POTENTIAL INHIBITORY EFFECT OF WHEY ON HYPOCHOLESTEROLEMIC ACTIVITY OF *LACTOBACILLIS ACIDOPHILUS*

By

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Bachelor of Science in Agriculture

University of Tennessee-Martin

Martin, Tennessee

1988

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May 1991

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ACKNOWLEDGEMENTS

I would like to thank Dr. Stanley Gilliland for serving as my major advisor. My appreciation is also extended to Dr. Charles Maxwell and Dr. Thomas Rehberger for serving on the Advisory Committee and for their guidance and assistance.

I would like to extend many thanks to Mary Cooke and Paul Swartz. Their knowledge and assistance was invaluable to this study.

Many thanks go to faculty members, fellow graduate students and others at OSU who have given of themselves to make my experience here enjoyable and memorable.

Special thanks go to Freddie Gant and David Bishop for their friendship and never-ending support.

Appreciation is extended to the Wisconsin Milk Marketing Board for providing partial support for this study.

Finally, I wish to thank my parents, Gordon and Linda Dodge for their support in my pursuit of this degree as well as in all my other endeavors.

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CHAPTER I

INTRODUCTION

Individuals with elevated blood cholesterol levels are at increased risk for developing atherosclerosis and subsequently coronary heart disease, the leading cause of death in the United States. At the present time the main methods used to control serum cholesterol levels are diet modification (low-fat, low-cholesterol) and the use of drugs. Although these are both effective methods, many people have difficulty maintaining the required diet and most drugs are not without adverse side effects.

Lactobacillus acidophilus is commonly found in the intestines of humans and animals. Some strains of *L. acidophilus* can assimilate cholesterol during growth under conditions expected to exist in the gastrointestinal tract. Studies have shown that the consumption of such strains in milk or in yogurt can reduce serum cholesterol levels in experimental animals. The consumption of dried sweet whey has also been reported to lower serum cholesterol levels in experimental animals.

Since both *L. acidophilus* and whey have been shown to have hypocholesterolemic properties, this study was undertaken to evaluate the possibility of a synergistic

effect and to further evaluate their singular effects on serum cholesterol levels. Young growing swine were used as the experimental model and were fed a high cholesterol diet to induce a hypercholesterolemic condition.

CHAPTER II

REVIEW OF LITERATURE

Origin and Transport of Cholesterol in the Body

Cholesterol is a steroid that serves many functions. It is an essential component of the cell membrane, the immediate precursor of steroid hormones, and the substrate required for bile acid formation (7,53). The cholesterol present in the body originates either from the diet or is endogenously synthesized. Although cholesterol can be synthesized by all tissues except red blood cells, the liver is the major site of its synthesis (32). Dietary cholesterol inhibits cholesterol biosynthesis by suppressing the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the catalyzing enzyme of the committing step in cholesterogensis (28).

Both endogenous and exogenous cholesterol are mainly absorbed in the small intestine. Specific lipoproteins transport cholesterol within the body (4,29,59). Low density lipoproteins (LDL) carry cholesterol in both free and esterified forms to non-hepatic tissues where they bind to receptors and the cholesterol is absorbed. High density

lipoproteins (HDL) carry only unesterified cholesterol away from peripheral tissues to the liver for excretion or catabolism (29,59).

Cholesterol and Coronary Heart Disease

Coronary heart disease is the leading cause of death in the United States and other developed countries (27,57). Epidemiologic studies have indicated that elevated cholesterol levels are highly correlated with the incidence of coronary heart disease. Additionally, the accumulation of LDL cholesterol is reported to contribute to the development of atherosclerosis, the leading cause of coronary heart disease (16,27,28,52). The most convincing evidence implicating cholesterol in the development of atherosclerosis is provided by a condition called familial hypercholesterolemia. In patients having this inherited disorder there is a single-locus gene mutation affecting the catabolism of LDL cholesterol resulting in dramatic increases in the development of atherosclerosis. Thus, elevated levels of LDL cholesterol in plasma are atherogenic (4,25,52). HDL cholesterol, on the other hand, protects against atherosclerosis. The reason why this is true, however, is still unclear (36,52). The best hypothesis for this phenomenon is that HDL cholesterol is involved in reverse cholesterol transport, the movement of cholesterol back to liver for excretion (25,52). Another possible

explanation is that HDL competes with LDL for a specific tissue receptor, inhibiting the uptake of LDL (52).

Hypocholesterolemic Effects of Milk and Milk Products

In the early 1970's Mann and Spoerry (31) suggested the presence of a factor in fermented bovine milk which lowers serum cholesterol. This was postulated after the serum cholesterol levels of 24 Maasai tribesmen fell after daily consumption of large quantities of fermented milk. Since then, there have been many reports on the hypocholesterolemic effects of milk, milk products, and cultured milk products in several animal species and humans (19,20,23,29,30,43,51,54). The hypocholesteremic factor(s) has been postulated as being 3-hydroxy-3-methyl glutaric acid (29), orotic acid (2,3), calcium (54), casein (51), or some metabolite(s) produced by organisms in fermented products (43,19).

Mann (30) fed whole milk, yogurt made from whole milk, or yogurt made from skim milk for twelve days to human subjects. Both types of yogurt produced statistically significant reductions in serum cholesterol compared to the control group but whole milk did not. Mann suggested that the fermentative action of the microorganisms responsible for fermentation during the manufacture of yogurt enhanced or produced the hypocholesteremic factor.

Howard and Marks (20) fed whole milk or reconstituted dried skim milk supplements to human volunteers. After two weeks those subjects receiving whole milk had significantly lower serum cholesterol than did those in the control group. Skim milk produced the same effect after one and two weeks. To help illustrate the effects of the milks, they fed a diet supplemented with butter containing the same amount of fat and cholesterol as the diet supplemented with whole milk and found a highly significant rise in serum cholesterol. Additionally, they gave subjects daily calcium supplements (equal to the content in milk) and found no hypocholesteremic effect. The authors concluded that the hypocholesteremic factor is in the nonfat component of milk and that calcium is not the factor.

Mann (29) postulated that 3-hydroxy-3-methyl glutaric acid was the hypocholesteremic factor when he adminstered radioactive acetate to human volunteers consuming yogurt and found that incorporation of acetate into serum cholesterol was inhibited during yogurt consumption. In a study to test this theory, rats were fed a high cholesterol diet with added skim milk powder or 3-hydroxy-3-methyl glutaric acid. The rats which received skimmed milk powder and 3-hydroxy-3-methyl glutaric acid had lower serum cholesterol levels than the rats on control diets and the effects of both were similar. Although this appeared to support the theory, the milk was not analyzed for 3-hydroxy-3-methyl glutaric acid content.

Orotic acid is present in milk in concentrations of 73-122 mg/l and in higher concentrations in dried milk and dried whey (45). Bernstein et al (2,3) found that both orotic acid and milk inhibited the biosynthesis of cholesterol, although orotic acid did not inhibit the biosynthesis as effectively. This led to the conclusion that orotic acid may be a hypocholesteremic factor in milk but not the only one.

In a study by McNamara et al (34), normolipidemic males consumed 8 oz. of yogurt twice daily for four weeks and 16 oz. daily of 2% milk (similar in composition to the yogurt mix) for another four weeks. No differences were found between the baseline, yogurt, and milk phases of the study. The authors concluded that in normolipidemic individuals, the use of yogurt to influence cholesterol was not effective. These results could be expected since the individuals were kept on a low-fat, low-cholesterol diet. The low initial levels of cholesterol would make it difficult to see any changes.

Stahelin et al (51) fed high fat diets supplemented with either skim milk, yogurt, or casein to pigs. The pigs receiving the skim milk had significantly lower total and HDL cholesterol levels than those in the control group. The pigs which received the yogurt also had lower total cholesterol but the effect was less significant; HDL cholesterol was reduced to the same extent as in the skim milk group. The pigs which received the casein did not show

a hypocholesteremic response. The authors concluded that milk was hypocholesteremic but that casein was not the hypocholesteremic factor and fermentation did not improve the hypocholesteremic response.

Kritchevsky et al (23) reaffirmed the hypocholesterolemic effect of milk in a study where rats were given either whole milk, skim milk, or water to drink. The group receiving water served as the control and all treatments were fed a stock diet. The group receiving water had significantly higher serum cholesterol levels than the milk groups.

Thompson et al (55), on the other hand, reported no effects of milk supplementation on LDL or HDL cholesterol levels. Human volunteers were given 1l daily of skim milk, 2% milk, whole milk, sweet acidophilus milk, buttermilk, or yogurt. There was no significant reductions in cholesterol in any of the groups although skim milk reduced levels by a larger percentage. However, changes in the cholesterol levels may not have been seen since the individuals in this study had normal or low serum cholesterol values.

In a study by Rao et al (43) rats were fed diets supplemented with water, skim milk, and thermophilus milk (milk fermented with *Streptococcus thermophilus*). Thermophilus milk significantly reduced total plasma cholesterol levels compared to both water and skim milk. When rats were fed diets supplemented with methanol solubles from whole thermophilus milk, plasma cholesterol levels were

significantly reduced compared to diets supplemented with solubles from non-fermented whole milk. The authors concluded that fermentation by *Streptococcus thermophilus* made the milk hypocholesteremic.

When Thakur and Jha ((54) fed rabbits high cholesterol diets supplemented with milk, yogurt or calcium, all groups exhibited reduced cholesterol levels. Yogurt and calcium, however, produced more significant effects than milk. They concluded that calcium may play a role in producing the hypocholesteremic response.

Hepner et al (19) found that both pasteurized and non-pasteurized yogurt reduced cholesterol in human volunteers. Milk also had a hypocholesteremic effect but to a lesser extent compared to the yogurts. It was concluded that yogurt is hypocholesteremic and milk may be.

Lactobacillus acidophilus as a Dietary Adjunct

Lactobacillus acidophilus is present in the gastrointestinal tract of many animal species including swine, rodents, and poultry and also in humans (5,11,12). Some strains of *L. acidophilus* are species specific as they bind only to the gastric epithelial surfaces in certain species (48). This may be mediated by protein receptors in the epithelial cells (48).

Lactose intolerance is a condition characterized by the inability to consume unfermented milk products without experiencing such discomforts as flatulence and diarrhea. This is due to an inability to digest lactose because of inadequate β-galactosidase production in the small intestine (10). The consumption of dairy products containing cells of *L. acidophilus* can make it possible for individuals with this disorder to consume dairy products by providing the lactase enzyme (22,49).

L. acidophilus is also able to produce substances which inhibit growth of such pathogens as *Escheria coli* and *Salmonella typhimurium*. Ooi et al (41) reported that hydrogen peroxide produced by *L. acidophilus* may inactivate the cytotoxin produced by *Clostridium difficule*. This cytotoxin is associated with diarrhea resulting from antibiotic drug therapy. Gilliland and Speck (12) reported that *L. acidophilus* inhibited the growth of *Staphylococcus aureus,* S. *typhimurium* and enteropathogenic E. *Coli*. They also noted that there were differences among strains of L. *acidophilus* in the intensity of the inhibition and also that the inhibition was not due solely to acid production since inhibition was observed when the pH of the growth medium was maintained at 6.5.

More recently McGroarty et al (32) reported that a strain of L. *acidophilus* produced a substance that inhibited growth of enteropathogenic E. *coli* when grown in MRS broth.

This activity was lost, however, when the broth was adjusted to pH 7.0.

L. *acidophilus* has also been shown to stimulate the immune response. Perdigon et al (42) fed milk supplemented with a mixture of L. *acidophilus* and L. *casei* to mice. This resulted in lymphocyte activation, an indicator of immune response.

Hypocholesterolemic Effect of *Lactobacillus acidophilus*

When Maasai tribesmen consumed large quantities of milk fermented with a "wild strain" of lactobacillus their serum cholesterol levels fell (31). This was true even when the milk was supplemented with a surfactant (Tween 20) that was thought to enhance lipid absorption.

Tortuero et al (56) found that the implantation of L. *acidophilus* in cecectomized and normal laying hens resulted in a significant reduction in serum cholesterol levels. Hens were fed a diet 0.2% added cholesterol and L. *acidophilus* was given three times at one month intervals in capsules containing $3x10^6$ organisms. Those receiving the L. *acidophilus* showed a significant decrease in cholesterolemia after one month.

Grunewald (17) fed rats a stock diet and drinking water containing no milk, 10% milk, or 10% milk fermented with L. *acidophilus*. After four weeks the rats receiving the L.

acidophilus milk had significantly lower serum cholesterol levels than the control or milk groups. She theorized that some metabolite(s) produced during fermentation produce the hypocholesteremic effect.

When human infants were fed humanized milk formula with and without supplementation of L. *acidophilus,* the infants receiving the L. *acidophilus* formula had significantly lower serum cholesterol levels. This was associated with an increase in the numbers of lactobacilli in their stools (18). These results suggested that high numbers of lactobacilli in the stool were directly correlated to low serum cholesterol levels and that lactobacilli in the intestinal tract may play a role in the control of serum cholesterol.

Gilliland et al (15) were the first to report the cholesterol assimilation abilities of L. *acidophilus*. They reported that in the presence of bile and anaerobic conditions, some strains could assimilate cholesterol although strain variations were dramatic. When pigs were fed a high cholesterol diet and given either an assimilating strain of L. *acidophilus* or a non-assimilating strain, the serum cholesterol levels of the pigs recieving the assimilating strain were significantly lower.

Danielson et al (8) fed mature boars a high cholesterol diet supplemented with yogurt fermented with a strain of L. *acidophilus* able to assimilate cholesterol. Analysis of weekly blood samples indicated that the acidophilus yogurt

significantly reduced total and LDL serum cholesterol. There was no effect on HDL cholesterol levels. The authors concluded that L. *acidophilus* yogurt could be used to reduce cholesterol but strain selection is crucial.

Mott et al (38) suggested that intestinal flora plays a decisive role in steroid metabolism. In this study germ-free pigs were raised in a sterile environment and at 2-3 weeks of age monocontaminated with L. *acidophilus*. One group was then allowed to develop normal intestinal flora. This group exhibited much lower serum cholesterol levels than those animals that remained in the sterile environment. Eyssen (10) also reported that conventional animals (normal flora) excrete more cholesterol than do germ-free animals.

Wycoff (60) reported that *L. acidophilus* had no effect on cholesterol levels in young pigs given *L. acidophilus* alone or in combination with dried whey. There was tremendous variation with respect to blood cholesterol levels among animals in the same treatments and this may have contributed to the lack of effect.

Hypocholesterolemic Effect of Whey

There have been several reports indicating the consumption of dried whey can reduce serum cholesterol levels. Norton et al (40) assigned four barrows to one of four diets having different levels of cholesterol and whey. Analysis of daily blood samples indicated that the addition

of cholesterol in the diet significantly increased serum cholesterol levels but the animal recieving a 40% whey diet had significantly lower cholesterol levels compared to the non-whey diet. HDL cholesterol was increased for the whey diet.

Stahelin et al (50) found that the feeding of 50% whey significantly decreased serum cholesterol levels in swine over a four week period. This decrease was also observed after the animals were taken off the whey diet for 4-weeks and then put back on the whey diet. The HDL cholesterol values were also significantly lowered in this study.

In a separate study by Stahelin et al (51), 48 swine were fed either whey , whey fermented with lactobacillus, or lactose for six weeks after a three week period of feeding a high fat diet. Whey and fermented whey lowered total cholesterol although not significantly. Fermented whey had a slightly greater hypocholesteremic effect than did non-fermented whey. Lactose did not precipitate a change in total cholesterol. The authors concluded that while whey does seem to reduce cholesterol levels, lactose is not the responsible factor.

Wycoff (60) found that the feeding of whey to young pigs had no effect on serum or HDL cholesterol values. However, excessive variation in serum cholesterol levels among animals may have masked any benefit from the whey.

Beames et al (1) studied the effects of whey and specific whey fraction on cholesterol levels in swine.

Although whey, whey proteins, and lactose caused reductions in serum cholesterol, they were not significant.

The Use of Drugs in Lowering Cholesterol Levels

The use of drug therapy in the treatment of hypercholesterolemia has been researched extensively. Although drug treatment is effective in reducing cholesterol levels they are not without side effects, some serious. Cholestyramine interferes with the enterohepatic circulation of bile acids by binding them and increasing their fecal excretion. The side effects associated with this drug, although not serious, include constipation, heartburn, and nausea (27). Probucol enhances LDL-cholesterol breakdown. Diarrhea is associated with this drug (9). Lovastatin and simvastatin are HMG-coA reductase inhibitors. The most common side effects of these drugs include headaches and constipation, and more serious ailments such as liver enzyme elevation, muscle pain, and eye lens opacity can occur (21,58).

Despite the fact that most side effects are mild and transient and that these drugs are effective, some individuals may not desire or be able to tolerate them. Therefore possible alternative treatments should be evaluated.

The Pig as a Model System

The pig is one of the most ideal animal models for cardiovascular research. Elevations in plasma cholesterol can be achieved readily by dietary manipulation since the pig is an omnivore by nature (6,40,50,59,60). Their lipoprotein structures are similar to man's as well as their coronary arterial and digestive systems (59,24,35,44). Swine also exhibit atherosclerotic tendencies similar to humans (23).

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CHAPTER III

Potential Inhibitory Effect of Whey on Hypocholesterolemic Activity of *Lactobacillus acidophilus*

ABSTRACT

This study was to evaluate the potential benefit of combining cells of *Lactobacillus acidophilus* ATCC 43121 and dried sweet whey as a dietary supplement to aid in controlling serum cholesterol. Four treatment groups of five week old Yorkshire gilts were fed a corn/soybean meal based diet with 0.2% added crystalline cholesterol. Diets for treatments (trt) 1 and 2 also contained 20% dried whey. Trt 1 and 3 received 50 ml sterile milk and trt 2 and 4 received 50 ml of milk containing cells of *L. acidophilus* 43121 prior to each feeding. Total and HDL serum cholesterol levels increased significantly ($P<.05$) in all treatment groups in two trials. In both trials, pigs receiving the whey diet had significantly greater increases ($P<.05$) in total cholesterol levels than those receiving the nonwhey diets. HDL cholesterol levels were not significantly influenced by treatment in either trial.

Analysis of feed samples showed the whey diet to be inhibitory to the growth of *L. acidophilus* 43121 compared to the nonwhey diet. Laboratory analysis of whey samples obtained from commercial sources revealed significant variation in inhibiting the growth activity of *L. acidophilus* 43121. Thus, care should be exercised in selecting dried whey as a dietary ingredient when using *L. acidophilus* as a dietary supplement. The inhibitory action of whey in such situations may prevent any benefit which requires growth of *L. acidophilus* in the intestine.

INTRODUCTION

Coronary heart disease is the leading cause of death in the United States (13,25). Recently it has been established that elevated total serum cholesterol levels, including low density lipoprotein (LDL) cholesterol levels, increase the risk of developing atherosclerosis, a major cause of coronary heart disease (9,13,14,23). Conversely, elevated levels of high density lipoprotein (HDL) cholesterol protects against coronary heart disease (16,23,27). LDL carries cholesterol to non-hepatic tissues where it then facilitates the transport of cholesterol into the cells. HDL appears to transport cholesterol out of tissues to the liver for excretion (13,16,27).

Although several drugs have proven effective in controlling serum cholesterol levels, most are not without

undesirable side effects (12,13,26). Some strains of *L. acidophilus* have the ability to assimilate cholesterol under conditions expected to exist in the gastrointestinal tract (6). The consumption of *L. acidophilus* in fermented and unfermented milk products has been shown to help control serum cholesterol levels in swine (4,6), laying hens (24), rats (10), and human infants (11). In particular, strain ATCC 43121 (formerly RP32) has been reported as being both bile resistant (important for growth in the intestine) and able to assimilate large amounts of cholesterol compared to other tested strains (6). The consumption of dried whey also has been shown to reduce serum cholesterol levels (2,18,21). The use of selected strains of *L. acidophilus* alone or in combination with whey as a dietary supplement may offer an alternative to the use of drugs to lower levels of serum cholesterol.

The objective of this study was to evaluate the effect of feeding cells of *L. acidophilus* ATCC 43121 and dried whey, separately and together, on total and HDL cholesterol levels in the serum of young growing swine fed a high cholesterol diet.

MATERIALS AND METHODS

Source and Maintenance of Lactobacillus acidophilus ATCC 43121

Lactobacillus acidophilus ATCC 43121 (formerly strain RP32) was obtained from the culture collection of the Dairy

Foods Microbiology Laboratory at Oklahoma State University. The culture was maintained by subculturing weekly (1% inoculum and 18 hr incubation at 37°C) in sterile Peptonized Milk Nutrient (PMN) broth (7) and storing at 4°C between transfers. The culture was subcultured in this way on two consecutive days immediately prior to use.

Preparation of Frozen Concentrated Cultures

Cells of *L. acidophilus* 43121 were grown in 4 l of PMN broth (1% inoculum) under constant agitation,at pH 5.0 (+0.1), and at 37°C in a 7 l fermenter (New Brunswick Scientific Co., Edison NY) for 18 hr as described by Gilliland and Rich (8). Cells were harvested by centrifugation (5000 x g at 0°C for 20 minutes). The cell pellets were resuspended in twice their weight of cold, sterile 10% reconstituted nonfat dry milk (NDM) and aseptically dispensed into sterile cryogenic vials in 2g portions. The vials were then frozen and stored in liquid nitrogen.

The storage stability and population of *L. acidophilus* 43121 was based on plate counts. The frozen concentrated cultures were thawed by placing the frozen vials in 1 l of tap water at room temperature for 5 min. The appropriate dilutions were prepared using sterile 0.1% peptone water (8). Dilutions were plated (pour plate method) on PMN agar (PMN broth + 1.5% agar) and PMNO agar (PMN agar + 0.1 percent oxgal) on day 0 (before freezing) and following

7,14,21, and 28 days of storage. Plates were incubated in a CO_2 enriched atmosphere at 37°C for 48 hr (8).

Feeding Trials

Two trials were conducted, each utilizing 24 5-week old Yorkshire gilts. Gilts were randomly assigned to individual pens equipped with automatic waterers and to 4 treatment groups (6 pigs/group). The first week, all pigs were fed a starter diet *ad libitum*. At the end of this week, pigs were weighed and for a one week adjustment period, all were fed a corn/soybean meal based diet (2.5% of body weight) without added cholesterol or whey (Table I). Pigs also received 50 ml of sterile reconstituted 10% NDM twice daily just prior to feeding the dry diet. The experimental period lasted 16 days and followed the adjustment period. All treatment groups received the corn/soybean meal diet containing 0.2% crystalline cholesterol. The diet for treatments 1 and 2, designated as whey diet, also contained 20% dried sweet whey (Table I). The nonwhey diet contained more corn, soybean meal, calcium carbonate, and dicalcium carbonate to compensate for nutrients contained in whey. All pigs were fed twice daily at 2.5% of their body weight. On days 0 and 7 of the experimental period, pigs were weighed and feed amounts were adjusted as needed. Any feed remaining two hours after feeding was removed and weighed. In addition to the dry diet, groups 1 and 3 were given 50ml of sterile reconstituted 10% NDM just prior to each feeding. Groups 2

and 4 received the same amount of reconstituted 10% NDM supplemented with 5 x 10^{10} cells of *L. acidophilus* 43121.

To prepare the milk containing *L. acidophilus* 43121, the required number of vials of frozen concentrated culture were thawed in 1 l of water at room temperature for 5 minutes and the appropriate amount added to the milk just prior to feeding. The milk was fed to the pigs in individual bowls.

Blood Collection and Analyses

Blood samples were taken on three consecutive days prior to the start of the experimental period (Period 1), on days 5,6, and 7 of the experimental period (Period 2), and on days 14,15, and 16 (Period 3). Samples were taken after a 12 h fast via vena cava puncture with Vacutainers (Becton Dickenson and Co., New Jersey) fitted with sterile 20 guage needles. Immediately following collection, the blood samples were placed on ice for transport to the laboratory. The samples were held at 4^{0}C for at least 4 hours and then the serum fractions were collected by centrifugation (3,000 x g) using Auto Iso-filters (Clay Adams, Parsippany NJ). Each serum sample was transferred to 5ml cryogenic vials and frozen at -20^{o}C until individual trials were completed. Duplicate serum samples were analyzed for total and HDL serum cholesterol using the Sigma enzymatic reagent kit (Sigma Chemical Co., St. Louis, MO.). The procedure for

spectrophotometers requiring greater than 1 ml volumes was followed (20). Values for both types of cholesterol were averaged over each three day period to obtain single values for each sampling period (Periods 1, 2, and 3). Increases in cholesterol levels were determined by subtracting Period 1 values from values for Periods 2 and 3.

Influence of Whey and Nonwhey Diets on Growth of Lactobacillus acidophilus ATCC 43121

Samples of both experimental diets were analyzed to determine if they were inhibitory to the growth of *L. acidophilus* 43121. Thirty grams of each feed sample and 100ml of sterile distilled water were mixed together on a magnetic stir plate for 15 min. at room temperature. The mixtures were transferred to 250ml centrifuge bottles and centrifuged (5000 x g) for 10 min. The supernatant fluids were collected and passed through sterile 0.45 micron pore membrane filters into sterile containers. Aliquots of the filtered supernatants were inoculated (1%) with a broth culture of *L. acidophilus* 43121 and incubated in a 37^{o}C water bath. Inoculated lactobacilli MRS broth (Difco Laboratories, Detroit, Michigan) was used as the control. The A_{620nm} was measured hourly and the increases plotted against incubation time. This procedure was repeated three times and the values averaged.

Influence of Dried Whey on Growth of Lactobacillus acidophilus ATCC 43121

Dried whey samples were obtained from three commercial sources. Samples from twelve different lots of whey were included. Six grams of each whey sample was mixed with 100 ml of sterile distilled water and centrifuged (procedure same as above for feed samples). The resulting 6% whey supernatants were used to determine the influence of the whey on the growth of *L. acidophilus* 43121. Five milliliters of lactobacilli MRS broth was mixed with 3 ml of sterile distilled water and 2 ml of the 6% whey supernatant to yield a liquid growth medium containing 1.2% whey solids. MRS broth was used as the control medium. These were inoculated (1%) with *L. acidophilus* 43121 and the A_{620nm} was measured initially and after 3 h incubation in a 37^0C water bath. Increases in A_{620nm} were used to compare growth. Three trials were performed in this manner and increases in A_{620nm} were averaged for each sample.

Statistical Analyses

Data from both feeding trials and the whey analyses was analyzed separately using the general linear models procedure from the Statistical Analysis System package (1). The least significant difference mean separation procedure was used to determine if statistically significant differences existed among the means of increases in serum cholesterol levels for the 4 treatment groups at periods 2 and 3. The same procedure was used to determine if

statistically significant differences occurred among the amounts of growth of *L. acidophilus* in broth containing the whey samples.

RESULTS

Preparation of Frozen Concentrated Cultures

The freezing and storage of *L. acidophilus* 43121 in liquid nitrogen did not affect the viability or bile resistance of the cells. This was determined by plate counts on PMN and PMNO agar. The ability of *L. acidophilus* to withstand long-term storage in liquid nitrogen has been documented in previous studies (7,8).

Feeding Trial 1

At the start of the experimental period, one pig was removed from treatment group 1 because it was unable to operate the waterer resulting in a reduction in feed intake. The remaining pigs were healthy throughout the trial and were consuming all feed in the allotted 2 hr periods. Weight gains did not differ among treatment groups. Total cholesterol levels increased at each sampling period for each treatment group, as expected from feeding a diet high in cholesterol (3,22). Mean increases in total cholesterol values are given in Table II. Supplementation with *L. acidophilus* 43121 had no significant effect on total cholesterol values, although those groups receiving *L.*

acidophilus 43121 tended to have smaller increases in total cholesterol than did those not receiving the organism. Pigs receiving the whey diets, however, had significantly higher increases in total choleterol levels than those on the nonwhey diet. ($P<.05$). There was a significant interaction ($P<.05$) between whey and *L. acidophilus* 43121 between Periods 2 and 3. This can be seen by comparing the increases in cholesterol values between periods 2 and 3 for the group receiving the nonwhey diet plus *L. acidophilus* 43121 (trt 4) and the group receiving the whey diet plus *L. acidophilus* 43121 (trt 2). The increase in cholesterol values in the trt 4 group was much less than in the trt 2 group.

HDL cholesterol values increased significantly ($P<.05$) in all four treatments groups during the experimental period (Table III). There were no significant differences in HDL cholesterol values between groups although treatment 2 tended to have larger increases in HDL cholesterol values than the other treatments. In the treatment groups recieving the nonwhey diets, the group supplemented with *L. acidophilus* 43121 tended to have smaller, although not significant ($P<.05$), increases in HDL cholesterol levels.

Feeding Trial 2

Throughout the trial all pigs remained healthy and weight gains were not significantly different among treatment groups.

Total cholesterol levels increased significantly across all periods in all four treatments (Table II). Supplementation with *L. acidophilus* 43121 had no significant effects on increases in total cholesterol values. However, the pigs receiving the whey diet had significantly larger ($P<.05$) increases in total cholesterol values compared to the nonwhey diet. HDL cholesterol values increased significantly ($P<.05$) in all four treatments (Table III). There were no significant differences among treatment groups.

Influence of Feed and Whey on Growth of Lactobacillus acidophilus 43121

The tendency toward higher cholesterol levels in the serum of pigs receiving the whey diet plus *L. acidophilus* 43121 compared to that in the pigs receiving the nonwhey diet plus *L. acidophilus* 43121 could indicate some inhibitory action by the whey on the growth of *L. acidophilus* 43121. Laboratory tests of water soluble materials extracted from both diets indicated some inhibition by the whey diet. As shown in Fig. 1, the aqueous extract of the whey diet inhibited the growth of *L. acidophilus* 43121 when compared to the aqueous extract of the nonwhey diet. After five hours of incubation the amount of growth in the extract from the whey diet was much less than in either the extract from the nonwhey diet or the MRS

broth control. This was a preliminary study and the results were not statistically analyzed.

Tests of whey samples from different commercial sources showed significant variation with respect to influencing growth of *L. acidophilus* 43121 (Table IV). Growth was significantly ($P<.05$) slower in samples 1,2,4,7,8,9, and 10 compared to the control and samples 3,5,11, and 12. Amounts of growth in samples 3,5,6,11, and 12 were not significantly different than in the control ($P<.05$). Growth in sample 9 was significantly less ($P<.05$) than in all other samples.

DISCUSSION

The latest U.S. Census (25) indicated heart disease as the leading cause of death in the United States. The Lipid Research Clinics Program (15) reported that high serum cholesterol levels increase the risk for the development of coronary heart disease. Thus, ways to reduce serum cholesterol levels are being sought. Several studies have reported that the consumption of *L.acidophilus* or whey can reduce serum cholesterol levels in animals (2,4,6,10,18,21) and in humans (11).

Pigs were chosen as an animal model because they have digestive and blood circulatory system similar to humans (16,17,19). *L. acidophilus* 43121 was chosen for use in this study because of its ability to assimilate cholesterol and also because it is of pig origin (6). In both feeding

trials, the addition of *L. acidophilus* 43121 had no significant effects on total cholesterol levels although there was a tendency for those receiving it to have smaller increases in cholesterol levels than those treatments not receiving it. Gilliland et al (6) reported that pigs fed *L. acidophilus* 43121 had significantly lower cholesterol levels than pigs receiving a nonassimilating strain of *L. acidophilus* or no lactobacilli. Wycoff (28) reported no significant influence of *L. acidophilus* 43121 when fed to young pigs. He suggested that the extensive day-to-day variation in blood cholesterol levels within treatments may have concealed any treatment effects. To minimize the influence of the day-to-day variations in the present study, blood samples were taken on three consecutive days and the cholesterol values averaged for Periods 1, 2, and 3.

In both feeding trials, the groups receiving whey had significantly greater increases in total cholesterol levels than those receiving the nonwhey diets. These findings are not in agreement with previous studies. Beames et al (2) reported that the addition of whey to the diet reduced cholesterol levels, although not significantly. Norton et al (18) reported significant reductions in serum cholesterol levels when whey was added to a high cholesterol diet. Wycoff (28) observed no significant influence of whey on cholesterol levels in young pigs. In none of these studies was the source the whey documented nor did they include

tests of the influence of whey on growth of the lactobacilli.

The tendency of whey to increase total cholesterol levels indicated the possibility of an inhibitory action by whey on the growth of *L. acidophilus* 43121 or other microorganisms present in the intestines that might influence cholesterol absorption. Tests of the aqueous extracts of the whey and nonwhey diets indicated that growth of *L. acidophilus* 43121 was suppressed by the whey when compared to the nonwhey diet. The influence of whey on total serum cholesterol levels is inconsistent among published studies (2,18,21,22) suggesting that dried whey from different sources may vary in their influence on serum cholesterol levels. The different types of whey analyzed in this study show significant variation with respect to the influence on the growth of *L. acidophilus* 43121. The results suggest the presence of an inhibitory substance(s) in some whey samples. The ability of some whey to inhibit the growth of lactobacilli may have been the cause of variable results reported in the literature on the effect of whey on serum cholesterol levels. Microorganisms in the intestines can reduce serum cholesterol levels (5). Thus anything that represses their growth may have an adverse effect by causing increases in serum cholesterol levels. Further research is needed to determine exactly what the inhibitory component(s) in certain types of whey is and how

this effects the growth of *L. acidophilus* under *in vivo* conditions.

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FIGURE 1

INFLUENCE OF WHEY AND NONWHEY DIETS ON GROWTH OF LACTOBACILLUS ACIDOPHILUS ATCC 43121

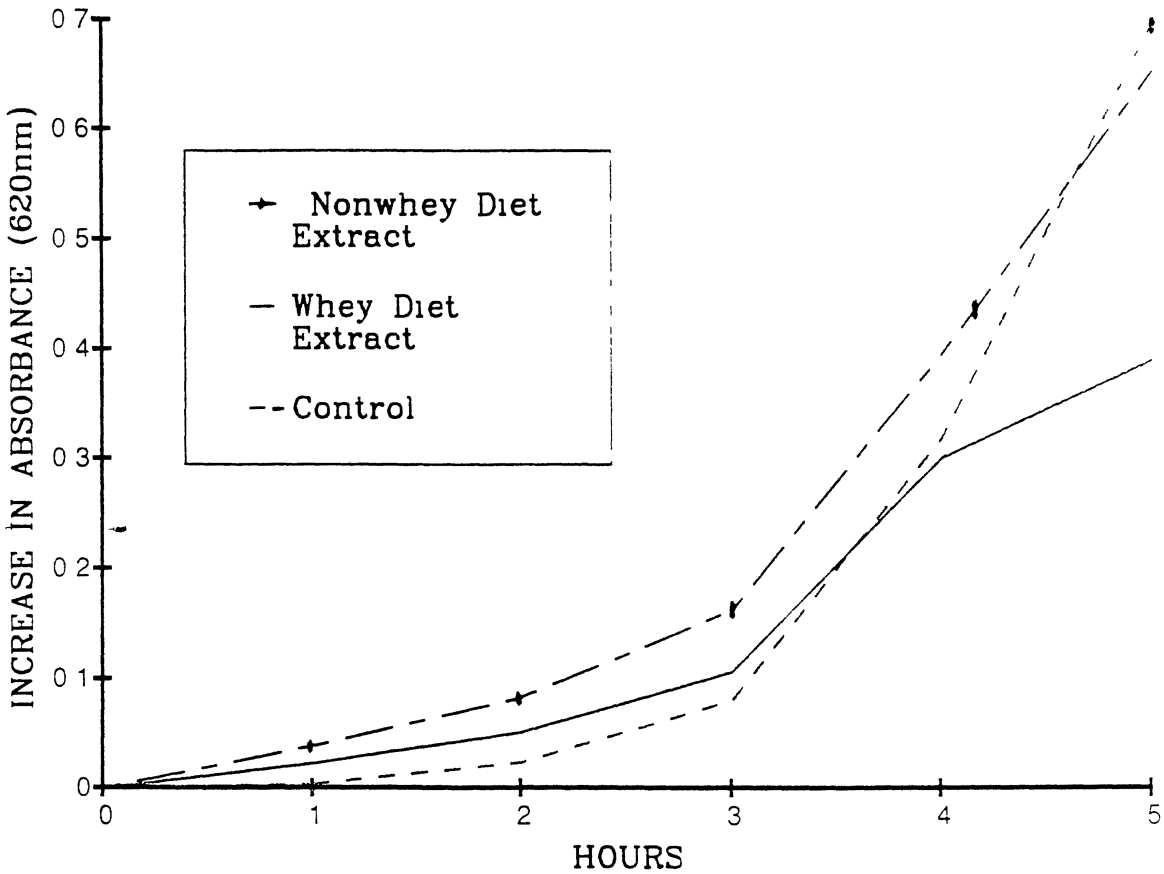

TABLE 1. Percentage Composition of Diets

Item	Adjustment Diet	Whey Diet	Nonwhey Diet
Corn	57.44	42.25	57.24
Soybean meal	31.40	27.00	31.40
Whey	-----	20.00	-----
Calcium carbonate	01.06	00.90	01.06
Dicalcium carbonate	01.95	01.50	01.95
Butter	07.50	07.50	07.50
Salt	00.25	00.25	00.25
Vitamin-trace mineral mix[a]	00.40	00.40	00.40
Cholesterol	-----	00.20	00.20

[a]Supplies 3628,874 IU vitamin A, 36,287.4 IU vitamin D, 1542.2 IU vitamin E, 1814.4mg pantothenic acid, 2449.4mg niacin, 362.9mg riboflavin, 299.4mg menadione, 1.8mg vitamin B_{12}, 36,287.4mg chlorine, 8.2mg selenium, 2.3g manganese, 8.2g iron, 0.91g copper, and 16.3mg iodine per kg of vitamin trace mineral mix

TABLE 2. Influence of feeding *Lactobacillus* acidophilus and whey on total serum cholesterol levels in pigs fed a high cholesterol diet

		Increase in Cholesterol (mg/dl)[1]			
		Trial 1		Trial 2	
Treatment		P2[2]	P3[3]	P2[2]	P3[3]
1	Whey	47.54±3.79	50.90±3.79[a]	20.13±3.62	36.21±3.62[a]
2	Whey + *L. acid.*	36.12±3.46	48.73±3.46[a]	21.19±3.62	35.59±3.62[a]
3	Nonwhey	22.08±3.46	40.05±3.46[b]	19.62±3.62	23.39±3.62[b]
4	Nonwhey + *L. acid.*	24.29±3.46	28.87±3.46[b]	20.38 3.62	25.21±3.62[b]

[1]each value represents the mean ± standard deviation from six pigs (except treatment 1 in trial 1 where n=5); values in the same column with different superscripts are significantly different (P<.05); values increased significantly from Period 1 to Period 2 and from Period 1 to Period 3 in all treatments (P<.05)
[2]Period 2 (midway in feeding trial)
[3]Period 3 (end of feeding trial)

TABLE 3. Influence of feeding *Lactobacillus* acidophilus and whey on HDL cholesterol levels in pigs fed a high cholesterol diet

		Increase in HDL (mg/dl)[1]			
		Trial 1		Trial 2	
Treatment		P2[2]	P3[3]	P2[2]	P3[3]
1	Whey	14.13±2.10	16.17±2.10	6.18±1.62	10.22±1.62
2	Whey + *L. acid.*	12.78±1.92	20.15±1.92	7.26±1.62	10.66±1.62
3	Nonwhey	9.09±1.92	17.76±1.92	5.79±1.62	8.97±1.62
4	Nonwhey + *L. acid.*	7.22±1.92	9.19±1.92	3.88±1.62	7.36±1.62

[1]each value represents the mean ± standard deviation from six pigs (except treatment 1 in trial 1 where n=5) ; there were no significant differences (P>.05) among treatments in either trial; values increased significantly from Period 1 to Period 2 and from Period 1 to Period 3 in all treatments (P<.05)
[2]Period 2 (midway in feeding trial)
[3]Period 3 (end of feeding trial)

TABLE 4. Influence of dried whey on the growth of *Lactobacillus acidophilus* ATCC 43121

Whey sample[1]	Increase in A_{620nm} after 3 hours
control	0.464^{c}
3	0.457^{cd}
12	0.454^{cde}
11	0.446^{cde}
5	0.446^{cde}
6	0.438^{cdef}
4	0.426^{defg}
2	0.423^{efgh}
8	0.409^{fghi}
1	0.398^{ghij}
10	0.394^{hij}
7	0.379^{ij}
9	0.368^{j}

[1]whey samples were obtained from commercial sources
[2]each value represents an average of three trials; means with the same superscript are not significantly different ($P<.05$)

CHAPTER IV

SUMMARY AND CONCLUSIONS

The increasing awareness of coronary heart disease and the associated risk factors has prompted much research. Among the risk factors associated with cardiovascular disease is elevated cholesterol levels (hypercholesterolemia). The current protocol for treating hypercholesterolemic individuals includes diet modification and medication. These methods, however, are not acceptable to some people and more importantly, most of the drugs have associated adverse side effects. The consumption of cells of *Lactobacillus acidophilus* (a common intestinal bacteria) and dried sweet whey have been shown to reduce or limit increases in serum cholesterol levels in experimental animal models. The purpose of this study was to evaluate the use of *L. acidophilus* ATCC 43121 (a strain with ability to assimilate cholesterol) and dried sweet whey, separately and together, as an alternate method to control cholesterol levels.

The results of this study were quite unexpected in that the supplementation of high cholesterol diets of six-week old pigs with whey did not limit increases in serum cholesterol levels. In fact, the pigs receiving the whey

diet, with or without *L. acidophilus* 43121, had significantly greater increases in total cholesterol levels than did those receiving the control diet. Furthermore, supplementation of the diets with *L. acidophilus* 43121 did not significantly effect total cholesterol levels, although pigs receiving these treatments did tend to have smaller increases in cholesterol levels than those in the other treatment groups. HDL levels were not significantly influenced by either whey or *L. acidophilus*.

The finding that whey did not reduce cholesterol levels alone or in conjuction with *L. acidophilus* 43121 prompted an investigation into the possibility that the whey may have been inhibitory to the growth of *L. acidophilus* 43121. Analysis of the diets used in the feeding trials indicated the water soluble portion of the whey diet inhibited the growth of *L. acidophilus* 43121 when compared to that of the nonwhey diet. Laboratory tests of individual whey samples from different commercial sources showed significant variation with respect to the influence on the growth *of L. acidophilus* 43121. Some were significantly inhibitory and some were not. The intensity of the inhibition varied among whey samples. These results suggest the presence of an inhibitory substance(s) in some lots of whey. Further research is needed to understand the mechanisms by which *L. acidophilus* and whey reduce serum cholesterol levels and also to understand how the inhibitory substance(s) effect the growth of *L. acidophilus* in vivo.

APPENDIXES

APPENDIX A

ASSIGNMENT AND AGE OF PIGS IN TRIAL 1

TABLE V

ASSIGNMENT OF PIGS TO TREATMENTS
FOR TRIAL 1

TRT 1		TRT2		TRT3		TRT4	
PEN NO.	EAR NOTCH	PEN NO.	EAR NOTCH	PEN NO.	EAR NOTCH	PEN NO.	EAR NOTCH
7	82-4	3	79-9	1	82-5	5	83-11
10	82-6	4	81-15	2	84-6	6	83-6
16	81-12	19	84-7	11	81-14	8	81-13
17	79-7	20	83-7	12	83-5	13	84-9
18	84-8	22	84-5	15	82-7	14	81-5
		24	80-11	21	83-8	23	80-10

TABLE VI

AGE OF PIGS USED IN TRIAL 1

LITTER NO.	BIRTHDATE	DAYS OF AGE AT ADJUSTMENT PERIOD	NUMBER OF PIGS IN TRIAL
79	5-23-88	44	2
80	5-24-88	43	2
81	5-25-88	42	5
82	5-25-88	42	4
83	5-26-88	41	5
84	5-27-88	40	5

APPENDIX B

ASSIGNMENT AND AGE OF PIGS IN TRIAL 2

TABLE VII

ASSIGNMENT OF PIGS TO TREATMENTS
FOR TRIAL 2

	TRT 1		TRT2		TRT3		TRT4
PEN NO.	EAR NOTCH	PEN NO.	EAR NOTCH	PEN NO.	EAR NOTCH	PEN NO.	EAR NOTCH
1	51-6	4	50-7	2	49-3	3	51-2
6	51-11	5	51-9	8	46-5	7	46-7
16	46-10	11	46-8	9	47-10	12	50-10
18	46-1	15	48-15	10	47-7	13	50-8
19	49-9	20	49-7	17	46-4	14	50-9
21	48-12	24	47-8	23	51-10	22	46-10

TABLE VIII

AGE OF PIGS USED IN TRIAL 2

LITTER NO.	BIRTHDATE	DAYS OF AGE AT ADJUSTMENT PERIOD	NUMBER OF PIGS IN TRIAL
46	9-6-88	41	7
47	9-6-88	41	3
48	9-6-88	41	2
49	9-7-88	40	3
50	9-7-88	40	4
51	9-8-88	39	5

APPENDIX C

INDIVIDUAL PIG DATA FOR TRIAL 1

TABLE IX

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE[a] (g)
1	3	initial			28.50	
		adjust.			33.75	353
		1(day -2)	77.38	53.74		
		1(day -1)	91.76	48.10		
		1(day 0)	99.80	61.59	36.00	
		$\bar{X}$	89.65	54.48		409
		2(day 5)	89.11	48.10		
		2(day 6)	102.52	54.63		
		2(day 7)	94.42	51.97		
		$\bar{X}$	95.35	51.57	43.50	494
		3(day 14)	108.00	60.64		
		3(day 15)	103.88	64.01		
		3(day 16)	99.80	65.49	53.50	
		$\bar{X}$	103.89	63.38		
2	3	initial			24.50	
		adjust			28.75	309
		1(day -2)	124.10	63.15		
		1(day -1)	99.12	58.35		
		1(day 0)	105.25	59.54	33.50	
		$\bar{X}$	109.50	60.35		380
		2(day 5)	165.82	78.45		
		2(day 6)	164.08	81.15		
		2(day 7)	171.07	83.88	39.25	
		$\bar{X}$	166.99	81.16		445
		3(day 14)	169.31	88.07		
		3(day 15)	174.60	70.63		
		3(day 16)	176.37	87.37	48.00	
		$\bar{X}$	173.42	82.02		
3	2	initial			18.50	
		adjust			20.25	200
		1(day -2)	77.37	26.72		
		1(day -1)	81.22	29.14		
		1(day 0)	90.42	36.35	23.00	
		$\bar{X}$	83.00	30.73		261
		2(day 5)	129.25	47.37		
		2(day 6)	132.11	53.34		
		2(day 7)	131.62	49.47	26.75	
		$\bar{X}$	130.99	50.05		304
		3(day 14)	138.04	49.89		
		3(day 15)	151.58	52.48		
		3(day 16)	146.94	54.68	32.50	
		$\bar{X}$	145.52	52.35		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
4	2	initial			26.25	
		adjust			32.25	300
		1(day -2)	87.89	51.97		
		1(day -1)	90.43	48.10		
		1(day 0)	97.10	57.82	35.50	
		$\bar{X}$	91.77	52.63		403
		2(day 5)	101.16	49.80		
		2(day 6)	117.77	62.07		
		2(day 7)	143.97	77.55	42.00	
		$\bar{X}$	120.97	63.14		477
		3(day 14)	139.49	71.62		
		3(day 15)	135.06	-----		
		3(day 16)	136.53	70.05	58.50	
		$\bar{X}$	137.02	70.83		
5	4	initial			21.00	
		adjust			18.00	170
		1(day -2)	75.46	33.94		
		1(day -1)	72.89	37.32		
		1(day 0)	62.76	31.03	25.00	
		$\bar{X}$	70.37	34.10		284
		2(day 5)	87.21	40.02		
		2(day 6)	99.28	44.81		
		2(day 7)	96.57	45.63	29.25	
		$\bar{X}$	94.35	43.49		332
		3(day 14)	96.57	48.55		
		3(day 15)	111.71	51.56		
		3(day 16)	111.71	53.31	35.00	
		$\bar{X}$	106.66	51.14		
6	4	initial			21.00	
		adjust			25.25	250
		1(day -2)	62.76	29.25		
		1(day -1)	67.80	32.47		
		1(day 0)	72.89	36.94	26.00	
		$\bar{X}$	67.82	33.12		295
		2(day 5)	78.04	42.79		
		2(day 6)	65.27	28.54		
		2(day 7)	64.02	28.89	31.75	
		$\bar{X}$	69.11	33.41		360
		3(day 14)	79.34	41.99		
		3(day 15)	80.64	41.20		
		3(day 16)	80.64	42.39	38.50	
		$\bar{X}$	80.20	41.86		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
7	1	initial			30.25	
		adjust			34.00	348
		1(day -2)	90.99	15.92		
		1(day -1)	114.00	18.91		
		1(day 0)	103.02	19.56	36.50	
		$\bar{X}$	102.67	18.13		411
		2(day 5)	148.76	17.46		
		2(day 6)	159.46	21.08		
		2(day 7)	139.80	22.48	43.00	
		$\bar{X}$	149.34	20.34		488
		3(day 14)	157.91	22.31		
		3(day 15)	142.77	22.31		
		3(day 16)	159.46	22.84	51.50	
		$\bar{X}$	153.38	22.48		
8	4	initial			16.25	
		adjust			22.00	250
		1(day -2)	84.45	17.78		
		1(day -1)	96.29	17.78		
		1(day 0)	105.74	19.07	24.00	
		$\bar{X}$	95.90	18.21		272
		2(day 5)	111.23	18.10		
		2(day 6)	111.23	17.00		
		2(day 7)	125.28	20.23	28.50	
		$\bar{X}$	115.91	18.44		323
		3(day 14)	119.60	19.90		
		3(day 15)	111.23	18.74		
		3(day 16)	118.19	19.56	35.00	
		$\bar{X}$	116.34	19.40		
10	1	initial			26.50	
		adjust			34.50	388
		1(day -2)	83.51	41.99		
		1(day -1)	100.01	49.83		
		1(day 0)	107.07	54.64	37.00	
		$\bar{X}$	96.86	48.82		420
		2(day 5)	127.44	89.78		
		2(day 6)	144.13	70.53		
		2(day 7)	134.95	72.09	44.25	
		$\bar{X}$	135.51	77.46		502
		3(day 14)	144.13	76.93		
		3(day 15)	142.59	64.97		
		3(day 16)	144.13	72.62	53.00	
		$\bar{X}$	143.62	71.51		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
11	3	initial			21.25	
		adjust			29.50	329
		1(day -2)	74.16	41.20		
		1(day -1)	78.14	45.22		
		1(day 0)	88.94	61.10	32.50	
		$\bar{X}$	80.42	49.17		369
		2(day 5)	87.58	56.00		
		2(day 6)	97.22	58.28		
		2(day 7)	111.36	64.97	39.00	
		$\bar{X}$	98.72	59.75		443
		3(day 14)	137.99	69.49		
		3(day 15)	128.94	72.62		
		3(day 16)	125.96	89.78	47.00	
		$\bar{X}$	130.96	77.30		
12	3	initial			18.75	
		adjust			21.25	209
		1(day -2)	52.73	27.52		
		1(day -1)	59.96	30.29		
		1(day 0)	81.15	37.93	24.50	
		$\bar{X}$	64.61	31.91		278
		2(day 5)	81.15	43.75		
		2(day 6)	82.43	44.15		
		2(day 7)	78.60	46.17	28.75	
		$\bar{X}$	80.73	44.69		326
		3(day 14)	92.82	48.23		
		3(day 15)	103.46	51.61		
		3(day 16)	104.81	53.78	35.00	
		$\bar{X}$	100.36	51.21		
13	4	initial			21.50	
		adjust			29.50	331
		1(day -2)	94.13	40.60		
		1(day -1)	88.89	43.35		
		1(day 0)	102.12	45.35	32.00	
		$\bar{X}$	95.05	43.10		363
		2(day 5)	129.88	53.34		
		2(day 6)	135.66	56.45		
		2(day 7)	129.88	54.66	37.75	
		$\bar{X}$	131.81	54.82		428
		3(day 14)	135.66	56.00		
		3(day 15)	134.21	52.91		
		3(day 16)	143.00	57.80	44.50	
		$\bar{X}$	137.62	55.57		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
14	4	initial			19.25	
		adjust			24.00	251
		1(day -2)	72.84	45.56		
		1(day -1)	70.21	39.90		
		1(day 0)	67.60	30.82	25.50	
		$\bar{X}$	70.22	38.76		289
		2(day 5)	97.22	48.94		
		2(day 6)	97.22	51.54		
		2(day 7)	101.41	52.86	30.00	
		$\bar{X}$	98.62	51.11		341
		3(day 14)	84.86	48.94		
		3(day 15)	91.68	50.23		
		3(day 16)	98.61	49.80	37.00	
		$\bar{X}$	91.72	49.65		
15	3	initial			26.50	
		adjust			30.00	299
		1(day -2)	70.21	34.13		
		1(day -1)	78.14	30.82		
		1(day 0)	76.81	33.39	31.50	
		$\bar{X}$	75.05	32.78		358
		2(day 5)	111.36	41.48		
		2(day 6)	88.94	41.48		
		2(day 7)	94.44	39.90	37.00	
		$\bar{X}$	98.25	40.96		420
		3(day 14)	112.80	43.91		
		3(day 15)	125.96	45.56		
		3(day 16)	117.14	48.94	47.00	
		$\bar{X}$	118.63	46.14		
16	1	initial			20.25	
		adjust			28.00	310
		1(day -2)	91.92	40.94		
		1(day -1)	79.54	33.77		
		1(day 0)	93.32	42.51	31.75	
		$\bar{X}$	88.26	39.08		360
		2(day 5)	120.72	43.71		
		2(day 6)	114.81	46.14		
		2(day 7)	122.20	49.89	36.75	
		$\bar{X}$	119.24	46.58		417
		3(day 14)	120.72	56.46		
		3(day 15)	129.72	56.01		
		3(day 16)	134.29	59.21	45.00	
		$\bar{X}$	128.24	57.23		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
17	1	initial			17.75	
		adjust			17.50	180
		1(day -2)	74.18	35.12		
		1(day -1)	75.49	31.20		
		1(day 0)	87.41	38.45	21.25	
		$\bar{X}$	79.03	34.93		241
		2(day 5)	152.41	51.22		
		2(day 6)	141.64	48.70		
		2(day 7)	155.53	50.37	23.50	
		$\bar{X}$	149.86	50.10		267
		3(day 14)	125.25	42.28		
		3(day 15)	129.66	44.64		
		3(day 16)	152.41	46.65	28.50	
		$\bar{X}$	135.77	44.52		
18	1	initial			20.00	
		adjust			25.00	254
		1(day -2)	77.46	29.24		
		1(day -1)	78.79	32.47		
		1(day 0)	95.00	36.56	27.50	
		$\bar{X}$	83.75	32.76		312
		2(day 5)	136.79	46.05		
		2(day 6)	132.28	52.00		
		2(day 7)	133.78	51.56	32.25	
		$\bar{X}$	134.28	49.86		366
		3(day 14)	152.16	64.48		
		3(day 15)	127.81	54.64		
		3(day 16)	152.16	57.36	40.00	
		$\bar{X}$	144.05	58.83		
19	2	initial			22.75	
		adjust			28.00	310
		1(day -2)	82.78	40.41		
		1(day -1)	93.62	42.39		
		1(day 0)	82.78	39.24	30.75	
		$\bar{X}$	86.40	40.68		349
		2(day 5)	113.25	47.71		
		2(day 6)	133.78	49.40		
		2(day 7)	127.81	53.31	36.50	
		$\bar{X}$	124.95	50.14		414
		3(day 14)	124.86	52.87		
		3(day 15)	120.47	55.09		
		3(day 16)	136.79	54.20	44.50	
		$\bar{X}$	127.37	54.05		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
20	2	initial			19.00	
		adjust			24.50	248
		1(day -2)	58.82	27.62		
		1(day -1)	66.43	29.72		
		1(day 0)	71.58	32.94	26.00	
		$\bar{X}$	65.61	30.09		295
		2(day 5)	98.29	42.85		
		2(day 6)	101.05	48.59		
		2(day 7)	96.91	50.29	29.50	
		$\bar{X}$	98.75	47.24		335
		3(day 14)	120.88	59.68		
		3(day 15)	117.99	63.00		
		3(day 16)	126.71	62.52	35.50	
		$\bar{X}$	121.86	61.73		
21	3	initial			16.25	
		adjust			18.75	191
		1(day -2)	48.86	24.21		
		1(day -1)	48.86	23.21		
		1(day 0)	58.82	27.27	21.75	
		$\bar{X}$	52.18	24.90		247
		2(day 5)	----	-----		
		2(day 6)	67.71	29.01		
		2(day 7)	60.08	31.50	23.50	
		$\bar{X}$	63.89	30.25		267
		3(day 14)	86.07	39.70		
		3(day 15)	82.07	40.48		
		3(day 16)	88.75	40.87	28.25	
		$\bar{X}$	85.63	40.35		
22	2	initial			14.75	
		adjust			20.50	189
		1(day -2)	109.43	48.23		
		1(day -1)	102.14	47.81		
		1(day 0)	116.84	51.18	21.75	
		$\bar{X}$	109.47	49.07		247
		2(day 5)	154.12	53.34		
		2(day 6)	150.89	59.18		
		2(day 7)	152.50	61.99	24.50	
		$\bar{X}$	152.50	58.17		278
		3(day 14)	157.36	66.35		
		3(day 15)	165.58	67.84		
		3(day 16)	168.91	68.34	30.75	
		$\bar{X}$	163.95	67.51		

TABLE IX (continued)

INDIVIDUAL PIG DATA FOR TRIAL 1

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
23	4	initial			19.50	
		adjust			24.50	257
		1(day -2)	64.43	31.70		
		1(day -1)	77.46	30.99		
		1(day 0)	73.51	44.15	27.75	
		$\bar{X}$	71.80	35.61		315
		2(day 5)	110.39	44.55		
		2(day 6)	101.93	45.35		
		2(day 7)	107.55	44.95	33.00	
		$\bar{X}$	106.62	44.95		375
		3(day 14)	108.97	44.55		
		3(day 15)	108.97	48.23		
		3(day 16)	116.12	28.55	41.00	
		$\bar{X}$	111.35	40.44		
24	2	initial			22.00	
		adjust			23.50	251
		1(day -2)	59.33	26.84		
		1(day -1)	58.06	26.84		
		1(day 0)	58.06	27.86	27.25	
		$\bar{X}$	58.48	27.18		309
		2(day 5)	84.12	37.18		
		2(day 6)	73.51	35.32		
		2(day 7)	92.25	42.56	31.00	
		$\bar{X}$	83.30	38.35		352
		3(day 14)	88.17	43.75		
		3(day 15)	85.47	44.55		
		3(day 16)	100.53	46.17	38.50	
		$\bar{X}$	91.39	44.82		

[a]values represent average values over each period

APPENDIX D

INDIVIDUAL PIG DATA FOR TRIAL 2

TABLE X

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE[a] (g)
1	1	initial			20.00	
		adjust.			19.00	150
		1(day -2)	87.07	38.48		
		1(day -1)	72.40	33.45		
		1(day 0)	85.42	37.69	20.50	
		$\bar{X}$	81.63	36.54		233
		2(day 5)	88.73	40.48		
		2(day 6)	100.44	39.68		
		2(day 7)	97.07	42.10	23.50	
		$\bar{X}$	95.41	40.75		267
		3(day 14)	115.87	44.58		
		3(day 15)	105.54	45.00		
		3(day 16)	117.61	45.42	27.50	
		$\bar{X}$	113.00	45.00		
2	3	initial			16.75	
		adjust			18.50	175
		1(day -2)	117.61	51.91		
		1(day -1)	97.07	46.26		
		1(day 0)	112.4	39.27	21.00	
		$\bar{X}$	109.02	45.82		238
		2(day 5)	121.10	40.08		
		2(day 6)	140.73	40.88		
		2(day 7)	140.73	39.67	24.75	
		$\bar{X}$	134.14	40.21		281
		3(day 14)	140.73	47.11		
		3(day 15)	129.94	44.16		
		3(day 16)	137.11	44.58	31.50	
		$\bar{X}$	135.92	45.28		
3	4	initial			20.25	
		adjust			25.25	291
		1(day -2)	117.67	42.28		
		1(day -1)	107.05	39.10		
		1(day 0)	137.65	46.80	28.25	
		$\bar{X}$	120.79	42.73		311
		2(day 5)	154.52	45.55		
		2(day 6)	133.97	43.90		
		2(day 7)	145.09	42.28	34.25	
		$\bar{X}$	144.53	43.91		360
		3(day 14)	167.99	48.92		
		3(day 15)	160.26	48.92		
		3(day 16)	160.26	53.29	42.52	
		$\bar{X}$	162.84	50.38		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
4	2	initial			26.25	
		adjust			30.50	340
		1(day -2)	145.09	44.31		
		1(day -1)	119.46	39.10		
		1(day 0)	128.49	42.28	34.50	
		$\bar{X}$	131.01	41.90		492
		2(day 5)	124.86	37.54		
		2(day 6)	160.26	43.49		
		2(day 7)	175.83	45.55	39.75	
		$\bar{X}$	153.65	42.20		451
		3(day 14)	191.83	48.92		
		3(day 15)	193.86	49.35		
		3(day 16)	177.81	51.09	50.50	
		$\bar{X}$	187.84	49.79		
5	2	initial			27.50	
		adjust			31.00	348
		1(day -2)	124.87	44.16		
		1(day -1)	123.16	44.58		
		1(day 0)	128.30	47.54	34.25	
		$\bar{X}$	125.44	45.43		389
		2(day 5)	114.69	45.00		
		2(day 6)	133.49	46.69		
		2(day 7)	156.58	53.71	42.00	
		$\bar{X}$	134.92	48.46		477
		3(day 14)	135.24	51.47		
		3(day 15)	140.50	52.36		
		3(day 16)	160.22	51.02	51.50	
		$\bar{X}$	145.32	51.62		
6	1	initial			14.75	
		adjust			17.50	183
		1(day -2)	106.36	45.00		
		1(day -1)	101.42	41.69		
		1(day 0)	119.76	45.00	20.50	
		$\bar{X}$	109.18	43.90		233
		2(day 5)	130.03	51.91		
		2(day 6)	121.46	49.27		
		2(day 7)	135.24	50.58	25.00	
		$\bar{X}$	128.91	50.59		284
		3(day 14)	147.59	55.54		
		3(day 15)	152.97	57.40		
		3(day 16)	152.97	56.93	31.50	
		$\bar{X}$	151.17	56.62		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
7	4	initial			12.50	
		adjust			16.75	177
		1(day -2)	125.62	46.73		
		1(day -1)	110.17	42.16		
		1(day 0)	100.11	37.77	17.75	
		$\bar{X}$	111.97	42.22		201
		2(day 5)	137.94	45.88		
		2(day 6)	141.51	46.30		
		2(day 7)	139.72	45.46	20.50	
		$\bar{X}$	139.72	45.88		233
		3(day 14)	145.10	45.46		
		3(day 15)	145.10	46.30		
		3(day 16)	157.86	49.30	24.50	
		$\bar{X}$	149.36	47.02		
8	3	initial			26.00	
		adjust			31.50	356
		1(day -2)	89.50	40.19		
		1(day -1)	86.65	36.56		
		1(day 0)	81.01	36.56	36.25	
		$\bar{X}$	85.72	37.77		411
		2(day 5)	105.47	44.80		
		2(day 6)	102.53	45.23		
		2(day 7)	111.42	46.52	41.75	
		$\bar{X}$	106.48	45.52		474
		3(day 14)	115.93	52.75		
		3(day 15)	105.48	48.71		
		3(day 16)	122.01	55.53	50.50	
		$\bar{X}$	114.47	52.33		
9	3	initial			18.50	
		adjust			22.00	231
		1(day -2)	71.29	35.76		
		1(day -1)	76.82	38.56		
		1(day 0)	81.01	40.61	23.25	
		$\bar{X}$	76.37	38.31		264
		2(day 5)	105.48	52.30		
		2(day 6)	115.93	55.53		
		2(day 7)	95.25	50.04	27.25	
		$\bar{X}$	105.55	52.62		309
		3(day 14)	106.95	54.60		
		3(day 15)	93.80	50.94		
		3(day 16)	105.48	53.67	33.25	
		$\bar{X}$	102.08	53.07		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
10	3	initial			21.25	
		adjust			18.00	191
		1(day -2)	81.74	28.17		
		1(day -1)	76.12	26.76		
		1(day 0)	77.52	28.89	19.75	
		$\bar{X}$	78.46	27.94		224
		2(day 5)	71.94	30.33		
		2(day 6)	60.97	24.66		
		2(day 7)	73.33	27.46	23.25	
		$\bar{X}$	68.75	27.48		264
		3(day 14)	83.16	36.69		
		3(day 15)	88.87	35.92		
		3(day 16)	90.31	37.85	28.25	
		$\bar{X}$	87.45	36.82		
11	2	initial			25.00	
		adjust			29.50	331
		1(day -2)	93.20	37.00		
		1(day -1)	84.58	35.07		
		1(day 0)	101.98	39.75	33.25	
		$\bar{X}$	93.26	37.27		377
		2(day 5)	106.43	38.17		
		2(day 6)	113.94	41.75		
		2(day 7)	112.43	43.80	39.50	
		$\bar{X}$	110.94	41.24		448
		3(day 14)	120.04	46.30		
		3(day 15)	120.04	48.00		
		3(day 16)	132.47	50.17	49.00	
		$\bar{X}$	124.18	48.16		
12	4	initial			31.25	
		adjust			32.50	351
		1(day -2)	86.01	29.12		
		1(day -1)	94.66	31.32		
		1(day 0)	127.77	38.56	35.00	
		$\bar{X}$	102.81	33.00		397
		2(day 5)	126.22	43.39		
		2(day 6)	137.22	45.04		
		2(day 7)	150.11	44.63	40.25	
		$\bar{X}$	137.85	44.35		457
		3(day 14)	150.11	46.30		
		3(day 15)	137.22	46.30		
		3(day 16)	140.41	44.63	50.50	
		$\bar{X}$	142.58	45.74		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
13	4	initial			20.75	
		adjust			25.00	277
		1(day -2)	106.95	40.19		
		1(day -1)	99.60	42.26		
		1(day 0)	104.00	43.10	27.25	
		$\bar{X}$	103.52	41.85		309
		2(day 5)	106.95	36.56		
		2(day 6)	95.25	32.65		
		2(day 7)	101.06	36.56	31.75	
		$\bar{X}$	101.09	35.25		360
		3(day 14)	93.80	39.78		
		3(day 15)	105.48	41.43		
		3(day 16)	98.15	41.02	39.25	
		$\bar{X}$	99.14	40.74		
14	4	initial			23.00	
		adjust			24.75	279
		1(day -2)	109.34	41.20		
		1(day -1)	117.23	43.20		
		1(day 0)	123.63	44.00	27.50	
		$\bar{X}$	116.74	42.80		312
		2(day 5)	118.82	43.60		
		2(day 6)	133.39	49.41		
		2(day 7)	138.34	50.69	32.50	
		$\bar{X}$	130.19	47.90		369
		3(day 14)	138.34	46.46		
		3(day 15)	138.34	48.14		
		3(day 16)	136.68	45.23	39.50	
		$\bar{X}$	137.79	46.61		
15	2	initial			19.00	
		adjust			22.75	251
		1(day -2)	100.04	30.67		
		1(day -1)	81.92	26.80		
		1(day 0)	95.45	31.03	25.75	
		$\bar{X}$	92.47	29.50		292
		2(day 5)	103.12	36.18		
		2(day 6)	106.22	35.43		
		2(day 7)	112.48	35.43	31.00	
		$\bar{X}$	107.28	35.68		352
		3(day 14)	112.48	39.24		
		3(day 15)	117.23	40.02		
		3(day 16)	125.25	40.41	38.50	
		$\bar{X}$	118.32	39.89		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
16	1	initial			17.50	
		adjust			21.75	245
		1(day -2)	93.44	39.52		
		1(day -1)	84.69	37.25		
		1(day 0)	96.40	41.05	24.25	
		$\bar{X}$	91.51	39.27		275
		2(day 5)	96.40	41.83		
		2(day 6)	102.36	42.61		
		2(day 7)	94.92	40.28	29.50	
		$\bar{X}$	97.89	41.57		335
		3(day 14)	97.88	41.83		
		3(day 15)	103.86	45.80		
		3(day 16)	105.36	43.40	35.50	
		$\bar{X}$	102.37	43.68		
17	3	initial			19.00	
		adjust			23.50	261
		1(day -2)	75.36	35.07		
		1(day -1)	84.02	33.88		
		1(day 0)	88.40	40.81	26.25	
		$\bar{X}$	82.59	36.59		298
		2(day 5)	117.11	51.33		
		2(day 6)	124.96	52.71		
		2(day 7)	123.38	54.58	30.50	
		$\bar{X}$	121.82	52.87		346
		3(day 14)	114.01	53.17		
		3(day 15)	112.47	50.87		
		3(day 16)	121.80	53.17	37.00	
		$\bar{X}$	116.09	52.40		
18	1	initial			12.25	
		adjust			17.25	184
		1(day -2)	73.94	40.81		
		1(day -1)	73.94	40.81		
		1(day 0)	73.94	38.32	19.50	
		$\bar{X}$	73.94	39.98		221
		2(day 5)	109.39	52.25		
		2(day 6)	103.30	48.61		
		2(day 7)	128.13	51.33	22.50	
		$\bar{X}$	113.61	50.73		255
		3(day 14)	131.32	53.64		
		3(day 15)	120.24	49.96		
		3(day 16)	132.93	52.25	27.50	
		$\bar{X}$	128.16	51.95		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
19	1	initial			18.50	
		adjust			19.25	207
		1(day -2)	91.14	32.70		
		1(day -1)	97.00	33.83		
		1(day 0)	104.41	38.48	21.50	
		$\bar{X}$	97.51	35.00		244
		2(day 5)	113.46	40.08		
		2(day 6)	122.69	44.58		
		2(day 7)	141.69	48.40	25.25	
		$\bar{X}$	125.95	44.35		287
		3(day 14)	138.47	50.58		
		3(day 15)	151.48	53.71		
		3(day 16)	144.93	53.71	31.00	
		$\bar{X}$	144.96	52.67		
20	2	initial			17.00	
		adjust			18.50	201
		1(day -2)	87.13	39.14		
		1(day -1)	69.40	33.13		
		1(day 0)	88.52	39.58	20.75	
		$\bar{X}$	81.68	37.29		235
		2(day 5)	96.96	44.06		
		2(day 6)	96.96	43.16		
		2(day 7)	108.47	46.37	24.50	
		$\bar{X}$	100.80	44.53		278
		3(day 14)	120.27	50.61		
		3(day 15)	114.33	48.24		
		3(day 16)	115.81	50.13	29.50	
		$\bar{X}$	116.80	49.66		
21	1	initial			14.00	
		adjust			10.50	119
		1(day -2)	82.41	44.43		
		1(day -1)	90.93	37.84		
		1(day 0)	90.93	46.13	14.25	
		$\bar{X}$	88.09	42.80		162
		2(day 5)	79.61	47.86		
		2(day 6)	102.53	45.28		
		2(day 7)	120.48	46.56	17.00	
		$\bar{X}$	100.87	46.57		193
		3(day 14)	115.93	47.43		
		3(day 15)	123.54	51.41		
		3(day 16)	118.96	47.86	20.25	
		$\bar{X}$	119.48	48.90		

TABLE X (continued)

INDIVIDUAL PIG DATA FOR TRIAL 2

PIG NO.	TRT	PERIOD	TSC (mg/dl)	HDL (mg/dl)	WT (lb)	INTAKE (g)
22	4	initial			24.25	
		adjust			30.00	322
		1(day -2)	87.91	30.89		
		1(day -1)	92.14	34.87		
		1(day 0)	102.15	36.49	33.50	
		$\bar{X}$	94.06	34.08		380
		2(day 5)	118.34	41.08		
		2(day 6)	116.84	43.66		
		2(day 7)	121.34	43.23	39.50	
		$\bar{X}$	118.84	42.66		448
		3(day 14)	100.71	49.00		
		3(day 15)	112.39	50.82		
		3(day 16)	115.35	51.28	48.50	
		$\bar{X}$	109.48	50.36		
23	3	initial			22.25	
		adjust			26.00	291
		1(day -2)	92.19	51.80		
		1(day -1)	83.33	39.63		
		1(day 0)	89.22	44.70		
		$\bar{X}$	88.25	45.38	29.25	332
		2(day 5)	90.71	43.84		
		2(day 6)	93.68	46.00		
		2(day 7)	119.77	52.26	34.25	
		$\bar{X}$	101.39	47.37		389
		3(day 14)	101.22	44.27		
		3(day 15)	102.74	48.20		
		3(day 16)	110.41	44.70	42.75	
		$\bar{X}$	104.79	45.72		
24	2	initial			19.00	
		adjust			22.25	231
		1(day -2)	68.90	33.60		
		1(day -1)	68.90	30.88		
		1(day 0)	80.41	38.81	24.00	
		$\bar{X}$	72.74	34.43		272
		2(day 5)	107.32	53.65		
		2(day 6)	122.93	61.84		
		2(day 7)	118.20	56.48	28.25	
		$\bar{X}$	116.15	57.32		321
		3(day 14)	116.63	50.44		
		3(day 15)	121.35	54.12		
		3(day 16)	115.07	47.31	34.50	
		$\bar{X}$	117.68	50.62		

[a]values represent average values for each period

APPENDIX E

TOTAL AND HDL CHOLESTEROL

LEAST SQUARES MEANS

TABLE XI

SERUM CHOLESTEROL LEAST SQUARES MEANS

	Cholesterol (mg/dl)[A]					
	Trial 1			Trial 2		
Treatment	P1	P2	P3	P1	P2	P3
1 Whey	90.11	137.65	141.01	90.31	110.44	126.52
2 Whey + *L. acid.*	82.46	118.58	131.19	99.43	120.62	135.02
3 Nonwhey	78.57	100.65	118.82	86.74	106.36	110.13
4 Nonwhey + *L. acid.*	78.46	102.74	107.32	108.32	128.70	133.53

[A]each value represents the mean from six pigs (except treatment 1 in trial 1 where n=5)

TABLE XII

HDL CHOLESTEROL LEAST SQUARES MEANS

		HDL Cholesterol (mg/dl)[A]					
		Trial 1			Trial 2		
Treatment		P1	P2	P3	P1	P2	P3
1	Whey	34.74	48.87	50.91	39.58	45.76	49.80
2	Whey + *L. acid.*	38.40	51.18	58.55	37.63	44.91	48.29
3	Nonwhey	42.26	51.40	60.07	38.30	44.34	47.61
4	Nonwhey + *L. acid.*	33.82	41.04	43.01	39.45	43.33	46.81

[A]each value represents the mean from six pigs (except treatment 1 in trial 1 where n=5)

VITA

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