EVALUATION OF YOUNG GROWING PIGS AS A MODEL TO STUDY HYPOCHOLESTEROLEMIC EFFECTS OF CONSUMING LACTOBACILLUS ACIDOPHILUS AND DRIED WHEY

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Stillwater, Oklahoma

1985

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
December, 1987

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ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Stanley Gilliland for his guidence and assistance throughout this study. I would like to extend my appreciation to Dr. H. G. Dolezal and Dr. E. D. Mitchell for serving on the Advisory Committee.

Many thanks go to Dr. Charles Maxwell for his advice and assistance with coordinating the animals used in this study and to the personnel at the Oklahoma State University Swine Farm for their valuble assistance.

Appreciation is extended to the faculty members, fellow graduate students, and laboratory personnel who have shared their knowledge, technical assistance, and friendship during my time at Oklahoma State University.

Finally, I wish to thank my wife Angela for her support, understanding, and help during this course of study. Also, I wish to thank my son, Joseph, for his patience and sacrifice during this course of study.

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

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Coronary heart disease has been identified as the major cause of death in the United States. Atherosclerosis has been implicated in the majority of these cases. Individuals with elevated levels of blood cholesterol (hypercholesterolemia) are considered to be at higher risk for developing atherosclerosis than those with normal levels. Thus individuals having hypercholesterolemia use medication or dietary modification in order to reduce serum cholesterol levels. The reductions achieved by dietary modifications may be minimal and most forms of medication currently used have undesirable side effects.

Lactobacillus acidophilus has been found to assimilate cholesterol under conditions expected to occur in the gastrointestinal tract. The cholesterol is apparently incorporated into the bacterial cells. Consumption of cells of selected strains of L. acidophilus can thus be beneficial in helping control the absorption of cholesterol from the intestine into the blood. Consumption of whey as well as other dairy products has also been shown to decrease blood cholesterol levels in humans and in animals.

If consumption of L. acidophilus alone or in conjunction with whey will lower serum cholesterol levels, this may provide an excellent method for individuals to reduce their risk of coronary heart disease. The purpose of this study was to evaluate the young growing pig as an animal model to examine the effects of consuming cells of L. acidophilus alone or in conjunction with dried whey on serum cholesterol levels.

CHAPTER II

REVIEW OF LITERATURE

Role of Cholesterol in the Body

Cholesterol is a natural and required component of the human body (63). The body uses cholesterol as a precursor for the steroid hormones, bile salts, and vitamin D. It also is incorporated into all cell membranes where it is involved in the regulation of membrane fluidity. Cholesterol is acquired by synthesis, which occurs primarily in the liver and in limited amounts in the intestine, or by absorbing undigested cholesterol from dietary sources. The synthesis of cholesterol is controlled by a feedback mechanism (63). Dietary cholesterol suppresses the synthesis of 3-hydroxy-3-methylglutaryl CoA reductase which catalyzes the committing step of cholesterol synthesis (11).

Within the body, specific plasma lipoprotein complexes transport cholesterol and cholesterol esters as well as other lipids. The complexes categorized based on their densities include: chylomicrons (<0.94 g/cm^3), very-low-density lipoproteins (VLDL) (0.94-1.006 g/cm^3), low-density lipoproteins (LDL) (1.006-1.063 g/cm^3), and high-density lipoproteins (HDL) (1.063-1.210 g/cm^3) (63). The LDL

complexes transport from one-half to two-thirds of the plasma cholesterol including both free and esterified forms (22). Unlike the LDL complexes, the HDL-cholesterol complexes contain only unesterified cholesterol (22). It has long been recognized that accelerated atherosclerosis is associated with the elevation of low-density lipoprotein cholesterol levels in the blood (18).

The low-density lipoproteins transport plasma cholesterol to non-hepatic tissues where specific receptors on cell membranes bind the LDL particles which allows the cells to take in the cholesterol complex (20). Excess cholesterol in a cell suppresses endogenous synthesis and may be re-esterified and stored. The high-density lipoproteins appear to transport cholesterol away from peripheral tissue to the liver for excretion (45).

Cholesterol and Coronary Heart Disease

The major cause of death in the United States is cardiovascular disease, which accounts for 49% of all deaths (67). The underlying cause of most cardiovascular disease is atherosclerosis (30,49). Atherosclerosis is an irregular thickening of the inner wall of the arteries that reduces the size of the lumen. The thickening is caused by the accumulation of plaque which consists of smooth muscle cells, connective tissue, and considerable deposits of lipid of which cholesteryl esters comprise the major part (20).

The most commonly used methods for decreasing the risk of coronary heart disease involve lowering blood cholesterol levels (22) by altering diets, administering drugs or both. However, both of these methods have limitations or repercussions. Many individuals have difficulty maintaining a low fat diet, and even more important, none of the drugs used for lowering blood cholesterol levels are without biochemical or systemic side effects (22).

Elevated levels of total cholesterol and low-density lipoprotein cholesterol increase the risk of coronary heart disease (21). A significant relationship exists between coronary heart disease and both cholesterol intake and plasma cholesterol levels (32). Much evidence suggests that the low density lipoprotein complexes are involved in atherogenesis (34, 35, 68), the basic process responsible for coronary heart disease (61). A report by the Lipid Research Clinic's Program (36, 37) from a seven year trial concludes that lowering total plasma cholesterol as well as low-density cholesterol could significantly reduce the risk of coronary heart disease (24%) or myocardial infarction (19%).

Hypocholesterolemic Effects of Milk, Milk Products, and Cultured Milk Products

Since the work by Mann and Spoerry (43) on the hypocholesterolemic effect of a cultured milk product, there

have been many reports of milk, milk products, and cultured milk products producing hypocholestrolemic effects in humans, pigs, rabbits, and rats. Several milk fractions or constituents have been shown to produce a hypocholesterolemic effect. The constituents proposed as the responsible factors include 3-hydroxy-3-methylglutarate (HMG) (41, 48), orotic acid (1, 4, 5), calcium (28),and lactose (25).

Orotic acid and 3-hydroxy-3-methylglutarate appear to exert their action by inhibiting cholesterol synthesis. In vitro and in vivo studies have shown that HMG (3) and orotic acid (4, 5) inhibit cholesterol synthesis from acetate in rat liver preparations.

Feeding trials using whey, skim milk, whole milk and yogurt have resulted in lowering of blood cholesterol levels in animals and human volunteers. Cultured products appear to produce more of an effect than do non-cultured products (23, 26, 41, 52, 54, 64). Levels of orotic acid in milk decrease during the fermentation of yogurt (51) and it is not known if HMG occurs in milk (54). These factors cannot account for the increased hypocholesterolemic effects of cultured milk products.

Mann and Spoerry (43) used Maasai tribesmen to test the hypothesis that a surfactant in the diet would cause hypercholesterolemia. The Maasai normally consume a diet consisting primarily of fermented milk and meat yet they exhibit a low incidence of coronary heart disease (42). Twenty four volunteers were given their usual diet of

fermented milk (fermented with a wild strain of Lactobacillus) for six days and on the seventh day, a steer was slaughtered and consumed. This was repeated for three weeks. The men were assigned to two treatment groups. For one the fermented milk was supplemented with a surfactant (Tween 20) and the other a placebo (pure olive oil). Both treatment groups, with and without a surfactant exhibited significant reductions in serum cholesterol levels during the three week trial. As milk consumption increased, as indicated by weight gain, serum cholesterol levels decreased. This was contrary to what was expected since their diet contained fat and cholesterol. This decrease in blood cholesterol was attributed to the consumption of the fermented milk. They concluded that some component of the fermented milk reduced serum cholesterol synthesis in the body

Mann (41) confirmed his previous report by showing that consumption of skim and whole milk yogurt resulted in reduced ($P<0.05$) serum cholesterol levels in humans during the twelve day trial. Synthesis of cholesterol from orally administered radioacetate was also reduced in human subjects consuming yogurt. Blood cholesterol levels slowly returned to normal after consumption of yogurt ceased. Whole fresh milk did not cause a reduction in blood cholesterol levels.

Fifty-four volunteers were used to test the effect of supplementing the diet with non-pasteurized yogurt, pasteurized yogurt, and 2 percent fat milk by Hepner et al.

(26). Both pasteurized and non-pasteurized yogurt significantly reduced serum cholesterol levels ($P<0.01$) during a twelve week trial where the diet was constantly supplemented with the milk products. Consumption of 2 percent fat milk caused a smaller reduction ($P<0.05$) in serum cholesterol levels than did supplementation with yogurt. Serum triglycerides were not significantly effected by any of the treatments.

In a report by Howard and Marks (29), both skim milk ($P<0.001$) and whole milk ($P<0.01$) exerted a significant hypocholesterolemic response on blood cholesterol in sixteen human volunteers. Seven volunteers receiving 2.4g calcium (calcium gluconate) (equivalent to 4 pints of milk) daily for 12 days did not exhibit any change in blood cholesterol levels, suggesting that calcium was not the hypocholesterolemic component.

Thompson (65) and Massey (44) saw no reduction in blood cholesterol levels of humans consuming fermented or non-fermented milk products. However, they saw no increase either, which might have been expected from the increases in fat and cholesterol intake which occurred in their studies.

Stahelin et al. (60) supplemented the high fat diet of growing swine with skim milk, yogurt, whey, fermented whey, casein, and lactose. During a five week trial, skim milk, whey, fermented whey, and yogurt lowered blood cholesterol levels compared to a high fat control diet. Only the effect

of the skim milk was significant. Lactose and casein had no effect on cholesterol levels.

The effect of whey and specific whey fractions on blood cholesterol levels in growing swine was studied by Beames et al. (2). Whey, whey protein and lactose produced a trend for reduced serum cholesterol during an eight week trial although it was not significant. A time by treatment interaction ($P<0.0001$) made interpretation of the data difficult.

Norton et al. (50) used four mature pigs to test the effect of whey on blood cholesterol levels with and without additional cholesterol supplementation. Pigs fed a diet supplemented with whey and cholesterol had a 15% ($P<0.001$) lower serum cholesterol level than pigs on a control diet without whey. Serum HDL cholesterol was significantly higher ($P<0.05$) in pigs consuming whey than the pigs on a non-whey diet, indicating that most of the reduction in serum cholesterol occurred in the LDL+VLDL fractions.

Thakur and Jha (64) found that serum cholesterol levels as well as the size of atherosclerotic lesions were reduced in rabbits fed a high cholesterol diet supplemented with milk, yogurt, and calcium. Yogurt and calcium reduced serum cholesterol levels to a greater extent than did milk ($P<0.001$). The lower level of serum cholesterol in the yogurt group than in the calcium group suggests that there may be hypocholestrolemic factors present other than calcium.

The effect of adding skim milk powder or B-hydroxy-B-methyl glutaric acid (HMG) to diets supplemented with cholesterol and fat for rats was studied by Nair and Mann (48). Rats which received skim milk powder and HMG with or with out cholesterol had reduced serum cholesterol compared to rats receiving control and control + cholesterol diets. The effect of the milk powder and HMG were similar. The authors suggested that the "Milk Factor" was HMG.

Supplementing the diets of rats with thermophilus milk (skim milk fermented by Streptococcus thermophilus) significantly reduced ($P<0.05$) total plasma cholesterol compared to control diets supplemented with water and skim milk in an investigation by Rao et al. (52). Consumption of diets supplemented with methanol solubles from whole milk fermented with Streptococcus thermophilus reduced plasma cholesterol ($P<0.05$) compared to diets supplemented with methanol solubles of nonfermented whole milk. They further reported that methanol solubles from skim milk and thermophilus milk inhibited cholesterolgenesis from acetate by rat liver homogenates in vitro.

Use of Lactobacillus acidophilus as a Dietary Adjunct to lower Serum Cholesterol

Several lactobacilli are used as dietary adjuncts in that they are present in various cultured products. However, not all of the lactobacilli are able to survive and

grow in the intestinal tract. Lactobacillus acidophilus normally inhabits the gastrointestinal tract of humans (7) where it can grow and help maintain a properly balanced microflora (13, 56, 57).

Several researchers have reported that the consumption of L. acidophilus resulted in lowered serum cholesterol levels. Torteuro (66) found that consumption of L. acidophilus by cecectomized and normal laying hens resulted in significantly lower blood cholesterol levels after one month. Harrison and Peate (24) found that human infants fed milk containing L. acidophilus had at day eight, significantly lower serum cholesterol levels ($P<0.001$), greater weight gains ($P<0.05$), and an increase in numbers of lactobacilli in their stools ($P<0.001$) compared to those infants consuming an unaltered milk formula. They offered no suggestion as to how L. acidophilus effected the reduction in serum cholesterol levels.

Mott (47) found that germ free pigs monocontaminated with L. acidophilus exhibited reduced serum cholesterol levels after subsequent development of a normal flora. This suggests that the intestinal flora can have a definite effect on blood cholesterol levels. Grunnewald (23) reported that rats fed milk fermented with L. acidophilus and methanol solubles from this fermented milk had lower serum cholesterol levels ($p<0.05$) compared to water or milk fed controls. She concluded that some factor was produced

during fermentation of the milk which reduced cholesterol synthesis in the rats.

Gilliland et al. (14) reported that some strains of L. acidophilus could assimilate cholesterol from a defined laboratory growth medium. The presence of bile and growth under anaerobic conditions were required for assimilation. They also found that supplementing a high cholesterol diet of growing pigs with cells of a strain capable of assimilating cholesterol from a laboratory medium significantly influenced serum cholesterol levels. The pigs receiving the cholesterol assimilating strain of L. acidophilus had significantly lower levels of serum cholesterol than did those receiving a non-cholesterol assimilating strain or the diet without any lactobacilli.

Pigs as Experimental Models for Cardiovascular Research

Pigs are one of the most suitable animal models for cardiovascular research. They have digestive and blood circulatory systems that are similar to humans, and they display atherosclerotic tendencies similar to humans (53). Diets high in cholesterol and fat induce increases in serum cholesterol levels (2, 10, 14, 50, 60) and aortic atherosclerosis (40, 15). The structure of the serum low-density lipoproteins and their apoproteins are similar in swine and humans (6, 12, 33, 39, 40). The lipoprotein receptors on liver membranes are similar in swine and humans (38). There

is a high correlation between aortic cholesterol and serum low-density lipoprotein levels in both swine and humans (27, 59).

CHAPTER III

MATERIALS AND METHODS

Selection of Strains of *Lactobacillus acidophilus* for Pig Feeding Trials

Strains of *Lactobacillus acidophilus* from the culture collection in the Dairy Foods Microbiology Laboratory at Oklahoma State University were screened as candidates for use as feed adjuncts in a pig feeding trial. The 14 strains were all isolates from swine intestines. The strains were screened for the the ability to assimilate cholesterol by the method of Gilliland et al. (14), except the assay broth contained 10 percent PPLO serum. The cholesterol assimilating ability of the cultures was based on the amount of cholesterol remaining in the spent broth following 24 hr of growth at 37°C. The method of Rudel and Morris (55) was used to quantitate cholesterol.

The bile tolerance of each strain was measured by the procedure of Gilliland et al. (14). The time required for the absorbance at 600 nanometers to increase by 0.3 units for each culture was measured.

The strains were also tested for antagonism against each other by the following procedure. One tenth ml of a

freshly prepared lactobacilli MRS broth (Difco) culture of each strain was added to 20 ml molten MRS agar (prepared by adding 1.5 percent Difco Bacto-Agar to MRS broth prior to sterilization) tempered to 45°C. Five ml of the seeded agar was added to each of three sterile petri dishes. Spent broth from an 18 hour MRS broth culture of each strain was adjusted to pH 6.5 with 1.0 N NaOH and filter sterilized into sterile screw cap tubes using Acrodisc filters with 0.45 micron pore size (Gelman Sciences Ann Arbor MI). Sterile paper discs (13 mm diameter) were saturated with the filter sterilized spent broth and placed onto the surface of seeded agar. Spent broth samples from five strains were assayed on each plate. A control disc prepared using sterile MRS broth was also included on each plate. The plates were incubated 24 hours at 37°C. The plates were observed for zones of inhibition around each disc.

Source and Maintenance of Selected Cultures

Lactobacillus acidophilus RP32 and Lactobacillus acidophilus RP42 were obtained from the culture collection in the Dairy Foods Microbiology Laboratory at Oklahoma State University. The cultures were propagated weekly (1 percent inocula and 18 hr incubation at 37°C) in pepsinized milk nutrient (PMN) broth (46) and stored at 4°C between transfers. Cultures were subcultured at least three times just prior to use.

Culture Identity

The identity of the two selected cultures was confirmed according to characteristics listed in the 8th edition of Bergey's Manual of Determinative Bacteriology (7) using the procedure of Gilliland and Speck (16). The substrates used for characterization were amygdalin, arabinose, arginine, cellobiose, esculin, galactose, glucose, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. The ability to grow at 15 and 45°C as well as the Gram stain morphology were also examined.

Preparation of Concentrated Cultures

Cell crops of L. acidophilus were grown in a 7-liter fermentor (New Brunswick Scientific Co., Edison NY) equipped with a combination pH electrode (Ingold Electrodes Inc., Wilmington MA). The crops were grown under constant agitation, pH, and temperature control. Broth temperature was controlled by immersing the fermentor in a water bath maintained at 37°C and pH was controlled by an automatic pH controller (New Brunswick Scientific Co., Edison NY).

The fermentor jar containing 3-liters of distilled water was sterilized by heating 45 minutes at 121°C. The

water was aseptically removed prior to the addition of the sterile growth medium.

Sterile PMN broth in 3 or 4 liter quantities was aseptically added to the fermentor and the temperature was adjusted to 37°C. The broth was adjusted to pH 5.0 with sterile 20 percent lactic acid before inoculation. The PMN broth was inoculated with 30-40 ml (1 percent) of a fresh 18 hour PMN broth culture of the desired strain of L. acidophilus. The broth was maintained at pH 5.0 with a 20 percent Na_2CO_3 in 20 percent NH_4OH neutralizer (46) during growth of the cultures.

The cell crop was removed, by vacuum, from the fermentor during the late exponential phase of growth (14-16 hours) into 1-liter sterile filter flasks and placed in an ice water bath for one hour. The culture was then dispensed into 250 ml sterile centrifuge bottles and centrifuged at 4080xg for 20 minutes at 0-1°C in a Sorvall RC5 Superspeed Refrigerated Centrifuge (DuPont Co., Newtown CN). The supernatant was discarded and the cell pellets were resuspended in two times their weight of sterile 10 percent reconstituted nonfat milk solids with the aid of sterile glass beads (0.4 cm in diameter). The resulting concentrated culture was then dispensed in 2 gram quantities into sterile cryogenic vials (DynaTech Laboratories Inc., Chantilly, VA). After dispensing, the vials were frozen and stored at -196°C in liquid nitrogen.

Evaluation of Concentrated Cultures

Numbers of lactobacilli were determined in the concentrated culture before freezing (day 0), at 24 hours post freezing (day 1) and at days 7, 14, 21, and 28 post freezing. The pour plate procedure using PMN and PMNO agars was employed. PMN agar was prepared by dissolving 1.5 percent Bacto-Agar (Difco) in PMN broth prior to sterilizing at 121^{o}C for 15 min. PMNO agar (PMN agar + 0.1 percent oxgall; Difco) was used to enumerate bile tolerant lactobacilli.

For all enumeration procedures, 99 ml dilution blanks containing 0.1 percent Bacto-Peptone (Difco) and 0.01 percent Antifoam Emulsion A (Sigma Chemical Co., St. Louis, MO.) in distilled water were used. Initial 1:100 dilutions of concentrated cultures were made by adding 1 gram of concentrated culture to 99 ml of diluent. The vials of concentrated cultures were thawed by submerging each in 1-liter of water at 25^{o}C for five minutes. Prior to opening, the vials were immersed in 95% ethanol and excess alcohol was removed by wiping with a Kim-Wipe laboratory wipe (Kimberly Clark Corp., Roswell, GA.)

Feeding Trial 1 Design

Six week old Yorkshire gilts weighing between 8.85 and 16.56 kg (avg=11.48 kg) were assigned to four groups (6/group) based on litter number and weight. Litter mates

were assigned to separate groups and the remaining animals were assigned so that each group had equal average weights. Each pig was housed in an individual pen. The pigs completed a seven day adjustment period prior to starting the experimental period. During the adjustment period, the pigs received a diet without added cholesterol twice daily (Table II) and 50 ml of sterile reconstituted 10 percent nonfat milk solids (NFMS) daily. Initially, they received 0.091 kg of feed per feeding but that was increased to 0.181 kg then to 0.272 kg per feeding as consumption needs increased during the adjustment period.

For the experimental period, the four groups were randomly assigned to one of four treatments (Table I) consisting of two rations supplemented with fat and cholesterol, one with and one without whey (Table II). All pigs received 50 ml of 10 percent NFMS each day. The milk for each pig in one group receiving each ration was supplemented with 2.5×10^{10} cells of L. acidophilus RP32 and 2.5×10^{10} cells of L. acidophilus RP42 per day. At the beginning of the experimental period, the pigs received 0.408 kg of feed per feeding which increased as consumption needs increased. At the end of the trial, the pigs were receiving 0.862 kg feed per feeding. Any feed left two hours after each feeding was removed, weighed and the amount consumed was recorded.

On days 0, 7, 14, 21, and 28, the pigs were weighed and blood samples were taken.

TABLE I

EXPERIMENTAL TREATMENTS FOR TRIAL I

Group 1	Nonwhey diet (S-244) and 50ml sterile 10 percent NFMS
Group 2	Nonwhey diet (S-244) and 50ml 10 percent NFMS containing $5x10^{10}$ cells of *Lactobacillus acidophilus*[a]
Group 3	Whey diet (S-245) and 50ml sterile 10 percent NFMS
Group 4	Whey diet (S-245) and 50ml 10 percent NFMS containing $5x10^{10}$ cells of *Lactobacillus acidophilus*[a]

[a]The milk was prepared by adding equal numbers of *Lactobacillus acidophilus* RP32 and *Lactobacillus acidophilus* RP42 from frozen concentrated cultures to sterile 10 percent NFMS. The final concentration was $5x10^{10}$ cells per 50ml.

TABLE II

PERCENTAGE COMPOSITION OF ADJUSTMENT AND TRIAL DIETS FOR TRIAL 1

Item	Adjustment diet S-237	Experimental diets S-244	S-245
Corn	48.48	55.24	17.49
Soybean Meal	28.62	33.50	22.80
Whey	20.09	-----	50.00
Butter	-----	7.50	7.50
Calcium Carbonate	0.73	1.10	0.60
Dicalcium Phosphate	1.46	1.85	0.80
Vitamin Mineral TM[a]	0.38	0.40	0.40
Salt	0.25	0.25	0.25
Cholesterol[b]	-----	0.16	0.16
Calculated composition			
Crude Protein	19.27	19.63	17.60
Lysine	1.10	1.10	1.10
Calcium	0.85	0.91	0.91
Phosphorus	0.70	0.68	8.68

[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 Mn, 8.2g Zn, 8.2g Fe, 0.91g Cu, 16.3mg I per kilogram of vitamin-mineral TM

[b] Purity at least equivalent to USP (Sigma Chemical Co St. Louis MO.)

Blood Collection and Analysis

Venous blood samples (10 ml volume) were collected on the appropriate days after a 12 hour fast by vena cave puncture using the method described by Carle and Dewhist (8). Sterile 10 ml draw Vacutainers with no additives, fitted with 3.81 cm 20 gauge sterile needles (Becton Dickinson, Rutherford, NJ.), were used. Immediately following collection, the blood samples were chilled on ice for transportation to the laboratory. The samples were held four hours at 4^{o}C then centrifuged at 3000xg for 10 minutes at 4^{o}C using the Vacutainer tubes and Auto Iso-Filters (Clay Adams, Parsippany, NJ.).

The serum was transferred to clean screw capped tubes and thoroughly mixed. Aliquots were removed for analysis and the remaining serum was frozen and stored in cryogenic vials (DynaTech Laboratories Inc., Chantilly, VA) at -20^{o}C.

The serum was immediately assayed for total cholesterol and HDL cholesterol using the enzymatic reagent kit from Sigma Chemical Co.(St. Louis MO.). A phosphotungstate precipitating reagent (Sigma Chemical Co. St. Louis MO.) was used in the HDL cholesterol analysis. The manual procedure for spectrophotometers requiring reaction volumes greater than 1 ml was used (58).

Feeding Trial 2 Design

Five week old Yorkshire gilts weighing 6.58 to 10.66 kg (avg=8.51 kg) were assigned to four treatment groups of five each (Table III) and housed as in the previous trial. The pigs had a seven day adjustment period prior to the start of the experimental period. Pigs in groups 1 and 2 received a diet without dried whey and those in groups 3 and 4 received the diet containing whey (Table IV). They each received 0.295 kg of the ration per feeding (two feedings per day). Additionally each animal was fed 50 ml of sterile 10 percent NFMS once daily. The adjustment rations for each group were the same as the experimental rations but did not contain added crystalline cholesterol.

During the experimental period, the diets with added cholesterol were fed (Table IV). Pigs in all groups were also fed 50 ml 10% NFMS once daily just prior to the morning feeding. The milk for pigs in groups 2 and 4 was supplemented with $5x10^{10}$ cells of L. acidophilus RP32 from concentrated cultures which had been stored at $-196^{o}C$. During week 1 the pigs received 0.408 kg per feeding, in week 2, they received 0.499 kg per feeding and in week 3, they received 0.590 kg per feeding. Any feed left two hours after each feeding was removed and the amount consumed was determined and recorded. On days 0, 5, 10, 15, and 20, the pigs were weighed and blood samples were taken.

TABLE III

EXPERIMENTAL TREATMENTS FOR TRIAL 2

Group 1	Nonwhey diets (S-247) and 50ml sterile 10 percent NFMS
Group 2	Nonwhey diets (S-247) and 50ml 10 percent NFMS containing $5x10^{10}$ cells of *Lactobacillus acidophilus* RP32[a]
Group 3	Whey diet (S-246) and 50ml sterile 10 percent NFMS
Group 4	Whey diet (S-246) and 50ml 10 percent NFMS containing $5x10^{10}$ cells of *Lactobacillus acidophilus* RP32[a]

[a]The milk was prepared by adding cells of *Lactobacillus acidophilus* RP32 from frozen concentrated cultures to sterile 10 percent NFMS. The final concentration was $5x10^{10}$ cells per 50ml.

TABLE IV

PERCENTAGE COMPOSITION OF ADJUSTMENT AND TRIAL DIETS FOR TRIAL 2[a]

Item	Diets	
	S-246	S-247
Corn	42.48	57.28
Soybean Meal	27.00	31.40
Whey	20.00	-----
Butter	7.50	7.50
Calcium Carbonate	0.90	1.06
Dicalcium Phosphate	1.50	1.95
Vitamin Mineral TM[b]	0.40	0.40
Salt	0.25	0.25
Cholesterol[c]	0.16	0.16
Calculated composition		
Crude Protein	18.03	18.88
Lysine	1.04	1.04
Calcium	0.92	0.92
Phosphorus	0.69	0.69

[a]Adjustment rations were the same as the experimental diets but did not contain added cholesterol

[b]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 Mn, 8.2g Zn, 8.2g Fe, 0.91g Cu, 16.3 mg I per kilogram of vitamin-mineral TM

[c]Purity of at least USP (Sigma Chemical Co.,St. Louis MO.)

Blood Collection and Analysis

On the appropriate days, 10 ml venous blood samples were collected after a 12 hour fast, and prepared by the procedure used in the previous trial. After preparation, the serum was frozen in cryogenic vials at -20^{o}C for later analysis.

When the trial was completed, all samples for each animal were assayed together. Before analysis, the serum samples were thawed at room temperature and mixed a minimum of thirty seconds on a Vortex-Genie mixer (Scientific Industries Inc., Bohemia NY.) before dispensing for analysis. The samples were analyzed by the methods used in the previous trial.

Statistical Analysis

Total and HDL serum cholesterol data as well as feed intake and pig weight data were analyzed using the general linear models procedure from the Statistical Analysis System (31). The least significant difference mean separation procedure was used to determine statistical significance among means of serum total and HDL cholesterol, feed intake and pig weights for the four treatment groups at the five sampling periods of each trial (62). Simple and partial correlation coefficients were determined to evaluate relationships among variables in the trials (62).

CHAPTER IV

RESULTS

Screening of Cultures

The amounts of cholesterol assimilated by each of 14 candidate strains of Lactobacillus acidophilus isolated from intestines of pigs are shown in Table V. The results are expressed as the amount (ug/ml) assimilated from the broth medium during 24 hours at 37°C. The strains varied substantially in their ability to remove cholesterol from the growth medium. Strains 1-3, RP32, RP42, and RP43 exhibited the most uptake of cholesterol. Strain GP4A was the least active of the 14 strains. Other strains were intermediate.

Strains GP1A, GP3A, RP32, and RP42 were the most bile resistant of the 14 strains tested in that they grew faster in MRS broth containing 0.3% oxgall (MRSO) than did the other strains (Table VI). This was indicated by the time needed for the growing culture to increase the absorbance of the growth medium by 0.3 units at 600 nanometers. Strains 149C, A4, GP1A, and GP3A appeared to grow as well as or more rapidly in MRSO than in the control (MRS) broth. All the other strains tested appeared to be somewhat inhibited by

TABLE V

ASSIMILATION OF CHOLESTEROL BY SELECTED STRAINS OF LACTOBACILLUS ACIDOPHILUS

Strain	Cholesterol Assimilated (ug per ml of broth)[ab]
1-3	93.8
149-C	63.8
A1	29.0
A3	31.6
A4	28.2
C1-3	47.0
C1-6	35.4
GP1A	27.4
GP3A	38.8
GP4A	4.8
RP32	87.4
RP34	25.0
RP42	94.2
RP43	95.0

[a]Each value is the average of three trials
[b]Difference between cholesterol concentration in uninoculated control broth and in spent broth following 24 hours of growth of the test strain

TABLE VI

BILE TOLERENCE OF SELECTED STRAINS OF LACTOBACILLUS ACIDOPHILUS

Strain	Hours for Culture to Increase A_{600} by 0.3 Units[a]	
	Control (MRS) broth	MRS broth +0.3% Oxgall
1-3	4.99	6.63
149C	4.13	4.15
A1	4.38	4.52
A3	3.94	4.18
A4	4.12	4.07
C1-3	3.97	5.65
C1-6	5.40	>7.42[b]
GP1A	4.45	3.92
GP3A	4.02	3.80
GP4A	4.17	4.52
RP32	2.45	2.76
RP34	4.23	>5.62
RP42	3.37	3.66
RP43	4.20	4.88

[a]Each value is the average of three trials
[b]Readings were made at 0-8 hours. One or more value was greater than 8 hours in which case the value 8 was used to obtain the mean value

the presence of oxgall. Strains C1-6 and RP34 were most sensitive to the bile. Strain RP32 which exhibited the most rapid growth of all strains in MRSO broth also grew fastest in MRS broth. However the rapidity of growth in the MRS broth was not a good predictor of rapidity of growth in MRSO broth. For example, strain C1-3 which appeared to grow almost as rapidly as strain GP3A in MRS broth grew much slower than GP3A in MRSO broth. No clear association between bile resistance (Table VI) and ability to assimilate cholesterol (Table V) was indicated.

All strains except 149-C produced some antagonistic action toward at least one other culture tested (Table VII). However, strain 149-C was inhibited by spent broth from 8 of the other strains. Growth of strains 149-C, C1-3, and C1-6 was inhibited by the majority of the other cultures indicating that these strains may be sensitive to the antagonistic action of a wide variety of strains of L. acidophilus. However none of the other strains tested were sensitive to spent broth from any of the 14 strains.

Strains RP32 and RP42 were selected for use in pig feeding trials based on their bile tolerance, cholesterol assimilating ability and production of (or resistance to) antagonistic interactions with other strains. The identity characteristics of these two strains are listed in Table VIII. They were confirmed to be Lactobacillus acidophilus. Both exhibited differences in fermentation patterns but they more closely matched the characteristics of L. acidophilus

TABLE VII

DISC ASSAY TO TEST FOR INHIBITORY INTERACTIONS AMONG STRAINS OF *LACTOBACILLUS ACIDOPHILUS*

Strain Tested for Sensitivity	Code	Spent Broth pH 6.5[a]													
		a	b	c	d	e	f	g	h	i	j	k	l	m	n
1-3	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-
149-C	b	+	-	+	+	+	+	+	-	+	-	+	-	-	-
A1	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A3	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A4	e	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C1-3	f	+	-	+	+	+	-	-	+	+	+	-	+	+	+
C1-6	g	-	-	+	+	+	-	-	+	+	+	-	+	+	+
GP1A	h	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GP3A	i	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GP4A	j	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RP32	k	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RP34	l	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RP42	m	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RP43	n	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] The spent broth of each strain was assayed against each strain three times; - = presence of inhibition; + = absence of inhibition

TABLE VIII

IDENTITY CHARACTERISTICS OF *LACTOBACILLUS ACIDOPHILUS* CULTURES USED IN PIG FEEDING TRIALS

TEST	Bergey's[a]	RP32	RP42
Gram Stain	+	+	+
Cellular Morphology	rods	rods	rods
Catalase	-	-	-
Growth at 15°C	-	-	-
Growth at 45°C	+	+	+
NH_3 from Arginine	-	-	-
Hydrolysis of Esculin	+	+	+
Acid from:			
Amygdalin	+	+	-
Arabinose	-	-	-
Cellobiose	+	+	+
Galactose	+	-	+
Glucose	+	+	+
Lactose	+	+	+
Maltose	+	+	+
Mannitol	-	-	-
Mannose	+	-	-
Melezitose	-	-	-
Melibiose	+	+	+
Raffinose	+	+	+
Rhamnose	-	-	-
Salicin	+	+	-
Sorbitol	-	-	-
Sucrose	+	+	+
Trehalose	+	+	-
Xylose	-	-	-

[a] The characteristics of *L. acidophilus* as listed in *Bergey's Manual of Determinative Bacteriology* 8th edition.

listed in the 8th edition of Bergey's Manual of Determinative Bacteriology (7) than any other species listed. Strain RP32 differed in that it did not ferment galactose or mannose. Strain RP42 did not ferment amygdalin, mannose, salicin, or trehalose.

The survivability of L. acidophilus RP32 and RP42 in concentrated cultures is shown in Table IX. Neither strain exhibited much decline in total viability (PMN agar counts) over the 28 day storage period. There also was no appreciable decline in numbers of bile resistant lactobacilli (PMNO agar counts). Thus the cultures were stable in frozen storage at -196^{o}C.

Feeding Trial 1

In trial 1, 24 six week old Yorkshire gilts were fed for 28 days on rations containing added cholesterol. Six animals were assigned to each of the 4 treatment groups (Table I). Animals in treatments 2 and 4 received $5x10^{10}$ cells L. acidophilus per day. Animals in treatments 3 and 4 received a 50% whey ration while those in treatments 1 and 2 received a nonwhey ration. Both were corn and soybean meal based rations (Table II).

Blood samples were drawn at 7 day intervals during the trial for total and HDL serum cholesterol analysis. Cholesterol levels are expressed as mg cholesterol per 100ml serum (Tables X and XI). Serum total and HDL cholesterol levels in all treatment groups increased significantly by

TABLE IX

SURVIVAL OF LACTOBACILLUS ACIDOPHILUS RP32 AND RP42 IN CONCENTRATED CULTURES DURING STORAGE AT -196°C

Days at -196°C	$\log_{10}$ CFU/g Concentrate			
	L. acidophilus RP32[a]		L. acidophilus RP42[b]	
	PMN[c]	PMNO[c]	PMN[c]	PMNO[c]
0	10.45 + 0.44	10.42 + 0.42	10.35 + 0.11	10.32 + 0.09
1	10.41 + 0.42	10.40 + 0.40	10.30 + 0.14	10.33 + 0.08
7	10.38 + 0.43	10.34 + 0.44	10.32 + 0.09	10.29 + 0.08
14	10.48 + 0.42	10.41 + 0.39	10.37 + 0.11	10.34 + 0.09
21	10.42 + 0.43	10.41 + 0.46	10.30 + 0.05	10.29 + 0.05
28	10.53 + 0.38	10.53 + 0.37	10.31 + 0.17	10.29 + 0.17

[a] Each value is the mean from seven batches
[b] Each value is the mean from four batches
[c] PMN = pepsinized milk nutrient agar; PMNO = PMN agar + 0.1% oxgall

TABLE X

SERUM TOTAL CHOLESTEROL LEVELS
AT SEVEN DAY INTERVALS
FOR TRIAL 1

	mg Cholesterol/dl Serum[a]				
	Days on Trial				
Treatment	0	7	14	21	28
1	83.35 ± 13.24	96.20 ± 13.51	104.50 ± 6.29[b]	101.10 ± 10.41	140.43 ± 17.35[e]
2	79.12 ± 8.50	88.95 ± 12.02	107.07 ± 17.77[b]	102.00 ± 17.49[b]	133.27 ± 26.80[e]
3	80.77 ± 6.33	88.75 ± 12.29	110.26 ± 19.47[c]	118.10 ± 14.17[d]	138.26 ± 20.37[e]
4	83.00 ± 9.30	92.09 ± 12.19	109.39 ± 17.96[c]	119.26 ± 22.68[d]	145.91 ± 31.86[e]

[a] Each value is a mean from six pigs ± standard deviation

[bcde] Superscript indicates level of significant difference of the value from the day 0 value. b = (P<0.05), c = (P<0.01), d = (P<0.001), e = (P<0.0001)

TABLE XI

SERUM HDL CHOLESTEROL LEVELS AT
SEVEN DAY INTERVALS
FOR TRIAL 1

	mg HDL Cholesterol/dl Serum[a]				
	Days on Trial				
	0	7	14	21	28
Treatment					
1	29.57 ± 5.17	32.88 ± 6.07	37.00 ± 3.03[b]	39.85 ± 4.67[c]	48.62 ± 4.8[e]
2	31.23 ± 7.65	31.24 ± 5.22	40.64 ± 4.39[c]	40.94 ± 4.85[c]	50.89 ± 4.06[e]*
3	30.00 ± 6.81	31.98 ± 4.57	38.25 ± 6.20[c]	43.11 ± 7.20[e]	46.21 ± 4.17[e]
4	32.00 ± 6.00	30.68 ± 3.65	39.89 ± 4.83[b]	42.25 ± 6.28[c]	43.70 ± 4.40[e]*

[a] Each value is a mean from six pigs ± standard deviation

bcde Superscript indicates level of significant difference of the value from the day 0 value; b = ($P<0.05$), c = ($P<0.01$), d = ($P<0.001$), e = ($P<0.0001$)

* Treatment values differ significantly ($P<0.05$)

day 14 of the trial from a baseline value at day 0. Serum total cholesterol levels were not significantly different among treatment groups at any of the 5 sampling periods (Table X). There was a significant difference in serum HDL cholesterol levels between groups 2 and 4 at day 28 ($P<0.05$) (Table XI). There was considerable variation in serum total cholesterol levels within all the treatment groups as indicated by the large standard deviations. This large variation between individual pigs within each treatment group may have masked any differences due to the treatments.

Pig weights did not differ significantly ($P>0.05$) among treatment groups at any of the 5 test periods of the trial (Table XII). Group means of pig weights did increase during each trial period however.

Feed intake data for each 7 day period showed that feed intake was not significantly different ($P>0.05$) among the 4 treatment groups. Group mean feed intake did increase throughout the trial (Table XIII).

Simple correlation coefficients were determined for the variables in the trial (Table XIV). "Days on trial" was significantly correlated with pig weights, HDL and total serum cholesterol levels ($P<0.0001$). Total and HDL serum cholesterol levels were significantly correlated with pig weights ($P<0.0001$) and total serum cholesterol levels were correlated with HDL serum cholesterol levels ($P<0.0001$). Partial correlation coefficients were significant between total and HDL serum cholesterol ($P<0.01$), between pig

TABLE XII

PIG WEIGHTS AT SEVEN DAY INTERVALS FOR TRIAL 1[a]

	Kg Bodyweight[b]				
	Days on Trial				
	0	7	14	21	28
Treatment					
1	12.53 ± 2.53	16.46 ± 2.72	21.39 ± 2.85	26.50 ± 3.66	29.79 ± 2.98
2	12.64 ± 2.26	16.37 ± 3.17	20.73 ± 3.64	24.83 ± 4.16	28.77 ± 4.40
3	12.76 ± 1.90	16.27 ± 2.23	20.89 ± 2.49	25.44 ± 2.62	30.41 ± 2.98
4	12.61 ± 1.77	15.80 ± 1.82	20.56 ± 2.03	24.23 ± 1.91	29.39 ± 2.02

[a] Group means did not differ significantly between treatments at any sampling period.
[b] Each value is the mean value from six pigs ± standard deviation

TABLE XIII

FEED INTAKE DURING SEVEN DAY INTERVALS FOR TRIAL 1[a]

Treatment	kg Feed Intake for 7 Day Period[b]			
	Days on Trial			
	1-7	8-14	15-21	22-28
1	5.60 ± 0.55	7.77 ± 0.42	9.61 ± 0.37	9.82 ± 0.82
2	4.98 ± 0.82	6.42 ± 1.11	8.51 ± 1.11	9.25 ± 0.80
3	5.33 ± 0.76	7.06 ± 1.04	9.09 ± 0.99	9.80 ± 0.91
4	5.22 ± 0.74	6.82 ± 1.08	8.53 ± 1.19	9.11 ± 1.18

[a] Intake values did not differ significantly between any of the treatment groups in any of the trial periods

b Each value is the mean from six pigs ± standard deviation

TABLE XIV

SIMPLE CORRELATION COEFFICIENTS
FOR TRIAL 1

	Pig Weights	HDL Cholesterol	Total Cholesterol	Feed Intake	Days on Feed
Days on Trial	0.899[a]	0.755[a]	0.719[a]	0.070	1.000
Feed Intake	0.117	0.074	0.098	1.000	
Total Cholesterol	0.663[a]	0.679[a]	1.000		
HDL Cholesterol	0.752[a]	1.000			
Pig Weights	1.000				

[a] Values are significant (P<0.0001)

weights and HDL serum cholesterol ($P<0.01$) and between pig weights and feed intake ($P<0.05$) (Table XV).

Feeding Trial 2

Feeding trial 2 involved 20 five week old Yorkshire gilts. Five animals were assigned to each of the 4 treatment groups (Table III). Animals in treatment groups 1 and 2 received a diet without whey and those in groups 3 and 4 received a diet containing 20% whey. Both experimental diets contained added cholesterol (Table IV). Pigs in groups 2 and 4 also were fed $5x10^{10}$ cells L. acidophilus RP32 per day.

The pigs were on trial for 20 days and blood samples were taken at 5 day intervals. A significant increase in serum total cholesterol was not observed for any group during the trial nor were there any significant differences among treatments at any of the five sampling periods (Table XVI). An overall increase was not observed for HDL cholesterol levels (Table XVII). There was a significant difference between treatment groups 1 and 3 on day 15 of the trial ($P<0.05$). This, however, does not appear to indicate a trend towards consistent differences in serum HDL cholesterol levels for the different treatment groups.

Mean pig weights for each treatment group increased at each collection period (Table XVIII). The weights did not,

TABLE XV

PARTIAL CORRELATION COEFFICIENTS
FOR TRIAL 1

	Feed Intake	Pig Weights	HDL Cholesterol	Total Cholesterol
Total Cholesterol	0.050	0.106	0.289[b]	1.000
HDL Cholesterol	-0.091	0.310[a]	1.000	
Pig Weights	0.228[b]	1.000		
Feed Intake	1.000			

[a] Values were significant (P<0.01)
[b] Values were signigicant (P<0.05)

TABLE XVI

SERUM TOTAL CHOLESTEROL LEVELS
AT FIVE DAY INTERVALS
FOR TRIAL 2[a]

	mg Cholesterol/dl Serum[b]				
	Days on Trial				
Treatment	0	5	10	15	20
1	79.20 ± 20.59	73.64 ± 19.78	80.84 ± 44.17	81.74 ± 24.69	84.42 ± 26.36
2	76.70 ± 13.56	72.24 ± 8.44	89.55 ± 22.42	84.27 ± 28.03	77.13 ± 26.57
3	76.95 ± 21.97	65.54 ± 4.62	81.37 ± 16.00	73.37 ± 15.67	79.54 ± 13.69
4	79.69 ± 9.00	64.10 ± 7.05	87.18 ± 14.15	91.78 ± 19.45	90.28 ± 13.85

[a] No significant increase from day 0 values occured nor were any significant differences between treatment group means observed at any of the sampling periods.
[b] Each value is the mean from five pigs ± standard deviation

TABLE XVII

SERUM HDL CHOLESTEROL LEVELS AT
FIVE DAY INTERVALS
FOR TRIAL 2

	mg HDL Cholesterol/dl Serum[a]				
	Days on Trial				
Treatment	0	5	10	15	20
1	32.64 ± 5.67	31.44 ± 5.96	32.32 ± 6.75	36.62 ± 7.78*	36.04 ± 5.39
2	28.75 ± 6.24	30.02 ± 5.21	36.52 ± 4.33[b]	35.10 ± 5.43	32.91 ± 7.63
3	29.64 ± 6.32	26.22 ± 5.62	32.08 ± 5.36	28.10 ± 6.61*	31.10 ± 5.38
4	27.23 ± 6.63	26.52 ± 4.65	33.72 ± 7.67	34.03 ± 5.62	34.78 ± 6.13

[a] Each value is the mean from five pigs ± standard deviation

Superscript indicates level of significant difference of the value from the day 0 value; b = (P<0.05), c = (P<0.01), d = (P<0.001), e = (P<0.0001)

* Treatment values differ significantly (P<0.05)

TABLE XVIII

PIG WEIGHTS AT FIVE DAY INTERVALS FOR TRIAL 2[a]

	Kg Bodyweight[b]				
	Days on Trial				
Treatment	0	5	10	15	20
1	8.32 ± 1.53	9.23 ± 2.19	10.07 ± 1.66	12.00 ± 1.52	15.56 ± 1.85
2	8.71 ± 1.00	9.00 ± 1.42	10.07 ± 1.57	11.32 ± 2.29	14.58 ± 2.81
3	8.66 ± 1.13	9.57 ± 1.38	11.25 ± 2.36	12.66 ± 1.78	15.42 ± 3.56
4	8.71 ± 1.25	9.93 ± 1.29	11.11 ± 2.39	12.86 ± 2.65	16.74 ± 2.86

[a] Group means did not differ significantly between treatments at any sampling period.
b Each value is the mean from five pigs ± standard deviation

however, differ significantly among treatment groups at any of the five weighing times.

Feed intakes were not significantly different for any of the treatments during the four, five-day test periods (Table XIX). Feed intakes increased throughout the trial, however, they were far below the intakes observed in trial 1.

In trial 2, days on trial were significantly correlated with pig weights ($P<0.0001$) and serum total cholesterol was correlated with HDL cholesterol levels ($P<0.0001$) (Table XX). Days on trial was negatively correlated with feed intake ($P<0.0001$).

Significant partial correlation coefficients were found between HDL and total serum cholesterol ($P<0.0001$) and between feed intake and pig weights ($P<0.05$) (Table XXI). Serum total or HDL cholesterol were not significantly correlated with feed intake in either trial. This lack of correlation and the partial correlation coefficients suggest that factors other than feed intake may have had an effect on blood cholesterol levels.

TABLE XIX

FEED INTAKE DURING FIVE DAY INTERVALS FOR TRIAL 2[a]

Treatment	kg Feed Intake for 5 Day Period[b]			
	Days on Trial			
	1-5	6-10	11-15	16-20
1	1.47 ± 0.81	2.22 ± 0.59	3.18 ± 0.86	4.27 ± 0.53
2	1.51 ± 0.24	2.20 ± 0.94	2.87 ± 1.06	3.69 ± 1.19
3	1.73 ± 0.93	2.62 ± 0.88	3.02 ± 1.16	3.92 ± 1.30
4	1.45 ± 0.67	2.62 ± 1.08	3.69 ± 0.88	4.60 ± 1.00

[a] Intake values did not differ significantly between any of the treatment groups in any of the trial periods

[b] Each value is the mean from five pigs ± standard deviation

TABLE XX

SIMPLE CORRELATION COEFFICIENTS
FOR TRIAL 2

	Pig Weights	HDL Cholesterol	Total Cholesterol	Feed Intake	Days on Feed
Days on Trial	0.740[a]	0.246	0.083	-0.600[a]	1.000
Feed Intake	-0.234	0.272	0.003	1.000	
Total Cholesterol	0.118	0.702[a]	1.000		
HDL Cholesterol	0.181	1.000			
Pig Weights	1.000				

[a] Values are significant ($P<0.0001$)

TABLE XXI

PARTIAL CORRELATION COEFFICIENTS
FOR TRIAL 2

	Feed Intake	Pig Weights	HDL Cholesterol	Total Cholesterol
Total Cholesterol	0.163	0.099	0.721[a]	1.000
HDL Cholesterol	-0.039	0.072	1.000	
Pig Weights	0.292[b]	1.000		
Feed Intake	1.000			

[a] Values were significant (P<0.0001).

[b] Values were significant (P<0.05).

CHAPTER V

DISCUSSION

There was much variation among the 14 cultures of L. acidophilus tested in respect to bile tolerance, ability to assimilate cholesterol and production of (or resistance to) inhibition of other strains (Tables V, VI, VII). This emphasizes the need for careful culture selection. Just being a L. acidophilus culture is not enough. Any culture selected as a dietary adjunct must have the properties to enable it to produce the desired function(s) in the host.

Any strain of L acidophilus chosen for use as a dietary adjunct to lower serum cholesterol levels must possess certain characteristics. The culture must be able to survive and grow under conditions found in the intestine. It must be able to grow in the presence of bile (17) and be able to assimilate cholesterol under conditions likely to be found in the intestine (14). Furthermore it should not be easily inhibited by organisms occurring in the existing intestinal flora. Since L. acidophilus exhibits host specificity (13), any strain selected as an adjunct should be one capable of surviving and functioning in the desired host. Thus it should be one originally isolated from the specie for which it is to be used. Both strains selected in

this study were isolated from pig intestines. They were bile resistant and were not inhibited by other strains of L. acidophilus. They also produced maximal reductions in cholesterol concentrations in a laboratory growth medium.

Both L. acidophilus and dietary whey have been shown to reduce blood cholesterol in animal models and in human infants. Gilliland et al.(14) were able to reduce serum cholesterol in growing swine by supplementing their diet with a cholesterol assimilating strain of L. acidophilus. In their report, variations in blood cholesterol levels among animals were much smaller within treatment groups (as indicated by group standard deviations) than in either trial in the present study.

The high variations in blood cholesterol within treatment groups such as observed in the present study must be reduced in subsequent trials. The variation may have been caused by differences in the ages (Tables XXIII and XXV) and weights (Tables XII and XVIII) among animals at the start of the trials. Differences in feed intake and weight gains may produce individual variations in blood cholesterol levels. However, the lack of significant differences in either feed intake or pig weights among treatments suggests that this may not have occurred in the present study. Normal variation would be expected to occur within animals in each treatment group. However, the variations observed in the trial by Gilliland et al. (14) were much less than in the present study.

Carroll and Hamilton (9) indicated that pigs undergo a reduction in plasma cholesterol within one week after weaning, however by the third week the cholesterol levels stabilize at near previous levels. Such a phenomenon may have influenced the results seen in the present trial. Early weaned pigs (3 weeks of age) do not appear to undergo as drastic a reduction (9). Since some of the animals in the second trial were less than five weeks old (Table XXV), this too may have influenced the results.

Beames et al. (2) observed a trend towards lower blood cholesterol in growing swine consuming a ration containing 50% whey and 0.5% cholesterol compared to pigs on a nonwhey ration which contained 0.5% cholesterol. Using mature animals on an elevated cholesterol (0.5%) diet, Norton et al. (50) were able to significantly decrease their serum cholesterol by supplementing the diet with 40% whey. Although total serum cholesterol was reduced, HDL serum cholesterol increased compared to animals on a control diet.

Both trials in the present study as well as the work by Beames et al. (2) involved animals that were considerably younger than those used by Norton et al. (50). Beames et al. (2) did not get a significant reduction in serum cholesterol levels as did Norton et al. (50), with the more mature animals. The reduction in serum cholesterol which can be achieved by adding whey in the diet may not be as pronounced in the younger, less mature animals.

Feed intake was much less for the smaller animals in the second trial than in the first which suggests that the animals may not have consumed enough of the diet containing cholesterol to produce an elevation in their cholesterol levels. The animals in the second trial were younger than those in the first. This may have adversely influenced their adaptation to the dry diet and thus resulted in greatly reduced feed intake.

Weights of the pigs at the end of trial 1 were 10 kg greater than those at the end of the trial in the study by Gilliland et al. (14). Since the cell numbers of lactobacilli fed daily were the same in both trials, the number fed may need to be increased as animal weight increases in order to produce the same response. Differences in growth rates of the young pigs may also be a factor.

In order to use growing swine as an experimental model for cholesterol related studies, the model must be better defined. Studies need to be conducted to determine what levels of dietary cholesterol elicit a consistent increase in serum cholesterol levels among experimental animals. Excessive cholesterol intake most likely will negate detection of any positive responses due to consumption of cells of L. acidophilus or dried whey. The level of whey intake required to produce a lowering response in the growing pig needs to be determined. Also, the optimum cell numbers of L. acidophilus needed daily should be determined. The many unanswered questions produced by

the present study point out the need for further research. A longer adjustment period following weaning for the younger animals may decrease their variability. Alternatively, mature animals (ie. full grown) may not express as much variability. Furthermore, such a nongrowing animal may more accurately model the adult human.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Elevated levels of serum cholesterol are considered one of the major risk factors contributing to coronary heart disease. Consumption of either cells of L. acidophilus or dairy whey has been shown to limit increases in blood cholesterol levels in animals on a high cholesterol diet. A further understanding is needed to determine if these methods of dietary supplementation would provide an alternative method for individuals to control elevated blood cholesterol levels. The purpose of this study was to evaluate the young growing pig as a model to determine the singular and combined effects of consuming cells of L. acidophilus and dried sweet dairy whey on serum cholesterol levels.

Fourteen strains of L. acidophilus were screened as candidates to use as adjuncts in pig feeding trials. Strains RP32 and RP42 were chosen because they more actively assimilated cholesterol from a growth medium and were more bile resistant than other strains tested. Furthermore, they were resistant to antagonistic inhibition produced by other strains of L. acidophilus tested.

Supplementation of the diet with cells of L. acidophilus, dried whey, or a combination of the two did not

influence elevated serum cholesterol levels of six week old pigs on a high cholesterol diet. Five week old pigs used in a similar trial on a high fat and cholesterol diet without L. acidophilus or whey did not exhibit an increase in blood cholesterol as expected. Supplementation of the younger pig's diet with whey or L. acidophilus RP32 did not cause a reduction in blood cholesterol levels. Excessive variation in blood cholesterol levels for pigs in each treatment group in both trials likely concealed any effects due to treatments. Because the younger pigs either did not exhibit an increase in blood cholesterol levels or exhibited excessive variation among animals, they may not be a model for cholesterol related studies.

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APPENDIX A

ASSIGNMENT AND AGE OF PIGS IN TRIAL 1

TABLE XXII

ASSIGNMENT OF PIGS TO TREATMENTS
FOR TRIAL 1

Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Ear Notch		Ear Notch		Ear Notch		Ear Notch
Pig no	Litter Ind	Pig no	Litter Ind	Pig no	Litter Ind	Pig no	Litter Ind
1	83 - 1	2	82 - 5	4	78 - 4	6	78 - 7
8	78 - 9	3	83 - 4	7	80 - 0	10	78 - 6
13	82 - 1	5	77 - 9	9	83 - 2	19	82 - 2
16	78 -11	14	78 - 5	11	4 - 6	21	80 - 5
17	81 - 9	15	4 - 5	12	82 - 3	22	81 -14
24	77 -11	20	81 -11	18	81 -13	23	83 - 4

TABLE XXIII

AGE OF PIGS USED IN TRIAL 1

Litter No.	Birth Date	Days of age at Beginning of Adjustment period	Number of pigs In Trial
77	5-19-1986	53	2
78	5-21-1986	51	6
80	5-27-1986	45	2
81	5-28-1986	44	4
83	5-30-1986	42	4
82	5-30-1986	42	4
4	6-05 1986	36	2

APPENDIX B

ASSIGNMENT AND AGE OF PIGS IN TRIAL 2

TABLE XXIV

ASSIGNMENT OF PIGS TO TREATMENTS
FOR TRIAL 2

Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Ear Notch		Ear Notch		Ear Notch		Ear Notch
Pig no	Litter Ind	Pig no	Litter Ind	Pig no	Litter Ind	Pig no	Litter Ind
1	55 - 3	7	54 -13	6	53 - 9	4	54 -10
3	52 - 7	8	52 -10	11	54 -12	12	57 - 8
5	55 - 9	10	57 -10	15	53 - 7	16	53 - 4
9	54 -11	14	55 -10	17	57 - 9	18	58 8
13	53 - 6	22	53 - 8	19	52 - 8	20	52 - 6

TABLE XXV

AGE OF PIGS USED IN TRIAL 2

Litter No.	Birth Date	Days of age at Beginning of Adjustment period	Number of pigs In Trial
52	9-03-1986	41	4
53	9-04-1986	40	5
54	9-06-1986	38	4
55	9-09-1986	35	3
57	9-12-1986	32	3
58	9-12-1986	32	1

APPENDIX C

INDIVIDUAL PIG DATA

FOR TRIAL 1

TABLE XXVI

INDIVIDUAL PIG DATA FOR TRIAL 1

Pig No.	Day of Trial	Treat-ment	Total Chol-esterol	HDL Chol-esterol	Pig Weights	Feed Intake
1	0	1	77.02	29.81	17.01	
2	0	2	69.19	25.29	10.77	
3	0	2	91.16	40.15	16.56	
4	0	3	86.62	37.68	13.15	
5	0	2	82.83	35.73	13.15	
6	0	4	82.58	30.16	11.68	
7	0	3	89.90	32.72	11.57	
8	0	1	73.99	28.39	11.34	
9	0	3	78.03	34.05	16.33	
10	0	4	82.83	41.13	12.70	
11	0	3	73.23	31.31	11.00	
12	0	3	76.52	25.47	12.02	
13	0	1	72.73	24.15	10.09	
14	0	2	84.09	36.35	13.27	
15	0	2	70.45	30.07	10.32	
16	0	1	97.98	38.21	13.83	
17	0	1	86.11	24.85	10.89	
18	0	3	80.30	18.75	12.47	
19	0	4	86.87	33.17	11.34	
20	0	2	77.02	19.81	11.79	
21	0	4	98.48	35.20	12.02	
22	0	4	73.48	28.83	11.79	
23	0	4	73.74	23.53	16.10	
24	0	1	104.29	32.02	12.02	
1	7	1	84.14	29.60	20.87	5.15
2	7	2	85.93	27.90	14.29	4.48
3	7	2	85.68	35.03	21.09	5.56
4	7	3	91.18	35.22	17.12	5.84
5	7	2	104.22	39.00	18.37	5.99
6	7	4	80.56	25.22	15.54	5.87
7	7	3	106.65	37.22	16.56	5.95
8	7	1	104.73	38.66	15.99	5.99
9	7	3	71.87	32.88	19.73	5.66
10	7	4	86.70	33.83	15.99	5.48
11	7	3	84.02	33.64	13.15	4.04
12	7	3	81.59	27.37	14.74	4.75
13	7	1	80.05	26.35	13.15	4.65
14	7	2	83.38	32.66	17.35	5.41
15	7	2	72.51	27.45	12.36	3.77
16	7	1	112.40	41.68	17.69	5.82
17	7	1	88.87	28.39	14.29	5.61

TABLE XXVI (Continued)

Pig No.	Day of Trial	Treat-ment	Total Chol-esterol	HDL Chol-esterol	Pig Weights	Feed Intake
18	7	3	97.19	25.52	16.33	5.71
19	7	4	106.91	30.92	13.27	3.98
20	7	2	101.92	25.41	14.74	4.67
21	7	4	103.07	34.01	16.22	5.71
22	7	4	97.70	27.33	14.97	5.62
23	7	4	77.62	32.77	18.82	4.68
24	7	1	107.03	32.58	16.78	5.95
1	14	1	94.21	36.86	25.40	7.98
2	14	2	125.07	40.58	17.46	4.96
3	14	2	99.45	41.82	25.17	7.36
4	14	3	115.98	47.77	21.21	6.90
5	14	2	133.33	46.75	24.49	7.98
6	14	4	86.64	33.73	20.98	7.88
7	14	3	131.54	41.91	22.34	7.94
8	14	1	110.61	36.60	21.43	7.95
9	14	3	95.73	37.59	24.27	7.89
10	14	4	96.28	44.14	20.41	6.69
11	14	3	88.29	38.91	17.24	5.27
12	14	3	96.69	31.93	18.94	6.58
13	14	1	106.06	37.07	17.80	6.92
14	14	2	95.73	40.71	21.77	6.41
15	14	2	88.98	40.92	16.56	5.56
16	14	1	105.92	42.55	22.91	7.90
17	14	1	100.14	35.45	18.48	7.98
18	14	3	133.33	31.38	21.32	7.79
19	14	4	120.52	38.87	17.12	4.96
20	14	2	99.86	33.05	18.94	6.25
21	14	4	134.71	43.54	21.32	7.14
22	14	4	118.04	34.72	20.19	7.82
23	14	4	100.14	44.31	23.36	6.44
24	14	1	110.06	33.48	22.34	7.87
1	21	1	98.55	45.26	30.62	10.01
2	21	2	121.49	41.68	22.00	7.78
3	21	2	105.79	43.47	30.16	9.97
4	21	3	110.58	46.53	26.08	8.85
5	21	2	122.49	42.40	29.03	9.41
6	21	4	97.33	37.07	24.95	10.14
7	21	3	137.86	39.12	27.22	9.94
8	21	1	100.11	37.16	25.40	9.43
9	21	3	106.79	50.54	28.58	9.78
10	21	4	121.05	51.90	24.04	8.59
11	21	3	102.23	50.63	21.55	7.52
12	21	3	131.29	38.78	23.13	8.49
13	21	1	114.48	42.19	22.23	8.96

TABLE XXVI (Continued)

Pig No.	Day of Trial	Treatment	Total Cholesterol	HDL Cholesterol	Pig Weights	Feed Intake
14	21	2	93.76	45.09	25.63	8.83
15	21	2	81.29	41.59	19.73	6.93
16	21	1	89.98	43.68	27.22	9.84
17	21	1	90.98	32.86	22.91	9.78
18	21	3	119.82	33.07	26.08	9.97
19	21	4	114.59	37.24	20.87	7.11
20	21	2	87.19	31.41	22.45	8.17
21	21	4	158.80	48.32	25.17	8.52
22	21	4	125.95	39.29	23.81	9.52
23	21	4	97.88	39.67	26.54	7.35
24	21	1	112.47	37.93	30.62	9.66
1	28	1	125.70	42.93	33.57	8.60
2	28	2	175.58	55.45	24.72	8.32
3	28	2	126.98	52.36	33.68	8.48
4	28	3	133.12	47.79	29.82	8.84
5	28	2	145.40	53.71	33.57	10.42
6	28	4	118.16	40.64	29.82	11.20
7	28	3	165.73	52.70	33.34	10.58
8	28	1	135.17	47.75	28.80	9.87
9	28	3	121.48	47.71	33.34	10.56
10	28	4	123.40	42.42	28.80	8.86
11	28	3	112.02	45.08	25.86	8.78
12	28	3	141.18	43.18	28.35	9.34
13	28	1	146.04	50.50	25.86	9.97
14	28	2	123.66	51.81	30.05	9.78
15	28	2	94.25	47.24	24.04	9.01
16	28	1	132.23	54.64	31.87	10.65
17	28	1	130.43	43.27	27.22	9.16
18	28	3	156.01	40.77	31.75	10.69
19	28	4	178.13	48.38	26.42	8.58
20	28	2	133.76	44.79	26.54	9.46
21	28	4	182.48	48.85	30.96	9.72
22	28	4	161.64	37.60	28.24	8.19
23	28	4	111.64	44.28	32.09	8.08
24	28	1	173.02	52.61	31.41	10.69

APPENDIX D

INDIVIDUAL PIG DATA

FOR TRIAL 2

TABLE XXVII

INDIVIDUAL PIG DATA FOR TRIAL 2

Pig No.	Day of Trial	Treatment	Total Cholesterol	HDL Cholesterol	Pig Weights	Feed Intake
1	0	1	91.27	35.04	8.85	
1	5	1	73.50	28.93	9.07	0.56
1	10	1	79.96	30.93	9.41	1.39
1	15	1	95.48	40.98	11.34	2.45
1	20	1	94.68	36.46	15.54	4.28
7	0	2	58.05	21.21	8.28	
7	5	2	74.81	31.61	8.96	1.69
7	10	2	110.17	35.28	11.34	3.41
7	15	2	77.28	31.23	12.70	3.56
7	20	2	51.02	24.35	16.10	5.03
6	0	3	58.18	24.01	8.16	
6	5	3	68.32	26.43	8.85	1.33
6	10	3	107.63	34.40	9.19	1.96
6	15	3	69.59	26.60	9.07	1.37
6	20	3	87.72	30.25	11.11	1.81
4	0	4	79.39	32.30	8.16	
4	5	4	60.15	29.11	10.55	2.09
4	10	4	70.32	24.86	10.66	2.77
4	15	4	87.12	31.36	10.55	3.42
4	20	4	74.27	33.36	14.52	3.27
3	0	1	62.00	35.67	7.48	
3	5	1	60.50	36.62	9.19	2.04
3	10	1	43.60	29.04	10.77	2.89
3	15	1	55.41	31.02	13.61	4.64
3	20	1	71.89	34.99	16.78	5.12
8	0	2	93.19	37.73	9.53	
8	5	2	63.08	34.91	11.34	1.81
8	10	2	63.40	32.67	13.27	2.58
8	15	2	72.17	35.74	14.63	4.37
8	20	2	51.72	25.61	18.82	4.73
11	0	3	48.48	22.96	7.82	
11	5	3	64.54	31.48	9.64	1.96
11	10	3	64.16	27.47	10.09	2.66
11	15	3	48.13	19.81	12.47	3.22
11	20	3	55.69	23.67	15.54	4.6
12	0	4	64.37	28.78	9.07	
12	5	4	63.98	28.02	9.30	1.05
12	10	4	94.23	41.80	10.09	2.06
12	15	4	100.92	42.30	12.25	3.36
12	20	4	106.88	45.42	16.44	4.63

TABLE XXVII (Continued)

Pig No.	Day of Trial	Treatment	Total Cholesterol	HDL Cholesterol	Pig Weights	Feed Intake
5	0	1	52.75	23.65	7.48	
5	5	1	56.05	24.67	8.05	1.25
5	10	1	43.76	24.25	9.30	2.04
5	15	1	71.08	35.98	10.43	2.68
5	20	1	65.47	37.34	14.17	4.01
10	0	2	73.05	31.67	9.98	
10	5	2	85.10	34.31	8.96	1.45
10	10	2	67.09	33.45	8.73	0.85
10	15	2	61.25	29.27	10.32	1.91
10	20	2	83.71	41.92	12.70	2.23
15	0	3	96.46	37.75	8.39	
15	5	3	70.56	29.77	8.39	0.68
15	10	3	78.83	39.99	11.57	2.07
15	15	3	88.48	37.34	12.81	2.82
15	20	3	83.54	29.90	15.08	3.71
16	0	4	85.10	16.11	7.37	
16	5	4	76.11	30.28	8.39	0.89
16	10	4	99.78	38.25	8.39	1.07
16	15	4	68.59	30.81	10.77	2.56
16	20	4	85.30	32.52	14.06	3.97
9	0	1	89.63	30.78	7.03	
9	5	1	71.69	28.25	7.03	0.96
9	10	1	85.15	35.32	8.28	2.14
9	15	1	69.14	27.80	10.55	3.26
9	20	1	63.94	28.18	13.38	3.7
14	0	2	73.05	26.38	8.16	
14	5	2	71.30	26.32	7.94	1.36
14	10	2	105.20	43.42	7.94	1.96
14	15	2	77.65	35.93	9.75	2.42
14	20	2	84.71	35.38	13.04	3.61
17	0	3	89.45	33.85	8.28	
17	5	3	65.48	26.50	9.07	1.53
17	10	3	75.82	27.06	10.21	2.3
17	15	3	78.65	31.36	12.25	3.04
17	20	3	89.27	33.25	14.40	4.25
18	0	4	82.85	32.13	8.28	
18	5	4	62.04	26.64	9.64	1.75
18	10	4	73.45	26.20	11.57	3.42
18	15	4	82.49	28.49	13.72	4.31
18	20	4	82.45	29.63	17.46	5.4
13	0	1	100.33	38.05	10.77	
13	5	1	106.50	38.71	12.81	2.55
13	10	1	151.72	42.08	12.59	2.66

TABLE XXVII (Continued)

Pig No.	Day of Trial	Treat-ment	Total Chol-esterol	HDL Chol-esterol	Pig Weights	Feed Intake
13	15	1	117.58	47.30	14.06	2.89
13	20	1	126.13	43.21	17.92	4.28
22	0	2	86.16	26.75	7.60	
22	5	2	66.91	22.96	7.82	1.24
22	10	2	101.87	37.77	9.07	2.31
22	15	2	132.99	43.35	9.19	2.08
22	20	2	114.50	37.30	12.25	2.87
19	0	3	92.16	29.62	10.66	
19	5	3	58.78	16.94	11.91	3.81
19	10	3	80.39	31.46	15.20	4.12
19	15	3	82.00	25.35	16.67	4.65
19	20	3	81.49	38.43	20.98	5.26
20	0	4	86.74	26.87	10.66	
20	5	4	58.23	18.57	11.79	2.46
20	10	4	98.13	37.50	14.86	3.77
20	15	4	119.78	37.17	17.01	4.81
20	20	4	102.49	32.97	21.21	5.71

VITA

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Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF YOUNG GROWING PIGS AS A MODEL TO STUDY HYPOCHOLESTEROLEMIC EFFECTS OF CONSUMING LACTOBACILLUS ACIDOPHILUS AND DRIED WHEY

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