The effect of varying levels of dietary calcium upon lipid excretion and deposition in swine
by Arthur Leo Hecker

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University

Abstract:
One trial involving 24 pigs was conducted to investigate the effect of varying levels of dietary calcium upon lipid excretion and deposition in swine. Phosphorus and calcium were maintained in a 1:1 ratio in all diets. Calcium levels, 0.4, 0.8 and 1.2 percent, and calcium sources, limestone and defluorninated rock phosphate, appeared to be satisfactory for normal growth. Although the effect was nonsignificant, the 0.8 percent dietary calcium level appeared to induce the highest average daily gain. Treatment had no effect on feed efficiency. Average daily gain did not demonstrate any significant difference in the,efficacy of the two calcium sources. The serum calcium level was significantly (P< .05) affected by the calcium source and 0.8 percent calcium intake level; Increased dietary calcium intake had no effect on fecal lipid levels. The diets used in the present investigation contained no added fats.

These diets contained only low melting point fats which are not subject to interference by calcium as are the high melting point fats. The lack of significance could be due to this reason. Data revealed that increased calcium ingestion caused a significant (P< .01) drop in' serum cholesterol but not in total serum lipids. Liver and heart total lipid levels showed -no effect due to treatment. The limestone calcium source when compared to defluorinated rock phosphate appeared to cause a significant (P< 0.5) decrease in heart cholesterol content. The limestone calcium source supplied the calcium in the form of calcium carbonate compared to tricalcium phosphate from the defluorinated rock phosphate. This difference in anion ,form may explain the difference in effect due to source here , and elsewhere in the study. Fecal, tissue and serum lipid phosphorus remained unaffected by treatment.

The investigation indicated that gilts had a lower (P<.05) serum cholesterol level than did the barrows. The sex hormones may be involved in this difference. At the 0.8 percent dietary calcium level, sex again appeared to introduce some effect (P<.05) causing an opposite flucuation in the heart cholesterol content between barrows and gilts.

There was a significant (P<.05) three-way interaction found in analyzing the effect of calcium level, calcium source and sex on serum lipid phosphorus.. As there were no significant,effects due to main treatments on this parameter, it is suspected that this interaction may have been due to random variation.

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ABSTRACT

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Increased dietary calcium intake had no effect on fecal lipid levels. The diets used in the present investigation contained no added fats. These diets contained only low melting point fats which are not subject to interference by calcium as are the high melting point fats. The lack of significance could be due to this reason. Data revealed that increased calcium ingestion caused a significant ($P < .01$) drop in serum cholesterol but not in total serum lipids. Liver and heart total lipid levels showed no effect due to treatment. The limestone calcium source when compared to defluorinated rock phosphate appeared to cause a significant ($P < 0.5$) decrease in heart cholesterol content. The limestone calcium source supplied the calcium in the form of calcium carbonate compared to tricalcium phosphate from the defluorinated rock phosphate. This difference in anion form may explain the difference in effect due to source here and elsewhere in the study. Fecal, tissue and serum lipid phosphorus remained unaffected by treatment.

The investigation indicated that gilts had a lower ($P < .05$) serum cholesterol level than did the barrows. The sex hormones may be involved in this difference. At the 0.8 percent dietary calcium level, sex again appeared to introduce some effect ($P < .05$) causing an opposite flucuation in the heart cholesterol content between barrows and gilts.

There was a significant ($P < .05$) three-way interaction found in analyzing the effect of calcium level, calcium source and sex on serum lipid phosphorus. As there were no significant effects due to main treatments on this parameter, it is suspected that this interaction may have been due to random variation.

INTRODUCTION

Experimental animal nutrition has transformed from an age of new discovery into one of interrelationship exploration. While much of our current knowledge has resulted from concentrated research on specific nutritional problems of animals and man, equally important discoveries have evolved from basic studies of the physiological and biochemical changes in the animal organism as affected by various dietary variables.

The nutritional and physiological importance of certain minerals has been recognized since the early 1800's. It has only been in the last fifty years, however, that a relationship has been demonstrated between minerals and other nutrients. It is known that proper calcium utilization depends upon not only the presence of phosphorus and vitamin D but also upon the availability of adequate amounts of them. With the advent of added fats in animal diets, an interrelationship involving calcium and lipids has evolved. Extended research has disclosed evidence supporting the theory that increased dietary calcium levels have an inhibitory effect upon lipid utilization.

Recent developments have served to stress the interrelationships between human and animal nutrition. Nutritional data from swine investigations may be extrapolated to human nutrition, since the nutritional requirements and digestive systems of man and swine are quite similar. High energy diets, as are now being fed to swine, approach the caloric value and origin of those consumed by man.

An effective reduction of fat absorption might be desirable in reference to human nutrition. In a human population accustomed to a high caloric and relatively high fat intake, it would seem difficult to change dietary habits; therefore, if a significant portion of ingested fat could be rendered nonabsorbable, it would probably constitute an aid in effecting weight loss or preventing weight gain. This calcium-lipid relationship takes on additional importance when you consider that certain lipids are suspected to be the crucial substances responsible for the development of arteriosclerosis in man. An effective control of serum lipid types and levels would be very advantageous.

This investigation was designed to study the effects of varying levels of dietary calcium upon lipid excretion and deposition in swine.

REVIEW OF LITERATURE

Calcium-Phosphorus Requirements of Swine

Dunlop (1935) stated that the true requirement of an animal for calcium and phosphorus is dependent on growth rate and efficiency of feed conversion. His investigations indicated that a swine diet with calcium and phosphorus levels of 0.45 percent of the dry matter was optimum for average daily gains of 1.0 to 1.4 pounds per day and efficiency of 3 to 4 pounds of dry matter per pound of gain. Low calcium intakes did not change blood calcium levels.

Aubel *et al.* (1936) established the minimum phosphorus requirements of swine at 0.27 to 0.30 percent of the diet. Aubel and Hughes (1937) found that pigs receiving rations of 0.51 percent phosphorus and 0.79 percent calcium had the best appetites, gains, bone formation and efficiency of gain. Blood levels of serum inorganic phosphorus dropped on low phosphorus diets from an initial 5.5 milligram percent to 2.6 milligram percent, while serum calcium level was maintained or increased. There was no effect on blood phosphorus at 0.23 percent dietary phosphorus or above. It was observed that carcasses of pigs fed low levels of phosphorus carried more fat than pigs receiving higher levels, though there was no consistent effect on dressing percent.

Theiler *et al.* (1937) conducted experiments with swine on the effect of rations deficient in calcium or phosphorus or both. The rations were considered deficient at 0.1 percent calcium and adequate at 0.2 percent. Theiler stated that serum calcium was not lowered by low levels of calcium in the diet.

Swine rations containing 0.1 percent calcium were considered deficient by Aubel et al. (1941). Pigs exhibited poor appetites, diarrhea, became coarse haired and nervous. One half of the pigs recovered after the 10th week and were fairly normal at the end of twenty-four weeks except in weight. The remainder showed continual disturbances and developed paralysis of the hindquarters. A small percentage of pigs receiving 0.25 percent dietary calcium showed deficiency symptoms and those appearing normal had soft bones when sacrificed. Blood calcium dropped intially and then rose to normal on the low calcium diets. Pigs were able to overcome the deficiency as they matured, indicating requirements to be greater in young pigs and decreasing with maturity.

Miller et al. (1960) reported that 0.6 percent calcium in the presence of 0.5 percent phosphorus supported maximum growth and feed response in baby pigs. Analysis of humerus ash, calcium, phosphorus, specific gravity and the breaking strength of femurs, however, showed that the minimum calcium level for maximum skeletal formation was 0.8 percent of the ration.

Rutledge et al. (1961) fed early weaned pigs practical corn-soybean meal diets containing 0.4, 0.6, 0.8 or 1.0 percent calcium and 0.6 percent phosphorus. Highly significant linear trends toward increased ash and calcium content of femur samples and of humeri breaking strength resulted from increases in dietary calcium. Rate of gain, feed efficiency and serum calcium and phosphorus levels were not affected significantly by calcium content of the diet. Radiographs of femurs and humeri indicated marked increases in degree of calcification and bone density with increases in dietary calcium. The minimum calcium level suggested was 0.8 percent of the ration.

Calcium and phosphorus requirements of growing-finishing swine fed rations fortified with vitamins, trace minerals, and an antibiotic were investigated by Chapman et al. (1962). Dietary levels of calcium and phosphorus ranged from 0.2 through 0.8 and 0.2 through 0.7 percent, respectively. Rate of gain decreased as calcium content increased when the diet contained 0.2 or 0.3 percent phosphorus, thus demonstrating the importance of a narrow calcium-phosphorus ratio when the dietary level of phosphorus is low. As the dietary phosphorus level increased, there was a significant increase in both femur weight and femur fat content. Increasing phosphorus to 0.6 percent of the diet resulted in increased gains to 100 pounds for all levels of calcium fed. There was a highly significant increase in femur weight with increases in dietary calcium or phosphorus, though there were no differences in calcium or phosphorus content of femurs due to treatment. There was a significant correlation between increasing calcium and phosphorus levels and femur breaking strength. Dietary requirements, to assure maximum rate of gain and optimum skeletal development, appeared to be 0.8 percent calcium and 0.6 percent phosphorus for the pig from 25 to 100 pounds, and 0.7 percent calcium and 0.5 percent phosphorus from 100 to 200 pounds.

Calcium-Phosphorus Interrelationship

Forbes (1914) was one of the first to demonstrate that a phosphorus deficiency was rendered more acute by adding calcium carbonate to diets low in phosphorus.

A close relationship between calcium and phosphorus intake and calcium retention was observed by Haag and Palmer (1927). These workers reported 70 to 80 percent retention by pigs on diets containing 0.34 percent calcium and 0.65 percent phosphorus. Their data indicated that this level of calcium was below the point where maximum retention occurred. Results were quite variable with phosphorus, and it was postulated that low retention was due to the low calcium content of the diet.

Bethke et al. (1932) emphasized the importance of calcium-phosphorus ratios in rat diets for optimum bone development. Increasing the calcium-phosphorus ration from 1 to 5 caused progressive decreases in growth, bone ash and serum inorganic phosphorus. Changing the ration from 1 to 0.25 depressed growth and ash content of femurs and serum calcium was depressed, whereas serum inorganic phosphorus was increased. The most favorable ratio was two parts calcium to one part phosphorus.

Haldt et al. (1939) observed that when large amounts of calcium and phosphorus were added to rat diets, practically all excess calcium was recovered from the feces, whereas phosphorus in the feces was considerably less than the intake. Excess phosphorus and little more than normal amounts of calcium were excreted through the kidneys. The large amount of calcium and phosphorus interfered to a slight extent with the utilization of nutrients.

Data presented by Vermeulen (1959) showed that rats fed extra high calcium levels excreted greater amounts through the urine. Formation of calcium phosphate stones was inhibited at high levels of calcium, and a profound drop in urine phosphorus was observed. Addition of phosphorus to high-calcium diets produced an increase in urine phosphate and a drop in urine calcium.

Clark and Cordero (1964) reported that with rats, in the absence of dietary phosphorus, urinary calcium was constant regardless of calcium intake and unless phosphorus intake was high, increasing calcium intake did not increase calcium retention. On a constant and adequate calcium diet, however, increasing dietary phosphorus increased calcium retention. When rats were fed calcium-free diets, urinary phosphorus accounted for as much as 75 percent of ingested phosphorus. Data indicated that level of phosphorus in the diet was as important as that of calcium for promoting calcium retention.

Zimmerman et al. (1963) conducted a series of experiments to determine the optimum calcium and phosphorus levels in high-milk-product rations for maximum body weight gain, efficiency of feed utilization and metatarsal calcification of swine 2 to 7 weeks of age. Low phosphorus levels and high calcium levels in the rations slowed the rate of body weight gain. In general, calcium-phosphorus ratios of 1.6:1.0 or wider adversely influenced gains. Calcium and phosphorus appeared to independently influence the efficiency of feed utilization. Calcium levels above 0.8 percent of the diet reduced feed efficiency, whereas phosphorus up to approximately 0.6 percent of the ration improved efficiency of feed utilization. The highest levels of calcium (1.0 percent) and phosphorus

(0.7 percent) in the ration produced maximum responses in metatarsal calcification; however, the largest increments of response come between the lower levels, especially with regard to phosphorus. Although high calcium levels in the ration lowered serum chlortetracycline values, it was demonstrated that this was not the cause of the adverse effect of high calcium intakes on body weight gains.

Effect of Source on Utilization of Calcium and Phosphorus

Thomas et al. (1933) conducted an investigation comparing the relative efficacy of ground limestone, steamed bone meal, and Dicapho (a commercial dicalcium phosphate supplement) as calcium supplements to rations for growing and fattening pigs. Data were collected in three metabolism experiments designed to determine the efficacy of the three calcium sources fed at equivalent low and high levels of calcium intake as supplements to basal rations deficient in calcium. Vitamin D was left out in an attempt to augment the possibility of detecting significant differences in the efficiency with which these compounds met the calcium requirements of the pig. The metabolism study showed that for equivalent quantities of calcium from each source, fed at either low or high levels of intake, calcium and phosphorus retention was essentially the same. A feedlot experiment comparing the three calcium sources was conducted concurrently with the metabolism trials. The data collected in this part of the study did not demonstrate any significant differences in the efficacy of the calcium supplements compared.

McCampbell and Aubel (1934) conducted several trials in an attempt to make a comparison between calcium carbonate and calcium sulfate (gypsum) as a source of calcium in swine rations. All pigs were fed the same basal ration: linseed oil meal, wheat shorts, and corn, to equal the amount consumed by the pig eating the least. There were no significant differences in gains, and there appeared to be no effect on the appetite or physical condition of the pigs. This study indicated that calcium sulfate could be used as an effective substitute for calcium carbonate as a source of calcium for swine rations.

The effect of pulverized limestone and dicalcium phosphate supplements on the nutritive value of dairy cattle feed were studied by Colovos et al. (1958). Results of these experiments indicated that pulverized limestone, when added to both all roughage and mixed dairy rations, decreased the digestibility of both protein and energy in dairy heifers. Similar amounts of dicalcium phosphate did not have this effect. The feeding of dicalcium phosphate along with the pulverized limestone decreased the depressing effects of the limestone. These results indicated that the feeding of calcium in excess of requirements, particularly in wide calcium:phosphorus ratios, may reduce the digestibilities of protein and energy. There was nothing in this data to suggest any involvement of the carbonate radical as the causative agent in the decreased digestibilities of protein and energy.

Data collected by Combs and Wallace (1962) showed little evidence of a dietary calcium source effect on average daily gain, feed consumption or feed efficiency in growing pigs. The source of calcium did not significantly affect the apparent digestibility of ether extract. In three experiments where calcium levels of 0.40 and 0.88 percent were compared, the higher level significantly depressed growth rate. Similar differences in growth rate were also obtained with rations containing either 0.52 or 0.72 percent calcium. When ration calcium was increased by increments of 0.10 from 0.40 to 0.80 percent, daily gains tended to decrease linearly with increasing calcium. In all experiments, the most efficient utilization of feed occurred with low calcium rations. Neither rate nor efficiency of gain were significantly influenced by the sources of supplementary calcium used in this study or by varying the diet concentration of chlortetracycline. Contrasting results were obtained with apparent digestibility determinations. In one experiment, the digestion coefficients for dry matter, ether extract and crude protein were comparable with pigs that received either 0.40 or 0.88 percent dietary calcium. The 0.88 percent calcium in another experiment significantly reduced digestibility of ether extract and crude protein. Dry matter and crude protein digestibilities were significantly lowered when gypsum rather than ground limestone or oyster shell flour supplied the supplemental calcium.

Fleishmann <u>et al</u>. (1967) compared the effect of several different sources of calcium on serum and fecal lipid levels. He found that irregardless of calcium sources; calcium carbonate, dibasic phosphate, calcium gluconate or Beta-glycerophosphate, an increase in intake caused a significant lowering of serum cholesterol level. Triglycerides were lowered only by carbonate and gluconate. The Beta-glycerophosphate yielded unexpected results in that it caused a significant decrease in serum cholesterol at the 0.2 percent level but an increase at the 0.7 percent level. Fecal lipid excretion was significantly elevated at the 0.7 percent calcium level with no difference shown between sources. All salts except Beta-glycerophosphate significantly increased fecal bile acid excretion at the 0.2 percent level. The authors postulated that the hypolipemic action of dietary calcium may be partially anion dependent.

Studies conducted by Chapman et al. (1955) concluded that there was some effect due to different sources of phosphorus. Eight phosphorus treatments were fed with and without chlortetracycline. Diets were formulated so that each had the same calcium and phosphorus level and ratio. Percentages of calcium and phosphorus used in the experiment were 0.7 and 0.5, respectively. Steamed bonemeal, dicalcium phosphate, colloidal clay and plant phosphorus represented the different phosphorus sources. The experiment was conducted in two phases, one extending through the first eight weeks and the second running from the eighth week weight to 200 pounds live animal weight. Data from the first period showed no significant difference in rate of gain, average daily feed intake or feed efficiency. The source of phosphorus had no significant effect on either average daily feed intake or feed efficiency; however, average daily gains were significantly reduced in those pigs receiving collodial clay compared with those fed steamed bonemeal or dicalcium phosphate.

Pigs receiving the higher levels of phosphorus from the inorganic phosphorus supplements ate significantly less feed per day and required significantly less feed per pound of gain than those receiving more of the phosphorus from plant sources. In all cases there was no significance due to the presence of antibiotics in the ration. In direct contrast to the initial eight week period, the second phase of the experiment demonstrated a small but significant increase in average daily gain due to the presence of the antibiotic. Once again those pigs receiving colloidal clay gained significantly slower than those receiving either steamed bonemeal or dicalcium phosphate, but there was little difference in the rates of gain between pigs receiving the latter two supplements. The daily feed intake of pigs fed colloidal clay was significantly less than for pigs fed dicalcium phosphate. The difference between colloidal clay and steamed bonemeal was just short of significance. The feed efficiency of the pigs receiving colloidal clay was significantly greater than that of pigs fed dicalcium phosphate. There was no effect on rate of gain when the phosphorus level was increased; however, the amount of feed required per pound of gain and the average daily feed intake were significantly decreased by increased phosphorus consumption. Considering both phases of the trial, the performance of pigs by the all-plant phosphorus rations was progressively improved as the content of phosphorus from plant sources was decreased and replaced by an equivalent amount of phosphorus from dicalcium phosphate. These studies indicated a significant decrease in the rate of gain and feed efficiency when phosphorus was supplied entirely by plant sources.

After comparing dicalcium phosphate, steamed bonemeal and defluorinated rock phosphate as sources of dietary phosphorus for pigs, Van Zante et al. (1967) reported no difference in bone ash between the three sources. However, at low levels deficiency symptoms developed in the pigs on steamed bonemeal and defluorinated rock phosphate.

Interrelationship Between Calcium, Protein and Carbohydrates

Woodman et al. (1937) found no evidence to warrant the conclusion that mineral metabolism was affected by higher levels of protein. On the other hand, McCance et al. (1942) showed that in healthy, adult human subjects, increasing protein intake increased calcium and magnesium absorption. Lehman and Pollak (1942) observed that alpha-amino acids increased the solubility of calcium salts and proposed that their presence aided in mineral absorption. Frandsen et al. (1954) reported calcium utilization and bone mineralization were greatly reduced in rats fed low protein or low quality protein rations. Lowery et al. (1962) demonstrated that the apparent protein digestibility of practical-type swine rations was not influenced by the addition of fat.

Lactose feeding has been shown to bring about changes in the pH of the contents of the whole intestinal tract below the duodenum (Robinson and Duncan, 1931) and to favor the development of an acidophilic flora (Rettger and Cheplin, 1923). The bacteriological studies of Cannon and McNease (1923) and Cruickshank (1928) might be cited as favoring the view that any effect of lactose on calcium metabolism is a specific one and not the result merely of an increased solubility of calcium salts in the more acid medium.

The influence of lactose in the diet on the utilization of calcium and phosphorus was studied by French and Cowgill (1937). The data obtained from young animals indicated that the presence of lactose in the diet favorably influenced the utilization of calcium and phosphorus; although this was not observed with mature animals. They were unable to determine whether this favorable action of lactose was due to increased absorption from or diminished excretion into the intestine. Even though they were unable to prove the exact mode of action, it did appear that a diminished excretion of calcium into the intestine was the most plausable. They very definitely confirmed with dogs that immaturity played a role in the effect of lactose on the utilization of calcium.

Kline et al. (1932) reported lactose to have a favorable effect on calcium absorption and skeleton building in chicks fed a rachitic ration. These workers postulated that the acid effect produced in the intestine by lactose was responsible for improved calcium utilization. Neither maltose nor citrate were of value in aiding calcium absorption or increasing intestinal acidity. Recent work of Charley and Saltman (1963) has strengthened these findings. In experiments with rats, they proposed evidence for the chelation of calcium ions by sugars. Lactose and other polyols prevented or delayed the precipitation of calcium ions in the presence of bicarbonate buffer. A charged calcium-lactose complex was demonstrated by electrophoresis and calcium-45. Greenwald et al. (1963) published somewhat conflicting data. They reported lactose had little effect on absorption and excretion of calcium in humans. Radioisotope studies carried out in a single patient indicated that absorption of calcium-45 was only slightly improved by lactose.

Lengemann et al. (1959) found that lactose enhanced the absorption of oral doses of calcium whether administered as chloride, lactate, gluconate or acetate. Even though direct derivatives of lactose were not able to increase calcium absorption, many other sugars were. Lactose increased calcium absorption both in the presence and absence of vitamin D. The greatest absorption took place in the duodenum and jejunum. Antibiotics had no effect on the rate of absorption, thus indicating that bacterial fermentation is not involved in the absorption mechanism.

Lloyd et al. (1961) found that by increasing the calcium level from 1.2 percent to 4.0 percent in the diets of young pigs, average daily gains and apparent digestibility of total carbohydrate were significantly decreased, but no other adverse effects were observed.

Interrelationship Between Calcium and Fat

A relationship between calcium and fat was shown in the work of Givens (1917). This author reported that poor utilization of dietary fats or fatty acids increased the excretion of calcium. Bosworth et al. (1918) pointed out the importance of calcium in the diet of human infants because of the elimination of calcium soaps when cows' milk was fed in place of breast feeding. Elimination of calcium soaps in the stools increased directly in proportion to the degree of solubility of diet calcium.

Because the infant cannot absorb the excess calcium from cow's milk, insoluble soaps are formed with the nonvolatile free fatty acids.

Telfer (1930) indicated that the higher calcium content of cows' milk as compared with human milk is the factor that leads to the greater loss of calcium soaps in the stools of bottle fed infants.

Holt et al. (1919a, 1919b) concluded from a study using infants, that a high calcium intake did not necessarily cause a large loss of fat in the feces. In a later study, Holt et al. (1920) found that the excretion of calcium in the feces was not related to the excretion of total fat, but some relationship existed to excretion of fat as a soap.

Telfer (1921) made the following postulation: if fatty acids are in excess, the calcium is excreted as soap; if alkalinity predominates, calcium phosphate is formed; if both fats and phosphate are low in the diet, calcium may be excreted as carbonate. These facts tend to indicate that the lower fatty acids readily dissolve calcium carbonate and calcium phosphate. In a later study, Telfer (1923) found that in the presence of higher fatty acids and their soluble soaps, a secondary reaction may then result in the formation of insoluble calcium soaps and the liberation of carbonic and phosphoric acids.

Boyd et al. (1932) stated that loss of calcium through insoluble calcium soap complexes depended upon other factors other than the amounts and nature of calcium compounds and fats in the diet. One such factor discussed was intestinal acidity. The addition of fat to a diet containing calcium ion as calcium chloride increased intestinal acidity and increased absorption of calcium and phosphorus thus decreasing the loss of soaps in the feces. These authors reported that young rats capable of storing 30 to 40 milligrams of calcium daily, utilized 25, 38, and 90 percent of calcium soaps of stearic, palmitic and oleic acids, respectively. When calcium intake was lowered, greater utilization of calcium stearate and calcium palmitate occurred.

The previous references all support the theory that there is an insoluble calcium soap complex formed where dietary calcium and fat levels are relatively high. In direct contrast to this theory is an early report by Klinke (1928). He reported that calcium-fatty acid-soap complexes were found to form soluble complexes with bile salts. He demonstrated this by excluding bile from the intestine of a dog which resulted in greater fecal calcium. Beznak (1931) supported Klinke when he found that by adding sodium taurocholate to diets high in fat, calcium absorption was increased. A possible explantion was postulated by Verzar (1936). He suggested that through the hydrotropic action of bile, bile acids are able to complex with calcium and fatty acids, thus making them water soluble.

Haldt (1939) demonstrated that rats fed high calcium and phosphorus diets had lower dry body weights than those fed normal calcium and phosphorus levels. A lowered body fat content was assumed to account for the weight loss.

Bassett et al. (1939) reported that a reduction in calcium intake facilitated the absorption of fatty acids. Increasing levels of dietary fat, increased calcium loss at the expense of stored calcium. Ingestion of alkaline phosphatase, a calcium salt or both caused an increase in total fecal fat.

In a study of the effects of calcium, manganese and other mineral elements upon the metabolism of lipids in dairy cattle, Ward and Reid (1948) found that all groups excreted similar proportions of the total lipid intake regardless of the mineral supplement received.

Wells and Cooper (1958) found that both 2 and 4 percent calcium chloride levels inhibited cholesterol absorption when the diet was supplemented with lactose, while a minimum of 4 percent calcium chloride was required to produce a similar effect on a diet based on sucrose. Calcium ion in the form of 2.2 percent calcium carbonate was less inhibitory than 2 percent calcium choloride. Calcium chloride had no effect when added to fat-free diets containing bile salts.

Carroll and Richards (1958) reported that the calcium content of the diet played an important role in determining the digestiblity of fatty acids in rats. The poor digestibility of oleic, linoleic and erucic acid relative to their corresponding triglycerides was shown to be associated with increased fecal excretion of calcium and phosphorus in the lipid fraction extractable with nuetral ether.

Fedde _et al_. (1960 reported that apparent absorption coeffients of fats by chicks were similar for either 10 or 20 percent dietary fat levels, were high for safflower oil, corn oil and hog grease, and were low for rendered beef fat. The apparent absorbability of tallow increased from 53 percent at one week of age to 80 percent at 12 weeks of age and was increased when 0.5 percent or more of dietary ox bile was added. The digestibility was apparently not dependent upon growth rate or feed intake and was decreased by high dietary calcium and increased by reducing the calcium intake.

An investigation conducted by Drenick (1961) using human subjects indicated that increased dietary calcium caused increased fecal fat. However, results involving other soap-forming agents such as aluminum hydroxide and magnesium sulfate were inconclusive.

Yacowitz (1962) reported a 100 percent increase in fecal fat in human subjects by raising the calcium level 2.66 grams above normal daily intake. Calcium soaps made up the major portion of the excreted fatty acids. It was also reported that serum cholesterol was reduced. This was in agreement with an investigation conducted by Iacona _et al._ (1960). He reported that dietary calcium was hypocholesterolemic in rabbits. In a later study, Iacona (1964) demonstrated that on a diet deficient in calcium, rabbits exhibited elevated plasma cholesterol.

Studies of Combs and Wallace (1962) with practical rations for young pigs containing 3 percent lard found no effect on the digestibility of either extract by doubling the calcium intake in one trial, while in a second trial, digestibility of ether extract was decreased from 64 to 52 percent. Average daily gain of pigs fed the lower calcium level was significantly higher than for those fed the high calcium level in the latter trial.

Yacowitz (1965) was able to demonstrate that increased calcium ingestion, in humans of both sexes, caused a significant decrease in both serum cholesterol and serum triglycerides. Both fecal dry matter and fecal lipid were increased. The exact mechanism of the hypolipemic action of calcium was undetermined.

In an attempt to elucidate the hypocholesterolemic and hypotriglyceridemic action of dietary calcium, Fleischman et al. (1966) found that blood lipids decreased with increasing dietary calcium with the major decrease occurring at the 0.2 percent calcium level. Some tissue lipids decreased and none increased. Fecal lipids were significantly increased up to the 1.2 percent dietary calcium level. Fecal fatty acids became progressively more saturated with increased calcium intake, but the preferential excretion of saturated fatty acids did not appear to be sufficient to alter significantly the blood fatty acids. Fecal bile acids significantly increased at the 1.2 percent calcium level. This data indicated that the lowering of blood cholesterol by increased dietary calcium is mediated in part by an increased excretion of bile acids.

At the conclusion of a long term study, fleischman et al. (1967) found that serum total lipids, phospholipids, cholesterol and triglycerides decreased significantly with increased calcium intake, often to levels equal to or lower than the control diet. This significance was based on a comparison to a control ration composed of 4 percent fat, 1.15 percent calcium and 0.76 percent phosphorus. Fecal lipids increased singificantly between the 0.2 percent and 1.2 percent level, primarily because of an increase in the free fatty acids. The excretion of lipid phosphorus was unaffected by increasing dietary calcium, except at the 2 percent calcium level or by increasing dietary fat levels. Fecal bile acids rose significantly at the 0.2 percent calcium level, with no further increase due to increased dietary calcium. Excretion of 3-Beta-hydroxysterol increased as dietary calcium increased and was, at the higher calcium levels, found to equal all the exogenous cholesterol, whereas fecal lipid excretion was found to account for the weight gain differences between the various calcium levels. No significant changes were noted in liver total lipid, cholesterol, triglyceride or free fatty acid due to an increase in dietary calcium. There was a decrease at the 1.2 percent calcium level in phospholipid, no further decrease was noted at the 2 percent level. All liver lipids were significantly higher ($P < .05$) with the high fat diet than with the control regardless of calcium level. Similar results were noted with the heart lipids, except that dietary fat concentration did not affect heart lipid concentration.

Bhattacharyya _et al._ (1968) reported that in humans a low calcium level in a saturated fat diet caused serum cholesterol to be significantly higher than a low calcium level in a polyunsaturated diet. The addition of 2 grams of calcium daily caused a decrease in serum cholesterol and an increase in fecal fatty acid excretion. No such changes were produced when calcium was added to the polyunsaturated diet but bile excretion increased.

The beneficial effects of dietary fat upon calcium assimilation as found by Holt and Fales (1922) and the curative effect of vitamin-free fat upon rickets as found by Zucker and Barnett (1922-23) possibly depended upon a more favorable pH of the intestinal contents, produced by the fatty food. Boyd and Lyman (1929) have shown that the utilization of calcium soaps may be high. Seventy to 80 percent of calcium oleate was absorbed. In direct contrast to these results, Cronheim and Muller (1908); Bahrdt (1910); Rothberg (1907); and Birk (1907), found that as the amount of fat in the diet increased above a moderate level, calcium soaps in the stools also increased.

Jones (1940) found that by adding lard at levels of 5 to 25 percent in the diet of chicks he could induce definite antirachitic properties which were not associated with the non-saponifiable fraction. Lard increased calcification on diets containing 1.1 or 0.38 percent calcium, but the effect was less pronounced when the diet was low in phosphorus. Oleic acid increased calcification also, but sodium oleate and calcium soaps showed no antirachitic action.

French (1942) demonstrated excellent growth in rats fed diets containing 5, 15 and 28 percent fat, but 45 percent of fat in the diet resulted in less growth. The high fat level exerted a definite depressive effect on the growth rate as well as feed consumption. The utilization of calcium was depressed moderately and consistently in the order of the increasing fat content of the diets from 5 to 28 percent, and then decreased considerably for the 45 percent fat diet. A more acid condition in the intestine resulted from the 5 percent fat diet than from diets richer in fat. The utilization of calcium paralleled the acidity of the intestional tract, the most efficient utilization accompanying the most acid reaction. The most efficient calcium utilization was obtained from the diet containing 1 gram of fat to 0.06 gram of calcium; and the efficiency of calcium utilization decreased in the order of the increase in the ratio of dietary fat to calcium. It was suggested that there are two basic physiological relationships involving fat and calcium: (1) the acidity of the intestinal tract, and (2) the formation of readily absorbable bile-fatty acid-calcium complexes.

Calverly and Kennedy (1949) studied various sources of fat in relation to calcium utilization. These authors concluded that poorly absorbed fats, long chain fats and saturated fatty acids increased fecal calcium, probably through formation of insoluble calcium soaps. The more soluble triglycerides resulted in less calcium excretion. Excretion of calcium and phosphorus in urine was affected indirectly by the presence of fat. When fecal calcium increased, there was a decrease in urinary calcium accompaned by a marked increase in urinary phosphorus. Inclusion of 5 percent fat in a normal diet well supplied with calcium and phosphorus in an optimum ratio, did not affect bone formation in healthy growing rats.

Newman et al. (1967) reported that added tallow in swine rations had no consistent effect on calcium digestibility or dietary phosphorus. Endogenous fecal calcium loss was not affected by the level of dietary tallow. Increased dietary calcium resulted in a reduction of the digestion coefficient of ether extract.

The melting point of fat has long been considered one of the factors affecting the digestibility of fats. Cheng et al. (1949) found an inverse relationship between melting point and digestibility of triglycerides with rats. Deuel (194 in a study of 34 vegetable fats melting below 50 degrees centrigrade, found that only two had coefficients of digestibility below 95. It was also found that in the case of animal fats, 18 different varieties melting under 50 degrees centigrade were found to be digestible to the extent of 93 percent or more in man. This work is supported by the results of Langworthy and Holmes, (1915); Holmes, (1919); and Deuel and Holmes, (1922). However, Lyman (1917) suggested that the melting point is not the only factor in determining digestibility but that the fatty acid makeup of the ester is also an important consideration. Cheng et al. (1949) demonstrated that calcium and magnesium did not significantly influence the digestibility of low melting fat, although they decreased markedly that a higher melting triglycerides and hydrogenated fats. These results were supported by data collected by Crockett and Deuel (1947).

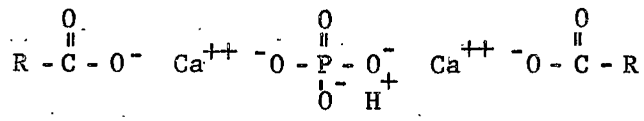
Investigations conducted by Werner and Lutwak (1963) demonstrated that primary lipid absorption is influenced both by the nature of dietary lipid and by dietary calcium concentration. The absorption increases as the melting point decreases, and is greater when triglycerides rather than free fatty acids of the same chain length are fed.

The incorporation of calcium-45 and phosphorus-32 in broiler femurs was studied by Bruggemann et al. (1961). Their data indicated that incorporation of calcium and phosphorus diminished in groups fed high calcium and 8 percent tallow. It was suggested that the metabolism of calcium and phosphorus on high calcium rations was not only dependent on the formation and excretion of calcium soaps but that there was a more specific interrelationship between fat and these minerals. The higher calcium diet decreased calcium-45 content in the bone by 20 percent. Tallow increased the calcium-45 content by 18 percent on the low calcium diet and decreased it by 50 percent on the high-calcium diet.

Swell et al. (1956_a) demonstrated the presence of a calcium-oleat-phosphate complex. In the case of dietary corn oil and olive oil, the phosphorus-containing lipid accounted for approximately 10 percent of the total lipid excretion. Oleic acid greatly increased the lipid-phosphorus excretion and there was a corresponding drop in the absorption of oleic acid. The study indicated that unsaturated fatty acids were more effective than saturated fatty acids in the formation of the calcium-fatty acid-phosphate complex. The data were in direct contrast to the belief that unsaturation causes better absorption. An oleic acid diet impaired calcium and phosphorus absorption. It was determined that 50 percent of the calcium and 50 percent of the phosphorus were lost in the feces as part of the calcium-fatty acid-phosphate complex.

Swell et al. (1956_b) found that rats fed a 25 percent oleic acid diet excreted large amounts of a phosphorus-containing lipid in their feces. The lipid resembled phospholipid in that it contained phosphorus and could be precipitated from chloroform solutions by acetone.

Characterization of the lipid revealed that it was a salt with the following composition: 2 atoms of calcium++, 1 mole of phosphate-, and 2 moles of oleic acid. The following is the proposed structure of the lipid:



Phosphorus-32 studies indicated that the lipid was formed in the gut from dietary phosphorus, calcium and oleic acid. The incorporation of phosphorus-32 into the lipid was about 15 times greater when the isotope was given in the diet than when given introperitoneally. Thus, there would appear to be no turnover of phosphorus in the lipid and its formation appeared to be strictly exogenous in nature. The formation of this lipid greatly impaired the absorption of oleic acid.

Kim and Ivy (1952) and Pihl (1955) reported that rats fed an oleic acid diet excreted large amounts of phospholipid in their feces. Kim and Ivy (1952) also pointed out that palmitic acid and corn oil were found to have a mild stimulatory action on phospholipid excretion.

EXPERIMENTAL

Twenty-four 8-week-old crossbred Hampshire-Poland China pigs weighing approximately 20.4 kilograms were allotted equally to 6 pens on the basis of weight, litter and sex. Diets were formulated to contain calcium levels of 0.4, 0.8 and 1.2 percent. Two sources of calcium, ground limestone and defluorinated rock phosphate were used. The calcium to phosphorus ratio was maintained at 1:1, and all diets were fed *ad libitum*. The pigs were fed a 14 percent protein grower diet during the initial 30 days of the trial, by which time the pigs had reached an average weight of 45.4 kilograms. During the remainder of the investigation, the pigs were fed a 10 percent protein finisher diet. Diets and proximate analysis are described in Tables I and II, respectively. Pigs were weighed at 3-week intervals for the duration of the trial and total feed consumption by pen was recorded.

The pigs were sacrificed as they reached a weight of approximately 91 kilograms. Prior to sacrifice, blood samples were taken by vena cava puncture. Serum was obtained by centrifugation and frozen for later lipid and mineral analysis.

Intestinal acidity was determined after sacrifice to determine the approximate acidity of the intestinal contents existing during the experimental period. After sacrifice, the entire digestive tract was removed intact. The duodenum was removed and its contents expelled into a beaker for pH reading. The contents were thoroughly mixed before inserting the electrodes and determining the pH. This value was then checked by repeating the procedure. The middle 90 percent of the jejunum was removed next