THE EFFECT OF *LACTOBACILLUS ACIDOPHILUS* AND DIETARY CALCIUM ON SERUM CHOLESTEROL LEVEL IN SWINE

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Submitted to the Faculty of the Graduate College
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in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
May, 1994

OKLAHOMA STATE UNIVERSITY

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ACKNOWLEDGMENTS

I would like to express my sincere and deep gratitude to Dr. C. V. Maxwell for his patience, guidance, and support as my major adviser during my studies at Oklahoma State University. Special words of appreciation to Dr. S. E. Gilliland for his guidance and assistance throughout this study and for serving on my graduate committee. Appreciation is also extended to Dr. Archie C. Clutter and Dr. E. C. Nelson for their suggestions as members of my advisory committee.

Thanks are expressed to the National Dairy Promotion and Research Board for the funding of this research. Many thanks are also extended to Dr. David L. Weeks, Dr. Selwyn Barron, Dr. John Kirkpatrick, M. L. Rose, Kim Brock, Tim Cash, and Luis Diaz for their valuable assistance during this study.

A special thanks goes to the faculty members, fellow graduate students, secretaries, and laboratory personnel, who shared their knowledge, technical assistance, and friendship during my time at Oklahoma State University.

My love and gratitude go to my mother, sister and brothers for their constant love, support, and encouragement throughout my education. A very special note of thanks is extended to my husband, Horacio, for his support and understanding during this course of study.

Finally, I would like to dedicate this thesis to a very special person whose existence has enlightened and inspired my life, my son Horacio Enrique, with my deepest love.

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CHAPTER I

INTRODUCTION

Coronary heart disease has been considered the major cause of death in the United States (107). Persons with elevated cholesterol levels have greater risk of having heart disease than those with normal serum cholesterol levels. Reducing blood cholesterol levels, especially that associated with low density lipoprotein (LDL), can significantly decrease the incidence of coronary heart disease in hypercholesterolemic persons (102, 103).

The intestinal microflora may influence serum cholesterol concentrations. Eyssen (27) reported that germ-free animals fed a high cholesterol diet accumulated twice as much cholesterol in the blood and liver as did conventional animals fed in a similar manner. Conventional animals excreted more cholesterol in the feces than germ-free animals. Studies using the pig as an animal model showed that dietary supplementation with a strain of *Lactobacillus acidophilus* that actively assimilated cholesterol during anaerobic growth reduced serum cholesterol levels of pigs fed a high cholesterol diet (11, 35). Furthermore, *in vitro* studies indicated that some species of lactobacilli present in the intestinal tract, including *L. acidophilus*, can deconjugate bile acids in anaerobic conditions (34, 114). Bile acids are a group of water-soluble acidic steroids with powerful detergent properties. They are formed in the liver from cholesterol, conjugated with glycine and taurine, stored in the gallbladder, and released into the duodenum where they assist in the absorption of fat and fat soluble vitamins. These conjugated bile acids are then reabsorbed from the small intestine and transported back to the liver for reuse (98). This process is known as enterohepatic circulation of

bile acids. Deconjugated bile acids do not support cholesterol absorption as well as conjugated bile acids (33, 53). In addition, increased deconjugation of bile acids could result in greater excretion of bile acids from the intestinal tract since free bile acids are less well reabsorbed than are bile acids conjugated with glycine or taurine (9). The decreased concentration of bile acids returning to the liver from the intestine would stimulate the synthesis of replacement bile acids from cholesterol. The ability of some strains of *L. acidophilus* to take up cholesterol during growth and to deconjugate bile acids offers two potential mechanisms for reducing the amount of cholesterol absorbed from the small intestine and therefore to reduce serum cholesterol levels in hypercholesterolemic persons.

Earlier epidemiological studies have shown a negative correlation between cardiovascular diseases and water hardness, especially with concentrations of calcium in the water (5, 93). It has also been reported in both human and animal studies that calcium may be hypocholesterolemic (18, 31, 55, 110, 118). However, the mechanism whereby this phenomenon occurs is still not clear.

The objectives of this study were to: (1) determine the effects of dietary cholesterol on the induction of hypercholesterolemia in pigs, and (2) investigate the effects and interactions of *L. acidophilus* and dietary calcium on total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and total bile acids in serum of pigs previously fed a high cholesterol diet.

CHAPTER II

REVIEW OF LITERATURE

Role of Cholesterol in the Body and Its Relationship With Cardiovascular Diseases

Cholesterol is one of the most common compounds found in the human body. It is present as free cholesterol in every cell membrane and esterified or in the free form inside the cell. The human body contains about 0.2% cholesterol and about two-thirds of the total body cholesterol is distributed equally in the brain and nervous system, muscle, and connective tissue plus body fluids other than blood (88, 98, 113). Most of the circulating cholesterol is derived from endogenous synthesis, which occurs principally in the liver and in limited amounts in the intestine; it also can be derived from dietary sources (88, 98, 99).

Cholesterol is the metabolic precursor of several substances that regulate a variety of physiological functions of critical importance to human and animal health such as bile acids, vitamin D and steroid hormones (20, 99, 113). In addition, it regulates cell fluidity when it is incorporated into cell membranes. The synthesis of cholesterol is controlled by a feedback mechanism by which dietary cholesterol inhibits the committing step of cholesterol synthesis (99).

Cholesterol is insoluble in water, and its transport in the blood is facilitated by being a part of lipoproteins. The lipoproteins of plasma which are classified by their hydrated densities are known as: (1) chylomicrons, which are composed primarily of triglycerides, and whose major functions is the transport of exogenous (dietary) lipids from intestine to the tissues; (2) very-low-density lipoproteins (VLDL), which contain

about 50% triglycerides and 22% cholesterol and which primarily transport endogenous lipids from the liver to tissues; (3) low-density lipoproteins (LDL), which contains most of the circulating cholesterol (46% of all LDL is cholesterol) and whose functions involve transport of cholesterol to nonhepatic tissue where receptors on these tissues bind the LDL-cholesterol complex and absorb cholesterol, and (4) high-density lipoproteins (HDL), whose major component is protein (50%) and which transport endogenous cholesterol from peripheral tissues to the liver (88, 98, 113). The last two lipoproteins (LDL, HDL) are the ones primarily involved in cholesterol transport. High levels of HDL and low levels of LDL are considered protective factors for coronary heart disease (43).

Despite the important role of cholesterol in normal human and animal physiology, several epidemiological, clinical and experimental studies have reported a positive correlation between serum cholesterol levels and cardiovascular diseases (56, 66, 102, 103). They indicated that persons having primary hypercholesterolemia have greater than average risk of having coronary heart disease than those with normal serum cholesterol levels (24). The National Heart and Lung Institute (104) reported that atherosclerosis is the major cause of most cardiovascular diseases. It accounts for about one half of all deaths in the U.S. every year (107). Atherosclerosis, the most common form of arteriosclerosis, is an irregular thickening of the inner wall of the arteries due to the accumulation of esterified cholesterol. This thickening is caused by the deposition of plaque which consists of smooth muscle cells, connective tissues, and considerable deposits of lipids of which cholesterol esters comprise the major part (39, 113).

In addition, elevated levels of LDL cholesterol is reported to contribute to the development of atherosclerosis (43). HDL cholesterol, on the other hand, protects against atherosclerosis. Reducing serum cholesterol levels, especially that associated with LDL, can decrease the incidence of coronary heart disease in hyper-

cholesterolemic persons (102, 103). Therefore, lowering serum cholesterol is very important for people having hypercholesterolemia.

There are several possible approaches to lower serum cholesterol levels. Grundy and Bilheimer (42) proposed the following: (1) inhibiting the absorption of cholesterol from the gut, (2) preventing reabsorption of bile acids in the normal enterohepatic circulation, and (3) inhibiting cholesterol synthesis. These can be achieved by diet therapy or through the administration of drugs. Dietary modifications appears to be the most favorable method for controlling cholesterol levels, although in some cases combining both methods may be necessary.

Many researchers have deep reservations about using drugs for treatment of hypercholesterolemia, because most drugs have side effects or the potential for side effects. The issue of the use of pharmacological agents is complicated by the fact that moderate reductions in cholesterol levels can be achieved in most patients with dietary changes alone. Although dietary modifications overall may not be as potent for lowering cholesterol , modifications of the diet can reduce the cost of medication and avoid the problem of side effects (43). However, in some situations like that of those patients with heterozygous familial hypercholesterolemia, pharmalogic lowering of cholesterol is justified.

Absorption of Cholesterol From the Small Intestine

Endogenous and exogenous cholesterol can be found within the lumen of the mammalian small intestine (79). Endogenous cholesterol is derived from bile and from the turnover of epithelial cells. Dietary cholesterol is primarily derived from animal sources with the relative amount depending on the nature of the diet. Unesterified biliary cholesterol output is 800-1200 mg daily compared to 400-500 mg of dietary cholesterol in adults (41). Biliary cholesterol is entirely unesterified, but a portion of

dietary cholesterol is esterified with fatty acids which is desterified by pancreatic cholesterol esterase (41).

The mechanism of cholesterol absorption is not completely understood, but much evidence indicates that bile acids are required, not only for micellar solubilization but also for mucosal uptake (51, 53, 88, 95). The concentration at which bile acids form micelles with lipolytic products is about 2 mM, and bile acids are present in concentrations three to five times higher throughout the proximal small intestine (88). As a consequence, most cholesterol is absorbed in the proximal part of the small intestine (25). Conjugated bile acids facilitate cholesterol absorption better than the deconjugated ones (33, 53). In fact, bile acids conjugated with taurine support better cholesterol absorption than those conjugated with glycine (33). However, glycine conjugated bile acids are predominant in man (22). During enterocyte transport, cholesterol is in part esterified with long chain fatty acids. Esterified cholesterol is transported in the hydrophobic center of the chylomicrom (88). It is generally accepted that dietary fats increase cholesterol absorption (94). This is accomplished probably by the fact that fats provide monoglycerides, fatty acids and phospholipids which are necessary for cholesterol solubilization in mixed micelles (117). In addition, fats provide fatty acids necessary for the mucosal esterification of cholesterol. Sylven and Borgstrom (94) reported that in rats the lymphatic transport of cholesterol increased proportionally with chain length of fatty acids within the range 6 to 18 carbon atoms. Kuksis et al. (63) suggested that the degree of saturation of fatty acids may also affect cholesterol absorption.

Relationship Between Dietary Cholesterol and Blood Cholesterol Concentrations

Dietary cholesterol has received particular attention because it is thought to increase blood cholesterol concentrations, which in turn may accelerate atherosclerosis.

Isotopic tracer studies indicate that cholesterol absorption rates vary from 20-80% in man (95). They postulated that since bile acids are required for cholesterol absorption, a relatively low pool size of bile acids could decrease absorption.

Grundy (41) suggested that the intestinal capacity for cholesterol absorption could be an important factor determining plasma cholesterol concentrations. He indicated that an increase of approximately 10% can be obtained by increasing dietary cholesterol in most people. In contrast, blocking cholesterol absorption by a variety of means can lower plasma cholesterol by 10-15% (40). Some reports indicated that plasma cholesterol is very sensitive to changes in dietary cholesterol at low cholesterol intakes but not at higher intakes (10, 46, 77). The mechanism whereby sensitivity changes with dietary intake is unclear and more studies are needed to determined the factors affecting plasma cholesterol values over a wide range of cholesterol intakes.

Eggen (26) and Lofland et al. (69) observed a widely variable response to dietary cholesterol in primates. They indicated that within the same species some animals are responders and others are nonresponders to dietary cholesterol. Human studies have provided conflicting results on the effect of dietary cholesterol on blood cholesterol levels. Mattson (77) studied the effects of increasing intakes of dietary cholesterol on plasma concentrations in young volunteer men. They found that all individuals responded with increases in plasma cholesterol and the degree of increase was remarkably uniform. In contrast, Dawber et al. (17) found no relationship between cholesterol intake and serum cholesterol concentrations. In fact, individuals who ingested 1.4 or 9 eggs per week had similar cholesterol concentrations. Another study involving subjects maintained in prison showed a direct relationship between dietary and serum cholesterol. The difference in results may reflect the stress of incarceration or a more constant diet other than amount of cholesterol.

In many animal species (pigs, Japanese quail, and primates), feeding a high cholesterol diet resulted in hypercholesterolemia (11, 35, 55, 69).

Enterohepatic Circulation of Bile Acids, Fecal Bile Acids Excretion, and Absorption of Bile Acids

Enterohepatic Circulation of Bile Acids

Bile acids are a group of water-soluble acidic steroids with powerful detergent properties. The main bile acids in human, cholic (40%), chenodeoxycholic (40%) and deoxycholic (10%) acids are formed in the liver from cholesterol, conjugated with the amino acids glycine and taurine, stored and concentrated in the gallbladder, and then released into the duodenum via the bile duct (85, 113). Within the intestinal lumen, bile acids interact with lipases (59) and assist the lipolysis and absorption of fats and fat-soluble vitamins, by formation of mixed micelles (50, 113). These bile acids are then reabsorbed from the small intestine and transported back to the liver via the portal vein for reuse. This process is known as enterohepatic circulation of bile acids. The small amount (less than 1 g/d) of bile acids that normally escape this recycling system are further metabolized by microorganism in the large intestine and excreted. This is the only route for cholesterol excretion from the body (113). Danielsson (13) reported that bile acids are found primarily in the conjugated form in bile, but when isolated from feces bile acids are found as free acids.

Although the majority of bile acids are extracted from portal blood by the liver, a fraction (10 to 60%) spills over into the peripheral blood (52). The serum level of bile acids thus represents the balance between intestinal absorption and hepatic uptake (108). Because plasma volume is relative constant, the serum bile acid level serves as a "flowmeter" to indicate the flux of bile acids presented to the liver (1, 52). Danielsson and Sjovall (14) indicated that the amount of bile acids returning to the liver regulates the conversion of cholesterol to bile acids (negative feedback). Therefore, the fecal loss of bile acids is counterbalanced by an enhanced synthesis of new bile acids from cholesterol.

Fecal Bile Acids Excretion

Many aerobic and anaerobic intestinal bacteria deconjugate bile acids (80) and may influence the enterohepatic circulation of bile acids. This action can result in high levels of bile acids being excreted in the feces (7). In fact, Eyssen et al. (27) and Kellogg and Wostmann (57) found the germ-free chicks and rats excrete 30 to 40% less bile acids through the feces than their conventional counterparts. Moreover, Eyssen et al. (27) indicated that the small intestine of germ-free rats contains at least twice the amount of bile acids as the small intestine of conventional rats. This could have important implications for the absorption of neutral sterols and fatty acids. Chickai et al. (9) reported that germ-free rats inoculated with *Bacteroids vulgatus, Bifidobacterium longum,* and *Clostridium romasun* excreted more bile acids than noninoculated germ-free animals or those inoculated with *E. coli,* which does not deconjugate bile acids. Conventional animals excreted a greater percentage of deconjugated bile acids than the germ free animals. The authors concluded that the presence of deconjugating microorganism increased fecal bile acid excretion and consequently resulted in decreased reabsorption of bile acids. Eyssen et al. (27) suggested that conventional animals can compensate for this increased loss of bile acids by a faster rate of oxidative catabolism of cholesterol into bile acids, which could result in a serum cholesterol lowering effect.

Absorption of Bile Acids

Bile acid absorption from the small intestine is part of the normal enterohepatic circulation. It is now well established that bile acid absorption is most efficient in the distal part of the small intestine (terminal ileum). This has been supported in many absorption studies (7, 64, 92) showing that active transport is confined to the distal ileum, whereas passive absorption occurs throughout the intestine. Active transport

prefered ionized molecules and was more efficient than passive diffusion which required nonionized molecules (7). Schiff et al. (92) reported that both conjugated and nonconjugated bile acids can be absorbed by active transport; however, free bile acids are not absorbed as well as conjugated bile acids.

The pKa of bile acids play an important role in their absorption. The unconjugated bile acids, which have a pKa of 5 are insoluble below a pH of about 7, depending on the bile acid (88). Conjugation with glycine or taurine lowers the pKa to 3.8 for glycine conjugates and to <1 for taurine conjugates (88). The effect of such conjugation is to decrease passive absorption in the biliary tract and small intestine as well as to lower the pH at which bile acids precipitate from solution (88). The pH along the small intestine varies from 4.5 to 6.0. Within this pH range, unconjugated bile acids will be unionized and passively absorbed while conjugated bile acids will be ionized and actively absorbed (19). If unconjugated bile acids are less efficiently absorbed from the ileum than conjugated bile acids, it is then possible that deconjugation could result in larger amounts of bile acids being excreted in the feces.

Role of Gut Microflora in Controlling Serum Cholesterol Levels

The intestinal tract is the most important route for eliminating cholesterol from the body (79). Serum cholesterol levels can be influenced by the intestinal microflora. Eyssen (27) reported that gnotobiotic rats, mice and chicks fed a high cholesterol diet accumulated twice as much cholesterol in the blood and liver as conventional animals fed and housed under similar conditions. In addition, less cholesterol and bile acids were excreted in the feces of germ-free animals than in the feces of their conventional counterparts. The author concluded that the intestinal microflora in some way may interfere with the efficiency of absorption of cholesterol from the small intestine.

Chickai et al. (9) monitored excretion of fecal bile acids for germ-free rats before and after inoculation with *Bacteriodes vulgatus, Bifidobacterium longum, Clostridium*

romasum or *Escherichia coli*. Fecal bile acid excretion increased after inoculation in all rats except those inoculated with *E. coli*, which does not deconjugate bile acids. A greater percentage of these excreted bile acids in the conventional animals were in the deconjugated form than in the germ-free animals. They speculated that free bile acids are excreted more rapidly than conjugated acids and that adhesion of these free bile acids to bacteria could be a cause for larger excretion of bile acids. This increased excretion of bile acids may be another mechanism whereby lowering serum cholesterol is achieved.

Experiments by Mott et al. (84) indicated that germ-free piglets mono-contaminated with *Lactobacillus acidophilus* exhibited reduced serum cholesterol levels after subsequent development of a normal flora. In addition, bile acid excretion was lower in these animals than in pigs not allowed to develop a normal flora. This experiment suggests that the intestinal flora can have a definitive effect on blood cholesterol and that *L. acidophilus* was not the only microorganism involved in lowering serum cholesterol levels. In fact, the researchers speculated that factors other than microbial metabolism of steroids in the gut are responsible for alterations in serum cholesterol and neutral steroid excretion. Recently, Norin et al. (86) conducted an experiment to evaluate the establishment of a strain of a species of either *Lactobacillus* or *Bifidobacterium* in germ-free mice, and to investigate the effect of the bacteria on the conversion of cholesterol to coprostanol, deconjugation of bilirubin glucoronides, transformation of bilirubin to urobilinogen, and degradation of mucin. Although both microorganisms were present in high numbers in the gastrointestinal tract, they were unable to mediate any alterations of the parameters investigated. The authors concluded that the benefit of such microbes when used as dietary supplements may be due to the interaction of these microorganism with other microbes and not to their direct influence on the metabolism of the host itself. However, the strains used in this study were not selected on the basis of their abilities to take up cholesterol or to

deconjugate bile acids. Gilliland (36) suggested that in choosing a culture selected to provide a beneficial influence on serum cholesterol level, the culture should actively assimilate cholesterol during growth under the conditions existing in the gastro-intestinal tract.

Anticholesterolemic Effects of Cultured or Culture Containing Dairy Products

A beneficial influence of milk fermented with a wild strain of *Lactobacillus* on serum cholesterol was shown by Mann and Spoerry (74) in a study involving 24 Maasai tribesman. This study was conducted to test the hypothesis that a surfactant in the diet would cause hypercholesterolemia. Volunteers were assigned to two treatment groups. One group received fermented milk (4 to 5 l/d per man) plus a surfactant (Tween 20) which was thought to enhance lipid absorption and the other group received the same amount of milk plus a placebo (olive oil) for 6 consecutive days. On the seventh day a steer was slaughtered and consumed. This regime was followed for 21 days. As milk consumption increased weight gain increased but serum cholesterol levels decreased for both groups. Serum cholesterol levels were lower in men who gained the most weight. These findings were contrary to expectations since the surfactant was added to enhance cholesterol absorption. The researchers concluded that some component in the fermented milk impaired cholesterol synthesis in the body.

Mann (75) confirmed his previous study by showing that consumption of skim and whole milk yogurt resulted in significant reductions in serum cholesterol levels in human volunteers when compared to fresh whole milk over a twelve-day trial. Blood cholesterol levels slowly returned to normal after consumption of yogurt ceased. Mann (75) postulated that this effect was due to the factor produced or enhanced by the action of the starter culture bacteria during the fermentation process in the manufac-

tured yogurt. Likewise, Hepner et al. (47) reported that dietary supplementation with both pasteurized and nonpasteurized yogurt significantly reduced serum cholesterol levels in human volunteers during a twelve week feeding trial. They suggested that the hypocholesterolemic effect of yogurt was probably due to its significant calcium content, which supplied 864 mg/day.

Thakur and Jha (101) fed rabbits a high cholesterol diet supplemented with skim milk, yogurt or calcium for 16 weeks. All groups exhibited reduced serum cholesterol levels; however, yogurt and calcium produced greater effects than milk. This study suggests the calcium is hypocholesterolemic but other hypocholesterolemic factors may also be present. A similar study also reported that dietary supplementation of a high fat diet with skim milk or yogurt significantly reduced serum cholesterol values of growing pigs (97).

Rao et al. (89) evaluated the effect of milk, thermophilus milk (milk fermented with *Streptococus thermophilus)* and methanol solubles of the milks on hepatic cholesterogenesis and plasma cholesterol levels in rats. Rats fed the thermophilus milk had significantly lower serum cholesterol levels than rats fed diets supplemented with skim milk or water. In addition, diets supplemented with methanol solubles from the thermophilus milk significantly lowered plasma cholesterol levels when compared to methanol solubles from nonfermented milk. The authors concluded that the fermentation by S. *thermophilus* was at least partially responsible for the hypocholesterolemic effect of the milk.

Thompson et al. (105), on the other hand, reported that acidophilus yogurt, skim milk, and 2% fat milk had no effect on serum cholesterol levels. Human volunteers were given 1 L daily of these products. No significant reductions in cholesterol was observed in any of the groups. However, changes in cholesterol may not have been observed since individuals used in the study were not hypercholesterolemic.

While several positive results have been published regarding cholesterol-lowering attributes of fermented dairy products, the case remains unclear. Additional research is needed in order to clarify the possible hypocholesterolemic effect of yogurt. To date, most studies in which a beneficial effect of yogurt has been reported required the consumption of very large amounts of yogurt, which in some way make its consumption impractical in helping control serum cholesterol levels. However, as stated by Gilliland (37), "if a factor produced during the fermentation of yogurt is indeed responsible, there may be means for concentrating the active factor into reasonably usable volumes for practical use."

Hypocholesterolemic Actions of *Lactobacillus acidophilus*

Lactobacilli belong to the normal oropharyngeal and intestinal microflora in man. These microorganisms contribute to the stabilization of the microflora and maintain the colonization resistance against pathogens. Lactobacilli have been used as dietary supplements in order to prevent gastrointestinal disturbances. Claims have been made that certain strains of lactobacilli exert hypocholesterolemic actions (11, 35, 44, 45). In these studies supplementation of diets with *L. acidophilus* resulted in decreased serum cholesterol in humans and animal model studies. Mott et al. (84) found that germ-free pigs monocontaminated with *L. acidophilus* and allowed to develop normal flora exhibited reduced serum cholesterol levels, suggesting that the intestinal microflora have a beneficial effect in blood cholesterol. Likewise, Tortuero et al. (106) found that the implantation of *L. acidophilus* in normal and cacectomized laying hens also resulted in a significant reduction in serum cholesterol.

Grunewald et al. (44) used rats to study the effect of feeding skim milk fermented with *L. acidophilus* on blood cholesterol. After four weeks, rats consuming the fermented milk and the methanol solubles from this fermented milk had significantly lower serum cholesterol levels than did rats fed water or control milk. The authors

theorized that some factors were produced by *L. acidophilus* during fermentation that reduced serum cholesterol levels.

Infants receiving formula supplemented with *L. acidophilus* had significantly lower serum cholesterol levels and higher numbers of lactobacilli in their stool than those receiving a sterile milk formula (45). The decreases were associated with increased numbers of lactobacilli in their stools.

None of the studies previously cited considered the direct action of *L. acidophilus* on cholesterol in the gut. Gilliland et al. (35), however, reported that *L. acidophilus* could act directly on cholesterol. They demonstrated that *L. acidophilus*, when grown under anaerobic conditions in the presence of bile could remove cholesterol from laboratory media. In addition, they evaluated the ability of *L. acidophilus* to assimilate cholesterol *in vivo* by obtaining swine isolates of *L. acidophilus* that were both bile tolerant and had the ability to take up cholesterol. Results from screening tests indicated a large variation among strains in their ability to assimilate cholesterol. Growing pigs fed a high cholesterol (1500 mg/d) diet supplemented with the strain of *L. acidophilus* RP32 which assimilated the greatest amount of cholesterol in laboratory media had significantly lower levels of serum cholesterol than did pigs receiving the same diet either supplemented with a strain of *L. acidophilus* P47 that assimilated very little cholesterol or without lactobacilli. The authors concluded that the ingestion of a selected strain of *L. acidophilus* significantly reduced the extent to which a diet high in cholesterol can increase serum cholesterol in swine.

Danielsson et al. (11) confirmed the cholesterol-lowering effect of *L. acidophilus*. Mature boars were fed a high cholesterol diet for 56 days and then divided into two groups. One group continued receiving the high cholesterol diet plus acidophilus yogurt whereas the other received the high cholesterol diet only. After 56 additional days the animals receiving the acidophilus yogurt had significantly lower serum cholesterol levels than those in the control group. The researchers concluded that *L.*

acidophilus could be used to reduce serum cholesterol levels but strains should be appropriately selected.

Lin et al. (68), on the other hand, reported no effect of *L. acidophilus* on serum cholesterol levels. However, the strains of *L. acidophilus* used were not selected for the ability to assimilate cholesterol or for bile tolerance. Gilliland et al. (35) and Gilliland and Walker (38) found large variation among strains of *L. acidophilus* in their ability to take up cholesterol. Therefore, they suggested that only cultures that actively assimilate cholesterol during growth under conditions existing in the gut should be selected for use as dietary adjuncts to reduce serum cholesterol.

Recently, Klaver and Van der Meer (61) postulated that the assumed assimilation of cholesterol by *L. acidophilus* is not due to bacterial uptake of cholesterol, but it results from bacterial bile salt deconjugation activity. They reported that in the laboratory medium, cholesterol was precipitated with deconjugated bile salts. However, Walker and Gilliland (114) reported no correlation between the ability of strains of *L. acidophilus* to take up cholesterol and to deconjugate bile acids. These data suggested that the isolates most active in taking up cholesterol during growth were not necessarily the most active in deconjugating bile acids.

Deconjugation of Bile Acids by Intestinal Microflora

Biotransformation of bile acids by intestinal bacteria includes deconjugation, disulfation, dihydroxylation, oxidation-reduction, and epimerization (58, 71, 80). Of these, deconjugation activity is the most commonly observed. The majority of bacteria capable of deconjugating bile acids are anaerobic (67). Gilliland and Speck (34) reported that *L. acidophilus* can deconjugate bile acids.

Kobashi et al. (62) reported that *L. brevis* and *L. plantarum* would deconjugate glycocholate, *L. fermentum* would deconjugate both glycocholic and taurocholic acid, *L. xylosus* would deconjugate neither bile acids. However, only one strain of each species

was included in the study. Hill and Drasar (48) reported that twelve strains of *L. acidophilus* studied were incapable of deconjugating taurocholic acid. However, in others studies, *L. acidophilus* has been shown to be capable of deconjugating both glycocholic and taurocholic acid (15, 34, 76). In mice gastric lactobacilli are responsible for approximately 86% of total deconjugation of bile acids occurring in the ileum and 74% in the cecum (70).

Walker and Gilliland (114) found a considerable amount of variation among strains of *L. acidophilus* in the ability to deconjugate conjugated bile acids. The deconjugation activity required that the organism be under anaerobic conditions. Since conjugated bile acids are required for cholesterol absorption from the small intestine, the deconjugation of these bile acids by *L. acidophilus* may decrease the absorption of cholesterol and thus exert some benefit in helping control serum cholesterol levels (27).

Bile salt hydrolase is the enzyme responsible for the deconjugation of bile acids (76). It is produced by lactobacilli and other bacteria in the presence and absence of conjugated bile salts, but it is stimulated only by the conjugated acids.

Tannock et al. (100) used conventional (normal microflora), *Lactobacillus* free (lacked *Lactobacillus* and enterococci) and reconstituted *Lactobacillus* free (similar microflora to conventional but lacked *Lactobacillus*) to study the effect of lactobacilli on bile salt hydrolase activity in the intestinal tract. The bile salt hydrolase activity in the mice devoided of lactobacilli was 86% lower than the conventional mice; however, feeding of bile salt hydrolase positive strains of lactobacilli resulted in significantly increased levels of the enzyme. These scientists suggested that lactobacilli play the major role in the bile salt hydrolase activity in the intestinal tract.

Some studies have reported that there are bile salt hydrolase enzymes that can only deconjugate taurine-conjugated bile salts; however, there are others bile salt hydrolases that can deconjugated both taurine and glycine conjugates (62, 70).

Increased deconjugation of bile acids could result in greater excretion of bile acids from the intestinal tract since free bile acids are excreted more rapidly than the conjugated acids (9). This can be important because increased excretion of bile acids will create the need for the body to synthesize replacement bile acids. This would tend to reduce serum cholesterol levels because cholesterol is a precursor for bile acids.

Hypocholesterolemic Effects of Calcium

Earlier epidemiological studies by Schoder (93) and Biorck et al. (5) showed a negative correlation between cardiovascular diseases and the mineral content of drinking water, especially calcium. It has also been reported in both human and animal model studies that high calcium intakes have serum cholesterol lowering effects.

Fleischman et al. (29) fed rats a corn-soybean diet supplemented with beef tallow and 2% cholesterol. Calcium was added as calcium carbonate at 0.08, 0.2, and 1.2% levels. They observed that increasing calcium in the diet caused a decrease in cholesterol, phospholipid, and triglyceride levels in serum and concluded that this effect was mediated in part by increased excretion of bile acids, 3-hydroxy-sterols and fatty acids and not by a redistribution of lipids between the blood and tissue pools as indicated by Spritz et al. (96). Yacowitz et al. (118) fed rats corn oil or cocoa butter along with 0.08, 0.2, and 1.2% levels of calcium. They observed that calcium was hypocholesterolemic and hypotriglyceridemic with either fat, but the effects were more pronounced in the presence of saturated fat. In another study Fleischman et al. (31) found that feeding rats a diet supplemented with 2% calcium carbonate, calcium carbonate plus vitamin D_2 and oyster shell calcium plus vitamin D_2 for 21 days reduced serum cholesterol by 35, 27 and 22%, respectively, as compared with the control. Similarly, Hines et al. (49) reported a reduction in plasma cholesterol in goats supplemented with calcium.

Johnson et al. (55) used Japanese quails to study the effect of two levels of dietary calcium (0.8 and 1.6%) and two sources of proteins (casein and soybean) on plasma cholesterol levels in an eight-week feeding trial. Cholesterol was added at a level of 0.5% to all diets. Plasma cholesterol concentrations decreased as dietary calcium increased from 0.8 to 1.6% with either casein or soybean protein. However, 1.6% calcium was more hypocholesterolemic when casein was the protein source. Plasma HDL cholesterol levels did not differ significantly between treatment groups. Van der Meer et al. (110) reported that calcium supplementation inhibited the casein-induced hypercholesterolemia in rabbits, but it did not have any effect on the serum cholesterol concentration of rabbits fed the soy diet. The researchers speculated that the effect of calcium on lipid metabolism is dependent on the type of dietary protein. Foley et al. (32), on the other hand, reported that varying the dietary calcium level (1-3 times the recommended daily calcium intake) did not significantly affect the concentration of plasma cholesterol in young growing pigs fed a diet supplemented with 1.6% egg yolk (0.046% cholesterol on dry matter basis).

Human studies have also provided conflicting results on the cholesterol lowering effects of calcium. Consumption of two grams of calcium carbonate per day over a period of one year caused a significant decrease (25%) in serum cholesterol levels in hyperlipidemic men and women (4). The researchers suggested that calcium carbonate should be considered as a potential agent for usage in long-term studies designed to produce hypolipemia.

Bhattacharrya et al. (3) fed young men diets containing either saturated or polyunsaturated fat, each with two different levels of calcium. At the start they were given 40 Ci cholesterol ^{14}C intravenously. The saturated fat-low calcium diet increase serum cholesterol values as compared with the polyunsaturated fat-low calcium diet. Addition of two grams of calcium daily to the low calcium-saturated diet caused a

decrease of 8 mg/dl in serum cholesterol level. No change in serum cholesterol occurred in the polyunsaturated fat diet with calcium supplementation.

Recently, Denke et al. (18) studied the effect of two dietary calcium levels (410 mg/dl versus 2200 mg/dl) on fecal fatty acids excretion and serum lipids in healthy men with moderate hypercholesterolemia. The high calcium diet significantly reduced total cholesterol, LDL and apolipoprotein B concentration when compared with the low calcium diet. There was no change in HDL cholesterol or apolipoprotein A concentration. The authors concluded that calcium fortification was effective in lowering total and LDL cholesterol and may be an effective adjunct to cholesterol-lowering diet therapy. Contrary to these studies Mitchell et al. (82) found no change in serum cholesterol in six patients (five were osteoporotic) given either organic salts (calcium glycerophosphate or calcium glucono-galactogluconate) or skimmed milk.

Although most of the reports cited above have demonstrated a hypocholesterolemic effect of calcium, the exact mechanism is still not clear. However, one hypothesis that has gained some attention is that dietary calcium increases fecal excretion of bile acids and neutral steroids which is thought to interfere with absorption of bile acids (30, 91, 110, 118) and cholesterol (21, 115).

Effect of Calcium on Bile Acid and Cholesterol Excretion

Since calcium can bind bile acids (83, 116), dietary calcium may produce its hypocholesterolemic effect by binding bile acids and suppressing their reabsorption into the enterohepatic circulation. Consumption of cholestyramine, a bile acid sequestrant, resulted in a five to six fold increase in bile acid excretion, with a 17% reduction in serum cholesterol (78).

Packard and Shepher (87) indicated that in several animal species, serum cholesterol can be controlled by the enterohepatic circulation of bile acids. Van der Meer (109) hypothesized that bile acids and/or biliary micelles bind to insoluble

calcium phosphate in the lumen of the small intestine, which results in an increased excretion of bile acids and analogously because of decrease uptake, in an increased excretion of fat. Moreover, in an in vitro study Van der Meer and De Vries (111) demonstrated that glycine-conjugated dihydroxy bile acids, but not their taurine-conjugated counterparts, bind insoluble calcium phosphate. In preliminary experiments, they also found that the binding of unconjugated deoxycholate to insoluble calcium phosphate was analogous to that of glycodeoxycholate. This could have important implications for the absorption of cholesterol.

Fleischman et al. (29) fed a high fat, high cholesterol diet supplemented with 0.08, 0.2 or 1.2% of calcium to mature rats. Excretion of fecal bile acids increased three fold at the 0.2% calcium level, with no additional increase at the 1.2% calcium level. Similarly the major decrease in serum and tissue cholesterol occurred at the 0.2% calcium level. These results suggest that the lowering of blood cholesterol by increase in dietary calcium is mediated in part by increased excretion of bile acids. In a second study, Fleischman et al. (30) confirmed their previous results that calcium supplementation increased excretion of bile acids in rats concomitant with a decrease in serum cholesterol.

Yacowitz et al. (118) fed rats cocoa butter or corn oil with 0.08, 0.2, and 1.2% dietary calcium for three weeks. Fecal total lipids and bile acids increased significantly with both fats at the 1.2% calcium levels. This same calcium level resulted in a significantly lower serum cholesterol level. Saunders et al. (91) found that the supplementation with six grams of calcium carbonate per day caused a twofold increase in bile acids and fatty acid excretion in human volunteers. Similarly, Van der Meer et al. (112) reported that supplementation of dietary calcium increased excretion of fecal bile acids in healthy men. The authors speculated that this was due to the binding of calcium to intraluminal bile acids. In contrast to this study, some reports

have found no significant effect of dietary calcium on bile acid excretion (3, 6, 18), even though a cholesterol lowering effect was observed (3, 18).

A second mechanism that has been proposed by which calcium may exert its hypocholesterolemic effect is that it may interfere with cholesterol absorption from the gut. It is well documented that dietary calcium decreases digestibility of dietary fats, especially saturated fats (16, 23, 54) by removing free fatty acids from solution through the precipitation of insoluble fatty acids-calcium soaps. A reduction in saturated fat absorption could result in a reduction in blood cholesterol concentrations.

Wells and Cooper (115), in a rat study, reported that calcium failed to inhibit cholesterol absorption when fat was omitted from the diet. The authors suggested that calcium acts by forming insoluble complexes with fatty acids which decrease cholesterol absorption. Recently, Denke et al. (18) reported that high calcium diets produced a two-fold increase in fecal saturated fatty acids in men, but the observed serum cholesterol reduction could not be explained by the increased fat excretion alone. According to Fetcher et al. (28) a 2 to 3 grams reduction in saturated fat intake should result in a 0.05 to 0.06 mmol/l reduction in total serum cholesterol concentration which is lower than the 0.34 mmol/l observed in their study. Therefore, some other mechanism(s) must also be involved. The increase excretion of bile acids with calcium supplementation is a possible mechanism; however, in this study no calcium effect on bile acid excretion was observed.

The Pig as a Model for Cardiovascular Research

The pig is one of the most suitable models for cardiovascular research. Pigs are similar to man, not only in being omnivorous but also in having similar digestive and circulatory systems (8, 90). Hypercholesterolemia (2, 8, 35) and aortic atherosclerosis (72) can be induced by feeding diets high in cholesterol and fat. Their lipoprotein receptors on liver membranes are similar to those observed in man (60, 65, 73, 81).

REFERENCES

1. Ahlberg, J., B. Angelin, and I. Bjorkhem. 1977. Individual bile acids in portal venous and systemic blood serum of fasting man. Gastroenterology. 73:1377.

2. Beames, C. G. Jr., C. V. Maxwell, S. A. Norton, and G. A. Bond. 1986. Whey and cholesterol in swine. pp 1041-1046. In M. E. Tumbleson (Ed.). Swine in Biomedical Research. Vol. 2. Plenum Publishing Co. New York, N. Y.

3. Bhattacharyya, A. K., C. Thera, J. T. Anderson, F. Grande, and A. Keys. 1969. Dietary calcium and fat: effect on serum lipids and fecal excretion of cholesterol and its degradation products in man. Am. J. Clin. Nutr. 22:1161.

4. Bierenbaum, M. L., A. I. Fleischman, and R. I. Raichelson. 1972. Long-term human studies on the lipid effects of oral calcium. Lipids. 7:202.

5. Biorck, G., H. Bostrom, and A. Widstrom. 1965. On the relationship between water hardness and death rate in cardiovascular diseases. Acta Med. Scand. 178:239.

6. Borum, M. L., K. L. Shehan, H. Fromm, S. Jahangeer, M. K. Floor, and O. Alabaster. 1992. Fecal bile acid excretion and composition in response to changes in dietary wheat bran, fat and calcium in the rat. Lipids. 27:999.

7. Brandt, L. J., and L. H. Bernstein. 1976. Bile salts: their role in cholesterol synthesis, secretion and lithogenesis. Am. J. Gastroenterol. 65:1513.

8. Cevallos, W. H., and W. L. Holmes. 1979. Swine in atherosclerosis research. Atherosclerosis. 34:303.

9. Chickai, T., H. Nakao, and K. Uchida. 1987. Deconjugation of bile acids by human intestinal bacteria implanted in germ-free rats. Lipids. 22:669.

10. Connor, W. E., R. E. Hodges, and R. E. Bleiler. 1961. The serum lipids in men receiving high cholesterol and cholesterol-free diets. J. Clin. Invest. 40:894.

11. Danielson, A. D., E. R. Peo, K. M. Shahani, A. J. Lewis, P. J. Whalen, and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966.

12. Danielsson, H. 1963. Present status of research on catabolism and excretion of cholesterol. Advan. Lipid Res. 1:335.

13. Danielsson, H., P. Eneroth, K. Hellstrom, S. Lindstedt, and J. Sjovall. 1963. On the turnover and excretory products of cholic and chenodeoxycholic acid in man. J. Biol. Chem. 238:2299.

14. Danielsson, H., and J. Sjovall. 1975. Bile acids metabolism. Ann. Rev. Biochem. 44:233.

15. Dashkevicz, M. P., and S. D. Feighner. 1989. Development of a differential medium for bile salt hydrolase-active *Lactobacillus spp.* Appl. Environ. Microbiol. 55:11.

16. Davison, K. L., and W. Woods. 1962. Effect of calcium and magnesium upon digestibility of a ration containig corn oil by lambs. J. Anim. Sci. 22:27.

17. Dawber, T. R., R. J. Nickerson, F. N. Brand, and J. Pool. 1982. Eggs, serum cholesterol, and coronary heart disease. Am. J. Clin. Nutr. 36:617.

18. Denke, M. A., M. M. Fox, and M. C. Schulte. 1993. Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. J. Nutr. 123:1047.

19. Dietschy, J. M., H. S. Salomon, and M. D. Siperstein. 1966. Bile acid metabolism. I. Studies on the mechanism of intestinal transport. J. Clin. Invest. 45:832.

20. Dietschy, J. M., and J. D. Wilson. 1970. Regulation of cholesterol metabolism. N. Engl. J. Med. 282:1128.

21. Dougherty, R. M., and J. M. Iocono. 1979. Effects of dietary calcium on blood and tissue lipids, tissue phospholipids, calcium and magnesium levels in rabbits fed diets containing beef tallow. J. Nutr. 109:1934.

22. Dowling, R. H. 1972. Progress in gastroenterology: the enterohepatic circulation. Gastroenterology. 62:122.

23. Drenick, E. J. 1961. The influence of ingestion of calcium and other soap-forming substances on fecal fat. Gastroenterology. 41:242.

24. Dujovne, C.A., P. Krehbiel, S. Decoursey, B. Jackson, S. B. Chernoff, A. Pitterman, and M. Garty. 1984. Probucol with colestipol in the treatment of hypercholesterolemia. Ann. Intern. Med. 100:477.

25. Dulery, C., and D. Reisser. 1982. Intestinal absorption of biliary and exogenous cholesterol in the rat. Biochem. Biophys. Acta. 710:164.

26. Eggen, D. A. 1974. Cholesterol metabolism in rhesus monkey, squirrel monkey, and baboon. J. Lipid Res. 15:139.

27. Eyssen, H. 1973. Role of the gut microflora in metabolism of lipids and sterols. Proc. Nutr. Soc. 32:59.

28. Fetcher, E. S., N. Foster, J. T. Anderson, F. Grande, and A. Key. 1967. Quantitative estimation of diets to control serum cholesterol. Am. J. Clin. Nutr. 20:475.

29. Fleischman, A. I., H. Yacowitz, T. Hayton, and M. L. Bierenbaum. 1966. Effects of dietary calcium upon lipid metabolism in mature male rats fed beef tallow. *J. Nutr.* 88:255.

30. Fleischman, A. I., H. Yacowitz, T. Hayton, and M. L. Bierenbaum. 1967. Long-term studies on the hypolipemic effect of dietary calcium in mature male rats fed cocoa butter. J. Nutr. 91:151.

31. Fleischman, A. I., M. L. Bierenbaum, and P. H. Lenz. 1972. The hypolipidemic effect of calcium containing compounds and vitamin D_2 on the rat. Lipid. 7:263.

32. Foley, M. K., S. T. Galloway, C. M. Luhman, T. D. Faidlay, and D. C. Beitz. 1990. Influence of dietary calcium and cholecalciferol on composition of plasma lipids in young pigs. J. Nutr. 120:45.

33. Gallo-Torres, H. E., O. N. Miller, and J. G. Hamilton. 1969. A comparison of the effects of bile salts on the absorption of cholesterol from the intestine of the rat. Biochim. Biophys. Acta. 176:605.

34. Gilliland, S. E., and M. L. Speck. 1977. Deconjugation of bile acids by intestinal lactobacilli. App. Environ. Microbiol. 33:15.

35. Gilliland, S. E., C. R. Nelson, and C. V. Maxwell. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377.

36. Gilliland, S. E. 1988. Probiotics: Fact or fancy?. 8th International Biotechnology Symposium, Paris. Proceeding. Vol II:923.

37. Gilliland, S. E.. 1990. Health and nutritional benefits from lactic acid bacteria. FEMS Microbiology Reviews 87:175.

38. Gilliland, S. E., and D. K. Walker. 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in human. J. Dairy Sci. 73:905.

39. Goldsmith, D. P. J., and H. P. Jacobi. 1978. Atherogenesis in swine fed several types of lipids-cholesterol diets. Lipids. 13:174.

40. Grundy, S. M., E. H. Ahrens Jr., and J. Davignon. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. J. Lipid Res. 10:304.

41. Grundy, S. M. 1983. Absorption and metabolism of dietary cholesterol. Ann. Rev. Nutr. 3:71

42. Grundy, S. M., and D. W. Bilheimer. 1984. Low density lipoprotein receptors and enterohepatic circulation. In Paumgartner, G., A. Steihl, and W. Gerok (Eds.), Enterohepatic Circulation of Bile Acids and Sterol Metabolism. MIE Press Ltd., Boston, MA.

43. Grundy, S. M. 1986. Cholesterol and coronary heart disease. J. Am. Med. Assoc. 256:2849.

44. Grunewald, K. K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078.

45. Harrison, V. C., and G. Peat. 1975. Serum cholesterol and bowel flora in the newborn. Am. J. Clin. Nutr. 28:1351.

46. Hegsted, D. M., R. B. McGandy, M. L. Myers, and F. J. Stare. 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17:281.

47. Hepner, G., R. Fried, S. St. Jeor, L. Fusetti, and R. Morin. 1979. Hypocholesterolemic effect of yogurt and milk. Am. J. Clin. Nutr. 32:19.

48. Hill, M. J., and B. S. Drasar. 1968. Degradation of bile salts by human intestinal bacteria. *Gut*. 9:22.

49. Hines, T. G., N. L. Jacobson, D. C. Beitz, and E. T. Littledike. 1985. Dietary calcium and vitamin D: risk factors in the development of atherosclerosis in young goats. J. Nutr. 115:167.

50. Hofmann, A. F. 1976. Fat digestion: the interaction of lipid digestion products with micellar bile acids solutions. In K. Rammel and H. Goebell (Eds), Lipid Absorption: Biochemical and Clinical Aspects (pp. 3-18). MTA Press, Lancaster, UK.

51. Hofmann, A. F. 1977. The enterohepatic circulation of bile acids in man. Clin. Gastroenterol. 6:3.

52. Hofmann, A. F. 1988. Bile acids. In I. M. Arias, W. B. Jakoby, H. Popper, D. Schachter, and D. A. Shafritz (Eds). The Liver: Biology and Pathobiology. 2nd. Ed. Raven Press, Ltd., New York, NY.

53. Holt, P. R. 1972. The roles of bile acids during the process of normal fat and cholesterol absorption. Arch. Intern. Med. 130:574.

54. Iocono, J. M., and C. Ammerman. 1966. The effect of calcium in maintaining normal levels of serum cholesterol and phospholipids in rabbits during accute starvation. J. Nutr. 18:197.

55. Johnson, L. L., C. G. Beames Jr., and C. V. Maxwell. 1986. Effect of calcium and protein source on the plasma cholesterol of Japanese quail. Proc. Okla. Acad. Sci. 66:31.

56. Kannel, W. B., J. T. Doyle, A. M. Ostfeld, C. D. Jenkins, L. Kuller, R. N. Podell, and J. Stamler. 1984. Optimal resources for primary prevention of atherosclerotic diseases. Circ. 70:155A.

57. Kellogg, T. F., and B. S. Wostmann. 1969. Fecal neutral steroids and bile acids from germ free rats . J. Lipid Res. 10:495.

58. Kellogg, T. F. 1973. Bile acids. In P. P. Nair and D. Kritchevsk (Eds.). The Bile Acids Chemistry, Physiology and Metabolism. Vol. 2, Plenum Press, New York, NY.

59. Kimura, H., Y. Futami, S. Tauri, and T. Shinomiya. 1982. Activation of human pancreatic lipase activity by calcium and bile salts. J. Biochem. 92:243.

60. Knipping, G. M. J., G. M. Kostner, and A. Holasek. 1975. Studies on the composition of pig serum lipoproteins, isolation and characterization of different apoproteins. Biochem. Biophys. Acta. 393:88.

61. Klaver, F. A. M., and R. Van der Meer. 1993. The assumed assimilation of cholesterol by Lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59:1120.

62. Kobashi, K., I. Nishizawa, and T. Yamada. 1978. A new hydrolase specific for taurine-conjugates of bile acids. J. Biochem. 84:495.

63. Kuksis, A., J. J. Myher, K. Geher, G. J. L. Jones, J. Shepherd, C. J. Packard, J. D. Morrisett, O. D. Taunton, and A. M. Gotto. 1982. Effect of saturated and unsaturated fat diets on lipid profiles of plasma lipoproteins. Atherosclerosis. 41:221.

64. Lack, L., and I. M. Weiner. 1963. Intestinal absorption of bile salts and some biological implications. Fed. Proc. 22:1334.

65. Lee, K. T., D. N. Kim, and V. A. Thomas. 1986. Atherosclerosis in swine. In swine in cardiovascular research (H. C. Stanton and H. J. Mersman, Eds). CRC press, Inc, Baton Rouge, Fl.

66. Levy, R. I. 1981. Review: declining mortality in coronary heart disease. Arteriosclerosis. 1:312.

67. Lewis, R., and S. Gorbach. 1972. Modification of bile acids by intestinal bacteria. Arch. Intern. Med. 130:545.

68. Lin, S. Y., J. W. Ayres, W. Winkler,Jr., and W. E. Sandine. 1989. *Lactobacillus* effects on cholesterol: In vitro and In vivo results. J. Dairy Sci. 72:2885.

69. Lofland, H. B. Jr., T. B. Clarkson, R. W. Clair, and N. D. M. Lehner. 1972. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. J. Lipid Res. 13:39.

70. Lundeen, S. G., and D. C. Savage. 1990. Characterization and purification of bile salt hydrolase from *Lactobacillus sp.* strain 100-100. J. Bacter. 172: 4171.

71. MacDonald, I. A., V. D. Bokkenheuser, J. Winter, A. M. Mclernon, and E. H. Musbach. 1983. Degradation of steroids in human gut. J. Lipid Res. 24:675.

72. Mahley, R. W., K. H. Weisgraber, T. Innerarity, H. B. Brewer Jr., and G. Assmann. 1975. Swine lipoproteins and atherosclerosis: changes in lipoproteins and apoproteins induced by cholesterol feeding. Biochemistry. 14:2817.

73. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. J. Clin. Invest. 68:1197.

74. Mann, G. V., and A. Spoerry. 1974. Studies of surfactant and cholesteremia in the Maasai. Am. J. Clin. Nutr. 27:464.

75. Mann, C. V. 1977. A factor in yogurt which lowers cholesteremia in man. Atherosclerosis. 26:335.

76. Masuda, N. 1981. Deconjugation of bile salts by bacteroides and clostridium. Microbiol. Immunol. 25:1.

77. Mattson, F. H., B. A. Erickson, and A. M. Kligman. 1972. Effect of dietary cholesterol on serum cholesterol in man. J. Clin. Nutr. 25:589.

78. Moutafis, D. D., L. A. Simons, N. B. Myant, P. W. Adams, and V. Wynn. 1977. The effect of cholestyramine on the fecal excretion of bile acids and neutral steroids in familial hypercholesterolemia. Atherosclerosis. 26:329.

79. McIntyre, N., and K. J. Isselbacher. 1973. Role of the small intestine in cholesterol metabolism. *Amer. J. Clin. Nutr.* 26:647.

80. Midvedt, T., and A. Norman. 1967. Bile acid transformation by microbial strains belonging to the genera found in intestinal contents. Acta Pathol. Microbiol. Scand. 71:1341.

81. Miller, E. R., and D. E. Ullrey. 1987. The pig as a model for human nutrition. Ann. Rev. Nutr. 7:361.

82. Mitchell, W. D., T. Fyfe, and D. Smith. 1968. The effect of oral calcium on cholesterol metabolism. J. Athero. Res. 8:913.

83. Moore, E. W., L. Celic, and J. D. Ostrow. 1982. Interactions between ionized calcium and sodium taurocholate: bile salts are important buffers for prevention of calcium-containing gallstones. Gastroenterology. 83:1079.

84. Mott, G. E., R. W. Moore, H. E. Redmond, and R. Reiser. 1973. Lowering of serum cholesterol by intestinal bacteria in cholesterol-fed piglets. Lipids. 8:428.

85. Nair, P. P. 1973. Enzymes in bile acids metabolism. In P. P. Nair and D. Kritchevsky (Eds.), The Bile Acids Chemistry, Physiology and Metabolism. Vol. 2. Plenum Press, New York, NY.

86. Norin, K. E., A. Persson, H. Saxerholt, and T. Midtvedt. 1991. Establishment of *Lactobacillus* and *Bifidobacterium* species in germ free mice and their influence on some microflora-associated characteristics. Appl. Environ. Microbiol. 57:1850.

87. Packard, C. J., and J. Sheperd. 1982. The hepatobiliary axis and lipoprotein metabolism: effects of bile acid sequestrants and ileal bypass surgery. J. Lipid Res. 23:1081.

88. Pandol, S. J. 1991. Gastrointestinal system. In J. B. West (Ed). *Best and Taylor's Physiological Basis of Medical Practice.* 12th Ed. Williams & Wilkins, Baltimore, MD.

89. Rao, D. R., C. B. Chawan, and S. R. Pulusani. 1981. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. J. Food Sci. 46:1339.

90. Ratcliffe, H. L., and H. Luginbuhl. 1971. The domestic pig: a model for experimental atherosclerosis. Atherosclerosis. 13:133.

91. Saunders, D., J. Sillery, and R. Chapman. 1988. Effect of calcium carbonate and aluminium hydroxide on human intestinal function. Dig. Dis. Sci. 33: 409.

92. Schiff, E. R., N. C. Small, and J. M. Dietschy. 1972. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. J. Clin. Invest. 51:1351.

93. Schroder, H. A. 1960. Relation between mortality from cardiovascular disease and treated water supplies: variations in status in 163 largest municipalities of the United States. J. Am. Med. Assoc. 172:1902.

94. Sylven, C., and B. Borgstrom. 1969. Intestinal absorption and lymphatic transport of cholesterol in the rat: influence of fatty acid chain length of the carrier triglyceride. J. Lipid Res. 10:351.

95. Siperstein, M. D., I. L. Chaikoff, and W. O. Reinhardt. 1952. ^{14}C- cholesterol: obligatory function of bile in intestinal absorption of cholesterol. J. Biol. Chem. 198:111.

96. Spritz, N., E. H. Ahrens Jr., and S. Grundy. 1965. Sterol balance in man as plasma cholesterol concentrations are altered by exchanges of dietary fats. J. Clin. Inv. 44:1482.

97. Stahelin, H. B., G. Ritzel, M. Wanner, M. Jost, and H. Schneeberger. 1981. Hypocholesterolemic effect of milk constituents in growing swine. Int. J. Vit. Nut. Res. 51:198.

98. Steinberg, D. 1991. Metabolism. In J. B. West (Ed). Best and Taylor's Physiological Basis of Medical Practice. 12th Ed. Williams & Wilkins, Baltimore, MD.

99. Stryer, L. 1988. Biochemistry. (3rd. ed.) W. H. Freeman and Company. New York, N.Y.

100. Tannock, G. W., M. P. Dashkevicz, and S. D. Feighner. 1989. Lactobacilli and bile salt hydrolase in the murine intestinal tract. Appl. Environ. Microbiol. 55:1848.

101. Thakur, C. P., and A. N. Jha. 1981. Influence of milk, yoghurt and calcium on cholesterol and induced atherosclerosis in rabbits. Atherosclerosis. 39:211.

102. The Lipid Research Clinics Program. 1984. The lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. J. Am. Med. Assoc. 251 (3):351.

103. The Lipid Research Clinics Program. 1984. The lipid Research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart heart disease to cholesterol lowering. J. Am. Med. Assoc. 251 (3):365.

104. The National Heart and Lung institute Task Force on Atherosclerosis. 1971. Vol. 2. DHEW Publication No. (NIH) 72:219.

105. Thompson, L. U., D. Jenkins, M. A. VicAmer, R. Reichert, A. Jenkins, and J. Kanaulsky. 1982. The effect of fermented and unfermented milks on serum cholesterol. Am. J. Clin. Nutr. 36:1106.

106. Tortuero, F., A. Brenes, and J. Rioperez. 1975. The influence of intestinal (ceca) flora on serum and egg yolk cholesterol levels in laying hens. Poultry Sci. 54:1935.

107. United States Bureau of the Census. 1986. Statistical abstract of the United States: 1987. 107th Ed. Washington, D.C.

108. Van Berge-Henegouwen, G. P., and A. F. Hofmann. 1983. Systemic spill-over of bile acids. Eur. J. Clin. Invest. 13:433.

109. Van der Meer, R. 1983. Is the hypercholesterolemic effect of dietary casein related to its phosphorylation state?. Atherosclerosis. 49:339.

110. Van der Meer, R., H. De Vries, C. E. West, and H. De Waard. 1985. Casein-induced hypercholesterolemia in rabbits is calcium-dependent. Atherosclerosis. 56:139.

111. Van der Meer, R., and H. T. De Vries. 1985. Differential binding of glycine- and taurine-conjugated bile acids to insoluble calcium phosphate. Biochem. J. 229:265.

112. Van der Meer, R., J. W. M. Welberg, F. Kuipers, J. H. Kleibeuker, N. H. Mulder, D. S. M. L. Termant, R. J. Vonk, H. T. De Vries, and E. G. E. De Vries. 1990. Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate, and bile acids. Gastroenterology. 99:1653.

113. Voet D., and J. G. Voet. 1990. Biochemistry. John Wiley & Sons, Inc. New York, NY.

114. Walker, D. K., and S. E. Gilliland. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956.

115. Wells, W. W., and S. B. Cooper. 1958. Coprostanol formation II. The opposing action of dietary lactose and calcium on cholesterol absorption. Arch. Biochem. Biophys. 75:273.

116. Williamson, B. W. A., and I. W. Percy-Robb. 1979. The interaction of calcium ions with glycocholate micelles in aqueous solution. Biochem. J. 181:61.

117. Wilson, J. D., 1972. The role of bile acids in the overall regulation of steroid metabolism. Arch. Intern. Med. 130:493.

118. Yacowitz, H., A. I. Fleischman, R. T. Amsden, and M. L. Bierenbaum. 1967. Effects of dietary calcium upon lipid metabolism in rats fed saturated or unsaturated fat. J. Nutr. 92:389.

CHAPTER III

HYPOCHOLESTEROLEMIC ACTION OF *LACTOBACILLUS ACIDOPHILUS* AND CALCIUM: INTERFERENCE WITH ENTEROHEPATIC CIRCULATION OF BILE ACIDS

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ABSTRACT

The objective of the present study was to determine the effects of *Lactobacillus acidophilus* and dietary calcium on total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and total bile acids in swine. Thirty-three Yorkshire barrows (4 replicates; 92 kg) were surgically fitted with indwelling jugular catheters and ileal cannulas. Following recovery, the diet for all pigs was supplemented with .5% crystalline cholesterol for 14 d to cause an increase in serum cholesterol. Mean serum cholesterol concentrations increased 209.2 mg/dl (2.4 fold increase) during the cholesterol feeding period. On d 15 crystalline cholesterol was removed from the diet and pigs within each replicate were assigned to one of four treatments in a 2 x 2 factorial arrangement of treatments including 2 levels of calcium (.7% and 1.4%) with and without *L. acidophilus* ATCC 43121. These diets were fed at 12-h intervals for 15 consecutive days as in the previous period. Blood samples were collected daily from the jugular catheter just prior to the morning feeding and sera was analyzed for total cholesterol, HDL cholesterol, LDL cholesterol, and total bile acids. Main effect means are presented since no significant *L. acidophilus* x calcium interaction was observed for any of the variables tested. When averaged over days pigs fed *L. acidophilus* had an 11.8% ($P < .01$) lower total cholesterol concentration than pigs fed a diet without *L. acidophilus* (167.9 ± 5.2 vs 148.0 ± 5.3). Similarly, pigs fed 1.4% calcium had an 11.3% lower ($P < .01$) total cholesterol concentration when compared to those fed .7% calcium (167.5 ± 5.2 vs 148.5 ± 5.3). A significant day on treatment x calcium interaction was observed for total cholesterol. Pigs fed 1.4% calcium had a lower ($P < .01$) total cholesterol concentration during d 16 to 18 than

pigs fed .7% calcium. From d 19 to 23 the difference continued but the magnitude of the differences was reduced ($P < .05$ for d 19, 20, 22 and 23; $P < .1$ for d 21). Although there was no significant day on treatment x *L. acidophilus* interaction ($P > .6$), pigs fed a diet supplemented with *L. acidophilus* had lower total serum cholesterol each day on treatment than pigs fed a diet without *L. acidophilus*. An *L. acidophilus* x calcium interaction ($P < .05$) was observed in the rate of decline of total cholesterol. *L. acidophilus* had a significant effect ($P < .07$) on the rate of decline of total cholesterol at 1.4% calcium but not at .7% calcium. Neither *L. acidophilus* nor calcium had any effect on HDL cholesterol concentration. However, LDL cholesterol levels tended to decrease when pigs were fed *L. acidophilus* ($P < .09$) or 1.4% calcium ($P < .1$). In addition, serum bile acid level was reduced by 23.9% ($P < .06$) by feeding *L. acidophilus* and by 21.4% by feeding 1.4% calcium ($P < .08$) when compared to their controls. Moreover, total bile acid concentration was positively correlated ($P < .0001$) with total cholesterol concentration in pigs fed *L. acidophilus* or 1.4% calcium ($r = .55$ and $r = .48$, respectively). These data indicate that both *L. acidophilus* and calcium have hypocholesterolemic actions and suggest that these actions are probably mediated through alteration in the enterohepatic circulation of bile acids.

INTRODUCTION

The latest U.S. census (42) describes the number-one cause of death in America as heart disease. A large body of epidemiological and nutritional studies have indicated that high levels of total serum cholesterol and low density lipoprotein (LDL) cholesterol correlate highly with the incidence of coronary heart disease (23, 26, 28, 29). Thus, considerable research has been conducted to determine factors which are effective in lowering serum cholesterol levels including dietary modifications and pharmacological agents.

Several reports have indicated that consumption of certain cultured or culture containing dairy products supplemented with *Lactobacillus acidophilus* resulted in a reduction of serum cholesterol (7, 17, 20, 21, 41). However, not all strains of *L. acidophilus* were hypocholesterolemic (17, 27). Gilliland et al. (17) observed that some strains of *L. acidophilus* took up cholesterol during anaerobic growth in laboratory media and others did not. Pigs fed a diet high in cholesterol and supplemented with a strain of *L. acidophilus* that actively assimilated cholesterol had lower serum cholesterol levels than pigs fed a strain of *L. acidophilus* that did not assimilate cholesterol in laboratory media or pigs fed the same diet without lactobacilli (17). Another factor that may allow *L. acidophilus* to lower serum cholesterol is bile acid deconjugation activity.

Some species of lactobacilli present in the intestinal tract can deconjugate both taurocholic and glycocholic acids in anaerobic conditions (16). This deconjugation activity becomes important when we considered that deconjugated bile acids do not function as well as conjugated bile acids in the absorption of cholesterol (11). Increased deconjugation of bile acids could also result in greater excretion of bile acids

from the intestinal tract because free bile acids are less likely to be reabsorbed in the intestines than are conjugated bile acids (6). Increased excretion of bile acids stimulates the synthesis of replacement bile acids from cholesterol, thus providing the potential to reduce cholesterol levels in the body. Danielson and Sjovall (8) indicated that the synthesis of bile acids is homeostically regulated by the amount of bile acids returning to the liver.

The consumption of diets high in calcium also has hypocholesterolemic activity in humans (3, 4, 9), and in several animal species, including rats (12, 14), rabbits (44), Japanese quail (22), but not in swine (15). Additionally, it has been reported that excess dietary calcium either increased (12, 13, 35, 46) or had no effect (3, 5, 9) on fecal bile acid excretion. Van der Meer et al. (46) postulated that the excretion of fecal bile acids was increased by the binding of these acids to calcium phosphate, thus making them insoluble and suppressing their reabsorption into the enterohepatic circulation.

Because *L. acidophilus* can take up cholesterol and deconjugate bile acids and dietary calcium binds and increases excretion of bile acids, the interaction of these factors may offer a better alternative to decrease serum cholesterol levels in individuals suffering from primary hypercholesterolemia. The serum bile acid concentration is determined by "spillover" of bile acids reaching the liver from the intestine (43). Therefore, the intestinal absorption of bile acids is the major determinant of the serum bile acid level. For these reasons serum bile acid concentrations could be an effective means of evaluating the deconjugation and/or binding of bile acids by *L. acidophilus* and dietary calcium in the intestine. Serum bile acid measurements, for instance, have been used to determine the efficacy of bile-acid sequestrants such as cholestyramine (1) and colestipol (33) in the treatment of hypercholesterolemia.

The primary objective of the present study was to investigate the effect of *L. acidophilus* and dietary calcium on total cholesterol, high density lipoprotein (HDL)

cholesterol, LDL cholesterol, and total bile acids in serum of pigs previously fed a high cholesterol diet and to determine if any interaction exists between the effects of calcium and *L. acidophilus*. A second objective was to determine the effects of dietary cholesterol on the induction of hypercholesterolemia in pigs. The pig was used as a model because its omnivorous eating habits, cardiovascular physiology, and metabolism of cholesterol and serum lipoproteins resemble those of humans (24, 32).

MATERIALS AND METHODS

Animals and Diets

Each of 33 Yorkshire barrows (three replicates of 8 pigs and one replicate of 9 pigs) weighing approximately 92 kg was surgically fitted with an indwelling jugular catheter (Tygon plastic tubing, 0.050 ID × 0.090 OD; Fisher Scientific, Dallas, TX) in which the lumen was coated with tridodecylemethyl ammonium chloride-heparin complex (TDMAC Heparin complex 7% w/w; Polyscience Inc., Warrington, PA) to retard blood clotting. In addition, each pig was fitted surgically with a simple T-type cannula (25) in the distal ileum close to the ileocecal junction. In each replicate, surgery was performed over three consecutive days and then each pig was moved to individual metabolism crates in an environmentally controlled room. Pigs received intravenously 1 g of polycillin-N (sterile ampicillin sodium; Bristol Laboratories, Evansville, IN) and 1 cc of Naxcel (Ceftiofur sodium; The Upjohn Company, Kalamazoo, MI) per 45 kg of body weight at 12-h intervals for five consecutive days. During the 5-d convalescence period, pigs were fed a pelleted standard 13.5% crude protein diet containing no antibiotics (Table I).

During the first 3 d of the recovery period, ad libitum access to feed was provided. Starting on day 4, the ration was fed at 1.4% of body weight at 12-h intervals to facilitate training pigs to a meal feeding regime. Feed not consumed within

approximately 3 h following feeding was removed. After the recovery period, each pig was fed a corn-based diet supplemented with 20% nonfat dried milk and 0.5% crystalline cholesterol (5-cholesten-3-ol; Sigma chemical Co., St. Louis, MO) for an additional 14 d period at the same amount and frequency as in the last 2 d of the recovery period (Table II). In addition, each pig was fed 250 ml of sterile 10% reconstituted nonfat dried milk (NDM) just prior to feeding the dried ration. Feed not consumed was removed, weighed and the amount consumed was recorded. Pigs were weighed at the end of the 14-d period and feed intake adjusted accordingly.

Starting on day 15 of the experimental period, pigs within each replicate were assigned randomly in a 2 x 2 factorial arrangement to one of four treatments including two levels of calcium (.7% and 1.4%) with and without *L. acidophilus* ATCC 43121. The low calcium diet met the recommended daily requirement of calcium for finishing pigs (31). Calcium carbonate and deflourinate phosphate were added as the calcium and phosphorus sources. Pigs in Treatments 1 and 2 were fed the same diet fed during the first 14 d of the experimental period with the exception that crystalline cholesterol was deleted (Table 2). Treatment 1 was fed 250 ml of sterile milk at 12-h interval and Treatment 2 was fed the same amount of milk supplemented with 2.5×10^{11} cells of *L. acidophilus* ATCC 43121. Treatments 3 and 4 were fed the same basal diet used in Treatments 1 and 2 but with 1.4% calcium (Table II). Treatment 3 was fed 250 ml of sterile milk whereas treatment 4 received the same amount of milk supplemented with 2.5×10^{11} cells of *L. acidophilus* ATCC 43121. These diets were fed for 15 consecutive days and the feeding frequency and amount of feed continued the same as in the previous period. Ad libitum access to water was provided throughout the experiment.

The milk supplemented with *L. acidophilus* ATCC 43121 was prepared by thawing the required number of vials (2 g) of frozen (-196 °C) concentrated culture in water (50 ml per vial) at room temperature for approximately five minutes just prior to use. The appropriate amount (2.5×10^{11} cells) of culture was added to the milk just

prior to feeding. Numbers were confirmed by methods reported by Gilliland and Rich (19). The frozen concentrated culture was prepared from cells of *L. acidophilus* ATCC 43121 grown at pH 5 according to procedures of Gilliland and Rich (19). The culture was not stored greater than 30 days at -196 °C prior to use. No loss in numbers of viable cells was observed.

L. acidophilus ATCC 43121 was selected for use in this study because of its ability to assimilate cholesterol and to deconjugate bile acids (47). Additionally it originated from the intestinal contents of a pig and its original strain designation was RP32 (17).

Blood Collection and Analysis

Blood samples were taken daily from the indwelling jugular catheters just prior to the morning feeding. The sampling period started on the last 2 d of the convalescence period and continued for 29 more days after initiation of the trial. Catheters were flushed with approximately 3 ml of sterile saline solution (0.9% sodium chloride) containing 10 units/ml of heparin (1000 units/ml; Elkins-Sinn, Inc., Cherry Hill, NJ) after collection and at 6-h intervals. Immediately following collection, the tubes containing the blood samples were placed in an ice water bath and held at 4 °C for at least 3 h. The samples were then centrifuged for 20 min at 3000 × g and the serum was transfered into a screw cap vial and stored at -20 °C until analyses were done.

Serum samples were thawed for analysis by placing the vials in a water bath at 27 °C for approximately 5 min. Duplicate serum samples were analyzed for total and HDL cholesterol by using the Sigma enzymatic reagent kit (37). Concentrations of LDL cholesterol were calculated by difference between total and HDL cholesterol. Additionally, serum samples from alternate days (replicates 2 to 4) were analyzed for total bile acids using the Sigma Diagnostic Bile Acids Reagents (38).

Ileal Sample Collection and Analysis

Samples of ileal contents were taken daily (from d 13 to 29) via the ileal cannula starting 5 h following the morning feeding. Ileal samples were collected into plastic bags suspended from the ileum until 100 ml of digest was collected. Bags were changed at a maximun of 1 h intervals. After removal from the pig, samples were immediately frozen and stored at -20 °C. Unfortunately, analyses of ileal samples were not completed because the methods available were not sensitive enough to detect the low concentrations of conjugated bile acids occurring in ileal samples. We tried several different HPLC methods including one that was previously used to measure conjugated bile acids in fecal samples; however, too many extraction procedures were required which made it impractical for the analysis of the large number of samples obtained in this study.

Statistical Analysis

Separate analyses were conducted to evaluate data obtained during the cholesterol feeding period (d 1 to 14) and during the experimental period (d 15 to 29). Nonlinear equations were fitted to total, HDL, and LDL cholesterol concentrations to predict changes in these variables in response to cholesterol feeding using PROC NLIN of SAS (34) and solved using Marquardt's method. The model included the following prediction equation:

$$Y = d + a\,[1 - \exp(-b\,(day - 1))]$$

where Y is the dependent variable, d is the pretreatment value of Y, a represents the increase in average of Y over the length of the test, and b measures the rate of increase of Y. Total bile acid concentrations of d 1 and d 15 were compared by using the ESTIMATE statement of the GLM procedure of SAS (34).

Data obtained during the experimental period including serum total cholesterol, HDL cholesterol, LDL cholesterol, total bile acids, and feed intake were evaluated by least squares analysis of variance for repeated measures in a split plot design using the General Linear Models (GLM) procedure of SAS (34). Pigs were defined as the experimental unit with treatment as the main plot and sampling day (day on treatment) as a repeated measure (subplot). Treatment sums of squares were partitioned into the appropriate factorial arrangement of treatments. The effects of *L. acidophilus*, calcium and their interaction were tested using pig within replicate x *L. acidophilus* x calcium mean square as the error term. The effects of day on treatment, day on treatment x *L. acidophilus*, day on treatment x calcium and the three-way interaction were tested by the residual error. Specific differences between treatment means on individual days were determined using the PDIFF procedure of SAS (34) when significant interactions with day were observed. Because initial (d 15) sera values of total and LDL cholesterol slightly differed (although not statistically significant) among treatment groups, the d 15 values were used as a covariant.

The delay of decline in total cholesterol for each treatment was evaluated using the GLM procedure of SAS (34) after treatment means were adjusted to a common value on d 15. The following comparisons among means were evaluated using the ESTIMATE statement of the GLM procedure of SAS (34): d 15 versus d 16 and the average of d 15 and 16 versus d 17. In addition, an exponential function for total cholesterol concentration on day on treatment was fitted for the experimental period. The model fitted to data for each animal using PROC NLIN of SAS (34) and solved using the multivariate-secant (DUD) method was as follows:

$$Y = d + a\,[\exp(-b\,(\text{day} - 15))]$$

where Y is average total cholesterol, d is the asymptote, a represents the increase in cholesterol obtained during the cholesterol feeding period, and b measures the rate of decline of total cholesterol. After estimates of parameters d and b for each animal

were obtained, they were analyzed by least squares procedures [PROC GLM, SAS (34)] using a model that included the effects of replicate, *L. acidophilus*, calcium, and their interaction. In the presence of a significant *L. acidophilus* x calcium interaction, effects of *L. acidophilus* were tested at each level of calcium. These analyses determined the effects of *L. acidophilus* and calcium on exponential characteristics of cholesterol response. The relationship between total cholesterol and total bile acid concentrations within treatment was evaluated by simple correlation (Pearson correlation coefficients) analysis (34). Pig weight was analyzed by using the GLM procedure of SAS (34). The model included the effects of replicate, calcium, *L. acidophilus*, and calcium x *L. acidophilus*.

RESULTS

Body Weights, Feed Intake, and Cholesterol Intake

Although during the cholesterol feeding period all pigs were fed the same diet, data were analyzed by treatment group to determine if there were significant differences in pig weight, feed intake or cholesterol intake among treatment groups previous to the application of treatments. All pigs appeared to remain healthy over the length of the experiment. There were no significant differences in pig weight , feed intake or cholesterol intake among treatment groups during the cholesterol feeding period or in feed intake or pig weight during the experimental period (Table III).

Cholesterol Feeding Period

During the 14 d period when a diet high in cholesterol was fed, serum cholesterol level increased in all animals. Cholesterol concentration averaged 85.4 mg/dl (actual mean) on d 1 (just prior to the initiation of the cholesterol feeding period) and increased to 294.6 mg/dl on d 15, a rise of 209.2 mg/dl for the entire period. Some variation among pigs was observed in the magnitude of increase of cholesterol. Figure

1 presents a plot of the regression of total cholesterol concentration on day of cholesterol feeding and the observed means. The estimated regression equation fit the data very well. The error sum of squares for this fitted model was = 150.3 with 12 degrees of freedom (mean squares of error = 12.5). After beginning the high cholesterol diet, serum cholesterol levels increased rapidly at first and then more slowly with a tendency to reach a plateau by d 15. The upper and lower 95% confidence limits for the expected means are shown in Figure 2.

Figure 3 shows the daily changes in HDL cholesterol concentration in response to cholesterol feeding and the estimated regression equation. Concentrations of HDL cholesterol averaged 41.3 mg/dl on d 1 and 63.9 mg/dl on d 15, an increase of 54.7% for the entire 14-d cholesterol feeding period. The increase in LDL cholesterol concentrations was similar to the increase observed in total cholesterol levels. It averaged 42.7 mg/dl on d 1 and increased to 231.1 mg/dl on d 15, a rise of 188.4 mg/dl (Figure 4). The estimated regression equation fit the data well and the error sum of squares for the fitted model was 408.6 (mean squares of error = 34.0).

Total bile concentrations increased during the cholesterol feeding period. They averaged 8.6 μmol/l on d 1 and 10.7 μmol/l on d 15, an increase of 24% ($P < .09$) for the entire cholesterol feeding period.

Experimental Period

The effects of *L. acidophilus* and excess dietary calcium on serum total cholesterol, HDL cholesterol, LDL cholesterol and total bile acids during the overall 15-d experimental period (d 15 to 29) are presented in Table IV. Main effect means are presented since no significant *L. acidophilus* x calcium interaction was observed. When averaged over days pigs fed *L. acidophilus* had an 11.8% lower ($P < .01$) total cholesterol concentration than those fed a diet without *L. acidophilus* (167.9 ± 5.2 vs 148.0 ± 5.3). Similarly, pigs fed 1.4% calcium had an 11.3% lower ($P < .01$) total

cholesterol concentration than those fed .7% calcium (167.5 ± 5.2 vs 148.5 ± 5.3). No differences were observed between the two levels of calcium (P > .55) and the two levels of *L. acidophilus* (P > .7) for serum HDL cholesterol concentration. However, serum LDL cholesterol tended to decrease when pigs were fed *L. acidophilus* (P < .09) or 1.4% calcium (P < .1). The concentration of LDL cholesterol was reduced by 16.4% in pigs fed milk supplemented with *L. acidophilus* when compared to pigs fed milk without *L. acidophilus*, and by 14.6% in pigs fed the diet containing 1.4% calcium when compared to those fed the diet containing .7% calcium. These responses should be expected because LDL cholesterol concentration was calculated from total cholesterol and HDL cholesterol values. Pigs fed the diet with *L. acidophilus* also tended to have lower (P < .06) total serum bile acids than those fed the diet without *L. acidophilus*, and pigs fed the diet containing 1.4% calcium tended to have lower (P < .08) bile acid levels than those fed the diet containing .7% calcium. Serum bile acids concentration was reduced 23.9% by feeding *L. acidophilus* and 21.4% by feeding 1.4% calcium compared to the respective controls.

Daily concentration of serum total cholesterol in response to dietary calcium level and *L. acidophilus* are shown in Figure 5. Day on treatment had an effect (P < .0001) on total cholesterol for both control and treated pigs. Regardless of dietary calcium or *L. acidophilus* level, total cholesterol concentration decreased after cholesterol was removed from the diet; however, the decrease was greater for pigs fed the high level of calcium or for those fed *L. acidophilus*. There was a tendency for total cholesterol concentration to reach a plateau by d 20 in all treatment groups. No significant (P > .36) three-way interaction effect (day on treatment x *L. acidophilus* x calcium) was detected for total cholesterol concentrations; however, a day on treatment x calcium interaction (P < .06) was observed. Cholesterol level was lower in pigs fed 1.4% calcium than in pigs fed .7% calcium throughout the 15-d experimental period (Figure 5, Panel A), but as expected the greatest magnitude of difference occurred during the

first three days after the initiation of the experimental diets (d 16, 17, and 18; serum cholesterol reduction of 32.7, 47.1 and 42.3 mg/dl, respectively; $P < .01$). From d 19 through 23 (d 4 through 8 after initiation of experimental diets) the difference due to calcium level continued but the magnitudes of the differences were reduced (25.7 mg/dl for d 19, 23.5 mg/dl for d 20, 21.5 mg/dl for d 21, 23.4 mg/dl for d 23; $P < .05$; and 14.8 mg/dl for d 21; $P < .1$). There was no significant day on treatment x *L. acidophilus* interaction; pigs fed the diet supplemented with *L. acidophilus* had a lower total cholesterol concentration each day after d 15 while on test than pigs fed a diet without *L. acidophilus* (Figure 5, Panel B).

Day on treatment affected ($P < .0001$) LDL cholesterol concentration (Figure 6). Regardless of dietary treatment, LDL level decreased after d 15 but the decrease was greater for the animals receiving *L. acidophilus* or 1.4% calcium. However, these differences did not result in either a two-way (day on treatment x calcium or day on treatment x *L. acidophilus*; $P > .6$) or three-way (day on treatment x calcium x *L. acidophilus*; $P > .7$) interaction (Figure 6). By d 20 (5 d after initiation of experimental protocol) LDL cholesterol level was reduced by 62.4% in pigs fed .7% calcium and by 69.8% in pigs fed 1.4% calcium when compared to cholesterol level on d 15 (Figure 6, Panel A). Similarly, by d 20, LDL cholesterol level was reduced by 62.3% in pigs fed the diet without *L. acidophilus* and by 70.3% in pigs fed the diet supplemented with *L. acidophilus* when compared to the cholesterol level on d 15 (Figure 6, Panel B). A tendency for LDL concentration to reach a plateau by d 20 in both levels of calcium or *L. acidophilus* was observed (Figure 6). Concentration of HDL cholesterol was not affected by dietary calcium level or *L. acidophilus* during the experimental period (Figure 7). However, a gradual but significant decrease in HDL cholesterol level was observed in all groups over the 15 d experimental period (day on treatment effect $P < .01$). By the end of the experimental period, HDL cholesterol concentration was reduced by 19% in pigs fed .7% calcium and by 25.8% in pigs fed 1.4% calcium. In a

similar manner HDL cholesterol concentration was reduced by 18.4% in pigs fed the diet without *L. acidophilus* and by 26% in pigs fed the diet supplemented with *L. acidophilus*.

Daily concentrations of total serum bile acids in response to dietary calcium level and *L. acidophilus* are shown in Figure 8. Total bile acid concentrations were decreased in all treatment groups during the 15-d experimental period (day on treatment effect $P < .0001$). However, the decrease was greater in pigs fed 1.4% calcium than in pigs fed .7% calcium (Figure 8, panel A). Similarly, the reduction in total bile acid concentration was greater in pigs fed *L. acidophilus* than in pigs fed the diet without *L. acidophilus* (Figure 8, panel B). These differences in reduction resulted in a tendency for a day on treatment x calcium interaction ($P < .2$) and a day on treatment x *L. acidophilus* interaction ($P < .2$). In fact, during the overall 15-d experimental period pigs fed 1.4% calcium had 21.4% lower ($P < .08$) bile acid concentrations than pigs fed .7% calcium, and pigs fed *L. acidophilus* had 23.9% lower ($P < .06$) bile acid concentrations than those fed the diet without *L. acidophilus*.

Results of delay and rate of decline of total cholesterol analyses are shown in Table V. The level of serum cholesterol in pigs fed the diet supplemented with .7% calcium without *L. acidophilus* (Treatment 1) did not decline within the first day on treatment (d 15 vs d 16, $P > .9$). However, total cholesterol concentration in pigs fed .7% calcium with *L. acidophilus* (Treatment 2), 1.4% calcium without *L. acidophilus* (Treatment 3) or 1.4% calcium with *L. acidophilus* (Treatment 4) declined within the first day on treatment (d 15 vs d 16, $P < .01$). Comparisons of cholesterol concentration of the average of d 15 and d 16 (the first two days on experimental diets) versus d 17 showed that cholesterol level on d 17 was lower than the average cholesterol concentration for d 15 and 16 in all treatment groups ($P < .01$). Because of these results, the rate of decline of total cholesterol was estimated from d 16 to 29 on pigs fed .7% calcium without *L. acidophilus* and from d 15 to 29 in the other treatment

groups. An *L. acidophilus* x calcium interaction ($P < .05$) was observed for the rate of decline of total cholesterol (parameter b). *L. acidophilus* had an effect on the rate of decline of cholesterol at the high calcium level ($P < .07$) but not at the low calcium level ($P > .2$). The rate of decline of total cholesterol was greater ($P < .07$) in pigs fed the high calcium diet with *L. acidophilus* than in those fed the high calcium diet without *L. acidophilus* (.59 and .41, respectively; Table V). There was also an *L. acidophilus* x calcium interaction ($P < .01$) for parameter d (asymptote). The asymptote was lower ($P < .01$) in pigs fed the low calcium diet with *L. acidophilus* than in pigs fed the low calcium diet without *L. acidophilus* (91.9 mg/dl vs. 140.0 mg/dl). A different response was observed at the high calcium level. Pigs fed the high calcium diet with *L. acidophilus* have a higher asymptote ($P < .01$) than those fed the high calcium diet without *L. acidophilus* (123.9 mg/dl vs. 85.8 mg/dl).

Correlation analysis indicated that total cholesterol concentrations during d 15 to 29 were positively correlated with total bile acid concentration in *L. acidophilus* treated pigs ($r = .55$; $n = 89$; $P < .0001$) but not in control pigs ($r = .08$; $n = 94$; $P > .4$). In both .7% and 1.4% calcium groups, concentration of total cholesterol correlated positively with total bile acid concentration ($r = .20$; $n = 96$; $P < .05$ and $r = .48$; $n = 87$; $P < .0001$, respectively).

DISCUSSION AND CONCLUSIONS

Hypercholesterolemia was induced, as expected, by feeding a diet supplemented with .5% crystalline cholesterol and 10% butter. This ability to increase serum cholesterol by increasing dietary cholesterol in pigs has been reported in other studies (2, 17, 39). However, previous studies have provided only limited information concerning the daily increase in serum cholesterol in pigs fed diets containing high levels of cholesterol. The present study provides information which can be used for

future studies to predict the concentration of cholesterol after a certain number of days of feeding a diet containing .5% crystalline cholesterol and 10% butter.

High levels of serum total cholesterol and LDL cholesterol are strongly associated with an increased risk of coronary heart disease (23, 26, 28, 29). Reduction in total cholesterol and LDL cholesterol reduces the incidence of cardiovascular disease. Thus, ways to reduce serum cholesterol levels are being sought. This study shows that dietary supplementation of *L. acidophilus* reduced serum total cholesterol and LDL cholesterol in pigs previously fed a high cholesterol diet more than in pigs not receiving *L. acidophilus*. These findings agree with those reported in humans (21), swine (7, 17) and rats (20). Lin et al. (27), in contrast, found no effect of *L. acidophilus* on serum cholesterol levels. One possible explanation for these differences in results is that the strains of *L. acidophilus* used in their study (ATCC 4962) were not selected for the ability to take up cholesterol during growth nor for the ability to deconjugate bile acids. In fact, Gilliland and Walker (18) reported that *L. acidophilus* ATCC 4962 was only moderately active in taking up cholesterol and in bile resistance. Moreover, this strain was not very competitive against other lactobacilli. No data have been reported on its ability to deconjugate bile acids. These factors may have limited its ability to survive and grow in the intestinal tract and exert a beneficial effect in influencing serum cholesterol levels. We used *L. acidophilus* ATCC 43121 which is very active in taking up cholesterol and in deconjugating bile acids. Gilliland and Walker (18) suggested that only strains that actively assimilate (take up) cholesterol during growth should be selected for use as a dietary adjunct to reduce serum cholesterol.

Concentrations of HDL cholesterol were not affected by *L. acidophilus*. This result agrees with results reported by Danielson et al. (7) which indicated that *L. acidophilus* had no effect on HDL cholesterol but did have a beneficial effect in reducing both total cholesterol and LDL cholesterol levels.

Another mechanism by which *L. acidophilus* may influence serum cholesterol levels is by its ability to deconjugate bile acids. Deconjugated bile acids are less well absorbed from the small intestine than are the conjugated ones (36). Thus, deconjugation of bile acids in the small intestine could result in greater excretion of bile acids from the intestinal tract. This is especially true since free bile acids are excreted more rapidly than the conjugated ones (6). An increased excretion of bile acids should result in lower serum bile acids, which in turn would decrease the amount of bile acids reaching the liver for secretion back into the intestine in the enterohepatic circulation. To replace the excreted bile acids more would have to be synthesized from cholesterol in the liver for secretion into the intestine in the enterohepatic circulation. In the present study pigs fed *L. acidophilus* had lower ($P < .06$) serum bile acid concentrations than the control pigs, which suggests that greater deconjugation occurred in the small intestine of those fed the lactobacilli. The greater decline in serum cholesterol coupled with lower serum bile acids suggested that the deconjugating action of *L. acidophilus* is associated with its hypocholesterolemic effect.

Increasing the level of calcium from .7% to 1.4% in the diet also caused a reduction in serum cholesterol. These results are in agreement with research conducted with other species: humans (3, 4, 9), rabbits (44), Japanese quail (22), and rats (12, 13, 14). Foley and colleagues (15), on the other hand, reported no effect of calcium on plasma cholesterol of young growing pigs. This difference in findings may have been due to the differences in the level of cholesterol added to the diet. Foley et al. (15) fed pigs a diet supplemented with 1.6% egg yolk (.046% cholesterol on dry matter basis) which may have been too low to cause a hypercholesterolemic condition; thus no significant differences in cholesterol reductions were observed.

Concentrations of HDL cholesterol were not affected by dietary calcium level. These results agree with those reported in a human study by Denke et al. (9) which

indicated no effect of calcium level on HDL cholesterol, but an effect on total and LDL cholesterol.

Although most studies have reported a cholesterol lowering effect of calcium , the exact mechanism is still not clear. Excess dietary calcium may act by binding bile acids and suppressing their reabsorption into the enterohepatic circulation. In fact, we found that increasing the level of calcium from .7% to 1.4% in the diet caused a 21.4% ($P < .08$) reduction in total serum bile acids. Whether this effect on serum bile acid concentration is associated with the effect of calcium on fecal excretion of bile acids reported in other studies (12, 13, 35, 46) remains to be determined. Van der Meer et al. (45) reported that unconjugated and glycine conjugated bile acids bind to insoluble calcium phosphate better than taurine conjugated bile acids. Some researchers have also suggested that calcium may exert its cholesterol lowering effect by forming insoluble complexes with fatty acids or phopholipids, inhibiting cholesterol absorption (10, 30). Recently, Denke et al. (9) reported that high calcium diets produced a two-fold increase in fecal saturated fatty acids in man, but the observed serum cholesterol reduction could not be explained by the increased fat excretion alone.

The experimental design used in this study was selected to determine both the magnitude of reduction of serum cholesterol as well as the time of onset of treatment effects in reducing serum cholesterol in pigs with serum cholesterol elevated by feeding high dietary cholesterol. In the present study, serum cholesterol concentrations in pigs fed the diet supplemented with .7% calcium without *L. acidophilus* started to decline one day later than in pigs fed any of the other diets. In addition, the rate of decline of total cholesterol of pigs fed the high calcium diet with *L. acidophilus* was greater than the rate of decline of those fed the high calcium diet without *L. acidophilus*. However, no differences in the rate of decline were observed between the two levels of *L. acidophilus* at the low calcium level.

The model used in inducing hypercholesterolemia in all animals by feeding a high fat, high cholesterol diet and then quantitating both the magnitude and rate of reduction of serum cholesterol is an unique approach as a mean of evaluating factors which reduced serum cholesterol. Studies have shown effects of some factors in lowering plasma cholesterol in hypercholesterolemic individuals (2, 7, 14, 17, 22) but not in individuals with "normal" plasma level (40).

In summary, feeding a diet supplemented with .5% crystalline cholesterol and 10% butter for 14 d caused a 2.4 fold increase (209.2 mg/dl) in total serum cholesterol in 92-kg barrows. Supplementation of the diet with *L. acidophilus* and/or 1.4% dietary calcium decreased serum concentration of total cholesterol and LDL cholesterol but not HDL cholesterol in pigs previously fed a high cholesterol diet. Moreover, total serum bile acid concentration was also reduced by feeding *L. acidophilus* or 1.4% calcium. A significant correlation was observed between total serum cholesterol and total bile acids. These data support previous findings that *L. acidophilus* and dietary calcium have hypocholesterolemic actions and that these actions are probably mediated through interference of the enterohepatic circulation of bile acids. There is no apparent relationship between the ability of *L. acidophilus* to assimilate cholesterol and to deconjugate bile acids (47). However, results of the present study and that of Gilliland et al. (17) indicate that both actions are important in enabling *L. acidophilus* to exert hypocholesterolemic action. This study also indicates that the model used in inducing hypercholesterolemia and then quantitating both the magnitude and rate of reduction of serum cholesterol is a good model for evaluating factors that reduced serum cholesterol. Future work is needed to evaluate the effects of both *L. acidophilus* and excess dietary calcium on serum and fecal bile acids simultaneously. Furthermore, postprandial serum bile acids should also be evaluated.

REFERENCES

1. Angelin, B., I. Bjorkhem, K. Einarsson, and S. Ewerth. 1982. Cholestyramine treatment reduces postprandial but not fasting serum bile acid levels in human. Gastroenterology. 83:1097.

2. Beames, C. G. Jr., C. V. Maxwell, S. A. Norton, and G. A. Bond. 1986. Whey and cholesterol in swine. Pp. 1041-1046. In M. E. Tumbleson (Ed.). Swine in Biomedical Research. Vol. 2. Plenum Publishing Co. New York, N. Y.

3. Bhattacharyya, A. K., C. Thera, J. T. Anderson, F. Grande, and A. Keys. 1969. Dietary calcium and fat: effect on serum lipids and fecal excretion of cholesterol and its degradation products in man. Am. J. Clin. Nutr. 22:1161.

4. Bierenbaum, M. L., A. I. Fleischman, and R. I. Raichelson. 1972. Long-term human studies on lipid effect of oral calcium. Lipids. 7:202.

5. Borum, M. L., K. L. Shehan, H. Fromm, S. Jahangeer, M. K. Floor, and O. Alabaster. 1992. Fecal bile acid excretion and composition in response to changes in dietary wheat bran, fat and calcium in the rat. Lipids. 27:999.

6. Chickai, T., H. Nakao, and K. Uchida. 1987. Deconjugation of bile acids by human intestinal bacteria implanted in germ-free rats. Lipids. 22:669.

7. Danielson, A. D., E. R. Peo, K. M. Shahani, A. J. Lewis, P. J. Whalen, and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966.

8. Danielsson, H., and J. Sjovall. 1975. Bile acids metabolism. Ann. Rev. Biochem. 44:233.

9. Denke, M. A., M. M. Fox, and M. C. Schulte. 1993. Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men. J. Nutr. 123:1047.

10. Drenick, E. J. 1961. The influence of ingestion of calcium and other soap-forming substances on fecal fat. Gastroenterology. 41:242.

11. Eyssen, H. 1973. Role of the gut microflora in metabolism of lipids and sterols. Proc. Nutr. Soc. 32:59.

12. Fleischman, A. I., H. Yacowitz, T. Hayton, and M. L. Bierenbaum. 1966. Effects of dietary calcium upon lipid metabolism in mature male rats fed beef tallow. J. Nutr. 88:255.

13. Fleischman, A. I., H. Yacowitz, T. Hayton, and M. L. Bierenbaum. 1967. Long-term studies on the hypolipemic effect of dietary calcium in mature male rats fed cocoa butter. J. Nutr. 91:151.

14. Fleischman, A. I., M. L. Bierenbaum, and P. H. Lenz. 1972. The hypolipidemic effect of calcium containing compounds and vitamin D2 in the rat. Lipid. 7:263.

15. Foley, M. K., S. T. Galloway, C. M. Luhman, T. D. Faidlay, and D. C. Beitz. 1990. Influence of dietary calcium and cholecalciferol on composition of plasma lipids in young pigs. J. Nutr. 120:45.

16. Gilliland, S. E., and M. L. Speck. 1977. Deconjugation of bile acids by intestinal Lactobacilli. Appl. Environ. Microbiol. 33:15.

17. Gilliland, S. E., C. R. Nelson, and C. V. Maxwell. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49:377.

18. Gilliland, S. E., and D. K. Walker. 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in human. J. Dairy Sci. 73:905.

19. Gilliland, S. E., and C. N. Rich. 1990. Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* grown at different pH. J. Dairy Sci. 73: 1187.

20. Grunewald, K. K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078.

21. Harrison, V. C., and G. Peat. 1975. Serum cholesterol and bowel flora in the newborn. Am. J. Clin. Nutr. 28:1351.

22. Johnson, L. L., C. G. Beames Jr., and C. V. Maxwell. 1986. Effect of calcium and protein source on the plasma cholesterol of Japanese quail. Proc. Okla. Acad. Sci. 66:31.

23. Kannel, W. B., J. T. Doyle, A. M. Ostfeld, C. D. Jenkins, L. Kuller, R. N. Podell, and J. Stamler. 1984. Optimal resources for primary prevention of atherosclerotic diseases. Circ. 70:155A.

24. Knipping, G. M. J., G. M. Kostner, and A. Holasek. 1975. Studies on the composition of pig serum lipoproteins, isolation and characterization of different apoproteins. Biochem. Biophys. Acta. 393:88.

25. Komarek, R. J. 1981. Intestinal cannulation of cattle and sheep with T-shaped cannula designed for total digesta collection without externalizing digesta flow. J. Anim. Sci. 53:796.

26. Levy, R. I. 1981. Review: declining mortality in coronary heart disease. Arteriosclerosis. 1:312.

27. Lin, S. Y., J. W. Ayres, W. Winkler,Jr., and W. E. Sandine. 1989. *Lactobacillus* effects on cholesterol: In vitro and In vivo results. J. Dairy Sci. 72:2885.

28. Lipid Research Clinics Program. 1984. The lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. J. Am. Med. Assoc. 251 (3):351.

29 Lipid Research Clinics Program. 1984. The lipid Research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart heart disease to cholesterol lowering. J. Am. Med. Assoc. 251 (3):365.

30. Mitchell, W. D., T. Fyfe, and D. Smith. 1968. The effect of oral calcium on cholesterol metabolism. J. Athero. Res. 8:913.

31. National Research Council. 1988. Nutrient Requirements of Swine. 9th rev. ed. Natl. Acad. Sci., Washington, DC.

32. Ratcliffe, H. L., and H. Luginbuht.1971. The domestic pig: a model for experimental atherosclerosis. Atherosclerosis. 13:133.

33. Rossi, S. S., M. L. Wayne, R. B. Smith, C. E. Wright, N. A. Andreadis, and A. F. Hofmann. 1989. Effect of the bile-acid sequestrant colestipol on post pandrial serum bile acid concentration: evaluation by bioluminescent enzymic analysis. Aliment. Pharmacol. Therap. 3:41.

34. SAS. User's Guide: Statistics. Version 5 Edition. 1985. SAS Inst., Cary, NC.

35. Saunders, D., J. Sillery, and R. Chapman. 1988. Effect of calcium carbonate and aluminium hydroxide on human intestinal function. Dig. Dis. Sci. 33:409.

36. Schiff, E. R., N. C. Small, and J. M. Dietschy. 1972. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. J. Clin. Invest. 51:1351.

37. Sigma Technical Bulletin No. 352. 1991. Sigma Chemical Co., St. Louis MO.

38. Sigma Technical Bulletin No. 450. 1989. Sigma Chemical Co., St. Louis, MO.

39. Stahelin, H. B., G. Ritzel, M. Wanner, M. Jost, and H. Schneeberger. 1981. Hypocholesterolemic effect of milk constituents in growing swine. Int. J. Vit. Nutr. Res. 51:198.

40. Thompson, L. U., D. Jenkins, M. A. VicAmer, R. Reichert, A. Jenkins, and J. Kamulsky. 1982. The effect of fermented and unfermented milks on serum cholesterol . Am. J. Clin. Nutr. 36:1106.

41. Tortuero, F., A. Brenes, and J. Rioperez. 1975. The influence of intestinal (ceca) flora on serum and egg yolk cholesterol levels in laying hens. Poultry Sci. 54:1935.

42. United States Bureau of the Census. 1986. Statistical abstract of the United States: 1987. 107th Ed. Washington, D.C.

43. Van Berge-Henegouwen, G. P., and A. F. Hofmann. 1983. Systemic spill-over of bile acids. Eur. J. Clin. Invest. 13:433.

44. Van der Meer, R., H. De Vries, C. E. West, and H. De Waard. 1985. Casein-induced hypercholesterolemia in rabbits is calcium-dependent. Atherosclerosis. 56:139.

45. Van der Meer, R., and H. T. De Vries. 1985. Differential binding of glycine- and taurine- conjugated bile acids to insoluble calcium phosphate. Biochem. J. 229:265.

46. Van der Meer, R., J. W. M. Welberg, F. Kuipers, J. H. Kleibeuker, N. H. Mulder, D. S. M. L. Termont, R. J. Vonk, H. T. De Vries, and E. G. E. De Vries. 1990. Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate, and bile acids. Gastroenterology. 99:1653.

47. Walker, D. K., and S. E. Gilliland. 1993. Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76:956.

TABLE I

COMPOSITION OF DIET FED DURING THE RECOVERY PERIOD

Ingredient	Percentage in Ration[a]
Yellow Corn	83.30
Soybean Meal	14.50
Dicalcium Phosphate	.85
Calcium Carbonate	.85
Vitamin, TM premix[b]	.15
Salt	.35
Calculated Analysis (%)	
Crude Protein	13.50
Lysine	.63
Calcium	.58
Phosphorus	.48

[a]As fed basis.

[b]Supplied 2950 IU vitamin A, 330 IU vitamin D, 16.5 IU vitamin E, 14.85 mg pantothenic acid, 23.1 mg niacin, 3.96 mg riboflavin, 2.18 mg menadione, 16.5 ug vitamin B12, 66 mg choline, 0.17 mg selenium, 11.97 mg manganese, 59.88 mg zinc, 59.88 mg iron, 5.98 mg cooper and .12 mg iodine per kg of feed.

TABLE II

COMPOSITION OF EXPERIMENTAL DIETS

	Diets[a]		
	Days 1 to 14	Days 15 to 29	
Ingredient		.7% calcium	1.4% calcium
Yellow Corn	67.425	68.095	66.245
Nonfat dried Milk	20.00	20.00	20.00
Butter[b]	10.00	10.00	10.00
Lysine, Hcl	.03	.03	.03
Calcium Carbonate	.32	.15	2.00
Dicalcium Phosphate	1.15	--	--
Deflourinate Phosphate	--	1.15	1.15
Vitamin, TM premix[c]	.25	.25	.25
Salt	.30	.30	.30
Ethoxyquin	.025	.025	.025
Cholesterol[d]	.50	--	--
	100.00	100.00	100.00
Calculated Analysis (%)			
Crude Protein	12.36	12.41	12.26
Lysine	.70	.70	.70
Calcium	.70	.70	1.40
Phosphorus	.60	.60	.60

[a]As fed basis, each ingredient presented on percentage basis.

[b]Supply approximately .2 g cholesterol per kg feed.

[c]Supplied 4950 IU vitamin A, 550 IU vitamin D, 27.5 IU vitamin E, 24.75 mg pantothenic acid, 38.5 mg niacin, 6.6 mg riboflavin, 3.63 mg menadione, 27.5 ug vitamin B12, 110 mg choline, .30 mg selenium, 19.96 mg manganese, 99.79 mg zinc, 99.79 mg iron, 9.97 mg cooper and .20 mg iodine per kg of feed.

[d]Purity at least equivalent to USP/NF (Sigma Chemical Co., St. Louis, MO).

TABLE III

EFFECT OF *LACTOBACILLUS ACIDOPHILUS* AND CALCIUM ON PIG WEIGHT, FEED INTAKE, AND CHOLESTEROL INTAKE[a]

	Assigned Treatment Group				
	With *L. acidophilus*		Without *L. acidophilus*		
	.7% Ca	1.4% Ca	.7% Ca	1.4% Ca	SE
Day 1 to 14:					
Initial Weight, kg	93.92	91.08	91.69	93.86	3.5
Weight at d 14, kg	101.48	99.60	99.32	103.52	3.8
ADFI, g/kg body wt[b]	25.81	25.50	23.34	25.43	1.1
ADCI, g/kg body wt[b,c]	.13	.13	.12	.13	.005
Day 15 to 29:					
Weight at d 29, kg	111.08	109.77	110.00	113.35	3.8
ADFI, g/kg body wt	23.83	25.17	23.72	25.21	1.0

[a]Values are least squares means.

[b]ADFI = average daily feed intake; ADCI = average daily cholesterol intake.

[c]Include cholesterol from crystalline cholesterol and butter.

TABLE IV

EFFECT OF *LACTOBACILLUS ACIDOPHILUS* (LA) AND CALCIUM ON SERUM TOTAL CHOLESTEROL, HIGH DENSITY LIPOPROTEIN (HDL), LOW DENSITY LIPOPROTEIN (LDL) AND TOTAL BILE ACIDS OF PIGS PREVIOUSLY FED A HIGH CHOLESTEROL DIET (d 15 to 29)[a]

Item	*L. acidophilus*		Calcium Level	
	Without	With	.7%	1.4%
Total cholesterol, mg/dl[b,c]	167.9 ± 5.2	148.0 ± 5.3	167.5 ± 5.2	148.5 ± 5.3
HDL, mg/dl[d]	57.9 ± 4.7	55.1 ± 4.1	57.7 ± 4.2	55.3 ± 4.6
LDL, mg/dl[b,e]	114.6 ± 7.7	95.9 ± 7.7	113.5 ± 7.3	97.0 ± 7.6
Total bile acids, µmol/l[f]	9.2 ± .7	7.0 ± .9	9.1 ± .7	7.1 ± .8

[a]Values are least squares means ± SE. LA = *L. acidophilus*.

[b]Least square means adjusted for initial (d 15) sera values using covariate analysis.

[c]LA effect ($P < .01$); Ca effect ($P < .01$) and LA x Ca interaction effect ($P > .6$).

[d]LA effect ($P > .5$); Ca effect ($P > .7$) and LA x Ca interaction effect ($P > .3$).

[e]LA effect ($P < .09$); Ca effect ($P < .1$) and LA x Ca interaction effect ($P > .6$).

[f]LA effect ($P < .06$); Ca effect ($P < .08$) and LA x Ca interaction effect ($P > .8$).

TABLE V

DELAY AND RATE OF DECLINE OF TOTAL SERUM CHOLESTEROL OF PIGS PREVIOUSLY FED A HIGH CHOLESTEROL DIET

	Difference Between d 15 and 16		Difference Between Avg. d 15 and 16 and d 17		
Treatment[a]	mg/dl	Probability Level	mg/dl	Probability Level	Rate of Decline[b]
1	.12	P > .9	44.17	P < .01	.46
2	39.44	P < .01	50.37	P < .01	.36
3	37.90	P < .01	84.19	P < .01	.41
4	65.10	P < .01	77.60	P < .01	.59

[a]Treatment 1 = .7% calcium without *L. acidophilus;* Treatment 2 = .7% calcium with *L. acidophilus;* Treatment 3 = 1.4% calcium without *L. acidophilus;* Treatment 4 = 1.4% calcium with *L. acidophilus.*

[b]*L. acidophilus* effect at high calcium level (P < .07).

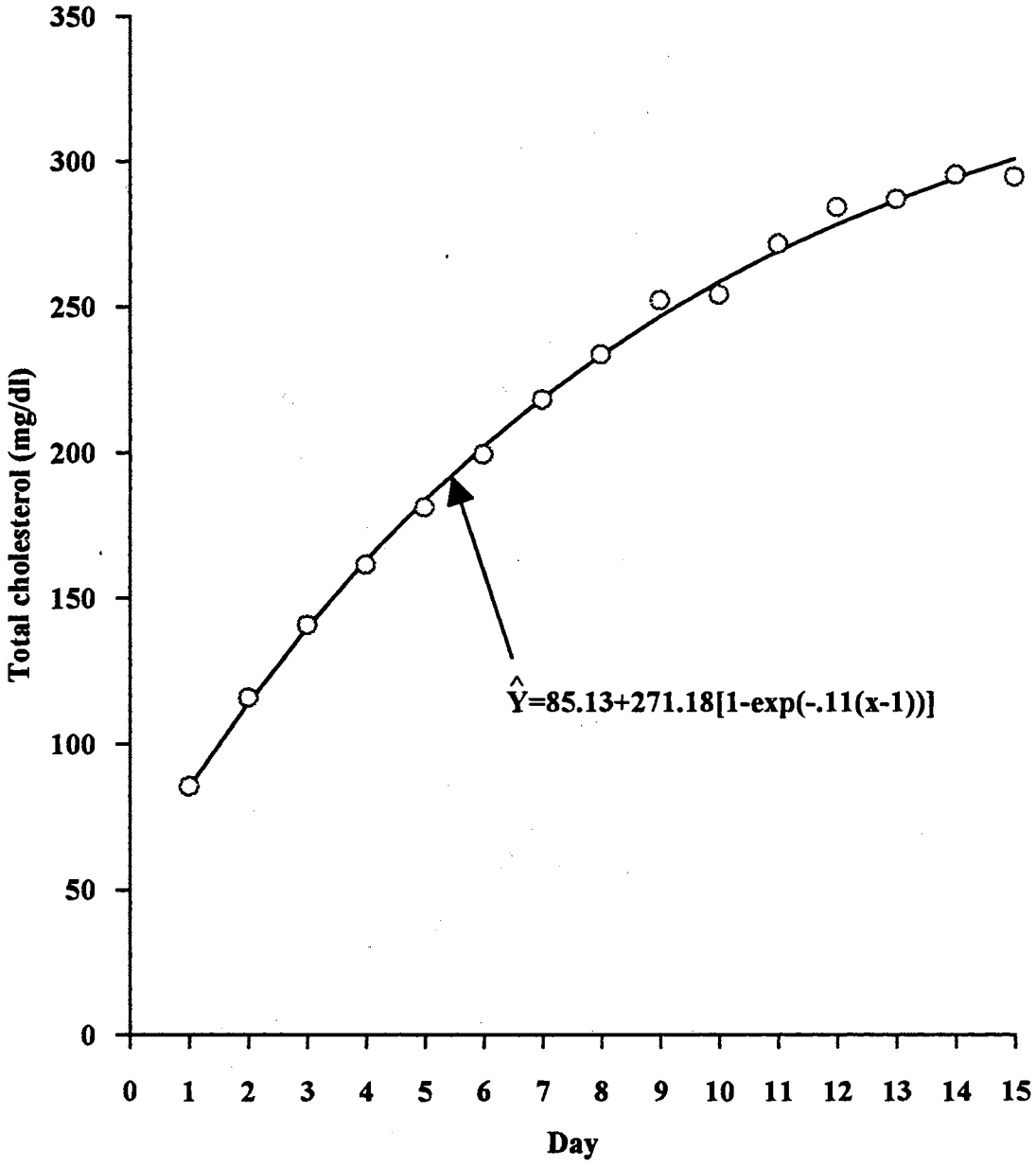

Figure 1. Regression of Total Cholesterol Concentration on Day of Cholesterol Feeding (o = Actual Means)

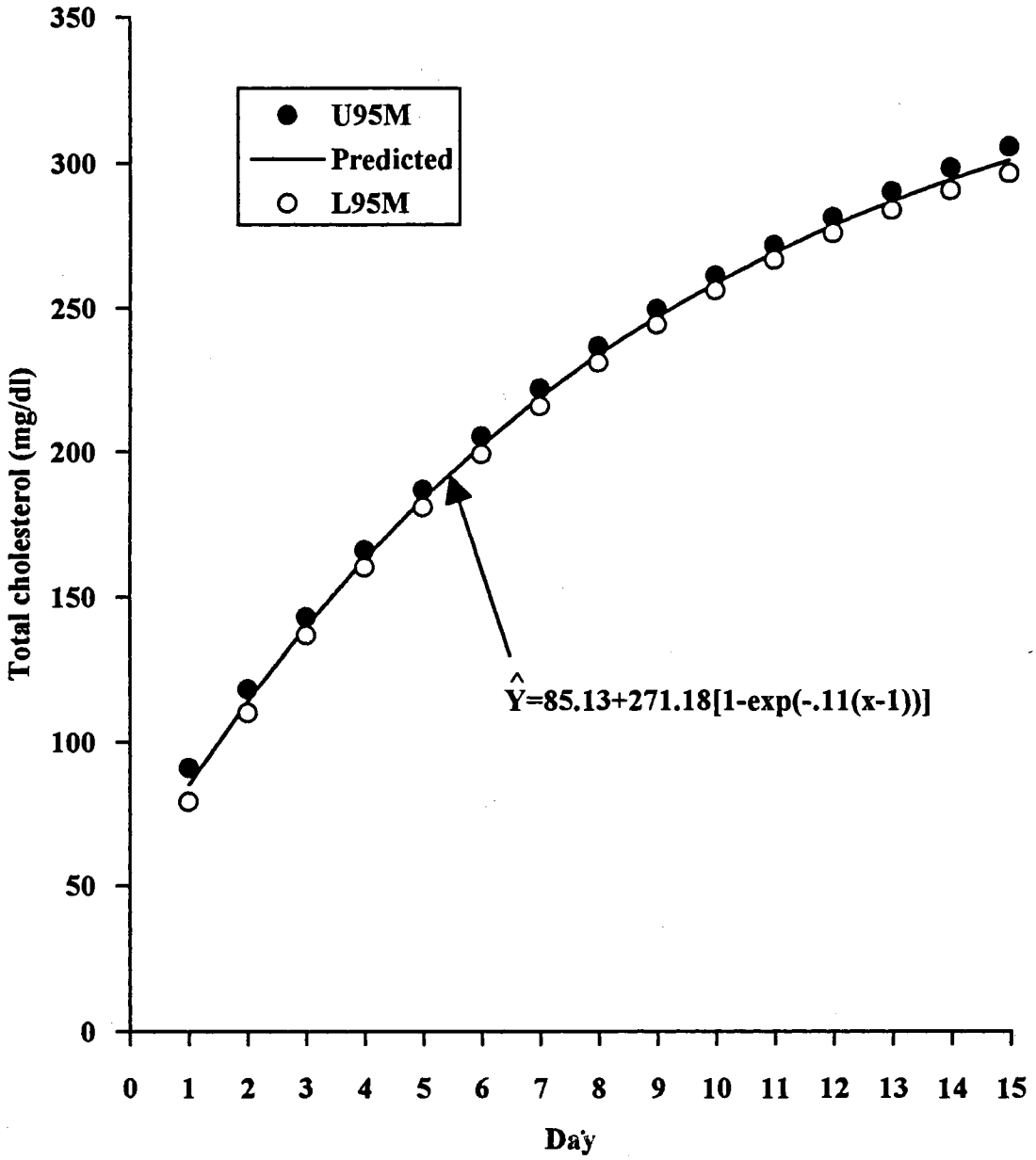

Figure 2. Regression of Total Cholesterol Concentration on Day of Cholesterol Feeding and Upper (•) and Lower (o) 95% Confidence Limits for the Expected Means

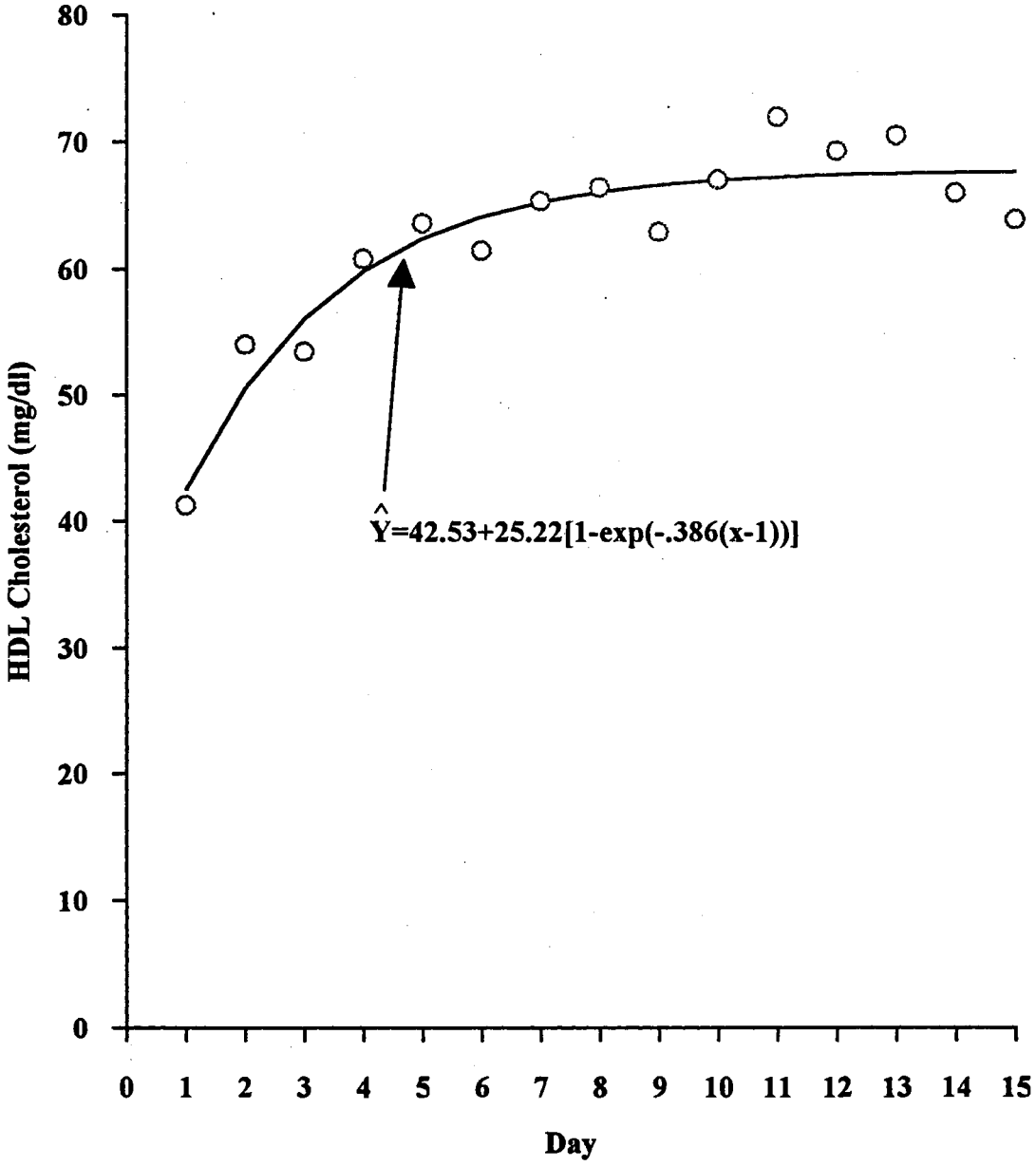

Figure 3. Regression of High Density Lipoprotein (HDL) Cholesterol Concentration on Day of Cholesterol Feeding (o = Actual Means)

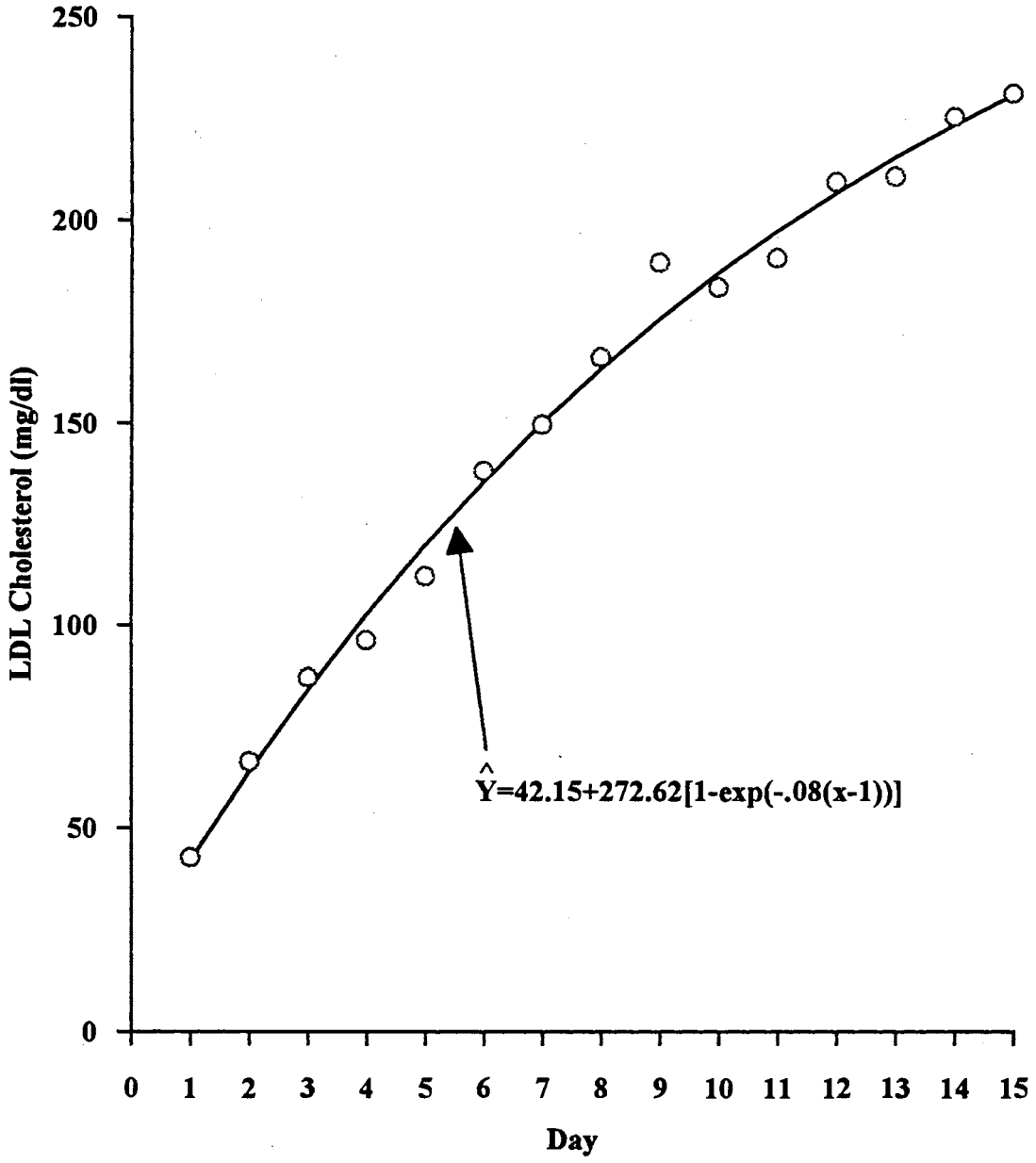

Figure 4. Regression of Low Density Lipoprotein (LDL) Cholesterol Concentration on Day of Cholesterol Feeding (o = Actual Means)

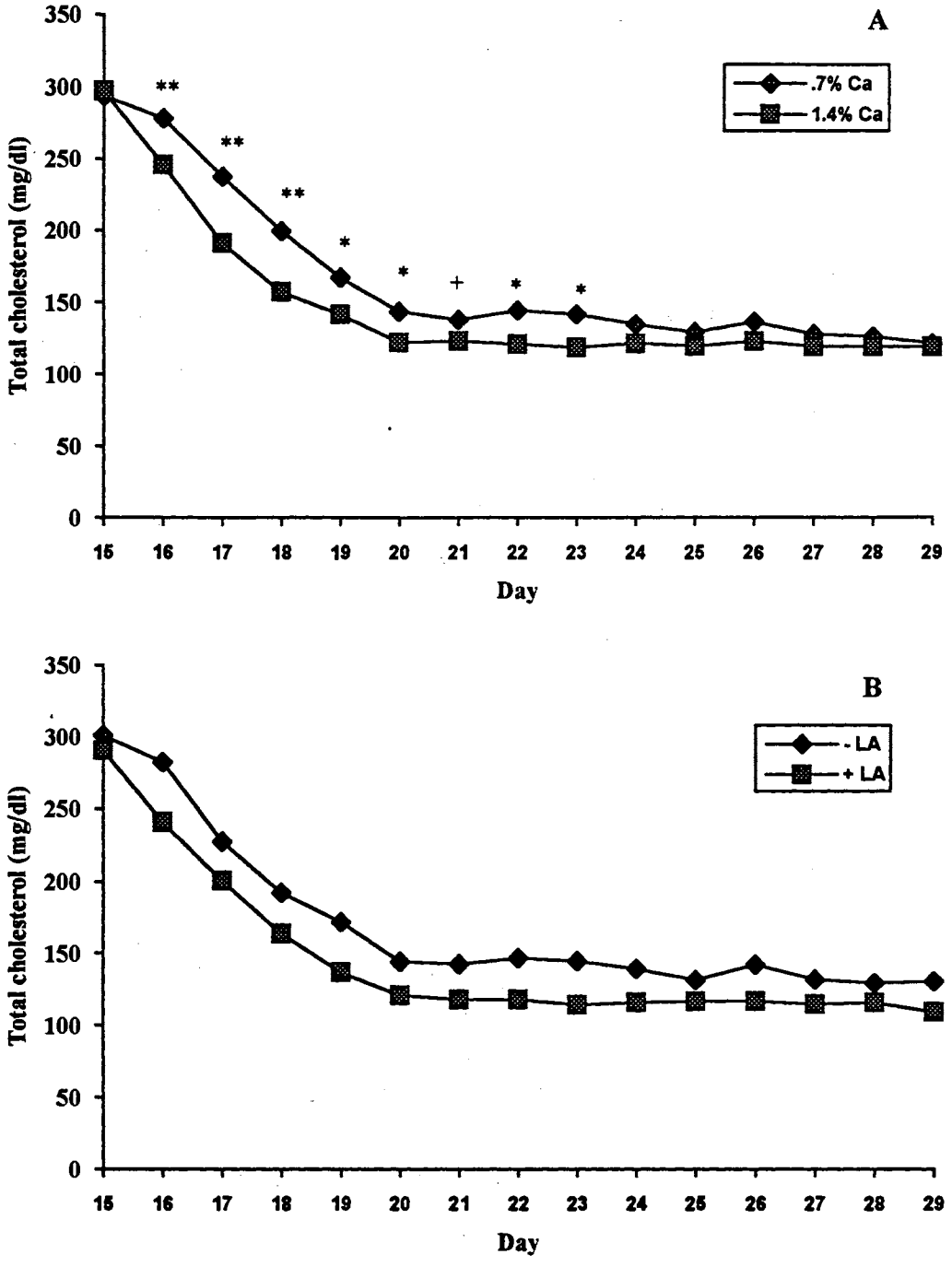

Figure 5. Effect of Dietary Calcium (Panel A) and *Lactobacillus acidophilus* (LA; Panel B) on Serum Total Cholesterol Level of Pigs Previously Fed a High Cholesterol Diet. Values are least squares means. Pooled SE = 7.7. Calcium effect ($P < .01$), *L. acidophilus* effect ($P < .01$), day on treatment effect ($P < .0001$), and day on treatment x calcium interaction effect ($P < .06$). **Means differ ($P < .01$) within day; *means differ ($P < .05$) within day; +means differ within day ($P < .1$).

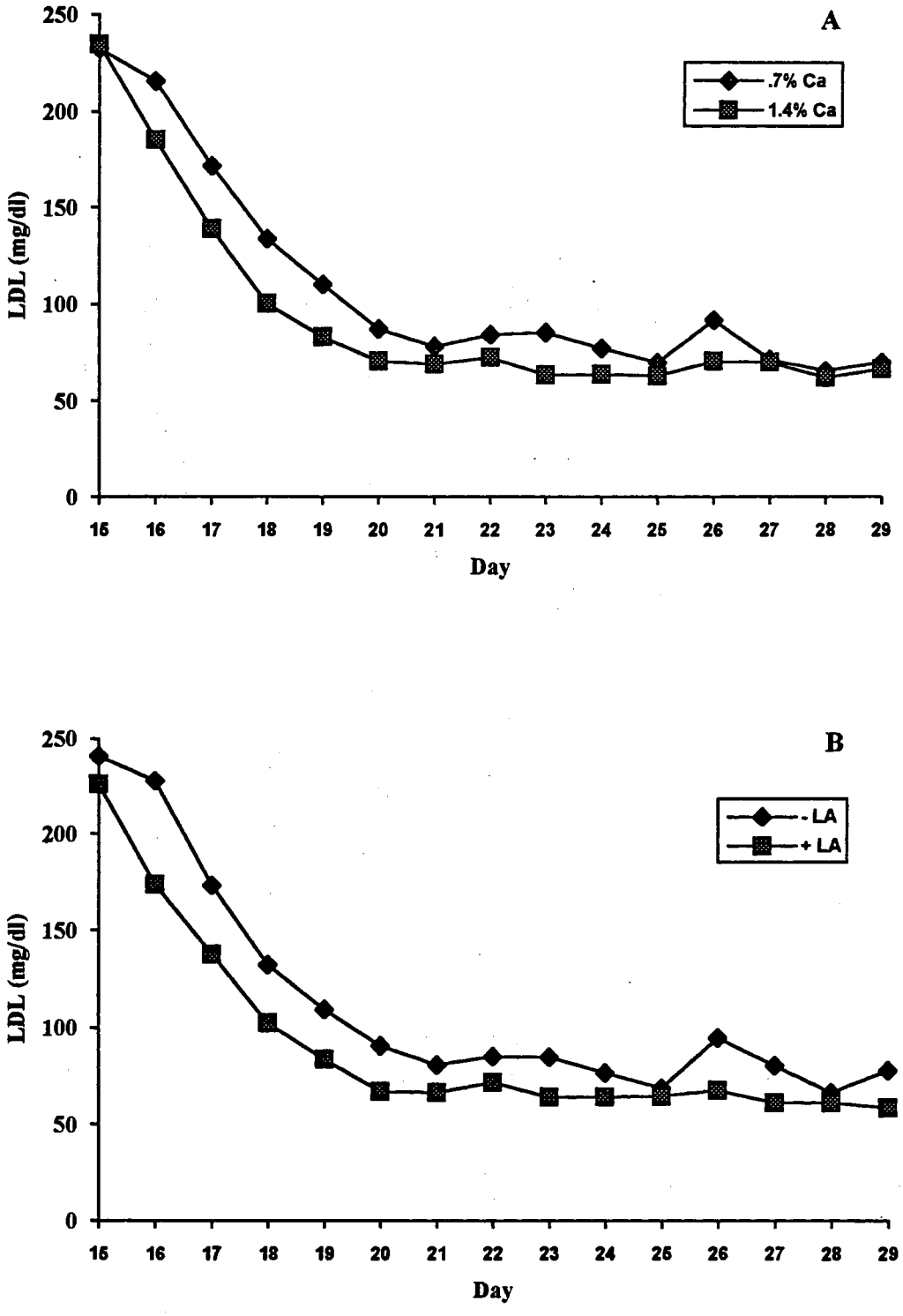

Figure 6. Effect of Dietary Calcum (Panel A) and *Lactobacillus acidophilus* (LA; Panel B) on Serum Low Density Lipoprotein (LDL) of Pigs Previously Fed a High Cholesterol Diet. Values are least squares means. Pooled SE = 9.6. Calcium effect ($P < .1$), *L. acidophilus* effect ($P < .09$), and day on treatment effect ($P < .0001$).

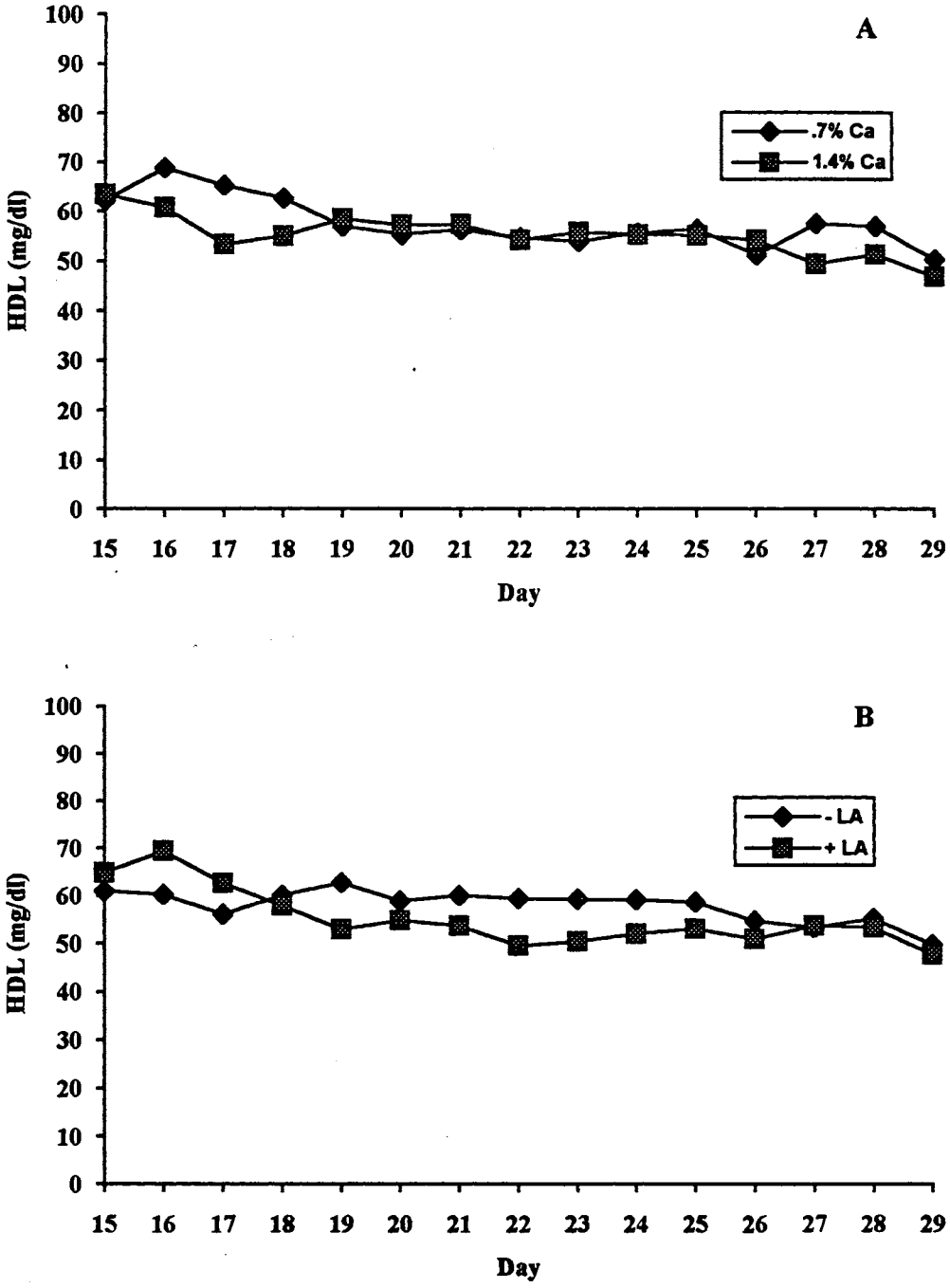

Figure 7. Effect of Dietary Calcium (Panel A) and *Lactobacillus acidophilus* (LA; Panel B) on Serum High Density Lipoprotein (HDL) of Pigs Previously Fed a High Cholesterol Diet. Values are least squares means. Pooled SE = 3.6. Day on treatment effect ($P < .01$).

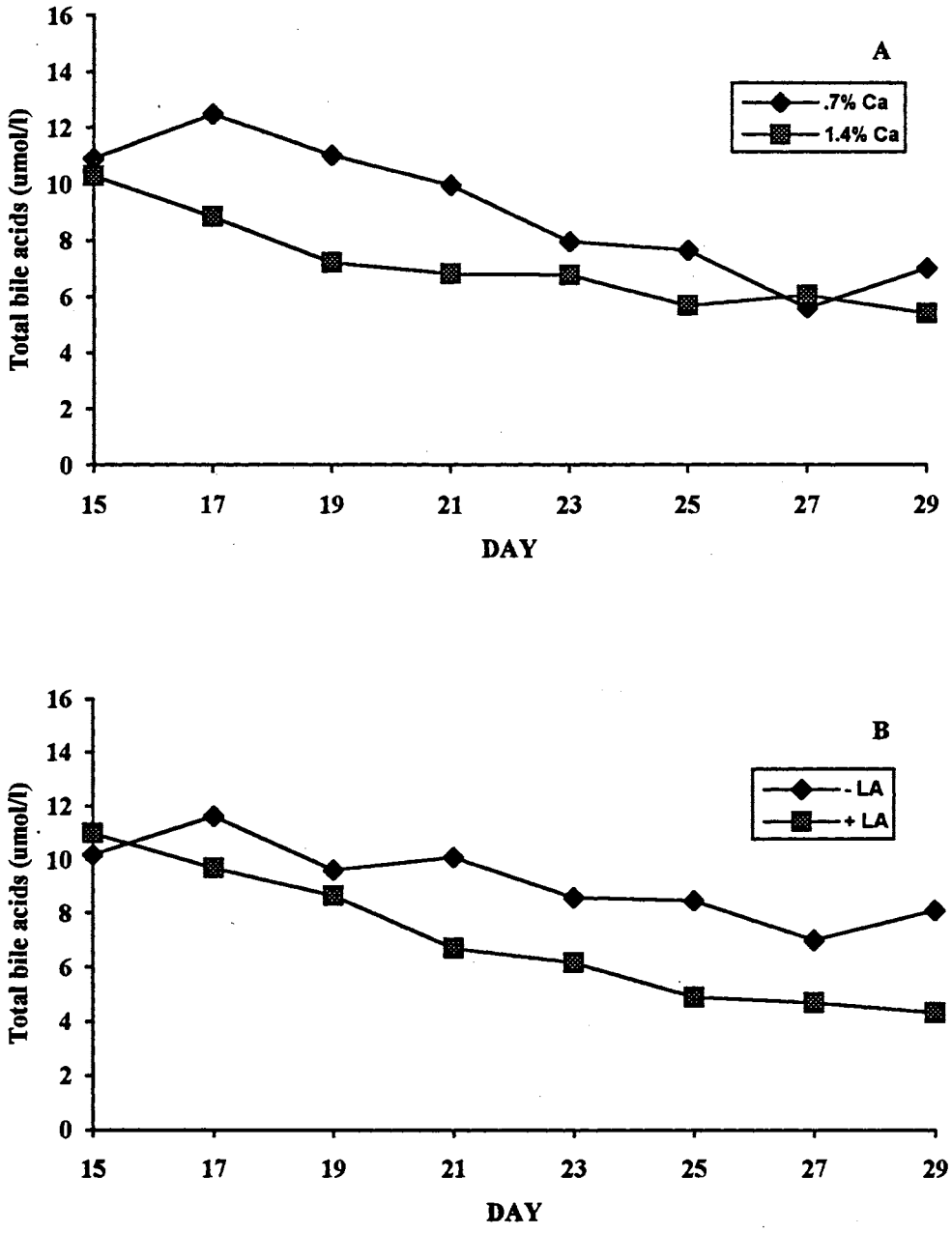

Figure 8. Effect of Dietary Calcium (Panel A) and *Lactobacillus acidophilus* (LA; Panel B) on Total Serum Bile Acid of Pigs Previously Fed a High Cholesterol Diet. Values are least squares means. Pooled SE = 1.0. Calcium effect ($P < .08$), *L. acidophilus* effect ($P < .06$), day on treatment effect ($P < .0001$), day on treatment x calcium interaction ($P < .2$), and day on treatment x *L. acidophilus* interaction ($P < .2$) effects.

APPENDIX A

ASSIGNMENT OF PIGS TO TREATMENT

TABLE VI

ASSIGNMENT OF PIGS TO TREATMENT

Treatment[a]	Replicate	Pig
1	1	5
1	1	8
1	2	14
1	2	17
1	3	18
1	3	19
1	3	22
1	4	33
1	4	35
2	1	2
2	1	3
2	2	9
2	2	11
2	3	21
2	3	23
2	4	31
2	4	34
3	1	6
3	1	7
3	2	12
3	2	13
3	3	20
3	3	25
3	4	29
3	4	32
4	1	1
4	1	4
4	2	10
4	2	16
4	3	24
4	3	26
4	4	27
4	4	28

[a]Treatment 1 = .7% calcium without *Lactobacillus acidophilus*, Treatment 2 = .7% calcium with *L. acidophilus*, Treatment 3 = 1.4% calcium without *L. acidophilus*, and Treatment 4 = 1.4% calcium with *L. acidophilus*.

APPENDIX B

INDIVIDUAL PIG DATA

TABLE VII

INDIVIDUAL PIG DATA OF TOTAL AND HDL CHOLESTEROL, AND FEED INTAKE

Pig No.	Treatment[a]	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
1	4	-2	54.0	27.1	
		-1	54.0	32.7	
		1	55.0	18.9	27.90
		2	85.3	49.1	12.24
		3	100.0	21.8	16.20
		4	122.3	38.3	21.07
		5	140.3	27.1	24.49
		6	164.5	19.3	13.95
		7	192.0	18.	19.61
		8	224.2	21.5	24.49
		9	248.4	24.9	27.90
		10	240.8	28.7	27.90
		11	250.3	35.1	23.51
		12	268.8		27.32
		13	263.1	34.2	25.27
		14	261.1	33.2	27.92
		15	244.2	33.2	27.96
		16	206.1	35.4	27.96
		17	134.6	39.4	27.96
		18	115.7	32.9	27.96
		19	96.7	27.5	27.96
		20	64.5	17.4	27.96
		21	87.2	46.	27.96
		22	80.1	27.8	27.96
		23	109.0	40.9	27.96
		24	89.1	37.0	27.96
		25	100.5	41.6	27.96
		26	105.2	50.8	27.96
		27	106.2	52.4	27.96
		28	106.2		27.96
		29	113.8		27.96
2	2	-2	65.4	36.3	
		-1	70.2	32.7	
		1	73.7	35.0	25.44
		2	121.9	28.8	18.38
		3	141.2	41.7	23.68
		4	174.5	44.5	28.07
		5	194.7		15.35

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
2	2	6	221.0	28.1	21.93
		7	250.8	32.9	28.07
		8	272.3	71.5	23.63
		9	292.0	39.0	28.07
		10	270.1	42.8	28.07
		11	321.4	92.2	28.07
		12	368.3	82.5	28.07
		13	363.1	44.3	28.07
		14	388.5	66.2	28.07
		15	357.1	55.9	28.02
		16	336.3	56.1	28.02
		17	251.3	26.5	27.37
		18	178.5	44.7	23.97
		19	148.2	30.9	25.02
		20	132.1	34.0	25.75
		21	112.3	36.6	25.75
		22	160.5		23.40
		23	130.7	62.3	28.02
		24	136.4	57.1	23.72
		25	132.4	78.9	23.56
		26	133.7		25.34
		27	144.0		24.53
		28	138.1	68.2	23.44
		29	135.5	50.6	22.19
3	2	-2	100.5	38.2	
		-1	86.0	41.7	
		1	90.0	57.6	23.10
		2	131.7	90.8	27.48
		3	186.4		20.68
		4	210.4		27.96
		5	266.5		27.96
		6	319.0	73.7	26.26
		7	360.2		27.96
		8	416.3	25.7	27.96
		9	425.8	12.9	22.62
		10	425.4		27.96
		11	493.2		27.48
		12	480.1		26.99
		13	504.1		19.22
		14	424.9	9.2	25.53

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
3	2	15	432.2	15.5	25.74
		16	407.7	17.9	25.02
		17	362.0	38.7	21.43
		18	337.6		16.95
		19	256.6	11.5	24.75
		20	224.0	7.3	25.74
		21	200.9		25.25
		22	220.4	12.1	21.17
		23	238.2	27.7	20.90
		24	230.6	47.	25.92
		25	214.4	41.3	23.77
		26	217.6		23.68
		27	211.3	44.2	27.98
		28	207.7	49.2	27.98
		29	165.6	57.4	18.12
4	4	-2	67.2	34.6	
		-1	68.5	34.9	
		1	63.6	37.0	28.02
		2	106.1	54.8	11.56
		3	108.7	27.6	22.41
		4	133.7	26.8	26.08
		5	156.1	32.9	26.75
		6	235.0	45.3	22.95
		7	215.9	42.4	28.02
		8	270.4		22.95
		9	224.9	10.2	25.91
		10	231.5	39.8	26.08
		11	272.0	42.3	28.02
		12	362.0	47.5	29.79
		13	344.6	50.8	24.14
		14	366.0	54.1	23.80
		15	349.8	31.1	25.33
		16	281.9	45.3	26.04
		17	186.7	35.6	26.43
		18	127.5	34.3	24.94
		19	114.1	37.7	25.94
		20	95.0	34.6	26.27
		21	80.5	33.4	24.04
		22	56.1		25.33
		23	97.5	69.2	25.61

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
4	4	24	95.7	49.6	25.02
		25		47.9	25.73
		26	96.5	44.0	21.49
		27	106.1		24.16
		28	102.3	47.0	24.78
		29	118.0	44.2	24.31
5	1	-2	77	28.3	
		-1	89.3	27.2	
		1	67.5	21.2	13.88
		2	97.8	21.6	16.83
		3	132.5	25.7	19.84
		4	160.3	16.4	13.74
		5	188.7	17.8	22.68
		6	239.8	21.7	
		7	208.2		17.56
		8	256.8	15.9	18.62
		9	268.5	11.8	23.50
		10	300.0	24.3	25.28
		11	325.9	26.6	22.28
		12	358.7	24.3	24.11
		13	386.2	29.7	23.90
		14	391.2	32.7	21.87
		15	427.3	24.7	24.54
		16	406.0	23.5	25.31
		17	392.5	28.7	21.62
		18	372.0	38.6	21.08
		19	330.9	24.1	20.73
		20	195.6	29.0	22.62
		21	184.9	17.6	6.54
		22	219.3	27.4	16.85
		23	280.5	26.7	20.23
		24	239.5	27.8	20.31
		25	211.7	32.8	16.23
		26	208.5		19.92
		27	205.1	54.7	24.92
		28	200.0	35.8	18.92
		29	196.2		20.15
6	3	-2	116.5		
		-1	102.4		
		1	116.4		27.78

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
6	3	2			16.76
		3			19.26
		4			24.87
		5			21.76
		6			24.07
		7			26.30
		8			23.15
		9			27.78
		10			27.78
		11			27.78
		12			24.35
		13	347.3		25.46
		14	327.3		27.78
		15	328.0		26.33
		16	296.2		27.83
		17	210.4		27.42
		18	226.4		28.00
		19	195.1		27.50
		20	94.0		25.92
		21	112.0		25.92
		22	161.8		27.17
		23	175.5		28.00
		24	200.0		28.00
		25	195.5		28.00
		26	183.5		25.75
		27	161.4		28.00
		28	171.0		25.67
		29	194.7		26.83
7	3	-2	67.0		
		-1		23.9	
		1	89.0	16.9	28.04
		2	72.1		25.80
		3	176.8	14.8	22.50
		4	237.4		24.29
		5	226.7	18.9	19.55
		6	256.4	17.9	20.00
		7	183.4		26.34
		8	262.6	12.0	20.45
		9	310.1	9.7	24.46
		10	311.4	18.1	22.68

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
7	3	11	379.3	21.3	20.89
		12	338.5		26.79
		13	339.9	65.6	23.84
		14	331.1	17.0	25.80
		15	376.8	35.7	25.92
		16	320.6	16.5	27.17
		17	232.8	19.3	24.50
		18	129.9	21.1	23.67
		19	150.3	24.8	27.42
		20	163.8		27.67
		21	176.8		24.50
		22	149.6		24.92
		23	143.5		26.17
		24	160.2		24.33
		25	141.5	37.3	27.02
		26	136.6		23.50
		27	198.2	27.6	25.50
		28	201.		23.92
		29	217.6	25.2	26.75
8	1	-2	103.3	34.2	
		-1	97.8	36.9	
		1	113.3	51.6	28.10
		2	133.7	44.2	24.10
		3	185.1	38.1	28.10
		4	195.6	32.3	28.10
		5	217.1	45.3	28.10
		6	243.9	60.0	28.10
		7	279.0	25.3	28.10
		8	308.8	25.3	28.10
		9	343.7	37.5	28.10
		10	350.8	24.1	28.10
		11	372.9	11.2	28.10
		12	383.4	36.3	28.10
		13	343.7	46.8	28.10
		14	392.8	19.5	28.10
		15	392.2	10.3	27.05
		16	432.6	22.3	25.62
		17	321.5	21.3	18.67
		18	256.0	21.5	23.24
		19	237.0	32.3	20.19

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
8	1	20	181.8	26.9	18.10
		21	150.3	35.4	22.48
		22	161.3	24.0	18.76
		23	177.4		23.71
		24	171.3		28.00
		25	160.8		28.00
		26	141.4		21.43
		27	128.7		26.86
		28	126.0	55.1	28.00
		29	156.9	43.9	27.24
9	2	-2	109.5	30.5	
		-1	113.0	30.4	
		1	113.2	31.3	27.93
		2	155.4	46.1	27.93
		3	172.0	54.3	27.93
		4	192.7	45.4	27.93
		5	221.9	44.8	27.93
		6	235.3	45.2	27.93
		7	259.2	49.8	27.93
		8	259.2	48.4	27.93
		9	279.5	47.0	27.93
		10	282.8	45.2	27.93
		11	311.8	52.2	27.93
		12	347.2	53.4	27.93
		13	335.2	50.1	27.93
		14	361.0	53.4	27.93
		15	317.4	53.2	28.00
		16	332.0	55.1	28.00
		17	298.2	50.8	28.00
		18	248.6	45.4	28.00
		19	214.3	41.6	28.00
		20	170.4	40.9	28.00
		21	186.6	36.3	20.90
		22	152.3	34.4	28.00
		23	137.3	40.0	8.20
		24	136.3	35.4	
		25	128.6	37.1	
		26	121.0	40.7	
		27	115.8	54.2	
		28			

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
9	2	29			
10	4	-2	83.9	31.1	
		-1	92.5	20.2	
		1	89.8	19.7	28.02
		2	147.3	24.0	28.02
		3	157.5	22.0	28.02
		4	194.9	28.2	28.02
		5	239.6		28.02
		6	259.3	30.8	28.02
		7	275.5	36.0	28.02
		8	273.9	30.0	28.02
		9	310.8	31.6	28.02
		10	296.8	32.1	28.02
		11	299.3	32.7	28.02
		12	351.6	38.7	28.02
		13	323.9	37.4	28.02
		14	342.1	38.5	28.02
		15	357.1	58.7	27.98
		16	305.6	31.9	27.98
		17	246.4	37.5	27.98
		18	199.7	35.0	27.98
		19	143.4	29.5	27.98
		20	138.2	31.0	27.98
		21	139.3	31.4	27.98
		22	130.1	27.5	27.98
		23	119.8	24.4	27.98
		24	120.6	23.3	27.98
		25	114.1	20.6	27.98
		26	131.4	24.5	27.98
		27	98.6	24.1	27.98
		28	117.0	21.3	27.98
		29	100.6	20.7	27.98
11	2	-2	83.7	40.9	
		-1	80.1	37.4	
		1	61.6	38.7	6.50
		2	63.6	42.9	2.80
		3	77.6	39.6	28.00
		4	105.5	35.4	28.00
		5	126.6	40.4	28.00
		6	159.1	46.6	28.00

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
11	2	7	201.6	57.4	28.00
		8	202.2	85.2	28.00
		9	233.4	59.8	28.00
		10	239.3	75.8	28.00
		11	269.0	68.6	28.00
		12	295.5	78.4	28.00
		13	287.4	70.1	28.00
		14	309.7	74.6	28.00
		15	295.8	68.7	27.91
		16	236.2	101.6	27.91
		17	249.7	82.7	25.12
		18	188.2	71.8	27.35
		19	168.8	58.7	17.21
		20	145.8	61.7	21.40
		21	121.8	57.9	10.98
		22	125.1	66.9	21.67
		23	112.9	64.7	27.91
		24	110.0	58.4	24.00
		25	110.2	57.5	23.26
		26	105.3	65.2	27.91
		27	100.0	61.8	27.91
		28	88.4	51.1	19.77
		29	85.8	41.3	27.91
12	3	-2	111.3	51.3	
		-1	110.7	52.4	
		1	113.4	49.6	28.02
		2	180.7	54.2	28.02
		3	221.7	55.0	28.02
		4	261.3	71.0	28.02
		5	296.2	60.5	28.02
		6	319.4	57.8	28.02
		7	351.8	81.9	28.02
		8	338.6	85.2	28.02
		9	357.5	52.4	28.02
		10	350.8	76.8	28.02
		11	361.2	60.2	28.02
		12	383.1	57.5	28.02
		13	399.1	94.5	28.02
		14	415.7		28.02
		15	418.4	42.4	28.16

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
12	3	16	380.9	51.6	28.16
		17	342.6	64.4	28.16
		18	311.7	73.5	18.16
		19	286.7	79.5	28.16
		20	261.7	83.6	28.16
		21	256.6	65.0	28.16
		22	218.9	62.1	15.92
		23	193.9	76.7	28.16
		24	179.6	67.2	23.30
		25	161.7	74.4	28.16
		26	153.8	61.0	28.16
		27	138.5	55.9	28.16
		28	145.1	54.5	28.16
		29	168.9	54.0	28.16
13	3	-2	101.9	60.3	
		-1	104.6	53.0	
		1	100.2	51.7	28.00
		2	162.9	62.5	28.00
		3	206.9		16.82
		4	250.9	63.4	28.00
		5	274.6	62.2	28.00
		6	277.9	57.5	28.00
		7	315.0	66.8	28.00
		8	308.4	53.3	28.00
		9	304.2	59.0	28.00
		10	295.0	60.5	28.00
		11	320.8	79.7	28.00
		12	319.2	57.7	28.00
		13	346.9	61.2	28.00
		14	342.6	68.4	28.00
		15	352.9	67.8	28.04
		16	314.1	61.9	28.04
		17	216.7	26.7	28.04
		18	194.3	75.4	28.04
		19	165.7	75.4	28.04
		20	148.4	68.4	28.04
		21	143.1	73.0	28.04
		22	146.9	39.8	28.04
		23	122.1	69.3	28.04
		24	137.1	73.7	28.04

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
13	3	25	125.8	67.1	28.04
		26	163.5	64.6	28.04
		27	116.2	62.2	28.04
		28	109.8	68.3	28.04
		29	102.5	66.5	28.04
14	1	-2	83.1	29.8	
		-1	78.8	33.6	
		1			27.98
		2	138.5		27.98
		3	198.2	34.2	27.98
		4	203.0	35.7	27.98
		5	232.0	33.3	27.98
		6	239.9	48.4	27.98
		7	257.8	40.3	27.98
		8	243.1	34.9	26.91
		9	281.2	43.7	27.98
		10	277.7	36.2	27.98
		11	313.1	38.2	27.98
		12	308.5	39.6	27.98
		13	312.6	36.3	27.98
		14	315.4	35.8	27.98
		15	340.5	37.6	25.14
		16	297.1	42.7	28.00
		17	221.5	38.5	28.00
		18	185.1	45.8	28.00
		19	134.5	40.6	28.00
		20	123.4	39.8	25.00
		21	126.5	43.6	26.24
		22	156.0	53.9	28.00
		23	142.0	56.2	28.00
		24	136.7	59.2	28.00
		25	131.3	62.1	28.00
		26	250.0	55.5	28.00
		27	137.8	46.5	28.00
		28	128.1	44.9	28.00
		29	122.7	46.8	28.00
16	4	-2	87.7	36.9	
		-1	82.5		
		1	77.3	35.4	28.00
		2	137.0	42.6	28.00

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
16	4	3	113.8	35.6	28.00
		4	177.0	41.7	28.02
		5	179.0	76.1	28.00
		6	225.8	49.3	28.00
		7	236.9	51.4	28.00
		8	248.8	50.9	28.00
		9	301.1	55.8	28.00
		10	284.3	56.7	28.00
		11	309.9		28.00
		12	298.9		28.00
		13	305.4	54.9	28.00
		14	326.2	55.7	28.00
		15	294.4	49.4	27.98
		16	147.3	56.5	27.98
		17	179.0	47.2	27.98
		18	125.4	42.8	27.98
		19	96.7	45.4	27.98
		20	78.4	75.6	27.98
		21	86.6	55.6	27.98
		22	85.4	41.6	27.98
		23	86.1	47.9	27.98
		24	78.4	48.4	27.98
		25	84.5	49.7	27.98
		26	91.5	49.5	27.98
		27	100.0	48.0	27.98
		28	94.5	41.3	27.98
		29	87.4	43.8	27.98
17	1	-2	77.5	50.6	
		-1	72.1	38.7	
		1	60.8	57.5	28.00
		2	86.7	44.5	28.00
		3	128.4	50.6	28.00
		4	138.8	44.5	28.00
		5	170.9	45.8	28.00
		6	186.9	45.1	28.00
		7	235.7	53.0	28.00
		8	243.3	54.3	28.00
		9	266.5	55.3	28.00
		10	266.1	60.5	28.00
		11	290.3	58.8	28.00

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
17	1	12	299.5	62.4	28.00
		13	307.1	71.9	28.00
		14	356.3	61.5	28.00
		15	342.3	55.7	28.04
		16	333.7	58.6	28.04
		17	297.4	53.3	28.04
		18	273.1	53.8	28.04
		19	214.2	72.5	28.04
		20	185.6	74.5	28.04
		21	167.6	67.5	12.16
		22	164.3	70.7	28.04
		23	145.8	78.3	28.04
		24	120.6	68.8	28.04
		25	110.2	70.1	28.04
		26	111.7	73.2	28.04
		27	107.9	74.5	28.04
		28	110.4	73.5	28.04
		29	101.2	38.2	28.04
18	1	-2	96.9	45.8	
		-1	100.6	28.0	
		1	85.3	29.5	13.72
		2	98.9	29.2	20.61
		3	109.5	31.4	7.32
		4	103.7	31.9	7.07
		5	110.6	32.2	17.07
		6	102.7	29.8	15.98
		7	121.1	32.2	15.12
		8	107.4	26.5	6.83
		9	134.2	31.6	9.51
		10	135.8	34.7	13.17
		11	123.7	58.4	15.12
		12	112.6	44.7	15.12
		13	129.3	38.2	11.34
		14	132.1	48.0	7.93
		15	141.6	39.7	28.00
		16	149.5	40.9	16.00
		17	142.1	40.2	22.55
		18	142.1	39.2	18.67
		19	100.0	51.2	22.42
		20	99.0	46.9	28.00

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
18	1	21	114.3	49.3	28.00
		22	114.2	57.0	26.67
		23	113.2	38.1	14.67
		24	106.9	54.3	28.00
		25	108.5	57.6	18.18
		26	116.9	44.3	24.00
		27	113.2	51.9	23.15
		28	110.5	36.4	24.85
		29	109.0	41.4	28.00
19	1	-2	102.2		
		-1	120.4	80.5	
		1	107.2		22.65
		2	133.6	85.2	25.69
		3	171.4		24.71
		4	168.7	74.8	16.37
		5	151.1	66.9	25.10
		6	207.2	68.1	26.76
		7	248.4	74.0	25.78
		8	246.7	71.7	27.84
		9	270.9	79.7	28.04
		10	286.6		25.98
		11	274.1		16.76
		12	240.7		19.51
		13	263.2		26.27
		14	267.0		17.75
		15	250.3		26.76
		16	267.6		24.32
		17	208.8		28.11
		18	149.5		26.85
		19	129.7		27.66
		20	121.5		28.11
		21	112.1		21.80
		22	126.4		25.23
		23	122.5		24.32
		24	120.4		23.15
		25	119.8		25.86
		26	124.2		25.68
		27	116.5		28.11
		28	125.3		21.58
		29	122.6		23.60

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
20	3	-2	85.0		
		-1	86.0		
		1	86.1	16.1	14.66
		2	113.4	21.1	27.97
		3	130.1		15.68
		4			22.20
		5			24.07
		6	146.7	20.6	19.49
		7	162.4	21.3	19.92
		8	210.9	25.4	22.97
		9	252.1	19.8	25.59
		10	266.1	20.0	27.97
		11	286.7	19.4	26.86
		12	296.4	19.8	25.42
		13	315.8	17.1	25.93
		14	340.0	14.1	19.92
		15	357.3	18.6	25.11
		16	304.9	20.7	19.24
		17	257.0	22.3	23.05
		18	196.4	25.2	18.70
		19	147.9		23.59
		20	124.2	49.5	9.00
		21	110.3	60.0	25.95
		22	103.0	63.3	20.76
		23	92.2	42.5	27.33
		24	97.0	49.5	26.56
		25	93.3	20.8	25.73
		26	104.3	17.4	21.15
		27	97.6	17.1	22.37
		28	103.0	22.9	20.84
		29	93.9	27.9	17.40
21	2	-2	92.5	35.9	
		-1	87.6	27.3	
		1	89.4	29.0	26.42
		2	122.5	28.2	18.53
		3	103.6	27.6	23.68
		4	95.9	22.8	28.00
		5	121.3	28.2	26.68
		6	135.5	32.6	28.00
		7	173.9	38.7	28.00

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
21	2	8	171.0	39.7	27.37
		9	214.8	43.3	27.05
		10	238.5	36.5	28.00
		11	258.6	42.2	27.89
		12	284.0	42.9	28.00
		13	311.9	45.1	28.00
		14	323.1	42.9	28.00
		15	323.7	46.4	27.96
		16	232.6	54.6	24.56
		17	228.8	45.8	27.96
		18	179.1	32.1	24.17
		19	127.4	44.2	21.84
		20	127.4	52.4	26.02
		21	124.2	50.1	26.50
		22	116.7	42.8	23.88
		23	103.3	32.0	26.41
		24	106.1	47.2	27.96
		25	120.5	45.1	25.63
		26	105.6	44.2	25.05
		27	101.4	42.2	24.66
		28	108.4	45.6	23.59
		29	95.8	33.2	27.96
22	1	-2	112.2	32.9	
		-1	119.6	33.7	
		1	114.9	31.7	27.92
		2	139.9	32.5	27.92
		3	172.4		18.49
		4	165.6	30.6	12.74
		5	152.7	24.6	23.02
		6	158.8	31.3	19.62
		7	185.1	22.9	20.85
		8	194.6	28.6	17.64
		9	234.5	22.1	15.19
		10	232.5	24.4	22.17
		11	203.4	28.5	18.87
		12	201.4	19.4	13.68
		13	195.3	16.7	21.79
		14	187.8	25.1	15.47
		15	185.4	20.6	23.50
		16	179.1	22.4	13.08

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
22	1	17	153.4	17.2	25.04
		18	150.7	13.5	12.74
		19	131.8	15.3	14.10
		20	133.1	17.3	10.94
		21	144.6		4.61
		22	139.9		
		23	129.1		14.02
		24			6.50
		25			9.40
		26	128.3	16.2	
		27	129.1	23.8	
		28		21.4	
		29		19.9	
23	2	-2	103.3	31.3	
		-1	96.1	26.5	
		1	100.0	32.2	23.12
		2	151.9	65.7	27.41
		3	177.9	90.5	26.44
		4	200.0	79.3	24.68
		5	205.9	44.8	26.93
		6	194.2	46.2	24.00
		7	199.4	45.3	22.93
		8	203.9	45.5	25.07
		9	216.9	46.9	26.54
		10	237.1	54.3	27.12
		11	263.6	54.4	26.54
		12	259.1	52.7	26.15
		13	271.4	67.8	24.98
		14	276.6	51.4	25.17
		15	296.5	49.9	26.58
		16	214.0	51.7	21.44
		17	180.5	50.3	24.77
		18	170.5	45.6	22.61
		19	150.0	38.4	18.42
		20	142.5	44.8	22.16
		21	140.5	40.3	20.54
		22	155.5	56.8	23.33
		23	138.5	39.2	19.19
		24	140.5	39.3	20.63
		25	140.0	33.4	21.17

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
23	2	26	133.0	58.7	24.68
		27	135.0	43.8	23.69
		28	133.5	41.1	23.60
		29	133.0	35.5	16.40
24	4	-2	90.7	25.9	
		-1	80.7	25.5	
		1	88.8	26.8	28.21
		2	108.2	36.4	28.21
		3	126.3	40.1	28.21
		4	140.0	63.0	32.05
		5	143.8	40.6	32.05
		6	146.9	43.3	32.05
		7	169.4	35.7	32.05
		8	192.5	49.8	32.05
		9	201.9	48.5	32.05
		10	228.8	74.8	32.05
		11	240.7	65.6	31.92
		12	230.7	54.1	32.05
		13	238.2	57.9	32.05
		14	220.0	62.2	32.05
		15	217.6	41.5	28.09
		16	156.9	39.4	28.09
		17	150.0	37.8	28.09
		18	103.8	54.1	28.09
		19	105.1	46.3	28.09
		20	104.4	45.6	28.09
		21	107.5	39.4	28.09
		22	96.9	47.4	28.09
		23	103.2	37.3	28.09
		24	107.6	44.6	28.09
		25	111.9	43.4	28.09
		26	98.8	56.2	28.09
		27	100.7	43.4	28.09
		28	100.0	52.6	28.09
		29	101.3	46.7	28.09
25	3	-2	148.5	76.0	
		-1	151.9	70.2	
		1	139.1		28.39
		2	129.6	65.1	28.39
		3	124.1		28.39

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
25	3	4	133.3	58.9	
		5	139.9	79.8	28.39
		6	152.5	71.3	26.46
		7	148.1	60.5	28.39
		8	153.6	55.1	28.39
		9	157.9	54.8	28.39
		10	154.7	61.3	28.39
		11	191.8	54.0	28.25
		12	208.8	65.2	28.39
		13	199.5	58.2	28.39
		14	227.3	66.2	28.13
		15	235.3	67.4	27.83
		16	207.1	72.3	25.78
		17	142.7	56.0	27.44
		18	120.8	69.0	28.06
		19	126.3	65.9	28.06
		20	121.9	69.9	28.06
		21	119.7	74.1	28.06
		22	119.7	85.6	28.06
		23	119.1	75.3	28.06
		24	120.2	70.7	28.06
		25	119.1	81.6	28.06
		26	118.1	84.0	25.67
		27	124.6	81.3	28.06
		28	118.6	80.5	28.06
		29	113.2	79.5	25.33
26	4	-2	80.2	24.9	
		-1	102.5	21.	
		1	93.5	21.3	14.37
		2	115.8	34.1	16.99
		3	134.0	32.0	14.93
		4	161.8	33.0	19.44
		5	147.9	38.9	20.00
		6	174.0	41.4	23.76
		7	206.1	41.7	21.22
		8	224.0	48.7	19.53
		9	231.5	44.6	22.25
		10	244.8	48.2	25.16
		11	249.7	72.3	25.82
		12	254.0	52.9	26.48

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
26	4	13	281.8	59.8	17.56
		14	273.9	47.3	10.14
		15	276.8	35.6	27.81
		16	206.7	38.0	13.82
		17	139.7	37.3	18.77
		18	127.3	30.6	16.58
		19	124.9	46.7	19.56
		20	104.9	26.7	15.44
		21	112.7	27.8	19.82
		22	110.9	32.1	19.21
		23	111.5	31.1	19.74
		24	112.2	34.8	17.28
		25	113.4	37.1	13.25
		26	130.3	41.3	15.96
		27	124.3	45.2	19.39
		28	109.7	48.5	20.88
		29	104.2		21.23
27	4	-2	67.4	46.3	
		-1	63.0	48.3	
		1	64.1	48.7	19.02
		2			27.45
		3	90.0		19.53
		4	115.8	88.4	25.91
		5	156.0	120.6	22.98
		6	175.0	113.2	24.35
		7	188.0	134.3	26.22
		8	187.5	138.4	23.32
		9	191.8	116.3	25.70
		10	218.5	129.2	25.28
		11	172.9	117.3	21.40
		12	171.3	100.5	22.85
		13	155.0	96.3	23.83
		14	155.2	95.3	24.04
		15	128.6	96.3	25.75
		16	113.6	82.6	25.65
		17	92.4	69.2	24.95
		18	119.0	69.4	25.00
		19	99.5	71.7	24.90
		20	85.3	68.2	19.95
		21	87.0	68.0	25.95

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
27	4	22	92.4	65.2	22.30
		23	90.8	60.3	23.85
		24	92.9	61.1	23.55
		25	83.7	67.5	21.20
		26	91.8	59.9	23.60
		27	87.5	67.4	23.00
		28	85.1	65.7	26.25
		29	82.6	59.5	28.00
28	4	-2	76.3	55.5	
		-1	61.8	55.2	
		1	80.9	84.5	26.64
		2	115.6	103.1	26.72
		3	163.0	144.1	27.24
		4	178.6	149.4	28.02
		5	217.9	145.0	25.52
		6	230.6	149.3	25.78
		7	258.4	149.3	25.30
		8	312.7	144.4	26.81
		9	320.8	147.6	26.64
		10	270.5	145.0	27.07
		11	319.1	149.6	27.50
		12	305.2	147.6	24.40
		13	306.5	149.4	24.31
		14	291.5	146.7	16.98
		15	292.9	149.4	21.12
		16	222.5	148.9	22.32
		17	151.4	105.3	28.00
		18	126.6	84.4	26.44
		19	129.5	91.6	21.96
		20	128.3	102.2	21.44
		21	116.3	100.1	17.28
		22	124.3	98.0	25.60
		23	90.8	83.0	13.80
		24	113.9	93.5	17.92
		25	120.1	89.2	18.12
		26	121.5	85.1	18.92
		27	122.9	81.0	20.92
		28	124.3	88.9	20.20
		29		84.9	28.00
29	3	-2	93.0	61.6	

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
29	3	-1	89.2	65.4	
		1	86.6	62.6	12.66
		2	96.8	71.2	27.57
		3	138.7	68.4	24.49
		4	171.5	121.5	28.04
		5	212.9	130.9	28.04
		6	243.5	131.9	24.44
		7	270.4	134.0	25.51
		8	269.4	128.2	26.73
		9	304.3	133.7	23.83
		10	324.7	134.2	24.39
		11	317.2	132.4	22.29
		12	331.2	133.2	24.77
		13	345.7	131.0	24.39
		14	339.8	134.8	22.24
		15	344.3	136.3	23.56
		16	323.7	134.8	22.63
		17	239.8	135.5	25.89
		18	166.1	110.5	26.61
		19	136.0	107.8	22.63
		20	114.5	92.4	20.59
		21	105.4	93.6	20.97
		22	122.0	94.1	22.84
		23	116.0	90.4	24.32
		24	110.8	86.7	25.89
		25	111.3	85.8	25.93
		26	120.4	90.8	21.74
		27	111.8	49.5	18.77
		28	117.7	87.3	25.00
		29	103.2	72.1	23.34
31	2	-2	82.0	58.9	
		-1	58.7	33.0	
		1	59.8	43.2	22.88
		2	94.7	46.1	26.08
		3	123.3	70.9	27.20
		4	110.1	97.4	16.56
		5	136.5	104.3	24.28
		6	148.7	96.0	23.76
		7	158.2	106.3	27.04
		8	175.7	117.4	24.32

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
31	2	9	160.8	131.5	26.32
		10	161.9		25.84
		11	163.0	118.2	23.24
		12	198.9	82.0	22.84
		13	188.1	82.7	22.96
		14	177.2	83.4	23.20
		15	199.6	100.4	19.89
		16		144.6	24.71
		17	172.0	144.6	22.78
		18	130.2		21.83
		19	113.8		22.40
		20	109.0		20.46
		21	103.7		21.90
		22	101.6	79.9	20.84
		23	87.8	76.2	19.32
		24	98.9	78.9	21.67
		25	93.1	81.0	20.03
		26	98.4	55.6	17.60
		27	97.1	78.1	13.16
		28	95.8	76.7	16.28
		29	89.4	76.1	14.14
32	3	-2		74.8	
		-1	42.0		
		1	64.1		23.69
		2	85.9		24.18
		3	99.5		19.91
		4	113.0	81.9	25.69
		5	161.5	82.6	22.13
		6	154.7	88.0	24.36
		7	178.1	64.5	23.96
		8	151.0	127.4	26.49
		9	149.0	133.3	24.53
		10	175.5	127.8	23.11
		11	168.8	144.3	26.44
		12	181.3	144.3	26.36
		13	188.5	145.9	26.31
		14	207.3	134.3	28.00
		15	182.2	119.8	24.34
		16	144.5	110.5	13.11
		17	128.1	101.1	23.61

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
32	3	18	122.4	98.2	25.94
		19	149.0	113.1	24.88
		20	125.4	91.7	25.98
		21	130.2	89.2	26.43
		22	136.1	86.6	26.76
		23	120.0	83.9	18.69
		24	124.9	88.0	8.85
		25	129.8	92.2	13.24
		26	120.2	85.9	21.72
		27	110.7	79.5	21.64
		28	101.7	65.9	22.62
		29	92.7	52.3	22.7
33	1	-2	61.1	47.9	
		-1	77.7	48.2	
		1	44.6	38.6	19.79
		2	70.5	45.2	20.93
		3	87.0	50.5	18.08
		4	91.2	61.2	25.39
		5	114.5	62.2	20.47
		6	104.1	67.1	21.50
		7	125.4	71.6	24.56
		8	176.2	84.2	24.25
		9	131.1	82.8	15.54
		10	130.6	94.6	21.71
		11	122.8	81.5	23.21
		12	129.0	83.0	24.66
		13	120.2		23.26
		14	111.4	74.9	21.97
		15	115.5	86.8	25.00
		16	125.4		23.85
		17	93.8	72.6	28.00
		18	92.3	73.6	28.00
		19	94.3	73.2	27.40
		20	83.9	64.9	26.15
		21	96.9	68.4	25.05
		22	93.3	72.0	23.75
		23	101.6	78.0	26.70
		24	99.5	71.4	27.50
		25	68.4		22.30
		26	72.5	62.0	13.50

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
33	1	27	75.6	65.1	20.70
		28	74.6	66.1	24.95
		29	73.1	61.5	22.40
34	2	-2	65.5	56.4	
		-1	61.5	48.8	
		1	62.0	47.8	20.70
		2	86.5	70.8	23.60
		3	112.0	87.6	27.70
		4	129.0	97.3	28.00
		5	129.0	110.5	28.00
		6	139.5	114.0	28.00
		7	153.5	129.2	28.00
		8	150.0	135.2	28.00
		9	153.5	148.7	28.00
		10	171.0	153.0	28.00
		11	181.5	147.9	26.00
		12			27.50
		13	210.5	150.3	28.00
		14	239.5	152.7	26.00
		15	266.4	151.3	27.24
		16	233.0	151.5	24.62
		17	185.5	153.4	28.00
		18	140.5	122.8	28.00
		19	101.5		28.00
		20	88.0		28.00
		21	87.5	84.6	28.00
		22	85.0	79.8	28.00
		23	76.0	71.7	26.38
		24	90.5	77.1	25.33
		25	93.0	77.5	20.29
		26	88.0	73.0	25.90
		27	87.0	85.3	21.71
		28	91.0	80.8	21.14
		29	86.5	77.0	28.00
35	1	-2	99.5	84.3	
		-1	91.0	76.3	
		1	82.0	73.7	14.04
		2	110.6	102.2	25.02
		3	137.6	128.7	25.79
		4	172.0	147.5	26.13

TABLE VII (Continued)

Pig No.	Treatment	Day on Trial	Total Cholesterol (mg/dl)	HDL (mg/dl)	Feed Intake (g/kg body wt)
35	1	5	191.5	163.0	20.26
		6	192.1	174.3	24.17
		7	221.7	178.2	27.28
		8	228.0	178.9	25.57
		9	296.3	177.5	20.00
		10	229.6	183.8	24.13
		11	257.7	182.6	22.34
		12	241.3	180.3	25.15
		13	246.0	180.3	25.87
		14	323.3	181.7	25.36
		15	281.1	180.3	26.77
		16	284.1	183.0	26.15
		17	247.1	180.8	22.15
		18	159.8		27.08
		19	157.1		25.69
		20	141.8	126.9	24.85
		21	134.9		25.31
		22	128.0		27.92
		23	135.4	126.7	24.77
		24	109.5		26.58
		25	124.3	116.2	24.62
		26	123.8		26.00
		27	129.1	117.9	26.54
		28	134.2	126.0	23.19
		29	125.9		27.08

[a]See footnote Table VI.

TABLE VIII

INDIVIDUAL PIG DATA OF TOTAL SERUM BILE ACIDS

Pig No.	Treatment[a]	Day on Trial	Total Bile Acids µmol/l
9	2	15	11.39
		17	
		19	7.85
		21	
		23	5.07
		25	1.20
		27	.25
		29	
10	4	15	8.94
		17	8.94
		19	5.03
		21	5.03
		23	6.42
		25	7.54
		27	3.07
		29	3.07
11	2	15	20.83
		17	20.38
		19	15.79
		21	12.34
		23	5.98
		25	4.28
		27	6.42
		29	4.83
12	3	15	12.86
		17	11.55
		19	6.76
		21	11.83
		23	6.20
		25	
		27	7.61
		29	8.17
13	3	15	6.12
		17	12.81
		19	3.06
		21	4.96
		23	4.02
		25	5.36
		27	6.89

TABLE VIII (Continued)

Pig No.	Treatment	Day on Trial	Total Bile Acids µmol/l
13	3	29	8.16
14	1	15	7.25
		17	3.37
		19	5.44
		21	8.03
		23	3.37
		25	
		27	3.89
		29	4.92
16	4	15	9.35
		17	4.56
		19	3.59
		21	4.80
		23	3.84
		25	1.92
		27	2.85
		29	2.41
17	1	15	13.37
		17	14.19
		19	15.01
		21	25.71
		23	8.74
		25	5.14
		27	5.40
		29	16.19
18	1	15	16.33
		17	20.84
		19	18.47
		21	11.08
		23	22.34
		25	27.18
		27	14.57
		29	12.40
19	1	15	8.82
		17	18.72
		19	16.85
		21	14.97
		23	6.42
		25	16.58
		27	5.08
		29	9.63

TABLE VIII (Continued)

Pig No.	Treatment	Day on Trial	Total Bile Acids µmol/l
20	3	15	15.99
		17	19.62
		19	
		21	14.36
		23	
		25	11.72
		27	14.79
		29	14.69
21	2	15	10.00
		17	17.03
		19	14.05
		21	8.11
		23	4.86
		25	6.49
		27	6.22
		29	8.92
22	1	15	7.65
		17	7.15
		19	8.97
		21	4.63
		23	8.97
		25	
		27	3.81
		29	
23	2	15	11.89
		17	13.24
		19	11.89
		21	6.22
		23	
		25	10.27
		27	7.03
		29	7.29
24	4	15	15.13
		17	3.08
		19	
		21	1.96
		23	5.72
		25	4.76
		27	2.24
		29	1.4
25	3	15	8.11

TABLE VIII (Continued)

Pig No.	Treatment	Day on Trial	Total Bile Acids µmol/l
25	3	17	7.84
		19	8.38
		21	
		23	10.80
		25	7.03
		27	5.95
		29	6.22
26	4	15	9.04
		17	3.56
		19	4.38
		21	5.75
		23	6.85
		25	4.66
		27	6.58
		29	5.32
27	4	15	6.92
		17	4.36
		19	10.25
		21	4.36
		23	6.92
		25	4.36
		27	4.87
		29	2.56
28	4	15	16.21
		17	16.48
		19	10.71
		21	8.67
		23	9.34
		25	2.18
		27	6.87
		29	3.30
29	3	15	8.12
		17	8.39
		19	8.89
		21	
		23	3.32
		25	7.28
		27	7.28
		29	5.93
31	2	15	8.50
		17	9.92

TABLE VIII (Continued)

Pig No.	Treatment	Day on Trial	Total Bile Acids µmol/l
		19	8.78
		21	9.92
		23	10.37
		25	7.98
31	2	27	7.98
		29	7.45
32	3	15	6.70
		17	5.05
		19	5.74
		21	
		23	4.37
		25	
		27	3.75
		29	3.55
33	1	15	14.36
		17	15.13
		19	8.46
		21	12.05
		23	14.36
		25	6.41
		27	6.92
		29	9.50
34	2	15	6.81
		17	4.63
		19	5.99
		21	7.36
		23	
		25	2.99
		27	1.85
		29	2.18
35	1	15	9.40
		17	9.54
		19	8.31
		21	7.08
		23	8.68
		25	4.36
		27	6.27
		29	3.54

[a]See footnote Table VI.

TABLE IX

INDIVIDUAL PIG WEIGHT

Treatment[a]	Replicate	Pig No.	Weight (kg)		
			Initial	Day 14	Day 29
1	1	5	111.82	118.18	128.18
	1	8	88.64	95.46	104.09
	2	14	85.46	95.46	107.27
	2	17	77.27	88.18	100.46
	3	18	74.55	75.00	84.55
	3	19	92.73	100.91	113.18
	3	22	96.36	106.36	113.18
	4	33	87.73	90.91	100.46
	4	35	106.82	118.18	132.27
2	1	2	103.64	112.27	124.55
	1	3	93.64	101.36	111.36
	2	9	79.09	90.91	100.00
	2	11	90.91	97.73	105.46
	3	21	86.36	93.64	105.46
	3	23	93.18	100.91	109.09
	4	31	113.64	119.55	125.46
	4	34	90.91	95.46	107.27
3	1	6	98.18	109.09	122.27
	1	7	101.82	109.09	117.73
	2	12	85.00	93.64	104.55
	2	13	88.64	97.27	107.27
	3	20	88.18	91.82	98.64
	3	25	70.46	81.82	93.18
	4	29	97.27	107.27	116.36
	4	32	102.27	110.91	116.36
4	1	1	93.18	102.73	113.64
	1	4	107.73	115.91	126.82
	2	10	75.91	87.73	98.64
	2	16	90.91	101.36	114.55
	3	24	70.91	80.91	90.91
	3	26	96.82	103.64	110.00
	4	27	87.73	90.91	100.46
	4	28	105.46	113.64	123.18

[a]See footnote Table VI.

VITA 2

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