		
0.1			720 ± 80	509 ± 37	615 ± 53[c]
0.4			509 ± 38	439 ± 36	474 ± 27[d]
1.0			569 ± 61	345 ± 23	457 ± 46[d]
4.0			357 ± 33	332 ± 36	345 ± 23[e]
8.0			319 ± 25	239 ± 35	279 ± 24[e]
Mean[2]			495 ± 35[a]	373 ± 22[b]	
LSD[3]					
	0.05	120			
	0.01	161			
Kidney					
0.1			236 ± 16	198 ± 4	217 ± 10
0.4			220 ± 10	217 ± 13	219 ± 8
1.0			207 ± 10	190 ± 15	199 ± 9
4.0			177 ± 11	204 ± 12	191 ± 9
8.0			188 ± 10	206 ± 33	197 ± 17
Mean[2]			206 ± 6	203 ± 8	
LSD[3]					
	0.05	43			
	0.01	58			

Source	Fat Level (%) 5	10	Mean[2]
	ug/gram dry weight[1]		
Spleen			
0.1	2372 ± 490	1006 ± 164	1689 ± 321[c]
0.4	1081 ± 128	830 ± 202	956 ± 117[d]
1.0	1362 ± 407	432 ± 23	897 ± 240[d]
4.0	852 ± 89	392 ± 56	622 ± 86[d]
8.0	594 ± 102	268 ± 43	431 ± 72[d]
Mean[2]	1252 ± 168[a]	586 ± 73[b]	
LSD[3]			
0.05 633			
0.01 853			
Testes			
0.1	95 ± 12	107 ± 14	101 ± 9
0.4	104 ± 8	107 ± 12	106 ± 7
1.0	100 ± 10	91 ± 4	96 ± 5
4.0	88 ± 7	102 ± 9	95 ± 6
8.0	106 ± 14	107 ± 11	107 ± 9
Mean[2]	99 ± 5	103 ± 5	
LSD[3]			
0.05 30			
0.01 41			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

($p < 0.05$) source and level of dietary fat effects were observed on spleen iron levels in the rat. Animals fed diets with P/S ratios of one had significantly ($p < 0.05$) higher spleen iron levels than those fed other diets. As the P/S ratio increased, spleen iron levels tended to decrease. Lowest spleen iron levels were observed in the animals fed P/S ratios of four and eight. Animals on the 5% dietary fat level had significantly ($p < 0.05$) higher spleen iron levels than animals fed 10% fat. Thus, increases in fat levels and dietary P/S ratios were associated with significant decreases in iron concentrations in the spleen. Neither P/S ratio nor level of dietary fat had an effect on the tissue iron levels. However, as the level of dietary fat increased, testes iron levels tended to increase in the rat. Testes iron deposition tended to be the highest in animals fed diets with P/S ratios of eight.

Tissue Zinc

Tissue zinc concentrations of animals fed different sources of dietary fatty acids (Experiment 1) are given in Table 7, and the statistical analysis of the data are found in Appendix C, Table C-9. Although dietary fat had no significant effect on liver zinc concentrations in rats, liver zinc levels tended to increase as the degree of unsaturation increased. Diets had significant ($p < 0.05$) effects on kidney zinc concentrations. Animals fed LCPFA as the dietary fat source had significantly ($p < 0.05$) lower kidney zinc concentrations than animals fed other fat sources, and this was observed in animals

Table 7

Tissue Zinc Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source		Fat Level (%) 5	10	Mean[2]
Liver		ug/gram dry weight[1]		
Corn oil		68 ± 3	71 ± 3	70 ± 2
SCSFA		68 ± 2	63 ± 2	66 ± 2
LCSFA		72 ± 4	67 ± 5	70 ± 3
LCMFA		73 ± 4	68 ± 3	71 ± 2
LCPFA		73 ± 4	76 ± 3	75 ± 3
Mean[2]		71 ± 2	69 ± 2	
LSD[3]				
0.05	9			
0.01	12			
Kidney				
Corn oil		93 ± 2	92 ± 2	93 ± 1[d]
SCSFA		94 ± 2	100 ± 2	97 ± 1[cd]
LCSFA		93 ± 5	95 ± 5	94 ± 4[cd]
LCMFA		95 ± 4	102 ± 4	99 ± 3[c]
LCPFA		83 ± 1	85 ± 5	84 ± 2[e]
Mean[2]		92 ± 2	95 ± 2	
LSD[3]				
0.05	7			
0.01	10			

Source	Fat Level (%) 5	10	Mean[2]
Spleen	ug/gram dry weight[1]		
Corn oil	93 ± 5	97 ± 3	95 ± 3
SCSFA	95 ± 4	91 ± 3	93 ± 2
LCSFA	92 ± 3	78 ± 16	85 ± 7
LCMFA	99 ± 3	109 ± 15	104 ± 7
LCPFA	92 ± 4	92 ± 4	92 ± 3
Mean[2]	94 ± 2	93 ± 4	
LSD[3]			
0.05 — 20			
0.01 — 27			
Testes			
Corn oil	198 ± 20	240 ± 7	219 ± 12^{cd}
SCSFA	222 ± 7	207 ± 12	215 ± 7^{cd}
LCSFA	235 ± 3	234 ± 5	235 ± 3^{c}
LCMFA	227 ± 5	222 ± 3	225 ± 3^{cd}
LCPFA	194 ± 9	218 ± 4	206 ± 6^{d}
Mean[2]	215 ± 5	224 ± 4	
LSD[3]			
0.05 — 26			
0.01 — 35			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

fed either level of fat. As the level of fat in the diet decreased, the zinc level in the kidney tended to increase in the animals on the SCSFA and LCMFA diets. There was no significant effect of diet on spleen zinc concentrations, but animals fed LCMFA tended to have higher spleen zinc levels than animals fed the other fatty acids. Tissue levels of animals fed LCSFA was found to be significantly higher than animals fed LCPFAs. When a 10% level of fat was included in the diet, testes zinc of all animals was similar regardless of source. Animals fed corn oil or LCPFA diets, however, showed marked increases in tissue zinc levels at the 10% dietary fat level. Testes zinc concentrations of animals fed SCSFA tended to decrease as the level of fat in the diet increased.

Tissue zinc concentrations of animals fed different P/S fat ratios are given in Table 8, and the statistical analyses of the data are given in Appendix C, Table C-10. P/S ratios did not significantly influence the uptake of zinc by the liver, kidney, spleen or testes in Experiment 2. Increases in dietary fat from 5% to 10% were associated with increases in zinc deposition in the liver and kidney and decreases in zinc levels in the spleen and testes. These differences, however, were not statistically significant.

Tissue Manganese

The effects of fatty acid sources at 5% and 10% dietary levels on manganese deposition are given in Table 9, and the analyses of variance of the data are presented in Appendix C, Table C-11. Animals

Table 8

Tissue Zinc Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

P/S Ratio		Fat Level (%)		
		5	10	Mean[2]
Liver		ug/gram dry weight[1]		
0.1		91 ± 8	101 ± 8	96 ± 6
0.4		99 ± 8	112 ± 14	106 ± 8
1.0		100 ± 8	103 ± 6	102 ± 5
4.0		96 ± 4	107 ± 14	101 ± 7
8.0		97 ± 5	104 ± 14	101 ± 7
Mean[2]		96 ± 3	105 ± 5	
LSD[3]				
0.05	19			
0.01	26			
Kidney				
0.1		139 ± 6	132 ± 8	136 ± 5
0.4		135 ± 5	143 ± 10	139 ± 6
1.0		133 ± 6	145 ± 9	139 ± 5
4.0		130 ± 10	165 ± 16	148 ± 11
8.0		139 ± 7	147 ± 16	143 ± 8
Mean[2]		135 ± 3	146 ± 5	
LSD[3]				
0.05	25			
0.01	33			

P/S Ratio			Fat Level (%) 5	10	Mean[2]
Spleen			ug/gram dry weight[1]		
0.1			131 ± 23	135 ± 40	133 ± 22
0.4			102 ± 26	109 ± 22	106 ± 16
1.0			175 ± 29	112 ± 33	143 ± 23
4.0			125 ± 31	107 ± 24	116 ± 19
8.0			117 ± 19	120 ± 22	119 ± 14
Mean[2]			130 ± 12	117 ± 12	
LSD[3]					
	0.05	45			
	0.01	61			
Testes					
0.1			251 ± 25	268 ± 15	260 ± 14
0.4			271 ± 12	291 ± 14	281 ± 9
1.0			285 ± 8	265 ± 3	275 ± 5
4.0			279 ± 12	264 ± 11	272 ± 8
8.0			281 ± 16	245 ± 24	265 ± 15
Mean[2]			273 ± 7	267 ± 7	
LSD[3]					
	0.05	41			
	0.01	55			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 9

Tissue Manganese Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source		Fat Level (%)		
		5	10	Mean[2]
Liver		ug/gram dry weight[1]		
Corn oil		2.19 ± 0.1	2.57 ± 0.2	2.38 ± 0.1[d]
SCSFA		4.26 ± 0.2	3.50 ± 0.2	3.88 ± 0.2[c]
LCSFA		2.35 ± 0.2	1.64 ± 0.2	2.00 ± 0.2[d]
LCMFA		3.63 ± 0.3	3.87 ± 0.2	3.75 ± 0.2[c]
LCPFA		3.14 ± 0.5	3.65 ± 0.3	3.40 ± 0.3[c]
Mean[2]		3.11 ± 0.2	3.05 ± 0.2	
LSD[3]				
0.05	0.8			
0.01	1.0			
Kidney				
Corn oil		2.35 ± 0.2	2.65 ± 0.3	2.50 ± 0.2[de]
SCSFA		3.42 ± 0.2	2.94 ± 0.5	3.18 ± 0.3[c]
LCSFA		2.71 ± 0.2	1.87 ± 0.5	2.29 ± 0.3[e]
LCMFA		2.72 ± 0.6	3.30 ± 0.5	3.01 ± 0.4[cd]
LCPFA		3.02 ± 0.3	3.41 ± 0.4	3.22 ± 0.2[c]
Mean[2]		3.06 ± 0.2	2.83 ± 0.2	
LSD[3]				
0.05	0.9			
0.01	1.1			

Source	Fat Level (%) 5	10	Mean[2]
Spleen	ug/gram dry weight[1]		
Corn oil	1.85 ± 0.2	1.69 ± 0.3	1.77 ± 0.2
SCSFA	1.40 ± 0.3	2.58 ± 0.2	1.99 ± 0.3
LCSFA	1.58 ± 0.2	1.92 ± 0.4	1.80 ± 0.2
LCMFA	1.45 ± 0.1	1.45 ± 0.1	1.45 ± 0.1
LCPFA	1.61 ± 0.3	2.04 ± 0.3	1.83 ± 0.2
Mean[2]	1.58 ± 0.1	1.94 ± 0.1	
LSD[3]			
0.05	0.7		
0.01	1.0		
Testes			
Corn oil	2.24 ± 0.2	2.60 ± 0.3	2.42 ± 0.2^{de}
SCSFA	2.85 ± 0.1	2.63 ± 0.5	2.74 ± 0.2^{cde}
LCSFA	2.27 ± 0.3	2.13 ± 0.2	2.20 ± 0.2^{e}
LCMFA	3.37 ± 0.4	2.97 ± 0.2	3.17 ± 0.2^{c}
LCPFA	2.83 ± 0.3	2.94 ± 0.3	2.87 ± 0.2^{cd}
Mean[2]	2.71 ± 0.2	2.65 ± 0.1	
LSD[3]			
0.05	0.6		
0.01	0.9		

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

fed diets containing SCSFA, LCMFA and LCPFA had significantly higher ($p < 0.05$) liver manganese concentrations than did animals fed diets containing corn oil or LCSFA. A level of 10% fat was associated with increases in liver manganese deposition in animals fed LCMFA and LCPFA diets. Diets containing LCSFA were generally associated with significantly lower kidney manganese levels when compared to animals fed SCSFA, LCMFA and LCPFA diets. Kidney manganese deposition was essentially the same in animals fed 5% or 10% dietary fat. Although not statistically significant, the presence of all fatty acids fed at 5% levels were associated with decreases in spleen manganese deposition when compared to the corn oil reference diet. The type of dietary fatty acid had a significant effect on the manganese concentrations of the testes of young rats in this study. Animals fed LCMFA and LCPFA had significantly higher testes manganese levels than animals on the LCPFA diet. Level of dietary fat had no effect on manganese concentrations in the testes regardless of fat source.

Tissue manganese concentrations of animals fed different P/S fat ratios are given in Table 10, and the statistical analysis of the data are found in Appendix C, Table C-12. Significant ($p < 0.05$) fat level effects were observed for liver manganese levels. Animals fed 10% fat had significantly higher liver manganese than animals fed 5% fat diets regardless of P/S ratio. Increases in dietary fat from 5% to 10% were associated with higher levels of kidney manganese deposition. Kidney manganese was the highest in animals fed a P/S ratio of 0.1 when the level of fat was 5%. A P/S ratio of eight, however,

Table 10

Tissue Manganese Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

P/S Ratio		Fat Level (%) 5	10	Mean[2]
Liver		ug/gram dry weight[1]		
0.1		4.16 ± 0.3	3.81 ± 0.3	3.99 ± 0.2
0.4		3.90 ± 0.3	4.44 ± 0.3	4.17 ± 0.2
1.0		3.57 ± 0.2	3.78 ± 0.2	3.68 ± 0.2
4.0		3.47 ± 0.2	4.41 ± 0.5	3.94 ± 0.3
8.0		3.13 ± 0.3	4.85 ± 0.5	3.99 ± 0.4
Mean[2]		3.65 ± 0.1[b]	4.26 ± 0.2[a]	
LSD[3]				
0.05	0.71			
0.01	0.95			
Kidney				
0.1		2.60 ± 0.1	2.51 ± 0.1	2.56 ± 0.1
0.4		2.37 ± 0.2	2.51 ± 0.3	2.44 ± 0.2
1.0		2.44 ± 0.3	2.63 ± 0.3	2.56 ± 0.2
4.0		2.53 ± 0.2	3.09 ± 0.4	2.81 ± 0.2
8.0		2.54 ± 0.2	3.22 ± 0.5	2.88 ± 0.3
Mean[2]		2.50 ± 0.1	2.79 ± 0.2	
LSD[3]				
0.05	0.65			
0.01	0.87			

P/S Ratio		Fat Level (%) 5	10	Mean[2]
Spleen		ug/gram dry weight[1]		
0.1		2.41 ± 0.5	1.87 ± 0.4	2.14 ± 0.3
0.4		3.16 ± 0.5	2.47 ± 0.5	2.82 ± 0.5
1.0		4.19 ± 1.2	4.08 ± 0.9	4.14 ± 1.0
4.0		3.05 ± 0.8	2.59 ± 0.5	2.82 ± 0.7
8.0		2.22 ± 0.5	2.81 ± 0.5	2.51 ± 0.3
Mean[2]		3.01 ± 0.6	2.76 ± 0.7	
LSD[3]				
0.05	1.97			
0.01	2.65			
Testes				
0.1		2.19 ± 0.2	2.92 ± 0.4	2.56 ± 0.3
0.4		2.56 ± 0.6	2.64 ± 0.4	2.60 ± 0.3
1.0		2.43 ± 1.2	2.77 ± 0.9	2.60 ± 0.2
4.0		2.62 ± 0.8	2.91 ± 0.5	2.77 ± 0.3
8.0		2.66 ± 0.5	2.67 ± 0.3	2.67 ± 0.3
Mean[2]		2.49 ± 0.2	2.78 ± 0.2	
LSD[3]				
0.05	0.95			
0.01	1.28			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

resulted in the highest amount of kidney manganese deposition when the level of fat was 10%. P/S ratios had little effect on spleen and testes manganese deposition in the rat. In the spleen, highest deposition of the mineral was found in rats fed diets of P/S ratios of one regardless of the level of dietary fat. Higher manganese levels were found at the 10% dietary fat level in the testes than at the 5% fat level.

Bone Calcium

The effects of fatty acids and corn oil on bone calcium deposition are given in Table 11. Based on the analysis of variance, there was no apparent effect of fat source on bone calcium levels (Appendix C, Table C-13). Femur calcium levels were higher in animals fed SCSFA at 5% and 10% levels. Increases in dietary fat level was associated with some increases in tibia calcium levels in animals fed various fatty acid containing diets.

The effects of P/S ratios on bone calcium deposition are given in Table 12. Analysis of the data (Appendix C, Table C-14) indicated that increasing the P/S ratios of the diets had no significant effect on bone calcium deposition. The calcium levels of both the femurs and tibias of animals fed 5% fat were essentially the same as those observed in animals fed 10% fat regardless of P/S ratio.

Bone Phosphorus

The effects of different fat sources and P/S ratios on bone phosphorus deposition are given in Tables 13 and 14, respectively.

Table 11

Bone Calcium Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

		Fat Level (%)		
Source		5	10	Mean[2]
Femur		mg/gram dry weight[1]		
Corn oil		142 ± 11	150 ± 8	146 ± 6
SCSFA		153 ± 6	278 ± 142	216 ± 70
LCSFA		143 ± 8	151 ± 10	147 ± 6
LCMFA		147 ± 15	145 ± 14	146 ± 10
LCPFA		151 ± 8	143 ± 8	147 ± 6
Mean[2]		147 ± 4	173 ± 28	
LSD[3]				
0.05	133			
0.01	179			
Tibia				
Corn oil		144 ± 4	128 ± 8	136 ± 5
SCSFA		133 ± 5	403 ± 173	268 ± 92
LCSFA		134 ± 6	127 ± 4	131 ± 4
LCMFA		128 ± 5	272 ± 134	200 ± 67
LCPFA		134 ± 7	130 ± 5	132 ± 4
Mean[2]		135 ± 3	212 ± 46	
LSD[3]				
0.05	190			
0.01	256			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 12

Bone Calcium Level of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

Ratio		Fat Level (%)		
		5	10	Mean[2]
Femur		mg/gram dry weight[1]		
0.1		133 ± 6	134 ± 6	134 ± 4
0.4		136 ± 4	149 ± 6	143 ± 4
1.0		145 ± 3	142 ± 4	144 ± 2
4.0		142 ± 4	144 ± 4	143 ± 3
8.0		135 ± 10	141 ± 7	138 ± 6
Mean[2]		138 ± 3	142 ± 2	
LSD[3]				
0.05	14			
0.01	19			
Tibia				
0.1		123 ± 5	127 ± 6	125 ± 4^{d}
0.4		126 ± 6	132 ± 7	129 ± 5^{cd}
1.0		121 ± 11	126 ± 5	124 ± 6^{d}
4.0		135 ± 7	137 ± 8	136 ± 5^{c}
8.0		135 ± 8	127 ± 3	131 ± 5^{cd}
Mean[2]		128 ± 3	130 ± 3	
LSD[3]				
0.05	14			
0.01	19			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 13

Bone Phosphorus Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

		Fat Level (%)		
Source		5	10	Mean[2]
Femur		mg/gram dry weight[1]		
Corn oil		119 ± 6	119 ± 9	119 ± 7
SCSFA		122 ± 7	98 ± 4	110 ± 6
LCSFA		114 ± 4	100 ± 7	107 ± 6
LCMFA		125 ± 6	112 ± 2	118 ± 4
LCPFA		106 ± 2	127 ± 8	116 ± 5
Mean[2]		117 ± 6		
LSD[3]				
0.05	17			
0.01	23			
Tibia				
Corn oil		102 ± 7	116 ± 5	109 ± 6^{cd}
SCSFA		105 ± 5	89 ± 8	97 ± 7^{d}
LCSFA		96 ± 11	103 ± 8	100 ± 10^{d}
LCMFA		113 ± 6	116 ± 8	114 ± 7^{cd}
LCPFA		121 ± 7	121 ± 7	121 ± 7^{c}
Mean[2]		107 ± 7	109 ± 7	
LSD[3]				
0.05	20			
0.01	27			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($\underline{p} < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 14

Bone Phosphorus Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

Ratio			Fat Level (%) 5	10	Mean[2]
Femur			mg/gram dry weight[1]		
0.1			74 ± 8	73 ± 6	74 ± 5
0.4			75 ± 5	68 ± 2	72 ± 3
1.0			95 ± 15	77 ± 7	86 ± 8
4.0			76 ± 5	80 ± 4	78 ± 3
8.0			79 ± 12	77 ± 4	78 ± 6
Mean[2]			80 ± 4	75 ± 2	
LSD[3]					
	0.05	20			
	0.01	27			
Tibia					
0.1			74 ± 7	86 ± 6	80 ± 5
0.4			81 ± 6	86 ± 7	83 ± 4
1.0			82 ± 6	84 ± 5	83 ± 4
4.0			74 ± 10	78 ± 6	76 ± 6
8.0			82 ± 7	80 ± 6	81 ± 4
Mean[2]			79 ± 3	82 ± 2	
LSD[3]					
	0.05	16			
	0.01	22			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Neither the type of fatty acid in the diet nor the P/S ratio of the diet had significant effect on the amount of phosphorus deposited in the femurs. Highest concentrations of bone phosphorus were found in femurs of animals fed diets containing unsaturated fatty acids. When the P/S ratio was a dietary variable (Experiment 2), the highest amount of femur phosphorus was found in rats fed a P/S ratio of 1.0. Dietary source of fatty acids had significant effects on tibia phosphorus levels. Animals fed diets containing LCPFA had significantly higher tibia phosphorus levels than animals fed SCSFA and LCSFA containing diets. Level of dietary fat had no effects on tibia levels of the mineral. Tibia phosphorus levels were higher in animals fed P/S ratios of 0.4, 1.0 or 8.0. Increasing the fat level to 10% was associated with decreases (except when the P/S ratio was 8) in tibia phosphorus deposition.

Bone Magnesium

The effects of different fat sources and different P/S ratios on bone magnesium deposition are given in Tables 15 and 16, respectively. No significant effects of fat source on P/S ratios were observed on bone magnesium deposition in young rats in this study (Appendix C, Tables C-17 and C-18, respectively). Femur magnesium levels tended to be higher in animals fed LCSFA, while animals fed SCSFA had the highest levels of magnesium in the tibias. Increasing the fat level to 10% was generally associated with slight decreases in femur magnesium deposition regardless of fat source. In the tibia

Table 15

Bone Magnesium Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source		Fat Level (%) 5	10	Mean[2]
Femur		mg/gram dry weight[1]		
Corn oil		4.30 ± 1.0	2.68 ± 1.0	3.5 ± 0.8
SCSFA		4.18 ± 0.4	3.22 ± 0.7	3.7 ± 0.4
LCSFA		5.10 ± 0.3	4.30 ± 0.2	4.7 ± 0.2
LCMFA		4.88 ± 0.4	4.19 ± 1.0	4.6 ± 0.5
LCPFA		4.44 ± 0.3	3.80 ± 1.0	4.1 ± 0.5
Mean[2]		4.6 ± 0.3	3.6 ± 0.4	
LSD[3]				
0.05	2.0			
0.01	2.7			
Tibia				
Corn oil		4.52 ± 1.1	4.45 ± 1.3	4.5 ± 0.8
SCSFA		4.99 ± 0.5	5.34 ± 0.9	5.2 ± 0.5
LCSFA		4.88 ± 0.6	2.66 ± 0.7	3.8 ± 0.5
LCMFA		3.88 ± 0.7	4.61 ± 0.6	4.3 ± 0.5
LCPFA		3.75 ± 0.8	3.31 ± 1.0	3.5 ± 0.6
Mean[2]		4.4 ± 0.3	4.1 ± 0.4	
LSD[3]				
0.05	2.3			
0.01	3.1			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 16

Bone Magnesium Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

Ratio			Fat Level (%) 5	10	Mean[2]
Femur			mg/gram dry weight[1]		
0.1			5.74 ± 0.3	5.82 ± 0.4	5.78 ± 0.2
0.4			6.00 ± 0.1	5.93 ± 1.6	5.97 ± 0.8
1.0			6.29 ± 0.4	5.98 ± 0.1	6.14 ± 0.2
4.0			5.56 ± 0.2	6.07 ± 0.1	5.82 ± 0.1
8.0			5.51 ± 0.2	5.91 ± 0.2	5.71 ± 0.2
Mean[2]			5.82 ± 0.1	5.94 ± 0.3	
LSD[3]					
	0.05	1.54			
	0.01	2.08			
Tibia					
0.1			4.92 ± 0.2	5.11 ± 0.3	5.02 ± 0.1
0.4			5.09 ± 0.2	5.08 ± 0.1	5.09 ± 0.1
1.0			4.96 ± 0.4	5.23 ± 0.2	5.10 ± 0.2
4.0			4.83 ± 0.1	5.47 ± 0.1	5.15 ± 0.1
8.0			5.47 ± 0.3	4.90 ± 0.1	5.19 ± 0.2
Mean[2]			5.05 ± 0.1	5.16 ± 0.1	
LSD[3]					
	0.05	0.51			
	0.01	0.68			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

an increase in fat to 10% was associated with increases in magnesium levels in rats fed SCSFA and LCMFA and decreases in magnesium levels in rats fed corn oil, LCSFA and LCPFA. When P/S ratios was a dietary variable, bone magnesium levels were slightly lower in the femurs of the animals.

Bone Zinc

The effects of different fat sources on bone zinc deposition are given in Table 17. The source of fat had a significant ($p < 0.05$) effect on femur zinc levels. The level of zinc found in the femurs of rats fed SCSFA was higher ($p < 0.05$) than the level of zinc found in the femurs of animals fed corn oil or LCPFA. An increase in fat level was associated with increases in femur zinc levels. Tibia zinc levels were significantly influenced by dietary source of fatty acid. Animals fed SCSFA, LCSFA and LCMFA had significantly higher tibia zinc levels than animals fed LCPFA. No significant differences in tibia zinc levels were observed for animals fed LCSFA and corn oil diets. Higher levels of dietary fat were associated with increases in tibia zinc levels.

The effects of P/S ratios on bone zinc deposition is given in Table 18. Although P/S ratios of 0.1, 0.4 and 4.0 were associated with higher femur zinc levels in rats than P/S ratios of 0.1 or 8.0, these differences were not statistically significant (Appendix C, Table C-20). Although animals receiving diets containing P/S ratios of 0.4 and 8.0 had higher tibia zinc levels than animals fed the other

Table 17

Bone Zinc Levels of Young Male Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source		Fat Level (%) 5	10	Mean[2]
Femur		mg/gram dry weight[1]		
Corn oil		176 ± 12	210 ± 19	193 ± 12[de]
SCSFA		222 ± 31	268 ± 19	243 ± 18[c]
LCSFA		240 ± 27	199 ± 10	220 ± 15[cd]
LCMFA		217 ± 22	238 ± 19	228 ± 14[cd]
LCPFA		179 ± 18	187 ± 18	183 ± 12[e]
Mean[2]		207 ± 11	219 ± 9	
LSD[3]				
0.05	48			
0.01	65			
Tibia				
Corn oil		207 ± 4	217 ± 17	212 ± 8[cd]
SCSFA		219 ± 29	263 ± 18	241 ± 18[c]
LCSFA		242 ± 32	214 ± 14	228 ± 17[c]
LCMFA		214 ± 15	231 ± 19	223 ± 12[c]
LCPFA		181 ± 14	181 ± 15	181 ± 10[d]
Mean[2]		213 ± 10	221 ± 8	
LSD[3]				
0.05	49			
0.01	66			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

Table 18

Bone Zinc Levels of Young Male Rats Fed Different P/S Ratios of Fat at Two Levels for Experiment 2

			Fat Level (%)		
Ratio			5	10	Mean[2]
Femur			mg/gram dry weight[1]		
0.1			469 ± 28	511 ± 25	490 ± 19
0.4			491 ± 41	526 ± 28	509 ± 24
1.0			515 ± 27	440 ± 9	478 ± 17
4.0			479 ± 25	541 ± 57	510 ± 31
8.0			452 ± 43	472 ± 27	462 ± 24
Mean[2]			481 ± 14	498 ± 15	
LSD[3]					
	0.05	84			
	0.01	113			
Tibia					
0.1			244 ± 41	281 ± 35	263 ± 26
0.4			313 ± 78	272 ± 32	293 ± 41
1.0			244 ± 25	245 ± 26	245 ± 17
4.0			244 ± 35	292 ± 29	268 ± 23
8.0			304 ± 38	244 ± 38	274 ± 27
Mean[2]			270 ± 21	267 ± 14	
LSD[3]					
	0.05	86			
	0.01	116			

[1]Each value is the mean of 6 animals ± SEM.

[2]Mean ± SEM of the main effects within the same experiment and within the same rows or column not sharing a common superscript are significantly different ($p < 0.05$). Absence of superscripts indicate no differences.

[3]Least significant differences at specified probability levels.

ratios, the effect of P/S ratio on the tibia zinc deposition was not statistically significant.

CHAPTER V

GENERAL DISCUSSION

Two experiments involving male weanling rats were conducted in this study to investigate the effects of saturated and unsaturated fatty acids and of P/S ratios on growth and tissue deposition of several minerals. In Experiment 1 saturated fatty acids in the diet significantly decreased growth in young rats. Highest growth rates were observed in the corn oil groups at both 5% and 10% levels of dietary fat. Zuniga (1987) also noted that when diets high in saturated fat (coconut and olive oil) were fed to rats, they grew slower than animals fed polyunsaturated fats (corn and safflower oil). Onderka and Kirksey (1975) and Sinthusek and Magee (1984) have also reported similar results in rats fed different dietary fats. Rats fed 5% dietary fat levels had significantly higher growth rates compared to animals consuming 10% fat levels. Although rats fed the 10% fat diet consumed less food than rats fed the 5% fat diet, highly significant dietary effects were observed after weight gains were adjusted for food intake by covariant analysis. Thus, weight gains between treatments observed were not due to differences in food consumption. Food consumption of animals on the SCSFA and LCSFA diets tended to be lower than animals on the LCMFA and LCPFA. Odor and unpalitability of the SCSFA and LCSFA diets may have contributed to the decrease in food intake in these groups.

In Experiment 2 animals were fed diets with different P/S ratios. It was observed that as the ratio of polyunsaturated to saturated fat (P/S) in the diet increased, weight gains of animals tended to increase expecially at the 5% fat level. Animals on the 10% fat level, except when the P/S ratio was eight, had higher weight gains than animals on the 5% dietary fat level. In comparison to animals in Experiment 1, animals fed the test diets in Experiment 2 had very similar feed intakes. Diets used in Experiment 2 were devoid of monounsaturated fatty acids and were not in compliance with recent research findings and recommendations (Mattson, 1989). The Nutrition Committee of the American Heart Association (1988) has recommended equal amounts of saturated, monounsaturated and polyunsaturated fats in the diet.

Results from both Experiments 1 and 2 indicated that neither the degree of unsaturation of fatty acids, the P/S ratio nor the level of fat in the diet had an effect on the hemoglobin and hematocrit levels of rats. Animals consuming polyunsaturated fat diets tended to have higher hemoglobin and hematocrit values. Zuniga (1987), Johnson et al. (1987), and Mohoney, Farmer and Hendricks (1980) have reported that animals on saturated fat diets have higher hematocrit and hemoglobin levels than animals on polyunsaturated fat diets. Hemoglobin levels of rats obtained from both experiments were within normal reported values (12-17 gms/dl) although animals in Experiment 1 had lower hemoglobin and hematocrit values than animals in Experiment 2.

Analysis of tissue copper levels revealed that type of dietary fat had highly significant effects on liver and kidney copper levels in the rat. Animals fed diets containing LCSFA had a higher liver, kidney and testes copper concentration than those fed other types of fatty acids in the diet. Observations obtained in this study are similar to those findings of Sinthusek and Magee (1984) who reported that increases in the saturation of dietary fat were associated with increases in liver copper concentrations but is in contradiction to observations of Onderka and Kirksey (1975) who reported higher liver copper levels in rats fed sunflower oil than in those fed coconut oil. Liver copper levels found in this study tend to be lower (15-30 ug/gm dry weight) than have been reported by other investigators (Cohen, Keen, Lonnerdal, & Hurley, 1985; Owen, 1972; Zuniga, 1987). Higher spleen copper levels were obtained in rats fed LCSFA diets. As the level of fat in the diet increased, spleen copper levels decreased in animals fed LCMFA and LCPFA and increased in animals fed LCSFA.

In Experiment 2, dietary P/S ratio had no effect on liver, spleen and testes copper levels in the rat. Animals on diets with P/S ratio = 0.1 (i.e., highly saturated fat diets) had higher tissue copper level than animals on other diets.

With regards to iron levels, animals fed LCSFA had significantly higher liver and kidney iron levels than those on corn oil or unsaturated fatty acid diets. Tissue levels of the mineral also tended to increase as the level of dietary fat increased. Results obtained in this experiment appear to be in agreement with those of

Onderka and Kirksey (1975) who reported higher kidney iron levels in animals fed polyunsaturated fats like safflower oil. As in the liver and kidney, spleen iron levels were highest in those animals fed LCSFA. As fat levels in the diet increased, spleen iron concentrations increased in animals fed saturated fatty acids as the dietary fat source. No significant dietary effects were observed on testes iron concentration, but animals fed saturated fatty acids tended to have higher tissue iron levels.

Significant dietary P/S ratio and level effects were observed for liver and spleen iron levels. As the P/S ratio of fat in the diet increased, levels of iron in the spleen and liver also increased indicating saturated fat diets may enhance tissue deposition or storage of iron. At higher levels of fat in the diet, liver and spleen iron levels were found to be lower. Neither P/S fat ratio nor level of dietary fat had an effect on the iron levels found in the kidney and the testes.

Dietary fatty acids had no effect on liver and spleen zinc levels, although animals on PUFA diets had higher liver zinc levels. Other investigators have also reported that liver zinc deposition was not affected by dietary fat source (Frimpong, 1982; Jones, 1985; Zuniga, 1987). LCPFA in the diet significantly decreased kidney zinc concentrations in the rat. This was observed at both 5% and 10% dietary fat levels. In the group of animals fed LCSFA, testes levels of zinc were found to be the highest at both the 5% and 10% dietary fat level. Animals consuming corn oil or LCPFA diets showed marked increase in testes zinc levels at the 10% dietary fat level.

In Experiment 2, anaimals on the 10% dietary fat level tended to have higher liver and kidney zinc levels than animals on the 5% fat diets. In the spleen and the testes neither dietary P/S ratio nor level of fat in the diet had an effect on tissue zinc levels. In the spleen significant variation in the tissue mineral levels were observed among the replicates. In both experiments zinc deposition in the liver and kidney were similar to reported values, but in the spleen and testes values obtained by our experiments were higher than normal reported values on a wet tissue basis for the rat (Hambridge et al., 1986).

Significant dietary fatty acid effects were observed for liver manganese levels in rats in Experiment 1. Animals fed SCSFA, LCMFA and LCPFA had higher levels of manganese in the liver than animals fed LCSFA. Increasing dietary fat level decreased manganese concentration in the liver in the groups of animals fed saturated fatty acids (SCSFA and LCSFA). Diet had significant effects on kidney and testes manganese levels but had no effects on spleen manganese levels. There appeared to be an apparent stimulation of manganese uptake by the testes of animals fed fatty acids which are liquid at room temperature (SCSFA, LCSFA and LCPFA). LCSFA (solid at room temperature) generally had a depressing effect on testes manganese deposition.

In Experiment 2, animals fed 10% fat diets had significantly higher liver and kidney manganese levels than the animals fed the 5%

fat diet, irrespective of P/S ratio. Neither dietary P/S ratio nor level of fat have an effect on spleen or testes manganese concentrations.

In Experiment 1 diet had no effect on femur and tibia levels of calcium, phosphorus, magnesium and zinc. In Experiment 2 animals on P/S = 1.0 diets had higher phosphorus levels in the femur and increasing dietary fat level seemed to have no effect on mineral levels in this bone. In the tibia animals on P/S = 1.0 diets had lower phosphorus levels than animals on P/S - 0.4, 1.0 or 8.0. In the tibia decreases in P/S ratios were associated with decreases in bone magnesium deposition. Dietary P/S ratios had no effect on bone calcium and zinc levels. Duration of both experiments may have contributed to the unclear results obtained as it takes longer periods of time to observe or induce dietary effects on bone mineral levels (Whitney, Hamilton, & Rolfes, 1990).

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Two experiments were used to investigate the effects of saturated and unsaturated fatty acids on growth and mineral status of young male rats. In the first experiment dietary fat sources included short-chain saturated (SCSFA), long-chain saturated (LCSFA), long-chain monounsaturated (LCMFA), long-chain polyunsaturated (LCPFA) fatty acids and corn oil fed at two levels (5% and 10%). Dietary factors used in the second experiment included five polyunsaturated/saturated (P/S) ratios. The length of each experiment was four weeks. Parameters used in both experiments to evaluate animal responses to various dietary regimens included food intake, weight gain, hematocrit, hemoglobin level, tissue (liver, kidney, spleen, testes) copper, iron, zinc, and manganese and bone (femur and tibia) calcium, phosphorus, magnesium and zinc concentrations.

Saturated fatty acids were found to significantly decrease weight gain. Animals fed corn oil (mainly unsaturated fatty acids) in the diets had significantly higher weight gains than animals fed the other fatty acid diets. Decreases in weight gains were associated with increases in dietary fat levels, regardless of the type of fatty acids. Significant dietary effects were observed on growth data after adjusting weight gain for food intake by covariant analysis. Although low weight gains may have been due to the lack of essential fatty acids in the diet or due to poor digestion and absorption, no signs of essential fatty acid deficiency were observed during the experimental periods.

Increases in the P/S ratios of the diets in Experiment 2 were associated with increases in weight gain. Higher weight gains were obtained in animals on diets in which fat was present at the 5% level. The observed effects of diet on weight gain could also have been related to the caloric density and essential fatty acid level of the test diets.

Neither dietary fatty acids, the P/S ratio nor the level of dietary fat was found to have an effect on hemoglobin or hematocrit levels. In contrast to results reported by other investigators, this study tended to indicate that animals on PUFA diets had higher hemoglobin and hematocrit levels.

Type of fatty acid in the diet had significant effects on copper deposition in the liver and kidney. Animals on LCSFA diets had higher liver and kidney copper levels than animals on the other test diets. This is in agreement with observations by other investigators who have reported higher liver and kidney copper levels in rats fed saturated sources of fat. Liver copper levels were found to be lower than values reported by other investigators. Increasing dietary level of fat from 5% to 10% resulted in decreased mineral deposition in liver and kidneys of animals. In the testes, highest copper levels were found in animals on LCSFA diets, and increases in fat level were associated with decreases in the testes copper levels regardless of fat source.

In the second experiment, P/S ratios and level of fat had no apparent effect on copper levels in the liver, kidney and spleen. Animals on diets with P/S ratio of 0.1 (10% fat level), however, had significantly higher kidney copper levels than animals on other test diets.

Saturated fatty acids in the diet had significant effects on liver and kidney iron levels. Animals fed LCSFA diets had significantly higher liver and kidney iron levels than animals on the unsaturated fatty acid diets. These findings are in agreement with other reports. Increases in dietary fat levels were associated with increases in liver and kidney iron levels. Highest spleen levels were found in animals fed LCSFA. Increasing dietary fat was observed to increase spleen iron concentrations in animals on saturated fat diets. As in the spleen, testes iron levels tended to be higher in animals fed saturated fatty acids.

In the second experiment, higher P/S ratios were associated with significantly lower liver and higher spleen iron in the rat. Levels of iron in these tissues decreased when dietary fat was increased. Neither P/S ratio nor dietary fat level had any effect on kidney and testes iron levels.

No significant dietary effects of fatty acids were observed on liver and spleen zinc. LCPFA diets were associated with significant decreases in kidney zinc levels regardless of dietary fat level. Animals on LCSFA diets at both 5% and 10% level had higher testes zinc content than animals fed other fatty acid diets.

Although P/S ratio was found not to have any effects on liver and kidney zinc levels, dietary fat levels were found to alter zinc levels in these tissues. In the spleen, highest zinc levels were found in animals fed diets of P/S ratios of one, and increasing dietary fat tended to decrease spleen zinc levels. In the testes, at the 5% level as the P/S ratio increases zinc levels tended to increase.

Animals fed SCSFA, LCMFA and LCPFA were found to have higher levels of liver manganese than animals fed LCSFA or corn oil. Increases in dietary fat were associated with decreases in liver manganese in animals on the saturated fatty acid diets and increases in levels of animals on the polyunsaturated fat diets. Neither dietary fatty acid nor level of fat had an effect on kidney, spleen and testes manganese levels in the rat.

Although P/S ratio had no effect on liver and kidney manganese levels, manganese levels tended to be higher at the 10% dietary fat level. Neither P/S ratio nor level of fat had an effect on spleen or testes manganese concentrations.

In Experiment 1, dietary fatty acid did not seem to influence femur and tibia levels of calcium, phosphorus, magnesium and zinc. In Experiment 2, data obtained for mineral content of the femur and tibia was unclear. P/S ratios tended to have an effect on some minerals and not on others. This could be due to the fact that it takes longer periods of time to obtain dietary effects on bone mineral levels.

Recommendations

Based on the data obtained by this and other studies (Zuniga, 1987), it seems that certain tissues have higher mineral levels than other tissues for a given mineral. This may be due to the physiological role of the tissue in the body. It would be of interest to study the dietary effects of fat on alteration of cell membrane structure and composition and how this effects mineral transportation into and outside the cell. Radio labeled fatty acids may be used to trace lipid incorporation into the membranes.

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APPENDIX A

COMPOSITION OF THE EXPERIMENTAL DIETS

Table A-1

Composition of Basal Diets

Ingredients	Percent Fat[a] 5.0	10.0
	gm/kg	
Fat source[b]	50	100
Dextrose	500	500
Cornstarch	220	170
Egg white solids	150	150
Mineral mix	40	40
Vitamin mix	20	20
Vitamin A and D mix	10	10
Cellulose	20	20
Zinc sulfate	0.01	0.01

[a]All fatty acids added in equal proportion in the diet.

[b]Experiment 1: SCSFA - Lauric and Myristic acids
LCSFA - Stearic and Palmitic acids
LCMFA - Oleic acid
LCPFA - Linoleic and Linolenic acids

Experiment 2: LCSFA - Stearic and Palmitic acids
LCPFA - Linoleic and Linolenic acids
Each added to obtain desired P/S ratios in the diet.

Table A-2

Composition of Mineral Mix, Wesson Modified Osborne-Mendel[a]

Constituents	Percent
Calcium carbonate	21.000
Cupric sulfate 5H2O	0.039
Ferric pyrophosphate	1.470
Manganous sulfate (anhydrous)	0.020
Magnesium sulfate (anhydrous)	9.000
Aluminum potassium sulfate 12 H2O	0.009
Potassium chloride	12.000
Potassium dihydrogen phosphate	31.000
Potassium iodide	0.005
Sodium chloride	10.500
Sodium fluoride	0.057
Tricalcium phosphate	14.900

[a]Teklad, Madison, WI

Table A-3

Composition of Vitamin Mix[a]

Constituents	Amount Per 2 kg Mix
	mg
Vitamin B-12	2
Biotin	20
Folic acid	100
Thiamin HCL	500
Pyridoxine HCL	500
	gm
Menadione (2 methyl naphthoquinone)	1
Riboflavin	1
Nicotinic acid	1
Calcium pantothenate	3
Para aminobenzoic acid	100
Inositol	100
Choline chloride	150
DL-Methionine	600
Cornstarch[b]	1040

[a]All vitamins and methionine were purchased from ICN Nutritional Biochemicals, Cleveland, OH.

[b]Teklad, Madison, WI.

Table A-4

Mineral Analysis of the Diets

Diet	Level (%)	Cu	Fe	Zn	Mn	Ca	P	Mn
		ug/gm				mg/gm		
Experiment 1								
Source								
Corn oil	5	9	94	67	5	4.8	1.3	1.0
	10	7	91	101	4	4.7	1.3	0.8
SCSFA	5	6	105	111	3	5.8	1.0	0.6
	10	13	103	140	3	5.1	1.4	0.7
LCSFA	5	16	103	66	4	5.6	1.7	1.2
	10	6	96	199	3	5.3	1.2	0.9
LCMFA	5	9	127	59	4	5.2	1.4	0.9
	10	9	99	68	4	5.1	1.3	1.1
LCPFA	5	10	94	74	3	5.1	1.3	1.3
	10	6	98	81	5	5.1	1.3	0.9
Experiment 2								
P/S Ratios								
0.1	5	10	96	31	3	4.9	1.4	1.0
	10	10	113	263	6	5.9	1.8	1.3
0.4	5	8	107	123	5	5.6	1.6	1.1
	10	6	96	49	3	5.2	1.4	1.0
1.0	5	7	91	33	3	4.9	1.2	0.9
	10	9	107	22	4	5.7	1.5	1.0
4.0	5	9	111	71	4	5.3	1.6	1.1
	10	8	95	54	2	5.1	1.6	1.0
8.0	5	9	91	37	4	4.6	1.3	0.8
	10	6	103	47	6	5.4	1.6	1.0

APPENDIX B

RAW DATA

Table B-1

Total Weight Gain of Rats in Experiments 1 and 2

Experimental Diets	Replicates 1	2	3	4	5	6	Mean
	Total Weight Gain in 4 Weeks (grams)						
Experiment 1							
1	203	196	167	180	238	160	191
2	139	146	139	157	156	142	147
3	151	146	122	143	168	144	146
4	144	158	141	162	143	142	148
5	166	148	160	132	165	180	159
6	185	200	156	170	189	212	185
7	148	145	78	67	104	96	106
8	123	149	119	126	140	131	131
9	159	156	142	156	158	158	155
10	144	151	166	133	154	169	153
Experiment 2							
1	107	83	91	95	75	98	92
2	95	80	90	86	85	87	87
3	100	84	88	112	82	99	94
4	108	106	108	96	94	94	101
5	117	115	106	122	104	111	113
6	99	91	97	110	115	99	102
7	102	105	117	84	93	99	100
8	102	101	101	97	102	115	103
9	114	102	131	99	76	97	103
10	84	97	102	92	90	87	92

Table B-2

Total Feed Consumption of Rats in Experiments 1 and 2

Experimental Diets	Replicates 1	2	3	4	5	6	Mean
	Total Feed Intake in 4 Weeks (grams)						
Experiment 1							
1	430	456	365	396	491	366	417
2	333	370	305	347	315	334	334
3	389	380	355	330	419	390	377
4	375	351	352	413	350	347	365
5	369	360	350	311	360	370	353
6	362	375	350	328	430	411	376
7	318	284	182	180	209	231	234
8	310	355	288	335	345	341	329
9	350	350	317	350	350	328	341
10	318	342	383	298	350	322	336
Experiment 2							
1	355	355	355	355	355	355	355
2	355	355	365	355	365	355	358
3	355	355	355	355	355	355	355
4	355	355	355	355	365	355	357
5	355	355	355	355	355	355	355
6	355	355	355	355	355	355	355
7	355	355	355	355	355	355	355
8	355	355	355	355	355	355	355
9	355	355	355	355	355	355	355
10	355	355	355	355	355	355	355

Table B-3

Blood Hemoglobin Concentration in Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	gm/dl						
Experiment 1							
1	13.9	13.7	12.9	11.1	12.5	13.4	12.9
2	12.0	12.5	13.2	11.4	9.8	12.6	11.9
3	11.5	12.5	11.2	11.6	12.2	11.9	11.8
4	12.5	14.0	11.9	10.9	11.7	12.3	12.2
5	11.1	12.9	11.1	11.9	11.9	11.8	11.8
6	11.3	12.0	12.3	10.5	12.0	12.2	11.7
7	11.8	11.5	11.8	12.1	12.3	11.1	11.8
8	13.1	11.0	13.6	11.4	11.5	11.2	12.0
9	12.5	13.1	11.3	11.0	14.0	12.7	12.4
10	11.5	11.8	13.6	13.0	12.1	10.9	12.2
Experiment 2							
1	14.6	14.7	15.8	14.7	15.1	14.9	15.0
2	12.3	13.2	16.1	14.7	15.8	15.8	14.7
3	13.9	14.5	15.1	13.4	14.6	14.3	14.3
4	13.4	15.1	14.4	13.9	18.8	14.9	15.1
5	13.6	13.2	12.9	11.3	14.1	13.5	13.1
6	11.3	13.4	14.3	15.4	13.0	12.3	13.3
7	13.4	14.3	14.3	12.7	13.8	16.1	14.1
8	13.3	13.7	16.0	13.9	14.3	12.3	13.9
9	14.1	13.4	12.6	19.2	14.3	15.1	14.8
10	14.1	15.7	14.9	13.1	14.1	14.4	14.4

Table B-4

Blood Hematocrits of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	Percent						
Experiment 1							
1	59	45	46	42	52	46	48
2	48	47	45	47	39	50	46
3	43	44	43	41	44	40	43
4	44	46	46	44	50	48	46
5	47	50	43	47	45	48	47
6	48	51	48	43	44	45	47
7	50	50	45	45	51	42	47
8	48	41	44	43	44	41	43
9	46	50	40	40	49	45	45
10	45	45	57	53	48	42	48
Experiment 2							
1	67	58	60	46	49	49	55
2	58	56	57	49	49	57	54
3	60	56	48	40	48	51	51
4	54	51	54	49	52	49	52
5	60	58	59	46	49	54	54
6	51	59	57	45	44	49	51
7	65	58	50	43	54	59	55
8	57	52	61	57	46	45	53
9	50	55	51	50	62	57	54
10	66	60	54	51	51	55	56

Table B-5

Liver Copper Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates 1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	9.0	8.4	9.3	9.9	8.4	9.0	8.9
2	11.0	11.0	9.4	10.1	12.2	9.7	10.6
3	9.7	12.3	12.5	8.5	11.7	13.1	11.3
4	11.8	9.2	10.6	10.5	9.8	10.3	10.4
5	7.5	10.7	9.1	10.8	10.2	8.6	9.5
6	8.9	8.6	10.8	8.9	11.5	7.1	9.3
7	8.4	11.1	10.1	9.9	9.0	7.9	9.4
8	8.7	11.0	9.1	10.8	13.8	13.8	11.2
9	9.7	11.6	6.8	10.8	9.2	7.9	9.3
10	8.1	7.6	7.7	9.4	10.3	7.3	8.4
Experiment 2							
1	7.4	7.5	8.9	6.6	8.9	8.0	7.9
2	7.5	7.8	8.5	7.5	9.0	7.6	8.0
3	10.5	7.6	7.3	7.7	7.9	6.1	7.8
4	5.4	7.6	6.6	6.5	7.4	7.0	6.7
5	7.8	5.9	13.2	5.6	6.0	7.0	7.6
6	7.5	9.6	9.3	6.9	8.1	9.5	8.5
7	8.3	7.7	7.0	6.2	7.7	9.1	7.6
8	8.5	8.3	9.6	7.8	8.1	7.0	8.2
9	9.0	7.3	7.7	7.9	8.5	10.1	8.4
10	8.2	8.4	8.9	8.6	10.2	8.6	8.8

Table B-6

Liver Iron Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	256	255	251	247	187	327	254
2	600	356	394	389	319	597	443
3	587	663	372	638	631	859	625
4	451	313	491	425	540	282	417
5	274	286	228	334	281	218	270
6	272	227	356	296	209	249	268
7	350	542	591	562	524	306	479
8	1004	954	707	1038	1026	1107	973
9	303	475	422	399	301	335	373
10	230	263	240	354	308	270	278
Experiment 2							
1	578	674	670	1039	857	504	720
2	473	510	358	494	608	610	509
3	394	543	623	834	507	511	569
4	439	274	269	315	418	429	357
5	230	344	418	303	304	315	319
6	408	433	622	502	478	611	509
7	390	396	308	521	483	537	439
8	269	317	426	343	395	320	345
9	367	269	275	421	228	434	332
10	173	279	380	156	185	258	239

Table B-7

Liver Zinc Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	68	69	70	81	59	64	68
2	64	68	73	64	75	65	68
3	65	70	89	69	68	68	72
4	84	62	60	78	82	71	73
5	62	66	73	86	83	67	73
6	74	64	70	66	86	66	71
7	60	67	63	60	72	56	63
8	49	64	61	68	81	80	67
9	68	75	63	73	71	57	68
10	61	75	79	87	79	75	76
Experiment 2							
1	74	60	92	98	117	102	91
2	70	82	93	114	120	116	99
3	98	83	73	105	127	112	100
4	89	84	91	110	99	100	96
5	74	96	100	96	103	114	97
6	85	103	80	89	131	115	101
7	74	95	93	118	124	171	112
8	82	93	106	123	114	101	103
9	90	88	89	139	77	159	107
10	66	74	77	128	140	141	104

Table B-8

Liver Manganese Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	2.12	2.25	1.95	2.51	2.21	2.10	2.19
2	4.55	5.18	3.99	4.07	3.49	4.29	4.26
3	1.78	2.61	2.38	2.95	2.05	2.34	2.35
4	3.62	2.69	4.19	3.11	4.63	3.57	3.63
5	2.74	3.40	4.84	3.95	2.90	0.99	3.14
6	2.15	2.46	2.90	3.36	2.62	1.95	2.57
7	3.94	4.08	3.44	3.26	3.65	2.65	3.50
8	1.23	1.63	1.39	1.16	2.03	2.41	1.64
9	3.46	3.72	3.75	4.98	3.64	3.66	3.87
10	3.68	4.83	2.76	3.41	3.83	3.37	3.65
Experiment 2							
1	3.54	3.61	4.14	4.07	5.26	4.36	4.16
2	3.30	3.26	3.32	4.73	4.79	4.02	3.90
3	2.43	3.87	3.90	3.47	4.04	3.68	3.57
4	3.37	2.88	2.92	3.78	4.06	3.78	3.47
5	3.47	2.73	2.51	2.74	4.47	2.87	3.13
6	3.18	4.13	3.10	3.56	4.55	4.35	3.81
7	3.70	4.43	3.35	4.82	5.57	4.74	4.44
8	3.50	3.53	3.30	4.58	4.43	3.34	3.78
9	4.30	3.40	3.63	4.72	3.82	6.57	4.41
10	4.74	3.73	3.81	4.72	7.18	4.92	4.85

Table B-9

Kidney Copper Concentration of Rats in Experiments 1 and 2

Experi-mental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	30.5	23.8	28.3	28.8	23.2	20.4	25.8
2	85.6	15.8	24.2	21.2	17.5	21.8	31.0
3	19.4	38.6	37.4	16.5	146.7	73.8	55.4
4	42.1	30.5	76.4	29.1	16.7	28.1	37.2
5	19.3	31.5	16.5	35.3	40.7	46.1	31.6
6	30.7	20.8	17.9	21.3	25.0	19.5	22.5
7	13.9	59.8	24.9	82.3	18.0	14.9	35.6
8	83.1	56.6	76.2	99.7	91.6	45.9	75.5
9	21.9	23.3	17.0	24.9	95.1	17.9	33.4
10	45.5	17.7	25.2	18.5	84.8	19.8	35.3
Experiment 2							
1	54.7	28.3	42.6	46.7	35.2	33.6	40.2
2	32.5	33.3	27.8	33.1	26.4	37.8	31.8
3	40.3	33.1	26.1	37.3	25.8	34.5	32.9
4	31.3	31.0	28.1	37.4	33.3	49.4	35.1
5	42.3	27.4	66.5	42.7	41.0	33.5	42.2
6	48.5	82.8	50.1	57.4	39.2	43.4	53.6
7	61.3	39.1	30.1	44.6	30.2	33.3	39.8
8	53.0	52.3	32.6	43.1	39.5	43.3	44.0
9	35.5	60.2	32.9	41.3	45.8	33.3	41.5
10	34.4	32.4	36.7	31.6	27.4	32.6	32.5

Table B-10

Kidney Iron Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	189	170	160	150	157	168	166
2	73	171	170	168	141	241	177
3	164	177	162	177	196	206	180
4	149	155	175	184	175	108	158
5	206	185	145	154	156	171	170
6	147	140	193	176	145	173	162
7	158	157	182	229	208	165	183
8	171	210	244	224	214	193	209
9	156	147	212	182	167	179	174
10	126	146	173	185	196	179	168
Experiment 2							
1	164	269	225	237	271	252	236
2	190	198	212	243	249	225	220
3	191	169	198	224	224	235	207
4	188	141	159	159	200	213	177
5	183	150	208	217	184	186	188
6	202	205	202	191	182	204	198
7	209	188	191	201	254	259	217
8	129	182	204	194	184	245	190
9	157	181	238	218	209	222	204
10	367	172	180	158	152	204	206

Table B-11

Kidney Zinc Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates 1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	84	96	92	92	101	90	93
2	90	92	92	94	101	98	94
3	73	95	99	87	92	113	93
4	87	93	97	92	114	90	95
5	81	80	89	81	84	85	83
6	86	88	92	91	99	92	92
7	95	97	96	104	104	102	100
8	74	95	101	87	107	108	95
9	92	98	110	94	118	99	102
10	66	82	94	86	95	86	85
Experiment 2							
1	123	156	129	131	154	142	139
2	111	135	132	140	147	145	135
3	117	127	127	129	149	152	133
4	111	111	116	135	178	129	130
5	169	131	134	144	130	124	139
6	113	150	117	163	119	132	132
7	128	124	132	135	151	192	143
8	129	131	122	169	143	176	145
9	150	143	135	171	241	151	165
10	122	126	118	150	145	220	147

Table B-12

Kidney Manganese Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	1.58	2.26	2.46	2.88	2.51	2.40	2.35
2	2.76	3.93	3.55	3.12	3.36	3.77	3.42
3	1.82	3.17	3.53	2.53	2.72	2.50	2.71
4	0.00	2.49	3.89	2.55	3.80	3.59	3.81
5	1.87	3.70	3.81	2.94	2.39	3.42	3.02
6	1.23	3.12	2.87	3.04	2.91	2.71	2.65
7	2.53	1.21	2.87	3.13	4.74	3.15	2.94
8	0.74	0.64	2.16	1.45	3.34	2.87	1.87
9	2.30	1.23	3.39	4.04	4.86	3.97	3.30
10	1.65	3.80	3.60	4.27	3.80	3.31	3.41
Experiment 2							
1	2.05	2.83	2.41	2.79	2.93	2.58	2.60
2	1.59	2.38	2.19	2.21	2.93	2.91	2.37
3	2.20	2.11	2.83	1.36	3.39	2.76	2.44
4	2.78	2.34	2.44	1.59	2.96	3.04	2.53
5	2.11	1.96	2.69	2.63	2.73	3.10	2.54
6	2.43	2.37	1.95	2.83	2.84	2.63	2.51
7	2.02	2.17	1.47	2.98	3.43	2.96	2.51
8	1.51	2.91	2.72	3.38	2.73	2.51	2.63
9	2.05	1.51	3.87	3.12	4.02	3.97	3.09
10	1.53	3.31	3.46	2.37	4.57	4.07	3.22

Table B-13

Spleen Copper Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	6.2	7.2	6.7	4.2	2.0	2.6	4.8
2	6.5	3.9	6.3	4.4	4.3	3.8	4.8
3	4.6	6.5	5.1	2.8	5.9	5.4	5.0
4	7.0	4.4	6.1	5.7	6.0	5.3	5.8
5	4.1	7.0	4.5	6.0	7.3	6.5	5.9
6	6.0	4.2	6.1	5.1	6.4	6.0	5.6
7	5.3	8.3	5.9	4.3	2.8	2.5	4.8
8	8.0	7.7	7.6	11.2	5.8	6.1	7.7
9	4.9	5.2	2.8	3.2	3.0	3.4	3.7
10	4.5	5.7	5.6	4.2	4.9	3.1	4.7
Experiment 2							
1	8.5	8.8	6.4	6.2	6.3	10.8	7.8
2	7.8	9.2	10.2	9.5	9.2	7.8	8.9
3	9.8	4.8	9.0	7.2	6.3	9.3	7.7
4	5.2	11.9	8.9	4.5	9.6	9.7	8.3
5	6.3	13.0	9.8	8.4	11.5	7.7	9.5
6	4.9	10.3	11.4	6.7	7.9	6.9	8.0
7	7.3	2.1	9.5	6.3	5.6	8.6	6.6
8	8.1	13.3	2.8	6.1	4.8	8.2	7.2
9	6.9	4.4	9.1	7.8	9.3	7.7	7.5
10	11.4	7.1	9.9	5.4	2.8	11.1	8.0

Table B-14

Spleen Iron Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	617	743	633	740	630	586	658
2	1169	1123	563	614	660	1420	925
3	1864	1585	682	1020	2520	1832	1584
4	962	858	2101	749	773	598	1007
5	697	822	491	655	670	601	656
6	584	873	788	1455	769	762	872
7	1144	1010	853	905	1111	1325	1058
8	1688	2238	1572	1250	974	2646	1728
9	948	803	1042	833	964	663	876
10	542	530	634	699	703	672	630
Experiment 2							
1	2034	4254	3077	2561	1281	1022	2372
2	1263	976	714	633	1078	1445	1081
3	1201	2452	2590	264	344	1319	1362
4	807	524	960	788	841	1189	852
5	294	543	784	903	711	327	594
6	1324	773	1386	1150	315	1086	1006
7	1165	702	625	1660	333	496	830
8	457	452	330	489	452	412	432
9	463	285	362	443	208	593	392
10	398	143	347	163	337	222	268

Table B-15

Spleen Zinc Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates 1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	92	109	100	98	79	78	93
2	97	78	94	102	106	95	95
3	91	82	101	84	99	94	92
4	104	89	92	99	103	106	99
5	82	87	89	89	109	97	92
6	105	84	91	103	103	95	97
7	88	99	88	86	83	100	91
8	83	92	76	93	87	109	78
9	86	91	97	94	181	102	109
10	90	95	93	85	82	109	92
Experiment 2							
1	64	66	192	143	188	134	131
2	26	00	76	158	115	137	102
3	59	191	241	169	250	139	175
4	26	48	156	180	120	218	125
5	42	87	172	147	138	115	117
6	25	52	136	183	295	121	135
7	73	41	189	84	139	129	109
8	00	27	137	183	191	137	112
9	23	44	127	156	139	155	107
10	85	36	99	163	169	167	120

Table B-16

Spleen Manganese Concentration of Rats in Experiments 1 and 2

Experi-mental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	1.54	1.81	1.67	2.79	1.97	1.30	1.85
2	1.62	0.98	0.00	1.46	1.06	1.89	1.40
3	1.52	1.63	1.26	1.40	0.99	2.67	1.58
4	1.75	1.48	1.53	1.41	1.21	1.33	1.45
5	1.37	1.75	0.00	1.49	1.81	1.62	1.61
6	1.50	1.40	3.03	1.11	1.28	1.19	1.69
7	1.76	3.31	2.94	2.16	2.78	2.50	2.58
8	2.08	1.54	1.89	0.00	2.91	1.21	1.92
9	1.23	1.30	1.39	1.57	1.51	1.70	1.45
10	1.51	1.89	1.87	2.12	3.27	1.56	2.04
Experiment 2							
1	0.00	0.00	2.14	0.00	0.00	2.69	2.41
2	0.00	0.00	0.00	3.16	0.00	0.00	3.16
3	7.87	0.00	0.00	2.40	0.00	2.31	4.19
4	0.00	0.00	2.23	4.50	0.00	2.43	3.05
5	0.00	0.00	2.45	0.00	2.29	1.92	2.22
6	0.00	0.00	2.27	1.67	0.00	1.72	1.87
7	0.00	0.00	0.00	0.00	2.78	2.16	2.47
8	0.00	2.66	5.49	0.00	0.00	0.00	4.08
9	0.00	0.00	0.00	2.60	0.00	2.58	2.59
10	0.00	0.00	0.00	0.00	2.81	0.00	2.81

Table B-17

Testes Copper Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	5	Mean
	ug/gram dry weight						
Experiment 1							
1	14.6	13.1	14.6	11.8	14.5	11.2	13.3
2	14.8	10.7	11.8	14.0	14.6	11.4	12.8
3	30.6	14.1	16.1	11.4	15.1	16.0	17.2
4	14.1	12.6	14.1	13.8	10.7	13.6	13.1
5	11.6	13.2	12.6	12.7	14.1	13.5	12.9
6	14.5	16.2	11.9	12.7	15.4	12.8	13.9
7	10.6	12.4	12.6	13.5	11.2	12.2	12.1
8	13.7	15.2	14.5	14.9	16.6	14.3	14.8
9	10.7	12.7	9.9	12.0	11.7	10.8	11.5
10	12.5	10.6	12.5	11.7	14.5	1119	12.3
Experiment 2							
1	16.0	19.0	7.8	12.0	12.9	13.0	13.5
2	13.9	38.3	22.1	13.4	13.5	13.5	19.1
3	17.9	27.0	18.8	13.5	12.7	13.5	17.2
4	14.8	21.2	20.2	13.7	12.7	12.5	15.8
5	16.3	19.7	23.2	12.8	13.3	12.3	16.2
6	18.4	23.9	30.5	13.1	12.4	13.6	18.6
7	19.3	14.4	21.9	11.4	12.3	13.8	15.5
8	14.8	15.8	24.5	12.5	14.4	13.0	15.8
9	15.3	23.7	18.0	10.9	13.9	12.7	15.8
10	14.4	21.0	23.1	11.0	12.6	15.7	16.3

Table B-18

Testes Iron Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates 1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	179	167	246	149	130	118	165
2	198	224	170	113	106	138	158
3	203	190	74	142	187	126	154
4	186	163	92	185	115	111	142
5	189	185	94	166	125	70	138
6	161	223	113	129	110	102	140
7	114	197	114	231	106	101	144
8	220	165	114	118	203	160	163
9	150	156	134	145	145	76	134
10	109	127	91	101	124	103	109
Experiment 2							
1	104	118	48	90	129	80	95
2	93	137	90	85	121	96	104
3	86	101	66	92	127	126	100
4	119	76	84	66	87	92	100
5	78	120	166	73	91	108	106
6	71	110	122	73	103	163	107
7	153	88	78	88	130	105	107
8	96	95	106	83	86	81	91
9	95	128	86	131	90	82	102
10	153	85	98	110	120	76	107

Table B-19

Testes Zinc Concentration of Rats in Experiments 1 and 2

Experi-mental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	229	100	209	237	209	205	198
2	211	226	236	239	229	190	222
3	227	243	228	229	239	243	235
4	218	239	235	239	207	226	227
5	195	208	211	207	154	190	194
6	217	269	232	232	251	243	240
7	196	201	184	216	183	260	207
8	224	253	221	234	238	233	234
9	221	215	220	233	228	217	222
10	227	212	205	228	220	214	218
Experiment 2							
1	286	281	126	262	285	268	251
2	262	217	287	275	285	298	271
3	296	259	291	270	275	315	285
4	257	249	261	249	286	325	279
5	255	300	215	308	291	315	281
6	240	248	244	269	267	339	268
7	261	272	258	343	322	291	291
8	259	260	258	274	268	268	265
9	255	237	258	240	299	292	264
10	289	256	285	239	134	286	248

Table B-20

Testes Manganese Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	2.08	2.62	3.16	1.48	1.74	2.35	2.24
2	3.13	3.13	3.14	2.47	2.70	2.54	2.85
3	1.57	2.56	3.71	1.63	1.89	2.28	2.27
4	5.26	2.51	3.13	2.90	2.67	3.77	3.37
5	3.04	3.46	3.51	2.00	1.92	3.06	2.83
6	2.17	3.08	3.51	1.50	2.28	3.04	2.60
7	2.45	1.77	4.58	1.35	2.39	3.25	2.63
8	1.87	2.53	2.55	1.42	2.16	2.26	2.13
9	3.35	2.68	3.55	2.74	2.60	2.89	2.97
10	3.12	3.02	2.93	2.07	2.52	3.96	2.94
Experiment 2							
1	2.09	2.72	1.94	1.50	2.72	2.17	2.19
2	2.32	5.00	3.28	1.41	1.43	1.92	2.56
3	2.34	3.38	2.82	2.13	2.11	1.80	2.43
4	3.96	3.02	2.53	2.41	2.12	1.67	2.62
5	2.83	2.57	4.97	1.62	1.66	2.31	2.66
6	4.24	2.76	4.07	1.45	2.07	2.91	2.92
7	4.60	2.40	3.13	1.76	2.30	1.62	2.64
8	2.96	2.37	5.16	1.66	2.87	1.62	2.77
9	2.19	5.47	3.13	2.18	2.99	1.50	2.91
10	2.71	3.11	3.56	1.84	2.97	1.85	2.67

Table B-21

Femur Calcium Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	mg/gram dry weight						
Experiment 1							
1	183	166	126	115	132	131	142
2	169	167	131	153	149	151	153
3	145	178	125	134	148	125	143
4	161	197	123	172	122	104	147
5	183	161	137	125	144	153	151
6	170	179	151	135	134	130	150
7	147	148	106	144	134	99	130
8	158	180	147	108	162	149	151
9	160	165	127	148	185	86	145
10	172	136	159	113	145	134	143
Experiment 2							
1	129	104	140	146	145	132	133
2	122	139	130	146	143	134	136
3	136	148	144	150	153	139	145
4	133	129	144	151	150	146	142
5	88	125	150	155	151	140	135
6	133	146	116	118	153	139	134
7	141	136	145	180	148	142	149
8	132	143	145	151	131	152	142
9	138	134	139	151	163	139	144
10	129	136	129	169	130	151	141

Table B-22

Femur Phosphorus Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	mg/gram dry weight						
Experiment 1							
1	112	93	118	126	123	141	119
2	95	126	147	123	110	132	122
3	100	112	109	126	106	127	114
4	119	109	136	118	146	121	125
5	105	112	98	100	106	109	106
6	131	121	96	95	155	115	119
7	103	111	81	97	101	97	98
8	100	106	72	123	88	112	100
9	111	104	110	110	118	120	112
10	113	136	103	157	116	138	127
Experiment 2							
1	66	63	100	99	53	66	74
2	53	80	87	84	72	76	75
3	70	166	89	70	96	78	95
4	58	81	88	78	82	66	76
5	59	83	133	62	81	57	79
6	71	83	73	51	90	69	73
7	70	73	73	68	59	64	68
8	64	89	83	64	62	101	77
9	73	73	85	82	98	71	80
10	65	85	79	88	64	82	77

Table B-23

Femur Magnesium Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	mg/gram dry weight						
Experiment 1							
1	6.07	8.56	0.00	2.55	4.64	4.00	5.16
2	3.61	5.15	4.26	5.35	2.94	3.77	4.18
3	5.53	3.83	4.88	5.12	6.27	4.96	5.10
4	5.18	5.60	4.60	5.69	4.92	3.31	4.88
5	4.66	4.32	4.23	5.65	3.87	3.93	4.44
6	5.37	3.32	0.00	3.72	0.00	3.67	4.02
7	5.45	3.66	3.15	3.90	0.00	3.18	3.87
8	4.55	4.19	4.95	4.13	3.77	4.22	4.30
9	4.17	7.08	5.14	0.00	5.74	3.01	5.03
10	7.69	4.36	3.80	2.82	0.00	4.15	4.56
Experiment 2							
1	5.70	4.38	6.45	6.06	5.91	5.94	5.74
2	5.80	5.75	5.95	6.36	6.48	5.64	6.00
3	7.73	6.10	6.15	6.83	5.85	5.10	6.29
4	5.85	5.31	5.70	5.79	6.19	4.52	5.56
5	4.29	5.56	5.88	6.33	5.45	5.53	5.51
6	6.01	6.29	5.02	4.50	6.63	6.45	5.82
7	6.50	6.06	5.88	5.78	5.50	5.88	5.93
8	6.13	6.32	5.61	5.98	5.69	6.12	5.98
9	6.10	6.02	6.09	6.15	6.28	5.80	6.07
10	5.64	6.03	5.29	6.13	5.88	6.40	5.90

Table B-24

Femur Zinc Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	211	202	156	133	185	171	176
2	177	258	273	313	209	101	222
3	240	357	237	217	235	154	240
4	303	224	194	244	197	142	217
5	220	230	179	106	183	157	179
6	215	284	161	189	171	240	210
7	327	276	294	234	252	197	268
8	218	209	198	152	208	211	199
9	283	298	216	204	246	181	238
10	231	215	213	113	157	192	187
Experiment 2							
1	456	351	516	485	552	455	469
2	362	437	454	555	648	492	491
3	495	634	451	468	532	510	515
4	429	387	477	547	533	503	479
5	309	407	431	525	614	424	452
6	481	594	457	443	531	559	511
7	520	485	471	647	560	471	526
8	453	400	449	457	436	449	440
9	526	500	435	821	503	464	541
10	492	442	388	589	451	473	472

Table B-25

Tibia Calcium Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	mg/gram dry weight						
Experiment 1							
1	130	146	144	147	140	159	144
2	144	122	149	131	116	135	133
3	116	158	121	140	127	140	134
4	130	129	112	145	134	117	128
5	143	151	151	115	117	125	134
6	139	161	120	127	113	108	128
7	113	135	92	148	125	98	119
8	133	116	128	116	142	127	127
9	145	138	105	151	154	94	113
10	120	143	115	123	142	135	130
Experiment 2							
1	107	127	133	110	139	124	123
2	98	131	124	145	129	131	126
3	68	137	130	128	136	124	121
4	112	127	129	162	135	146	135
5	117	121	124	133	150	167	135
6	118	137	125	107	127	146	127
7	110	127	124	143	129	158	132
8	108	116	128	129	137	139	126
9	117	127	118	161	149	152	137
10	122	119	119	129	134	138	127

Table B-26

Tibia Phosphorus Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	mg/gram dry weight						
Experiment 1							
1	109	107	83	87	130	99	102
2	92	113	90	114	100	120	105
3	41	122	94	104	106	107	96
4	100	98	139	112	115	114	113
5	123	109	100	110	134	148	121
6	110	115	130	99	126	131	116
7	104	92	80	53	103	103	89
8	82	124	93	99	94	129	103
9	103	118	84	124	124	145	116
10	156	113	113	117	117	112	121
Experiment 2							
1	74	85	99	60	56	70	74
2	72	104	93	68	77	73	81
3	61	96	99	84	71	82	82
4	85	93	99	54	79	35	74
5	73	55	98	97	88	84	82
6	83	84	101	80	67	103	86
7	100	89	97	87	83	50	84
8	82	82	92	93	64	92	84
9	93	66	97	66	75	69	78
10	82	78	91	70	62	99	80

Table B-27

Tibia Magnesium Concentration of Rats in Experiments 1 and 2

Experimental Diets	Replicates						
	1	2	3	4	5	6	Mean
	mg/gram dry weight						
Experiment 1							
1	0.00	7.08	4.66	5.92	3.25	6.22	5.43
2	5.16	6.84	5.24	5.56	3.91	3.25	4.99
3	4.57	7.61	3.80	4.90	3.73	4.67	4.88
4	7.02	4.07	4.16	1.65	3.19	3.17	3.88
5	5.04	0.00	4.60	3.80	5.30	3.75	4.50
6	8.29	7.82	0.00	4.43	2.32	3.86	5.34
7	5.31	7.89	3.87	8.33	3.81	2.80	5.34
8	1.55	2.94	4.56	3.64	3.24	0.00	3.19
9	4.40	3.33	4.40	5.41	7.05	3.06	4.61
10	5.16	4.71	0.00	5.02	4.96	0.00	4.97
Experiment 2							
1	4.66	4.90	5.18	4.35	5.50	4.93	4.92
2	4.14	5.33	4.76	5.70	5.50	5.12	5.09
3	3.16	5.56	5.13	5.69	5.79	4.41	4.96
4	4.37	4.69	4.95	4.81	5.34	4.83	4.83
5	4.76	4.90	5.29	5.63	5.79	6.44	5.47
6	4.74	5.26	4.64	4.41	5.71	5.92	5.11
7	5.10	4.95	4.95	5.17	5.15	5.15	5.08
8	4.93	4.78	4.90	5.21	5.76	5.79	5.23
9	5.43	5.58	5.04	5.29	5.68	5.79	5.47
10	5.00	4.76	4.87	4.62	5.08	5.08	4.90

Table B-28

Tibia Zinc Concentration of Rats in Experiments 1 and 2

Experi-mental Diets	Replicates						
	1	2	3	4	5	6	Mean
	ug/gram dry weight						
Experiment 1							
1	211	217	187	206	208	213	207
2	159	253	272	306	208	117	219
3	223	365	118	245	249	249	242
4	263	195	187	247	227	165	214
5	202	226	167	139	199	150	181
6	221	247	270	221	186	154	217
7	314	274	211	296	271	211	263
8	207	221	185	189	281	202	214
9	240	283	164	247	266	188	231
10	175	220	157	127	214	195	181
Experiment 2							
1	203	157	207	206	440	251	244
2	151	284	169	684	312	279	313
3	211	178	185	284	295	313	244
4	136	169	198	321	330	309	244
5	148	412	308	263	379	317	304
6	168	234	227	300	381	379	281
7	245	218	178	391	268	330	272
8	179	191	196	271	314	316	245
9	326	223	185	360	341	316	292
10	171	171	141	287	345	345	244

APPENDIX C

STATISTICAL ANALYSIS

Table C-1

Analysis of Variance of Growth Data of Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Total	59		48266			
Replicates	5		4234		847	
Diet	9		31517		3502	
Source		4		25756		6439[a]
Level		1		2088		2088[a]
Source x Level		4		3676		919[b]
Error	45		12510		278	
Covariant Analysis						
Total	59		48266			
Replicates	5		319		64	
Feed Intake	1		35360		35360[a]	
Diet	9		7529		837	
Source		4		6199		1550[a]
Level		1		879		879[a]
Source x Level		4		451		113
Error	44		5058		115	

[a]Highly significant ($p \leq 0.01$)

[b]Significant ($p \leq 0.05$)

Table C-2

Analysis of Variance of Growth Data of Rats Fed Different P/S Ratios at Two Levels of Fat for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Total	59		8002			
Replicates	5		922		184	
Diet	9		2975		331[a]	
Source		4		652		163
Level		1		112		112
Source x Level		4		2211		553[a]
Error	45		4104		91	
Covariant Analysis						
Total	59		8002			
Replicates	5		909		182	
Food Intake	1		254		254	
Diet	9		2741		2741[a]	
Source		4		557		139
Level		1		61		61
Source x Level		4		2123		531[a]
Error	44		4098		93	

[a]Highly significant ($\underline{p} \leq 0.01$)

Table C-3

Analysis of Variance of Hemoglobin and Hematocrit Data of Rats Fed Different Sources of Fat at Two Levels for Experiment 1

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square
Hemoglobin			
Total	59	49	
Replicates	5	6	1
Diet	9	9	0
Source	4	3	1
Level	1	1	1
Source x Level	4	5	1
Error	45	36	1
Hematocrit			
Total	59	924	
Replicates	5	84	17
Diet	9	196	22
Source	4	164	41
Level	1	1	1
Source x Level	4	31	8
Error	45	644	14

Table C-4

Analysis of Variance of Hemoglobin and Hematocrit Data of Rats Fed Different P/S Ratios at Two Levels of Fat for Experiment 2

Source of Variation	Degree of Freedon		Sum of Squares		Mean Square	
Hemoglobin						
Total	59		118			
Replicates	5		12		2	
Diet	9		24		3	
Source		4		9		2
Level		1		2		2
Source x Level		4		13		3
Error	45		82		2	
Hematocrit						
Total	59		4849			
Replicates	5		750		150	
Diet	9		765		85	
Source		4		91		102
Level		1		91		91
Source x Level		4		268		67
Error	45		3335		74	

Table C-5

Analysis of Variance of Tissue Copper From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		153			
Replicates	5		12		2	
Diet	9		50		6[a]	
Source		4		39		10[b]
Level		1		6		6
Source x Level		4		5		1
Error	45		90		2	
Kidney						
Total	59		46439		851	
Replicates	5		4253		1481	
Diet	9		13328		1481[a]	
Source		4		11935		2984[b]
Level		1		273		273
Source x Level		4		1120		280
Error	45		28858		641	
Spleen						
Total	59		168			
Replicates	5		18		4	
Diet	9		61		7	
Source		4		20		5[a]
Level		1		1		1
Source x Level		4		40		10[b]
Error	45		90		2	
Testes						
Total	59		452			
Replicates	5		29		6	
Diet	9		145		16[a]	
Source		4		116		29[b]
Level		1		14		14
Source x Level		4		15		4
Error	45		279		7	

[a]Significant ($\underline{p} \leq 0.05$)

[b]Highly significant ($\underline{p} \leq 0.01$)

Table C-6

Analysis of Variance of Tissue Copper From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		102			
Replicates	5		13		3	
Diet	9		18		2	
Source		4		3		1
Level		1		8		8
Source x Level		4		7		2
Error	45		71		2	
Kidney						
Total	59		7142			
Replicates	5		602		120	
Diet	9		2411		268[a]	
Source		4		903		226
Level		1		513		513[a]
Source x Level		4		995		249[a]
Error	45		4130		92	
Spleen						
Total	59		355			
Replicates	5		35		7	
Diet	9		37		4	
Source		4		10		3
Level		1		15		15
Source x Level		4		12		3
Error	45		284		6	
Testes						
Total	59		1705			
Replicates	5		939		188	
Diet	9		141		16	
Source		4		16		4
Level		1		0		0
Source x Level		4		125		31
Error	45		623		14	

[a]Significant ($p \leq 0.05$)

Table C-7

Analysis of Variance of Tissue Iron From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		3150872			
Replicates	5		23552		4710	
Diet	9		2663833		295981[a]	
Source		4		2290466		572617[a]
Level		1		78409		78409[a]
Source x Level		4		294958		73740[a]
Error	45		463485		10300	
Kidney						
Total	59		41867			
Replicates	5		3243		649	
Diet	9		11484		1276[b]	
Source		4		8030		2008[a]
Level		1		1251		1251
Source x Level		4		2203		551
Error	45		27140		603	
Spleen						
Total	59		14734744			
Replicates	5		323754		64751	
Diet	9		7703484		855942[a]	
Source		4		7397199		1849300[a]
Level		1		66800		66800
Source x Level		4		239485		59871
Error	45		6707506		149056	
Testes						
Total	59		109894			
Replicates	5		36037		7207	
Diet	9		14763		1640	
Source		4		9267		2317
Level		1		2653		2653
Source x Level		4		2843		711
Error	45		59094		1313	

[a]Highly significant ($p \leq 0.01$)

[b]Significant ($p \leq 0.05$)

Table C-8

Analysis of Variance of Tissue Iron From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		1690199			
Replicates	5		87042		17408	
Diet	9		1121721		124636[a]	
Source		4		801799		200445[a]
Level		1		223382		223382[a]
Source x Level		4		96560		24140
Error	45		481437		10699	
Kidney						
Total	59		86623			
Replicates	5		8513		1703	
Diet	9		16281		1809	
Source		4		7705		1926
Level		1		107		107
Source x Level		4		8469		2117
Error	45		61829		1374	
Spleen						
Total	59		35665987			
Replicates	5		1963176		392635	
Diet	9		20273015		2252557[a]	
Source		4		11027614		275690[a]
Level		1		6407894		640789[a]
Source x Level		4		2837507		70937
Error	45		13429795		298440	
Testes						
Total	59		36149			
Replicates	5		2763		553	
Diet	9		2775		308	
Source		4		1433		358
Level		1		308		308
Source x Level				1034		259
Error	45		30611		680	

[a]Highly significant ($p \leq 0.01$)

Table C-9

Analysis of Variance of Tissue Zinc From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		4327			
Replicates	5		776		155	
Diet	9		718		80	
Source		4		457		114
Level		1		42		42
Source x Level		4		219		55
Error	45		2832		63	
Kidney						
Total	59		5523			
Replicates	5		2037		407	
Diet	9		1825		203[a]	
Source		4		1591		398[a]
Level		1		121		121
Source x Level		4		113		28
Error	45		1660		37	
Spleen						
Total	59		19233			
Replicates	5		2089		418	
Diet	9		3195		355	
Source		4		2224		556
Level		1		14		14
Source x Level		4		957		239
Error	45		13948		310	
Testes						
Total	59		38030			
Replicates	5		1364		273	
Diet	9		13458		1495[b]	
Source		4		5590		1398[b]
Level		1		1198		1198
Source x Level		4		6670		1668[b]
Error	45		23208		516	

[a]Highly significant ($p \leq 0.01$)

[b]Significant ($p \leq 0.05$)

Table C-10

Analysis of Variance of Tissue Zinc From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		29972			
Replicates	5		15922		3184	
Diet	9		2021		2225[a]	
Source		4		634		159
Level		1		1206		1206[b]
Source x Level		4		181		45
Error	45		12029		267	
Kidney						
Total	59		35604			
Replicates	5		9460		1892	
Diet	9		5654		628	
Source		4		971		243
Level		1		1906		1906[b]
Source x Level		4		2777		694
Error	45		20490		455	
Spleen						
Total	59		255596			
Replicates	5		157429		31486	
Diet	9		29374		3264[b]	
Source		4		15014		3754
Level		1		1438		1438
Source x Level		4		12922		3231
Error	45		68793		1529	
Testes						
Total	59		81793			
Replicates	5		15779		3156	
Diet	9		10542		1171	
Source		4		3332		833
Level		1		544		544
Source x Level		4		6666		1667
Error	45		55473		1233	

[a]Highly significant ($\underline{p} \leq 0.01$)

[b]Significant ($\underline{p} \leq 0.05$)

Table C-11

Analysis of Variance of Tissue Manganese From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		60.14			
Replicates	5		2.31		0.46	
Diet	9		38.84		4.31[a]	
Source		4		34.21		8.55[a]
Level		1		0.07		0.07
Source x Level		4		4.56		1.14[b]
Error	45		18.99		0.42	
Kidney						
Total	59		58.42			
Replicates	5		21.37		4.27	
Diet	9		12.80		1.42[a]	
Source		4		8.42		2.11[a]
Level		1		0.01		0.01
Source x Level		4		4.37		1.09
Error	45		24.09		0.54	
Spleen						
Total	59		27.29			
Replicates	5		0.69		0.14	
Diet	9		8.72		0.97	
Source		4		1.25		0.31
Level		1		2.32		2.32
Source x Level		4		5.15		1.29
Error	45		17.87		0.40	
Testes						
Total	59		34.49			
Replicates	5		12.50		2.5	
Diet	9		8.00		0.9[a]	
Source		4		6.99		1.75[a]
Level		1		0.06		0.06
Source x Level		4		1.06		0.27
Error	45		13.87		0.31	

[a] Highly significant ($p \leq 0.01$)

[b] Significant ($p \leq 0.05$)

Table C-12

Analysis of Variance of Tissue Manganese From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Liver						
Total	59		45.99			
Replicates	5		14.95		2.99	
Diet	9		14.42		1.60[a]	
Source		4		1.54		0.39
Level		1		5.60		5.60[a]
Source x Level		4		7.28		1.82[a]
Error	45		16.54		0.37	
Kidney						
Total	59		28.09			
Replicates	5		9.98		2.00	
Diet	9		4.26		0.47	
Source		4		1.73		0.43
Level		1		1.31		1.31
Source x Level		4		1.22		0.31
Error	45		13.85		0.31	
Spleen						
Total	59		156.29			
Replicates	5		13.48		2.70	
Diet	9		13.00		1.44	
Source		4		8.64		2.16
Level		1		1.55		1.55
Source x Level		4		2.96		0.74
Error	45		129.67		2.88	
Testes						
Total	59		58.17			
Replicates	5		25.34		5.07	
Diet	9		1.53		0.17	
Source		4		0.32		0.08
Level		1		1.26		1.26
Source x Level		4		0.95		0.24
Error	45		30.30		0.67	

[a]Highly Significant ($p \leq 0.01$)

Table C-13

Analysis of Variance of Bone Calcium From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		729199			
Replicates	5		46977		9395	
Diet	9		93352		10372	
Source		4		46184		11546
Level		1		10428		10428
Source x Level		4		36740		9185
Error	45		588869		13086	
Tibia						
Total	59		1896017			
Replicates	5		223898		44780	
Diet	9		457812		50868	
Source		4		175512		43878
Level		1		90094		90094
Source x Level		4		192206		4805
Error	45		1214307		26985	

Table C-14

Analysis of Variance of Bone Calcium From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		11713			
Replicates	5		3646		729	
Diet	9		1560		173	
Source		4		913		228
Level		1		228		228
Source x Level		4		419		105
Error	45		6507		145	
Tibia						
Total	59		15699			
Replicates	5		7420		1484	
Diet	9		1711		190[a]	
Source		4		1260		315[a]
Level		1		40		40
Source x Level		4		411		103
Error	45		6567		146	

[a]Significant ($\underline{p} \leq 0.05$)

Table C-15

Analysis of Variance of Bone Phosphorus From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		16965			
Replicates	5		1485		297	
Diet	9		5554		617[a]	
Source		4		1374		344
Level		1		454		454
Source x Level		4		3726		932[b]
Error	45		9926		220	
Tibia						
Total	59		23753			
Replicates	5		3472		694	
Diet	9		6644		738[a]	
Source		4		4920		1230
Level		1		89		22
Source x Level		4		1735		434
Error	45		13637		303	

[a]Significant ($p \leq 0.05$)

[b]Highly significant ($p \leq 0.01$)

Table C-16

Analysis of Variance of Bone Phosphorus From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
<u>Femur</u>						
Total	59		20286			
Replicates	5		4256		851	
Diet	9		2685		298	
Source		4		1476		369
Level		1		358		358
Source x Level		4		851		213
Error	45		13344		297	
<u>Tibia</u>						
Total	59		13695			
Replicates	5		3759		752	
Diets	9		973		108	
Source		4		411		103
Level		1		219		219
Source x Level		4		343		86
Error	45		8963		199	

Table C-17

Analysis of Variance of Bone Magnesium From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		187			
Replicates	5		34		7	
Diet	9		28		3	
Source		4		13		3
Level		1		13		13
Source x Level		4		2		1
Error	45		125		3	
Tibia						
Total	59		258			
Replicates	5		34		7	
Diet	9		38		4	
Source		4		20		5
Level		1		2		2
Source x Level		4		16		4
Error	45		186		4	

Table C-18

Analysis of Variance of Bone Magnesium From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		111.10			
Replicates	5		11.14		2.23	
Diet	9		20.37		2.26	
Source		4		10.44		2.61
Level		1		3.28		3.28
Source x Level		4		6.65		1.66
Error	45		79.58		1.77	
Tibia						
Total	59		16.20			
Replicates	5		4.85		0.97	
Diet	9		2.71		0.30	
Source		4		0.20		0.05
Level		1		0.16		0.16
Source x Level		4		2.35		0.59[a]
Error	45		8.64		0.19	

[a]Significant ($p \leq 0.05$)

Table C-19

Analysis of Variance of Bone Zinc From Rats for Experiment 1

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		168452			
Replicates	5		47166		9433	
Diet	9		44120		4902	
Source		4		29134		7284[a]
Level		1		2369		2369
Source x Level		4		12617		3154
Error	45		77166		1715	
Tibia						
Total	59		144924			
Replicates	5		30348		6070	
Diet	9		33795		3755	
Source		4		24659		6165[b]
Level		1		1162		1162
Source x Level		4		7974		1994
Error	45		80782		1795	

[a]Highly significant ($\underline{p} \leq 0.01$)

[b]Significant ($\underline{p} \leq 0.05$)

Table C-20

Analysis of Variance of Bone Zinc From Rats for Experiment 2

Source of Variation	Degree of Freedom		Sum of Squares		Mean Square	
Femur						
Total	59		392567			
Replicates	5		97505		19501	
Diet	9		58401		6489	
Source		4		20169		5042
Level		1		4220		4220
Source x Level		4		34012		8503
Error	45		236660		5259	
Tibia						
Total	59		533165			
Replicates	5		243594		48719	
Diet	9		41958		4662	
Source		4		14600		3650
Level		1		170		170
Source x Level		4		27188		6797
Error	45		247614		5503	