EFFECT OF PECTIN AND OAT BRAN ON PORTAL SHORT CHAIN FATTY ACIDS AND CHOLESTEROL IN RATS

by

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TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF APPENDIX TABLES

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"Trust in the Lord with all your heart and do not lean on your own understanding. In all your ways acknowledge Him and He will make your paths straight."

Proverbs 3:5,6

INTRODUCTION

The inclusion of dietary fiber in the human diet has been shown to affect gastrointestinal function, glucose homeostasis, and serum lipid levels [1,2-8]. William Beaumont [9] in the early 1800's observed physiological properties of the human digestive system and discovered that vegetable fiber was beneficial to health. More recently, evidence suggests that the high incidence of colon cancer, coronary heart disease, diabetes mellitus, obesity, hypertension, and certain other diseases among western people is associated with a low intake of dietary fiber [1,10-18] and a high consumption of refined carbohydrates [10,19].

Epidemiological and nutritional studies have shown that an elevation of plasma lipids, especially cholesterol, is one of the most important risk factors leading to coronary heart disease [20-22]. Dietary fiber, particularly the water soluble type, may be protective against hypercholesterolemia [10] by significantly reducing plasma and liver total cholesterol and lipid levels [23]. One possible mechanism by which soluble fibers lower total cholesterol is by inhibition of hepatic cholesterol synthesis.

Certain soluble plant fibers, such as pectins and gums, are almost completely fermented by colonic bacteria to short

chain fatty acids (SCFA). Acetate, propionate, and butyrate are two, three, and four-carbon SCFA, respectively. The SCFA are almost completely absorbed, travel to the liver, and possibly affect cholesterol synthesis by decreasing HMG-CoA synthase and/or reductase activities. Chen et al. [24] found that liver and serum cholesterol levels were significantly reduced in rats fed a cholesterol-propionate supplemented diet versus cholesterol-fed rats. The two-fold purpose of this study was: (1) to study the effects of soluble and insoluble dietary fibers on the appearance of SCFA in the portal circulation, and hepatic and serum cholesterol concentrations and (2) to evaluate the dietary supplementation of SCFA on the same parameters, i.e. appearance of SCFA in portal circulation, and serum and hepatic cholesterol.

LITERATURE REVIEW

In order to fully understand dietary fiber a concise definition should be suggested. Several definitions of dietary fiber are in use today [25]. Dietary fiber has been described in physiological terms, i.e. "the sum of all plant polysaccharides and lignins that are not digested by human digestive enzymes" [26].

SOLUBLE vs INSOLUBLE DIETARY FIBER

Dietary fiber is categorized into insoluble and soluble components each having different physiological effects. The insoluble components are celluloses, hemicelluloses, and lignins. The carbohydrate components of insoluble fiber (cellulose and hemicellulose) have been shown to increase fecal bulk and decrease intestinal transit time. Cellulose from food sources is found primarily in wheat-based products while hemicelluloses are found in cereals and vegetables. The physiological effects of lignin, the only non-carbohydrate dietary fiber component, are not well-defined. Food related lignins are found in mature fruits and vegetables. The soluble components of dietary fiber, such as pectins and gums, have been shown to delay gastric emptying, slow glucose absorption, and reduce serum cholesterol. Pectins are found primarily in citrus fruits while gums are contained in legumes, oats, and barley [1].

In man, a hypocholesterolemic action has been demonstrated for guar gum, pectin, wheat straw (peat), and oat bran. Similar effects have also been reported for diets rich in fruits and vegetables, apples, and pulses (eg. chick peas and brown beans) [25]. This review will focus primarily on pectin and oat bran as soluble and cellulose and lignins as insoluble dietary fibers.

SOLUBLE PLANT FIBERS. Story et al. [27], compared the effects of 5% cellulose, lignin, or pectin in rats fed 0.5% cholesterol in semi-purified diets. Two other groups of rats were fed a cholesterol and fiber-free or cholesterol-containing and fiber-free diet. In comparison with the hepatic cholesterol (17.9 $\pm$ 3.4 mg/g) of rats fed a fiber-free diet containing cholesterol, the addition of cellulose to the diets reduced hepatic cholesterol 30% (12.6 $\pm$ 2.1 mg/g), lignin reduced hepatic cholesterol 66% (6.1 mg/g), and pectin, a soluble fiber, reduced hepatic cholesterol 75% (4.5 $\pm$ 0.7 mg/g).

Rolled oats have been found to lower serum cholesterol in man [28]. One-half of the oat groat is soluble [1] while one-third of the plant fiber consists of oat gum [23]. Oat bran has been shown to reduce serum low-density lipoprotein (LDL) cholesterol without affecting the high-density lipoprotein (HDL) cholesterol [4,29,30]. The LDL cholesterol is believed to be the most atherogenic of all

lipoproteins. An increase in LDL cholesterol probably shows the greatest risk of coronary heart disease in man. The HDL cholesterol is the agent which transports cholesterol to very low-density lipoproteins or back to the liver. Therefore, increased concentrations of HDL cholesterol may be beneficial [31]. Chen et al. [23] compared rats fed four diets containing 1% cholesterol and 36.5% oat bran (10% oat fiber) (OB), 10% oat gum (OG), 10% pectin (P), or 10% cellulose (C). Plasma total cholesterol values were significantly reduced in rats fed diets containing either oat bran (107 $\pm$ 8 mg/100 ml), oat gum (83 $\pm$ 5 mg/100 ml), or pectin (78 $\pm$ 6 mg/100 ml) compared to cellulose-fed rats (C = 140 $\pm$ 18 mg/100 ml). They also found that the plasma high-density lipoprotein (HDL) cholesterol levels were significantly higher in all three groups fed soluble-plant fibers. The largest increase was seen in the group fed oat gum where plasma HDL cholesterol values were 76% higher compared to cellulose-fed rats (C = 21 $\pm$ 1 mg/100 ml, OB = 34 $\pm$ 2 mg/100 ml, P = 34 $\pm$ 2 mg/100 ml, OG = 37 $\pm$ 2 mg/100 ml). All three soluble dietary fiber-fed groups had significantly lower liver cholesterol levels than the cellulose-fed rats (C = 57 $\pm$ 4 mg/g, OB = 31 $\pm$ 1 mg/g, OG = 15 $\pm$ 2 mg/g, P = 8 $\pm$ 1 mg/g). This study suggests that plasma and liver cholesterol-lowering effects of oat bran are due to its gum fraction.

INSOLUBLE PLANT FIBERS. While the soluble components of dietary fiber seem to have specific hypocholesterolemic effects, there is some disagreement whether the insoluble plant fiber components have any effect on plasma and liver total cholesterol levels. Kiriyama, Tsai, and Wells, and their associates [32-34] have shown that cellulose is ineffective in reducing and may even cause an accumulation of serum and liver cholesterol in rats fed semi-purified, cholesterol-containing or cholesterol-free diets. Yet, Story et al. [10,27] reported that cellulose and lignin did reduce liver cholesterol while serum cholesterol was not affected. Judd et al. [35] found that serum lipids were significantly reduced in rats fed 30 g lignin/day.

MECHANISMS BY WHICH PLANT FIBERS AFFECT CHOLESTEROL SYNTHESIS

The mechanisms by which certain soluble plant fibers lower serum cholesterol in humans and animals are still not determined [36]. The hypocholesterolemic effect of plant fibers may be related to the fiber-induced alterations of lipoprotein or bile acid metabolism, intestinal or pancreatic hormone secretion, intestinal absorption, or metabolic effect in the colon [36,37].

BILE ACID ADSORPTION. The viscous or gelling fibers and the lignins appear to have the greatest ability to bind bile acids [38,39]. Cookson et al. [40], found that alfalfa

fed to rabbits in sufficient quantities prevented the cholesterolemia and atherosclerotic lesions that were induced by feeding 600 mg cholesterol/day. This hypocholesterolemia was accompanied by a large increase in excretion of neutral steroids. According to Horlick et al. [41], this increase in excretion of neutral sterols (cholesterol) indicated that alfalfa interfered with cholesterol absorption. Kritchevsky et al. [42,43] reported a decrease in cholesterol absorption with alfalfa. They found that, when compared with cellulose in isocaloric, semi-purified diets, alfalfa resulted in a greater excretion of both neutral and acidic steroids (bile acids).

Lin et al. [44] discovered that rats fed 50 mg of cholesterol and 500 mg of pectin daily increased excretion of saponifiable lipids in the feces. Leveille et al. [39] reported a large increase in bile acids but not in neutral sterol excretion in rats fed pectin with 1% cholesterol. Kritchevsky et al. [43] found that alfalfa and other types of fiber normally found in animal diets bound large quantities of bile salts. Cellulose bound no bile salts.

In order to explain the hypocholesterolemic action of certain dietary fibers, one must not ignore the information about bile salt-binding. When certain dietary fibers bind bile acids in the small intestine, these bile acids are not available for interaction with cholesterol and other lipids

essential for micelle formation. This would cause an increased excretion of unabsorbed cholesterol. Also, these fiber-bound bile acids would be excreted, causing a loss of bile salts in the feces [10].

BACTERIAL FERMENTATION IN THE COLON. The bacterial ecosystem in the human is one of the most complex known. Fifteen major bacterial species have been isolated from the large intestine [45]. Over 90% of these colon bacteria are obligate anaerobes [45,46]. Many bacteria have the necessary enzymes (eg. cellulase and pectinesterase) to further complete digestion of dietary fiber [25] and obtain a major source of energy and carbon needed for growth and maintenance [25,46-49]. Bacterial metabolism of dietary fiber occurs primarily in the cecum of the rat and right colon in the human [1].

Substrate. Different dietary fibers are degraded to different degrees. Pure crystalline cellulose is very resistant to bacterial attack. Celluloses are degraded less extensively than other polysaccharides. Pectins and some hemicelluloses are completely fermented [25]. Whenever there is insufficient fermentable dietary polysaccharides [46] in the diet, bacteria may digest available host products and injure the mucosal surface. Unlike organs of the body, the microflora is not under direct neural or hormonal control. Control must be affected through the supply of both

endogenous and exogenous substrates [36].

End Products. According to McNeil [50], starch, cellulose, and hemicellulose are degraded by bacterial enzymes to pentoses and hexoses. Pyruvate is provided via glycolysis or the pentose phosphate pathway from subsequent metabolism and then changed to the end products SCFA, hydrogen gas, carbon dioxide, energy, and methane) [1,25,48-51]. Except for ammonia (fermentation product of aminosugars), the products that result from fermenting mucins are basically the same as those that result from fermenting plant polysaccharides [46].

Short Chain Fatty Acids. The SCFA produced are primarily acetate (approximately 60% of total SCFA absorbed), propionate (approximately 20% of total absorbed), and butyrate (approximately 16% absorbed) [51,52]. Branched four and five carbon fatty acids are of limited importance in monogastric animals [53]. All mammalian species studied, e.g. the rat, pig, horse, goat, and human, absorb SCFA [47,54-61]. These acids reduce the pH in the colon before being almost completely absorbed and drain into the portal vein and transported directly to the liver [25,46,47,62]. The mechanisms of absorption are basically the same for acetate, propionate, and butyrate. According to Demigne and Remesy [63], rats fed a high fiber diet (20% crude potato starch, 19% wheat bran, 5% apple pectin, and 5% carob gum)

produced 10-20% SCFA in the protonated form. These protonated acids are assumed to readily cross the hydrophobic matrix of cell membranes. Rats fed a fiber-free diet produced less than 1% SCFA in the protonated form. The enhanced efficiency of SCFA absorption with the high fiber diet might be due to a higher rate of transfer, adaptation of cecal surface, and/or blood flow.

Cholesterol Inhibition by Propionate. According to Story [64], the hypothesis that dietary fiber alters cholesterol levels by adsorbing bile acids is too simplistic. Different dietary fibers will exert different effects on acidic and neutral steroid excretion. This action may be independent of the fiber's effect on cholesterol synthesis.

The SCFA may affect cholesterol synthesis by decreasing 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) synthase and/or reductase activities. The synthesis of cholesterol from acetyl-CoA is mediated by the cytoplasmic form of HMG-CoA synthase [65]. The rate of cholesterol formation is highly responsive to the amount of cholesterol absorbed from the diet. This feedback mechanism is mediated by changes in the activity of HMG-CoA reductase. This enzyme catalyzes the formation of mevalonate and is the committed step in cholesterol biosynthesis [66].

Propionate [52] and straight chain fatty acids (5

carbon to 11 carbon) [67] have been shown to inhibit cholesterol synthesis in isolated rat hepatocytes. Propionate has been shown to lower hepatic acetyl-CoA concentrations [68] and increase hepatic citrate concentrations [69]. Bush and Milligan [70] demonstrated that HMG-CoA synthase in bovine liver incubations was inhibited by propionate. At concentrations of 15 and 30 mM of propionate, HMG-CoA synthase activity was inhibited by 30 and 58%, respectively. Prior to addition of propionate, the HMG-CoA synthase activity was 7.01 $\pm$ 0.38 disappearance of aceto-acetyl CoA μM/min per g liver. The HMG-CoA synthase plus 15 mM propionate gave an activity of 5.73 $\pm$ 0.08, while the addition of 30 mM propionate yielded an activity of 4.53 $\pm$ 0.51. Acetate, the predominate SCFA produced in the colon [52], is not well metabolized in the ruminant liver in the presence of propionate [71,72].

Hepatic Portal Venous Levels of SCFA. Research suggests that increasing soluble dietary fiber in the diet will increase hepatic portal venous concentrations of SCFA. Illman et al. [73], fed 10% citrus pectin (CP), 10% wheat bran (WB), or standard chow (SC) diet to three groups of rats. Hepatic portal blood concentrations of acetate in the pectin-fed group increased by 60% (WB = 0.42 $\pm$ 0.03 mM, CP = 0.67 $\pm$ 0.05 mM) and propionate by 100% (WB = 0.07 $\pm$ 0.01 mM, CP = 0.14 $\pm$ 0.03 mM) more than the wheat bran-fed rats.

Acetate increased 157% (WB = 0.42 ± 0.03 mM, SC = 1.08 ± 0.05 mM), propionate 200% (WB = 0.07 ± 0.01 mM, SC 0.21 ± 0.03 mM), and butyrate 209% (WB = 0.11 ± 0.02 mM, SC = 0.34 ± 0.05 mM) in the standard chow-fed rats compared to the wheat bran-fed group.

Both Storer et al. [74] and Illman and Topping [29] found greater hepatic portal venous levels of SCFA in rats fed 36.5% oat bran (OB) than in rats fed 10% cellulose (C). Illman et al. [73] reported a 3.5 fold increase in hepatic portal venous levels of propionate in the oat bran-fed group (0.29 ± 0.02 mM) as compared to the cellulose fed group (0.08 ± 0.01 mM). Storer et al. [74] also observed increases in hepatic portal venous levels of propionate (C = 0.11 ± 0.01 mM, OB = 0.22 ± 0.04 mM) and butyrate (C = 0.04 ± 0.01 mM, OB = 0.24 ± 0.05 mM) in the oat bran-fed group compared to the cellulose-fed rats.

Storer et al. [30] fed 10% gum arabic (GA) and 10% cellulose (C) to two different groups of rats and observed increases in hepatic portal venous plasma SCFA of 111% (C = 0.67 ± 0.04 mM, GA = 1.45 ± 0.05 mM), and 71% (C = 0.14 ± 0.02 mM, GA = 0.24 ± 0.05 mM) in rats fed gum arabic as compared to the cellulose-fed rats. Topping et al. [75] studied the effects of gum arabic (GA) and cellulose (C), separately and combined as a mixture. They found that a mixture of 7% gum arabic and 7% cellulose (GAC) had a

greater effect on the propionate (GAC = 0.31 ± 0.05 mM) and butyrate (GAC = 0.21 ± 0.02 mM) concentration in hepatic portal venous plasma than either 14% cellulose-fed rats (C = 0.15 ± 0.01 mM propionate, C = 0.08 ± 0.01 mM butyrate) and 14% gum arabic fed rats (GA = 0.22 ± 0.04 mM propionate, GA = 0.14 ± 0.02 mM butyrate). On the other hand, acetate levels were higher in the 14% gum arabic-fed rats than from either of the other two diet groups (C = 0.72 ± 0.04 mM, GAC = 0.80 ± 0.05 mM, GA = 1.11 ± 0.15 mM).

Propionate Supplementation. Since certain soluble fibers tend to increase SCFA, particularly propionate, some researchers decided to investigate supplementing the diet with this SCFA. Chen et al. [24] reported that 0.5% propionate added to diets that contained 0.3% cholesterol and 10% cellulose, and fed to rats, reduced serum cholesterol (cholesterol-fed rats (C) = 101 ± 5 mg/100 ml, cholesterol + propionate-fed rats (CP) = 87 ± 3 mg/100 ml), liver cholesterol (C = 4.31 ± 0.28 mg/g, CP = 3.56 ± 0.22 mg/g), and liver triglycerides (C = 9.17 ± 1.46 mg/g, CP = 5.87 ± 0.80 mg/g). Serum triglycerides on the other hand (C = 116 ± 13 mg/100 ml, CP = 116 ± 9 mg/100 ml) did not change. Thacker et al. [22] fed pigs diets based on barley, wheat, and soybean meal. They discovered after ten weeks that the addition of 5% propionic acid to these diets significantly reduced plasma cholesterol. Concentrations of

87 mg cholesterol/100 ml occurred when no propionate was added (NP) and 76 mg/100 ml when propionate was added to the diet (P). Increased cholesterol in backfat (NP = 175 mg/100 g, P = 214 mg/100 g) and decreased cholesterol concentrations in the kidney were found when propionate was added (NP = 393 mg/100 g, P = 350 mg/100 g) and in the liver (NP = 414 mg/100 g, P = 385 mg/100mg). This suggests that there may be reduced cholesterol transport from peripheral tissues to liver for excretion in bile in those pigs fed propionic acid-supplemented diets.

Certain soluble plant fibers, such as pectins and oat gums may be protective against hypercholesterolemia [10] by significantly reducing plasma and liver total cholesterol and lipid levels [23]. The mechanism by which soluble fiber accomplishes this hypocholesterolemia is not well-defined. Propionate, acetate, and butyrate (SCFA) are produced in the colon from the fermentative action of anaerobic bacteria on soluble dietary fiber. One possibility discussed here is that absorbed SCFA, especially propionate, will inhibit hepatic cholesterol biosynthesis by decreasing HMG-CoA synthase and/or reductase activities.

MATERIALS and METHODS

ANIMAL CARE

Seventy-five male Sprague-Dawley rats (214-224 g) were housed in individual stainless steel mesh-floored cages in a temperature (23-25 ^{o}C) and light controlled (12:12 h light:dark cycle) laboratory. Upon arrival, rats were acclimatized for one wk on standard laboratory chow. After one wk, they were randomly divided into five different treatment groups. Food intake and body weights were recorded at regular intervals.

Diets. Diets were provided in individual feed cups with stainless steel caps to prevent spillage. Each group was fed one of five different semi-purified diets for 28 d. Those diets differed in the type of fiber, based on cellulose (Control diet), pectin, oat bran, or supplementation with SCFA sodium acetate-control, or sodium propionate-control. The composition of the diets is shown in Table 1. Feed and water were allowed ad libitum for 28 d.

Tissue collection. At the end of the 28 d experimental period, rats were anesthetized (50 mg sodium pentobarbital/kg body weight) and terminated in the non-fasted state. Blood samples were obtained from both the hepatic portal vein and abdominal aorta. Livers were excised, blotted to remove excess blood, and weighed. Blood

TABLE 1

The composition of experimental diets

Ingredient	CONTROL	PECTIN	OAT BRAN	ACETATE	PROPIONATE
		g/100 g diet			
Casein[1]	15.0	15.0	15.0	15.0	15.0
DL-Methionine	0.2	0.2	0.2	0.2	0.2
Cornstarch	30.0	30.0	30.0	30.0	30.0
Sucrose	30.0	30.0	30.0	29.5	29.5
Corn Oil	10.0	10.0	10.0	10.0	10.0
Vitamin Mix[2]	1.0	1.0	1.0	1.0	1.0
Mineral Mix[3]	3.5	3.5	3.5	3.5	3.5
Cholesterol[4]	0.3	0.3	0.3	0.3	0.3
Cellulose[5]	10.0			10.0	10.0
Pectin[6]		10.0			
Oat bran[7]			10.0		
Sodium Acetate[8]				0.5	
Sodium Propionate[9]					0.5
Kcal/g diet	3.9	4.2	4.1	3.9	3.9

[1]Casein ICN Nutritional Biochemicals, Cleveland, OH). [2]AIN-76 Vitamin Mixture (#905454, ICN Nutritional Biochemicals, Cleveland, OH) composition: thiamine HCL, 0.6g; riboflavin,0.6g; pyridoxine HCL,0.7g; nicotinic acid, 0.003g; d-calcium pantothenate,0.0016g; folic acid,0.2g; d-biotin,0.02g; cyanocobalamin,0.001g; retinyl palmitate pre-mix,0.8g; dl-α-tocopheryl acetate pre-mix,0.02g cholecalciferol,0.0025g; menaquinone, 0.005g; sucrose,972.9g. [3]AIN-76 Mineral Mixture (#905455,ICN Nutritional Biochemicals, Cleveland, OH) contains: calcium phosphate dibasic,500.0g; sodium chloride,74.0g; potassium citrate monohydrate,220.0g; potassium sulfate,52.0g; magnesium oxide,24.0g; manganous carbonate (43-48% Mn); ferric citrate,6.0g; zinc carbonate (70% Zn),1.6g; cupric carbonate (53-55% Cu),0.3g; potassium iodate,0.01g; sodium selenite,0.01g; chromium potassium sulfate,0.55 g; sucrose,118.03g. [4]Cholesterol (ICN Nutritional Biochemicals, Cleveland, OH). [5]Alphacel Cellulose (#900453,ICN, Cleveland, OH). [6]Citrus Pectin Powder (P-9135, Sigma Chemical, St. Louis,MO). [7]Oat Bran (National Oats Company, Inc., Cedar Rapids, IA) composition: 57% carbohydrate, 20% protein, 10.5% moisture, 3.5% ash, 3.0% crude fiber. dietary fiber = 20%. [8]Sodium Acetate (Fisher Scientific, Fair Lawn, NJ). [9]Sodium Propionate (Fisher Scientific, Fair Lawn, NJ).

and liver samples were frozen at -5 °C until biochemical determinations.

BIOCHEMICAL ANALYSES on LIVER

Liver lipids. Liver lipids were determined by a modification of the Folch gravimetric method [76]. The initial extraction mixture was methylene chloride:methanol (2:1, v/v) rather than the choroform:methanol mixture used by Folch.

Hepatic samples weighing 1.5 g were homogenized in 20 ml methylene chloride:methanol for 30 s using a Polytron high-speed homogenizer (Brinkmann Instruments, Westburg, NY) in 50 ml plastic centrifuge tubes. Homogenates were shaken 5 min on an automatic shaker. Four ml of methanol were added to the homogenate; this blend was mixed on a vortex mixer, and centrifuged for 10 min. The supernatant was decanted to a 50 ml centrifuge tube. Eight ml of 0.73% NaCl were added and tubes were shaken 5 min on an automatic shaker. After a second centrifugation for 5 min, the top aqueous layer was aspirated off and 15 g of the bottom organic layer (containing the lipids) were pipetted into dried, pre-weighed aluminum dishes. The solvent was allowed to evaporate and the dishes were dried at 103 °C for 1 h. After cooling in a desiccator, dishes containing the lipid residue were weighed to the nearest mg. Lipid (mg lipid/g liver) was calculated by multiplying mg lipid in each dish

by 22.8/15 (15 g organic layer was withdrawn from a total of 22.8g), dividing by 1.5 g for weight of liver sample, and dividing by 1000 to convert mg to g.

Total cholesterol. Total liver cholesterol levels were determined by the ferric chloride-sulfuric acid reaction (Modified Leffler method) [77]. Two-tenths ml (in duplicate) of the organic layer from the lipid extraction procedure was pipetted into disposable 15 ml test tubes. Two-tenths ml of the standard (200 mg cholesterol/100 ml alcohol) was pipetted into another tube. Five ml of isopropyl alcohol were added rapidly to each of the tubes from an automatic pipettor and the mixtures blended thoroughly using a vortex mixer. One ml mixtures were transferred into 15 ml tubes (screw-type teflon coated cap). Three ml of glacial acetic acid were added and mixed using a vortex mixer. Then 3.3 ml of concentrated sulfuric acid-iron reagent mixture (3 ml sulfuric acid to 0.3 ml of a mixture of 2.5 g ferric chloride dissolved in 100 ml phosphoric acid) were allowed to drip slowly down the sides of the centrifuge tubes so that the acid would form a layer underneath the solutions. The centrifuge tubes were then immediately capped tightly and slowly inverted 5 times. Tubes were allowed to stand 10 min at room temperature to cool. The solutions were then transferred to cuvettes, and tapped to eliminate any bubbles. A Bausch and Lomb

Spectronic 20 colorimeter was zeroed using a blank at a wavelength of 560 nm. Absorbance was then measured on the standard and each sample. The absorbance reading of the sample was multiplied by the concentration of the standard (200 mg cholesterol/100 ml) and divided by the absorbance of the standard to obtain the concentration of the sample in mg/100 ml. This value was multiplied by 18/100 (organic layer was 18 ml) and divided by 1.5 g (weight of liver sample) to obtain mg cholesterol/g liver.

BIOCHEMICAL ANALYSES on BLOOD

Serum total cholesterol. Total cholesterol in arterial serum was determined using the Modified Leffler Method [77]. In determining serum cholesterol (in duplicate) the 0.2 ml blood serum samples plus 5 ml isopropyl alcohol were centrifuged 10 min (IEC Centra-7R refrigerated centrifuge, International Equipment Company) to remove all proteins. The rest of the procedure was the same as described for total cholesterol, except in calculating the final concentration in the sample. To obtain the concentration in the serum sample in mg/100 ml, the absorbance of the sample was multiplied by the concentration of the standard and divided by the absorbance of the standard.

SCFA analysis. The SCFA in whole hepatic portal venous

blood was determined by the gas chromatograph method of Reynolds et al. [78]. One ml of blood was pipetted into a 50 ml plastic centrifuge tube containing 2 ml internal standard (4 ml 0.0363 g 2-ethyl butyrate/100 ml diluted to 200 ml +15 µl Triton X-100 to equal 0.25 mM in the sample) and allowed to stand for 5 min. To this mixture, 2 ml 0.3N $Ba(OH)_2$ was added and allowed to stand for 5 min. Two ml of 5% $ZnSO_4$ was added, the solution mixed, and allowed to stand for 5 min. The mixture was centrifuged (Beckman J2-21) at 15,000 x g for 15 min to remove the proteins from solution. Five ml of the supernatant were pipetted slowly onto the top of two piggy-backed cation exchange columns, (materials: AG50W-X 8, 100-200 mesh in top cation-exchange column, Bio-Rex 5, 100-200 mesh in bottom anion-exchange column, and propylene Econo-Column Cat. no. 731-1550, Bio-Rad Laboratories, Richmond CA 94804) and allowed to drip through both columns. Columns were rinsed 3 times by automatically dispensing 1 ml water onto the top column and allowing each washing to drip completely through. The top column was then removed. The bottom column was rinsed twice with 1 ml of water. Five ml of 25 mM NaOH were added to the bottom column and collected in 30 ml beakers. This eluent was dried over night in a 65 ^{o}C oven.

The dried material was reconstituted in 1.5 ml 0.03 M oxalic/0.5 M formic acid and carefully transferred to 2 ml

crimp top glass injection vials and placed into an auto injector gas chromatograph (Hewlet Packard, model #5890). Values for acetate, propionate, isobutyrate, and butyrate were obtained in mM relative to the internal standard (0.25 mM 2-ethyl butyrate) from an electronic integrator (Hewlet Packard #3392).

Statistical Procedures. Data were analyzed using the SAS statistical computing package. Data were statistically evaluated by using ANOVA followed by the Duncan's Multiple Range test. The General Linear Model procedure was used to determine standard error mean.

RESULTS

BODY WEIGHT, FOOD INTAKE, AND FOOD EFFICIENCY

There were no significant differences in initial and final body weights, daily weight gains, and food efficiency ratios for the five groups of rats (Table 2). Average initial body weights, final body weights, and daily weight gains were 219 g, 344 g, and 4.4 g/d, respectively. The average food efficiency ratio was 6.4 g gained/100 kcal. Significant reductions ($P<0.0075$) in daily food intake occurred in pectin-fed rats compared to those fed sodium propionate supplemented and cellulose-control diets. Food intakes were significantly lower ($P<0.0075$) in oat bran-fed than cellulose-fed rats. However, food intakes for sodium acetate, and sodium propionate-supplemented control rats or for sodium acetate supplemented-control and oat bran-fed rats were not significantly different.

LIVER WEIGHTS, LIVER AND SERUM CHOLESTEROL

The pectin diet had significantly reduced liver weights ($P<0.0017$), liver lipids ($P<0.0001$), liver cholesterol ($P<0.0001$), and serum cholesterol ($P<0.007$) compared to cellulose, sodium acetate, and sodium propionate-fed rats (Table 3). However, liver weights of the oat bran-fed rats were significantly ($P<0.0017$) lower than those of rats fed sodium propionate diets, while liver weights were not

TABLE 2

Effect of dietary composition on body weight, food intake, and food efficiency[1]

Diet group	Initial weight	Final weight	Weight gain	Food intake[2]	FER[3]
	g	g	g/d	g/d	g/100 kcal
C[4]	219 ± 5	348 ± 5	4.7 ± 0.2	18.1 ± 0.4[a]	6.6 ± 0.2
P	223 ± 5	339 ± 5	4.2 ± 0.2	16.2 ± 0.4[c]	6.1 ± 0.2
OB	215 ± 5	343 ± 5	4.6 ± 0.2	16.5 ± 0.4[bc]	6.8 ± 0.2
SA	222 ± 5	345 ± 5	4.4 ± 0.2	17.3 ± 0.4[abc]	6.5 ± 0.2
SP	218 ± 5	342 ± 5	4.4 ± 0.2	17.5 ± 0.4[ab]	6.5 ± 0.2

[1]Values are means ± SEM. [2]Within animal groups, means with different superscript letters are significantly different ($P<.0075$). [3]FER = food efficiency ratio (g gained/100 kcal). [4]Abbreviations for diet groups are as follows: C=Cellulose-control, P=Pectin, OB=Oat bran, SA=Sodium acetate, and SP=Sodium propionate.

significantly different when comparing pectin, cellulose, and sodium acetate-fed rats.

Supplementing the cellulose-control diets with sodium acetate and sodium propionate did not significantly affect liver lipids and liver cholesterol. Liver lipid levels were significantly ($P<0.0001$) has in rats fed oat bran than those fed cellulose. Liver cholesterol values were significantly lower in oat bran-fed rats compared to the sodium acetate and sodium propionate supplemented control-fed rats. The pectin diet reduced liver lipids by 38%, while liver lipids were reduced by 14% in the oat bran diet compared to the cellulose diet. Liver cholesterol values were reduced 64% in pectin-fed rats compared to those rats fed cellulose. Oat

bran feeding did not significantly affect liver cholesterol values compared to cellulose-fed rats. Serum cholesterol values for oat bran-fed rats were not significantly different from the other four groups of rats. Yet, the addition of pectin to the diet resulted in a reduction of 15% in serum cholesterol.

TABLE 3

Effect of dietary composition on liver lipid, liver cholesterol, and serum cholesterol[1]

Diet group	Liver weight[2]	Liver lipid	Liver cholesterol	Serum cholesterol
	g	mg/g	mg/g	mg/100 ml
C[3]	14.8 ± 0.4^{ab}	68.6 ± 3.3^{a}	19.7 ± 1.6^{ab}	95.4 ± 4.0^{a}
P	13.1 ± 0.4^{c}	42.5 ± 3.3^{c}	7.1 ± 1.6^{c}	80.6 ± 4.0^{b}
OB	14.3 ± 0.4^{bc}	59.0 ± 3.4^{b}	15.3 ± 1.7^{b}	91.4 ± 4.2^{ab}
SA	14.9 ± 0.4^{ab}	74.1 ± 3.4^{a}	20.8 ± 1.8^{a}	93.6 ± 4.3^{a}
SP	15.6 ± 0.4^{a}	77.8 ± 3.4^{a}	21.3 ± 1.8^{a}	102.9 ± 4.2^{a}

[1]Values are means ± SEM. [2]Within animal groups, means with different superscript letters are significantly different ($P<0.0017$) for liver weight, ($P<0.0001$) for liver lipid and cholesterol, and ($P<0.007$) for serum cholesterol. [3]Abbreviations for diet groups are as follows: C=Cellulose-control, P=Pectin, OB=Oat bran, SA=Sodium acetate, and SP=Sodium propionate.

HEPATIC PORTAL SCFA CONCENTRATIONS

The group fed pectin as the dietary fiber was the only group in which propionic acid levels were significantly elevated ($P<0.0001$) in hepatic portal whole blood (Table 4). Propionic acid was elevated by 91%, and butyric acid by 75%

compared to those rats fed cellulose. The hepatic portal blood-butyrate was insignificantly increased by 38% in the oat bran-fed rats, respectively. No significant changes were observed in portal concentrations of acetate in any of the groups. The average ratio of acetate:propionate:butyrate was 9.54:0.32:0.10, respectively.

TABLE 4

Effect of dietary composition on short chain fatty acid concentrations in hepatic portal whole blood[1]

Diet group[3]	Short Chain Fatty Acids		
	Acetate	Propionate[2]	Butyrate
	mM		
C	3.705 ± 0.117	0.099 ± 0.008^{a}	0.031 ± 0.004^{b}
P	3.808 ± 0.117	0.189 ± 0.008^{b}	0.053 ± 0.004^{a}
OB	3.559 ± 0.130	0.117 ± 0.009^{a}	0.042 ± 0.005^{ab}
SA	3.753 ± 0.130	0.109 ± 0.009^{a}	0.037 ± 0.004^{b}
SP	3.851 ± 0.123	0.119 ± 0.009^{a}	0.038 ± 0.004^{b}

[1]Values are means ± SEM. [2]Within animal groups, means with different superscript letters are significantly different (P<0.0001) for propionate and (P<0.0049) for butyrate. [3]Abbreviations for diet groups are as follows: C=Cellulose-control, P=Pectin, OB=Oat bran, SA=Sodium acetate, and SP=Sodium propionate.

DISCUSSION

Male Sprague-Dawley rats were used in this study to (1) investigate the effects of soluble and insoluble dietary fiber on the production of portal SCFA, and serum and hepatic cholesterol and (2) to evaluate dietary SCFA supplementation on the same parameters.

Randomization of rats into five experimental groups resulted in initial body weights which were not significantly different. Although daily feed intakes were significantly different among some of the groups, these differences were not reflected in significant differences among final body weights, daily weight gains, or efficiency of food utilization. The range of values for each group within each parameter was very consistent suggesting that the groups were quite similar and the diets were well tolerated.

Pectin containing diets had the greatest influence on liver total lipids, and liver and serum cholesterol. These results agreed with those of Chen et al. [23] who also reported significant reductions of liver lipids, and liver and plasma cholesterol in rats fed 10% pectin compared to 10% cellulose-fed rats. Although oat bran produced liver lipid, serum and liver cholesterol-lowering trends similar to pectin, only liver lipids were significantly lower than those values reported for the control diet. Chen et al.

[23], on the other hand, did find significant reductions in liver lipids, and liver and serum cholesterol in rats fed 36.5% oat bran (10% oat gum) compared to 10% cellulose-fed rats. The differences between results that Chen et al. [23] found and those observed in our study may have been related to the amount of oat gum fed to our rats. Our oat bran diet contained 10% oat bran, which meant it had only 2% total oat fiber (see National Oats Company, Inc. formulation, Appendix Table 1). Since oat fiber consists of about 1/3 oat gum, which has been reported to be the hypocholesterolemic agent in oat bran [23], the low amount of oat gum may explain the differences between results we observed and those reported by Chen et al. [23].

Fats and cholesterol accumulated in the livers of all of our rats. The average amount of lipids contained in the livers was 64.4 mg/g. These values were high compared to Chen et al. [24] who reported average liver lipid values of 27.3 mg/g for animals fed similar diets but with adequate choline. The accumulation of liver lipid in our rats may be explained by the inadvertant ommission of choline from the experimental diets. The role of choline as a lipotropic agent, i.e. it decreases the rate of deposition of abnormal amounts of lipid in the liver and accelerates the removal of excess lipid from it, is well documented [80]. Therefore, when choline is lacking in the diet of rats, fats and

cholesterol esters accumulate in the liver [81].

The average amount of liver cholesterol for all five groups in our study was 16.84 mg/g. Chen et al. [24] found liver cholesterol values for animals fed propionate supplemented diets at 3.56 mg/g compared to cellulose-fed rats at 4.31 mg/g. In our study, pectin was more effective than oat bran in reducing both liver lipids and cholesterol which in turn was more effective than the cellulose-based diets.

The average liver weights of rats in our study (4.23 g/100 g body weight) were 24% heavier than the average liver weights (3.40 g/100 g body weight) reported by Schneeman et al. [79]. Diets in Schneeman's study, however, contained twice the cellulose and oat bran, and half the pectin compared with the diets in our study. This difference in liver weights is probably linked to the elevated amounts of lipids and cholesterol in the livers of our rats.

The oat bran-fed rats may have been provided with more choline than the other rats. For example, rolled oats provides 151 mg choline/100 g. This may be one reason why oat bran, which contained approximately 0.7% (2% oat fiber x 1/3 oat gum) oat gum, had a significantly greater effect on reducing liver lipids and liver cholesterol than the dietary SCFA-supplemented diets. This hypocholesterolemic effect of oat bran compared to the SCFA-supplemented diets also may be

related to the ability of the 2% total dietary fiber to bind bile acids and result in increased excretion of cholesterol-containing compounds. Illman et al. [29] did report that fecal bile acid and neutral sterol excretion increased in rats fed 36.5% oat bran (10% oat gum).

The acetate- and propionate-supplemented diets had little or no effect upon liver lipids, or liver and serum cholesterol when compared with the control diet. Thacker et al. [22] reported that supplementation in the diet of pigs with ten times as much propionate (5%) as was used in our study did not produce a change in liver cholesterol levels. However, he did observe a reduction in plasma and kidney cholesterol and an elevation of backfat cholesterol in the pigs fed 5% propionate-supplemented diets compared to the control-fed animals. He proposed that the increase in backfat cholesterol may have been due to a reduced cholesterol transport.

Pectin feeding in our study was more effective in elevating portal propionate in rats than the other diet groups. Butyrate was also elevated in pectin-fed rats compared to the rats fed the three cellulose-based diets. Illman et al. [29] reported a significant increase of portal acetate and propionate, but not butyrate in rats fed 10% citrus pectin compared to 10% wheat bran-fed rats. The effect of oat bran on portal butyrate was not as pronounced

as the pectin effect and was not significantly different from any of the diets. Oat bran was not effective in elevating portal propionate levels in rats compared to the cellulose-fed rats. Storer et al. [74], on the other hand, found significant increases in both portal butyrate and propionate in rats fed 36.5% oat bran compared to rats fed 10% cellulose. This discrepency is probably due to the small amount of water soluble oat gum in the diet of our rats.

Chen et al. [24] reported that dietary supplementation of propionate reduced liver and serum cholesterol. They suggested that the hypocholesterolemic effect of certain soluble fibers, such as pectin and oat bran, may be related to absorbed propionate, a bacterial degradation product of certain soluble dietary fibers in the colon, which inhibits hepatic cholesterol synthesis. In this study the supplementation of animal diets with either 0.5% acetate or propionate did not elevate portal SCFA and did not result in changes in hepatic or serum cholesterol compared to control rats fed cellulose.

The average ratio of portal acetate:propionate:butyrate in our study was 9.54:0.32:0.10, respectively. Cummings et al. [51] reported a ratio of 6.0:2.0:1.6. The higher amount of acetate and lower amount of propionate and butyrate may be due to our methodology in quantifying the SCFA. Pomare

et al. [82] reported that all of the formic acid that they checked was contaminated with acetic acid.

In summary, pectin in the basal diet reduced liver lipids, liver and serum cholesterols, and elevated portal propionate and butyrate levels more than any of the other experimental diets. Oat bran, the other soluble dietary fiber used, significantly reduced liver lipids. Although oat bran feeding resulted in slight reductions in liver and serum cholesterol, and slight elevations in portal propionate and butyrate concentration when compared to cellulose-fed rats these differences were not statistically significant. Dietary SCFA-supplementation did not significantly affect liver lipids, liver and serum cholesterol, and portal SCFA concentrations compared to the cellulose diet.

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APPENDIX

APPENDIX TABLE 1. Composition of oat bran

OAT BRAN

GENERAL REQUIREMENT: The product shall be manufactured in accordance with current good manufacturing practices promulgated under the Federal Food, Drug, and Cosmetic Act, and applicable State statutes and regulations.

DESCRIPTION: Oat Bran-type product will be manufactured by processing, grinding, and sifting groats obtained from clean, sound, white oats. There will be no additives or preservatives added.

PHYSICAL REQUIREMENT:

Particle Size:	
on US #14	10% maximum
on US #20	10 - 45%
on US #30	30 - 50%
on US #40	10 - 40%
through US #40	5% maximum
Foreign Material:	This product contains many small oat and barley hulls.

CHEMICAL REQUIREMENT:

Enzymes	Inactivated
Protein (DMB)	20.0% Minimum
Moisture	10.0% ± 1%
Ash	4.0% ± 1%
Fiber	3.0% ± 1%
Fat	6.0% ± 1%
Free Fatty Acid (NOCO Method)	30.00 cc Maximum
Purity	Irreducible Minimum
Total Dietary Fiber	18% - 23% Typical

ORGANOLEPTIC: The product must possess a natural oat flavor and odor, free from rancid, bitter, musty, sour or other undesirable flavor or odors.

MICROBIOLOGICAL:

Standard Plate Count	10,000/gram Maximum
Coliform	100/gram Maximum
E. coli	Less than 10/gram
Yeast Spores	100/gram Maximum
Mold Spores	100/gram Maximum
Coag. Pos. Staph	Negative
Salmonella	Negative

08/86

APPENDIX TABLE 2. Effect of dietary composition on body weight, food intake, and food efficiency

Diet group	Initial weight	Final weight	Weight gain	Food intake	FER
	g	g	g/d	g/d	g/100 kcal
Control					
1	210	355	5.2	17.9	7.4
2	207	346	5.0	18.1	7.0
3	215	318	3.7	16.0	5.9
4	213	340	4.5	18.2	6.4
5	207	329	4.4	17.0	6.6
6	222	338	4.1	16.2	6.5
7	207	350	5.1	18.0	7.3
8	233	343	3.9	16.8	6.0
9	222	353	4.7	18.4	6.5
10	221	358	4.9	17.4	7.2
11	234	340	3.8	17.3	5.6
12	226	357	4.7	19.7	6.1
13	256	378	4.4	19.0	5.9
14	240	398	5.6	21.2	6.8
15	170	315	6.3	20.9	7.7
	219 ± 5*	348 ± 5	4.7 ± 0.2	18.1 ± 0.4	6.6 ± 0.2
Pectin					
1	204	317	4.0	14.8	6.4
2	195	326	4.7	15.5	7.1
3	207	343	4.9	16.6	6.9
4	213	320	3.8	15.9	5.7
5	225	340	4.1	15.2	6.4
6	216	336	4.3	16.4	6.1
7	209	326	4.2	16.5	6.0
8	226	340	4.1	15.5	6.2
9	214	340	4.5	17.3	6.1
10	250	375	4.5	17.1	6.1
11	222	320	3.5	14.8	5.6
12	221	332	4.0	17.0	5.5
13	257	359	4.7	16.9	6.5
14	242	353	4.0	16.8	5.5
15	249	362	4.0	16.6	5.7
	223 ± 5	339 ± 5	4.2 ± 0.2	16.2 ± 0.4	6.1 ± 0.2

*Adjusted mean ± standard error mean computed by the GLM option of SAS.

APPENDIX TABLE 2. **Effect of dietary composition on body weight, food intake, and food efficiency**

Diet group	Initial weight	Final weight	Weight gain	Food intake	FER
	g	g	g/d	g/d	g/100 kcal
Oat bran					
1	200	345	5.2	16.5	7.7
2	216	346	4.6	15.8	7.2
3	----------	----------	DECEASED	----------	----------
4	204	351	5.3	16.7	7.7
5	221	353	4.7	16.9	6.8
6	206	328	4.4	14.8	7.2
7	235	366	4.7	19.2	5.9
8	220	346	4.5	15.8	7.0
9	185	318	4.8	15.1	7.7
10	231	353	4.4	15.8	6.8
11	229	346	4.2	15.9	6.4
12	243	374	4.7	17.9	6.4
13	235	330	3.4	15.6	5.3
14	214	306	3.3	14.7	5.5
15	170	345	6.3	20.9	7.3
	215 ± 5*	343 ± 5	4.6 ± 0.2	16.5 ± 0.4	6.8 ± 0.2
Acetate					
1	225	383	5.6	20.7	6.9
2	212	371	5.7	20.0	7.2
3	194	323	4.6	15.2	7.7
4	225	345	4.3	16.6	6.6
5	217	362	5.2	18.5	7.1
6	205	318	4.0	15.4	6.7
7	227	345	4.2	16.6	6.4
8	219	361	5.1	17.6	7.3
9	232	347	4.1	16.6	6.3
10	220	320	3.6	16.3	5.6
11	211	322	4.0	17.1	5.9
12	----------	----------	DECEASED	----------	----------
13	225	325	3.6	14.7	6.2
14	254	371	4.2	20.7	5.1
15	236	331	3.4	16.3	5.3
	222 ± 5	345 ± 5	4.4 ± 0.2	17.3 ± 0.4	6.5 ± 0.2

*Adjusted mean ± standard error mean computed by the GLM option of SAS.

APPENDIX TABLE 2. **Effect of dietary composition on body weight, food intake, and food efficiency**

Diet group	Initial weight	Final weight	Weight gain	Food intake	FER
	g	g	g/d	g/d	g/100 kcal
Propionate					
1	216	349	4.8	18.9	6.4
2	201	354	5.5	17.5	8.0
3	195	321	4.5	15.9	7.3
4	216	347	4.7	17.0	7.1
5	206	340	4.8	17.3	7.1
6	225	365	5.0	18.7	6.9
7	213	305	3.3	15.0	5.6
8	203	301	3.5	15.9	5.6
9	226	340	4.1	16.7	6.2
10	----------	----------	DECEASED	----------	----------
11	242	375	4.8	19.4	6.3
12	218	339	4.3	17.6	6.3
13	228	350	4.4	18.7	6.0
14	241	376	4.8	18.6	6.6
15	224	326	3.6	17.0	5.5
	218 ± 5*	342 ± 5	4.4 ± 0.2	17.5 ± 0.4	6.5 ± 0.2

*Adjusted mean ± standard error mean computed by the GLM option of SAS.

APPENDIX TABLE 3. Effect of dietary composition on liver lipid, liver cholesterol, and serum cholesterol

Diet group	Liver weight	Liver lipid	Liver cholesterol	Serum cholesterol
	g	g	mg/g	mg/100 ml
Control				
1	16.6	64.8	11.4	76.4
2	15.9	91.2	25.3	89.6
3	11.8	76.0	22.0	82.4
4	14.8	75.2	23.8	98.6
5	13.5	44.8	9.0	101.8
6	14.2	76.8	28.7	103.0
7	14.9	88.8	26.2	67.7
8	15.2	67.2	22.1	99.1
9	16.0	71.2	35.2	110.9
10	13.6	42.4	6.6	106.1
11	13.0	65.6	13.9	112.8
12	16.7	79.2	19.7	71.7
13	15.9	74.4	19.7	89.3
14	18.5	64.0	20.4	125.9
15	12.0	48.0	11.4	95.8
	14.8 ± 0.4*	68.6 ± 3.3	19.7 ± 1.6	95.4 ± 4.0
Pectin				
1	12.4	49.6	7.3	80.8
2	11.9	44.0	4.9	88.2
3	14.7	36.0	6.8	88.4
4	11.9	40.0	7.3	104.1
5	13.7	40.0	6.6	84.8
6	13.4	46.4	7.3	76.3
7	12.6	42.4	4.9	82.2
8	12.7	36.8	10.7	74.8
9	14.0	60.8	15.6	75.9
10	13.6	44.8	5.8	76.4
11	11.4	38.4	4.9	49.8
12	12.8	41.6	5.8	76.3
13	14.4	36.8	7.3	106.8
14	13.4	36.0	5.8	78.4
15	13.8	43.2	5.8	66.4
	13.1 ± 0.4	42.5 ± 3.3	7.1 ± 1.6	80.6 ± 4.0

*Adjusted mean ± standard error mean computed by the GLM option of SAS.

APPENDIX TABLE 3. Effect of dietary composition on liver lipid, liver cholesterol, and serum cholesterol

Diet group	Liver weight	Liver lipid	Liver cholesterol	Serum cholesterol
	g	g	mg/g	mg/100 ml
Oat bran				
1	15.1	53.6	9.0	100.4
2	16.3	76.8	24.6	93.9
3	----------DECEASED----------			
4	14.3	41.6	9.0	84.8
5	14.1	72.0	19.7	99.6
6	13.3	55.2	13.9	82.7
7	15.9	52.8	19.7	92.6
8	16.7	44.8	9.8	85.4
9	13.5	94.4	26.2	78.0
10	14.4	53.6	10.7	69.7
11	13.5	49.6	9.8	85.5
12	15.4	47.2	13.9	122.4
13	12.4	59.2	14.8	110.1
14	12.0	63.2	19.7	102.1
15	12.8	62.4	13.9	72.7
	14.3 ± 0.4*	59.0 ± 3.4	15.3 ± 1.7	91.4 ± 4.2
Acetate				
1	17.6	96.0	----**	119.4
2	16.7	109.6	36.8	108.2
3	13.3	58.4	6.6	94.9
4	15.5	65.6	18.0	81.6
5	16.3	74.4	19.7	----
6	13.0	69.6	18.0	97.3
7	15.3	65.6	28.2	84.2
8	15.9	92.0	27.0	113.2
9	14.1	71.2	19.7	56.4
10	13.4	64.8	18.0	78.9
11	13.7	57.6	20.4	91.2
12	----------DECEASED----------			
13	13.8	72.0	18.8	100.9
14	15.9	71.2	20.4	102.4
15	13.8	69.6	18.8	88.7
	14.9 ± 0.4	74.1 ± 3.4	20.8 ± 1.8	93.6 ± 4.3

*Adjusted mean ± standard error mean computed by the GLM option of SAS.
**These samples were lost.

APPENDIX TABLE 3. **Effect of dietary composition on liver lipid, liver cholesterol, and serum cholesterol**

Diet group	Liver weight	Liver lipid	Liver cholesterol	Serum cholesterol
	g	g	mg/g	mg/100 ml
Propionate				
1	16.7	94.4	33.5	112.3
2	19.6	81.6	----**	109.0
3	14.2	92.0	4.1	78.3
4	16.8	88.0	26.2	95.9
5	14.1	60.8	24.0	91.8
6	16.3	75.2	18.8	114.4
7	13.3	54.4	20.9	69.2
8	12.3	69.6	16.3	91.6
9	14.5	68.0	21.2	99.1
10	----------DECEASED----------			
11	18.1	76.8	20.4	107.2
12	16.3	80.8	18.8	110.9
13	17.8	92.8	23.8	111.4
14	14.8	79.2	19.7	110.9
15	13.6	76.0	29.4	138.8
	15.6 ± 0.4*	77.8 ± 3.4	21.3 ± 1.8	102.9 ± 4.2

*Adjusted mean ± standard error mean computed by the GLM option of SAS.
**These samples were lost.

APPENDIX TABLE 4. **Effect of dietary composition on short chain fatty acid concentrations in hepatic portal whole blood**

Diet	Short Chain Fatty Acids			
	Acetate	Propionate	Isobutyrate	Butyrate
			mM	
Control				
1	-----**	-----	-----	-----
2	-----	-----	-----	-----
3	-----	-----	-----	-----
4	-----	-----	-----	-----
5	4.181	0.077	0.009	0.029
6	4.638	0.080	0.010	0.026
7	3.251	0.101	0.013	0.035
8	3.890	0.133	0.015	0.058
9	3.611	0.129	0.013	0.035
10	3.433	0.079	0.012	0.019
11	3.620	0.093	0.013	0.023
12	3.055	0.093	0.011	0.029
13	3.595	0.099	0.011	0.032
14	3.880	0.100	0.010	0.024
15	3.601	0.103	0.010	0.026
	3.705 ± 0.117*	0.099 ± 0.008	0.012 ± 0.001	0.031 ± 0.004
Pectin				
1	-----	-----	-----	-----
2	-----	-----	-----	-----
3	-----	-----	-----	-----
4	4.755	0.241	0.012	0.059
5	3.945	0.154	0.012	0.086
6	4.087	0.196	0.014	0.076
7	3.401	0.194	0.015	0.065
8	3.650	0.245	0.014	0.035
9	3.741	0.185	0.009	0.055
10	3.755	0.184	0.012	0.019
11	-----	-----	-----	-----
12	3.508	0.240	0.011	0.062
13	3.403	0.108	0.009	0.047
14	3.787	0.146	0.009	0.047
15	3.856	0.180	0.008	0.035
	3.808 ± 0.117	0.189 ± 0.008	0.011 ± 0.001	0.053 ± 0.004

*Adjusted mean ± standard error mean computed by the GLM option of SAS.
**These samples were lost.

APPENDIX TABLE 4. Effect of dietary composition on short chain fatty acid concentrations in hepatic portal whole blood

Diet	Short Chain Fatty Acids			
	Acetate	Propionate	Isobutyrate	Butyrate
			mM	
Oat bran				
1	-----**	-----	-----	-----
2	-----	-----	-----	-----
3	----------DECEASED----------			
4	-----	-----	-----	-----
5	3.767	0.124	0.014	0.052
6	3.628	0.093	0.011	0.041
7	-----	-----	-----	-----
8	3.316	0.129	0.014	0.042
9	3.543	0.112	0.014	0.038
10	3.393	0.104	0.013	0.052
11	3.576	0.140	0.016	0.046
12	3.574	0.140	0.013	0.035
13	3.728	0.064	0.012	-----
14	-----	-----	-----	-----
15	3.502	0.148	0.009	0.032
	3.559 ± 0.130*	0.117 ± 0.009	0.013 ± 0.001	0.042 ± 0.005
Acetate				
1	-----	-----	-----	-----
2	-----	-----	-----	-----
3	-----	-----	-----	-----
4	-----	-----	-----	-----
5	-----	-----	-----	-----
6	3.626	0.096	0.011	0.041
7	4.031	0.133	0.018	0.066
8	4.407	0.114	0.012	0.049
9	3.568	0.106	0.013	0.037
10	3.828	0.118	0.014	0.033
11	3.190	0.107	0.015	0.028
12	----------DECEASED----------			
13	3.680	0.108	0.011	0.027
14	3.548	0.103	0.013	0.027
15	3.898	0.097	0.008	0.023
	3.753 ± 0.130	0.109 ± 0.009	0.013 ± 0.001	0.037 ± 0.004

*Adjusted mean ± standard error mean computed by the GLM option of SAS.
**These samples were lost.

APPENDIX TABLE 4. **Effect of dietary composition on short chain fatty acid concentrations in hepatic portal whole blood**

Diet	Short Chain Fatty Acids			
	Acetate	Propionate	Isobutyrate	Butyrate
			mM	
Propionate				
1	-----**	-----	-----	-----
2	-----	-----	-----	-----
3	-----	-----	-----	-----
4	-----	-----	-----	-----
5	4.601	0.103	0.011	0.044
6	3.656	0.106	0.012	0.044
7	3.708	0.124	0.016	0.041
8	3.391	0.147	0.017	0.057
9	3.887	0.103	0.012	0.037
10	----------DECEASED----------			
11	3.417	0.179	0.021	0.051
12	3.179	0.107	0.014	0.025
13	4.735	0.098	0.012	0.031
14	3.787	0.115	0.011	0.030
15	4.148	0.106	0.012	0.023
	3.851 ± 0.123*	0.119 ± 0.009	0.014 ± 0.001	0.038 ± 0.004

*Adjusted mean ±standard error mean computed by the GLM option of SAS.
**These samples were lost.

EFFECT OF PECTIN AND OAT BRAN ON PORTAL SHORT CHAIN FATTY ACIDS AND CHOLESTEROL IN RATS.

by

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B.S., Kansas State University, 1984

AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

The objective of this study was to evaluate the effects of various dietary fiber types and dietary SCFA-supplementation on the appearance of portal SCFA, hepatic and serum cholesterol, and liver lipids in 75 male Sprague-Dawley rats. These rats (215-223 g) were randomly assigned to five dietary treatment groups containing either 10% cellulose, pectin, oat bran, cellulose plus 0.5% sodium acetate, or cellulose plus 0.5% sodium propionate. These diets all contained 0.3% cholesterol, 0.2% methionine, 1.0% AIN-76 Vitamin Mixture, 3.5% AIN-76 Mineral Mixture, 10% corn oil, 15% casein, and 30% cornstarch and sucrose. Rats were fed ad libitum for 28 d and sacrificed in the fed state. No significant differences between groups existed for final body weight, daily weight gain, food efficiency, or portal acetate and butyrate concentrations. Dietary SCFA-supplementation did not affect liver cholesterol, liver lipids, or portal SCFA concentrations in rats compared to the cellulose-fed rats. Pectin feeding resulted in a 12.6 mg/g reduction in liver cholesterol and a 14.8 mg/100 ml reduction in serum cholesterol compared to the cellulose fed animals. Oat bran feeding produced a 6.0 mg/g reduction in liver cholesterol values compared to the SCFA-supplemented cellulose-fed rats. Both pectin and oat bran feeding resulted in reduced liver lipids (Pectin 42.5 $\pm$ 3.3 mg/g,

Oat bran 59.0 ± 3.4 mg/g) compared to the three cellulose-based diets (68.6 ± 3.3 - 77.8 ± 3.4 mg/g). Portal propionate (0.189 ± 0.008 mM/l) was elevated in animals fed pectin compared to the four other animal groups (0.099 ± 0.008 - 0.119 ± 0.009 mM/l). Similarly, pectin feeding resulted in elevated portal butyrate values (0.053 ± 0.004 mM/l) compared to the three cellulose-based diets (0.031 ± 0.004 - 0.038 ± 0.004 mM/l). These data suggest that the increased portal propionate concentrations in the pectin-fed rats may be associated with a hepatic and serum cholesterol lowering effect. However, the data are compromised from an accidental omission of choline in the diet which accounts for the presence of fatty livers.