Interaction between source of dietary fat and cereal grain fiber on lipid metabolism and growth in the chick

by Virginia Marcelina Martinez

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Home Economics

Montana State University

Abstract:
Elevated serum cholesterol, a risk factor for heart disease, is influenced by both dietary fats and fiber. Soluble dietary fiber has a hypocholester-olemic effect on animals and humans with elevated serum lipids. Waxy, hulless barley contains up to 15% β-glucans, a form of soluble fiber. A model for testing interaction of dietary fat and fiber was developed using the chick.

Two hundred eighty-eight broiler chicks were fed 23% protein diets containing either wheat or hulless barley and 10% palm oil, egg yolk, corn oil, butter, or tallow. Protein, vitamin and mineral supplements, and 1% cholesterol were included. Growth, feed consumption, lipid profiles of blood, excreta, and livers were measured or determined. Chicks fed wheat diets gained the most weight ($P < 0.05$) when palm oil, butter, tallow, or corn oil were fed. Lower ($P < 0.05$) feed/gain values were seen in all wheat fed chicks except for those fed egg yolk. Barley fed chicks had lower ($P < 0.05$) total plasma cholesterol and LDL-cholesterol than those fed wheat, with the highest ($P < 0.0001$) levels in chicks fed palm oil with wheat. HDL-cholesterol levels were highest for egg yolk diets, followed by barley with palm or corn oils; there were no differences in triglycerides (TG) between chicks fed barley or wheat with any fat source. LDL/HDL ratios were higher ($P < 0.05$) for wheat fed chicks than for barley fed chicks. Liver and body weights were greater ($P < 0.05$) for chicks fed wheat compared to barley, with only small differences in liver weights as percentage of body weight. Liver cholesterol was higher ($P < 0.0001$) for wheat fed chicks compared to those fed barley. Excreta dry matter was lower for barley fed chicks and excreta fats were higher when barley was fed. Barley had a hypocholester-olemic effect regardless of type of fat, particularly with palm oil, followed by tallow, corn oil, butter, and egg yolk. As fecal fat increased, plasma cholesterol decreased, indicating reduced fat absorption, accompanied by lower body weight gains.

Results suggest that barley dietary fiber is a hypocholesterolemic agent, particularly when fed with certain saturated fats.

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APPROVAL

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This thesis has been read by each member of the graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Elevated serum cholesterol, a risk factor for heart disease, is influenced by both dietary fats and fiber. Soluble dietary fiber has a hypocholesterolemic effect on animals and humans with elevated serum lipids. Waxy, hulless barley contains up to 15% β-glucans, a form of soluble fiber. A model for testing interaction of dietary fat and fiber was developed using the chick.

Two hundred eighty-eight broiler chicks were fed 23% protein diets containing either wheat or hulless barley and 10% palm oil, egg yolk, corn oil, butter, or tallow. Protein, vitamin and mineral supplements, and 1% cholesterol were included. Growth, feed consumption, lipid profiles of blood, excreta, and livers were measured or determined. Chicks fed wheat diets gained the most weight ($P < 0.05$) when palm oil, butter, tallow, or corn oil were fed. Lower ($P < 0.05$) feed/gain values were seen in all wheat fed chicks except for those fed egg yolk. Barley fed chicks had lower ($P < 0.05$) total plasma cholesterol and LDL-cholesterol than those fed wheat, with the highest ($P < 0.0001$) levels in chicks fed palm oil with wheat. HDL-cholesterol levels were highest for egg yolk diets, followed by barley with palm or corn oils; there were no differences in triglycerides (TG) between chicks fed barley or wheat with any fat source. LDL/HDL ratios were higher ($P < 0.05$) for wheat fed chicks than for barley fed chicks. Liver and body weights were greater ($P < 0.05$) for chicks fed wheat compared to barley, with only small differences in liver weights as percentage of body weight. Liver cholesterol was higher ($P < 0.0001$) for wheat fed chicks compared to those fed barley. Excreta dry matter was lower for barley fed chicks and excreta fats were higher when barley was fed. Barley had a hypocholesterolemic effect regardless of type of fat, particularly with palm oil, followed by tallow, corn oil, butter, and egg yolk. As fecal fat increased, plasma cholesterol decreased, indicating reduced fat absorption, accompanied by lower body weight gains.

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CHAPTER 1

INTRODUCTION

Coronary heart disease (CHD) is the number one cause of human deaths in the United States. The cost to survivors of this disease is financially prohibitive; therefore, ways to reduce the risks of CHD are being avidly sought. Elevated serum cholesterol is linked to atherosclerosis and is one of the major risk factors associated with myocardial infarction. Researchers have determined that dietary intervention, such as increased soluble dietary fiber intake, reduces hypercholesterolemia in animals and humans.

β-glucan, a component of the soluble dietary fiber found in oats and barley, is thought to be one of the active fractions of dietary fiber which decreases serum cholesterol. Therefore, barley cultivars that are high in this component are being intensively studied for dietary intervention. Hulless barley cultivars with waxy starch that contain up to 15% β-glucans have been identified. Researchers at Montana State University (MSU) fed various barley cultivars to chicks, rats and humans, and have determined that the waxy hulless barleys and high-fiber milling fractions of these barleys are very effective in lowering total serum cholesterol and low-density lipoproteins (LDL-cholesterol).

Barley is a major agricultural crop in Montana, ranking second to wheat in total production as a cereal grain. Currently, MSU is a leader in research on the health properties of barley. This effort also includes developing new markets for Montana-grown barley to further enhance the economy of Montana agriculture. Barley may be the alternate grain that consumers are seeking to increase the intake of soluble dietary fiber. Currently, several barley cultivars with high levels of soluble dietary fiber are being evaluated by the Nutrition Research Laboratory at MSU. Numerous products have been made with whole barley flour and refined barley flour obtained through a milling process. Additionally, the dietary fiber of barley can be concentrated or extracted and used as an additive in many foods.

The U.S. Department of Agriculture and the U.S. Department of Health, Education and Welfare established U.S. Dietary Guidelines for Americans and Dietary Goals for the U.S.A. in 1980. The guidelines of particular interest are: "Avoid too much fat, saturated fat, and cholesterol" and "eat foods with adequate starch and fiber." The goals of concern to dietitians include: "Increase the consumption of complex carbohydrates and 'naturally occurring' sugars from about 28 percent of energy intake to about 48 percent of energy intake"; "Reduce saturated fat consumption to account for about 10 percent of total energy intake, and balance that with polyunsaturated and monounsaturated fats, which should account for about 10 percent of energy intake each"; and "Reduce cholesterol consumption to about 300 milligrams a day."

Countries with lower incidences of CHD have diets higher in fiber and lower in fat than average diets in the U.S. The American Heart Association (AHA) developed a plan which closely resembles the eating patterns of countries with lower CHD. To prevent risk of CHD from elevated cholesterol, AHA recommends that most people consuming high fat diets (i.e., fat intake representing 35-40% of total calories) lower their fat intake to 30% of total calories.

There is enormous public interest in cholesterol education on dietary intervention as a control for elevated serum lipids. Barley research to decrease the risk of CHD can lead to improved health for the population at risk. Dietitians will have new information available to share with clients to improve their health.

Numerous studies have examined the effects of one dietary component, but few have considered that as one dietary component level is changed, so is another inversely altered. This research examines the effects of two dietary components -- fat and fiber -- recognizing that many other studies have identified either fat, fiber, cholesterol, or protein as individual factors. Evidence is growing to link low dietary fat and cholesterol intake and increased soluble fiber to the prevention of hypercholesterolemia.

The overall objective of this study was to develop a better understanding of the physiological effects of source and level of dietary fat and dietary fiber, and the interaction between fat and fiber. This research will enhance

nutritional knowledge as it applies to serum cholesterol and lipids in cardiovascular disease (CVD).

The specific objectives of this study were:

(1) To measure the effects of dietary fat from different sources and dietary fiber from cereal grains on lipid metabolism in hypercholesterolemic chicks.

(2) To determine if there is an interaction between type or source of dietary fat and barley fiber on lipid metabolism in hypercholesterolemic chicks.

CHAPTER 2

REVIEW OF LITERATURE

Coronary Heart Disease

Mortality, Survival, and Cost

Coronary heart disease due to atherosclerosis is the leading cause of death in the United States and, in addition, more than five million people have symptoms of the disease (Kuske & Feldman, 1987). Financial expenses to survivors of myocardial infarctions (MI) are often exorbitant (USDHHS, 1981). Treatment with two pharmacological bile acid binding resin drugs and oat bran were evaluated by Kinoian and Eisenberg (1988) for cost to society per year of life saved. Cholestyramine, cholestipol, and oat bran cost $117,400, $70,900, and $17,800, respectively. Dietary modification with oat bran which contains soluble dietary fiber was recommended as the most cost effective treatment.

Risk Factors for Myocardial Infarction

Nutrition related risk factors for heart disease include hypertension, elevated serum cholesterol above 200 mg/dl, and obesity of more than 30% above ideal body weight. Other risk factors include diabetes mellitus and

smoking (Kuske & Feldman, 1987). The risks of mortality associated with total serum cholesterol, low density lipoprotein (LDL-cholesterol), and high density lipoprotein (HDL-cholesterol) were assessed by Goldbourt et al. (1985). The components were measured in males who died of CHD. High total cholesterol was not associated with CHD death, and HDL-cholesterol was inversely associated with CHD mortality. Therefore, the authors suggested that a low HDL-cholesterol concentration appears to predict mortality more than high total cholesterol or high LDL-cholesterol.

As early as 1949, the Framingham study examined the adult population of a town to determine the rate of risk for CHD. As part of the Framingham study, Kannel et al. (1971) examined data to determine if high serum lipoproteins and cholesterol were risk factors for CHD. More than 5000 males and females with varying serum lipid levels and without CHD underwent a 14-year follow-up. About 10% of the subjects developed clinical manifestations of CHD. Elevated serum cholesterol was the best indicator of manifestation, even in individuals without other risk factors. Therefore, elevated serum cholesterol is considered a major risk factor for CHD.

Since research has proven that risk of CHD can have a dietary component, modified diets have been studied as a means of prevention and intervention.

Dietary Intervention

Dietary Fats

Some nutritional factors have been identified as more likely to lead to heart disease. Consequently, dietary intervention has been studied as a means of understanding prevention and therapy. Numerous studies on the effects of dietary fats on hyperlipidemia have been reviewed. Dietary fats and cholesterol have been studied for their hypo- and hypercholesterolemic effects on human serum cholesterol.

Human studies. Male subjects were fed saturated fatty acids (SAFAs) (C12-0 to 18-0), monounsaturated fatty acids (MOFAs), and dietary cholesterol (Keys & Parlin, 1966). Subjects fed MOFAs and stearic acid (C18-0) exhibited no serum cholesterol effects, while those fed polyunsaturated fatty acids (PUFAs) experienced depressed serum cholesterol. Keys and Parlin proposed that the lack of effect of stearic acid was by virtue of substitution of stearic acid (C18-0) for oleic acid (C18-1).

For five months, Baudet et al. (1984) studied Benedictine nuns with plasma cholesterol below and above 230 mg/dl. The sisters were fed either sunflower oil with a polyunsaturated fat-to-saturated fat (P:S) ratio of 1.75, peanut oil P:S .68, palm oil P:S .31, with 300 mg/dl cholesterol, or butter P:S .09 with 400 mg/dl cholesterol daily. Nuns fed palm oil, a saturated oil, had HDL-cholesterol and LDL-cholesterol levels identical to those fed sunflower

and peanut oils, while those fed the butter diet had elevated levels. The investigators concluded that composition of fat saturation rather than level of total fat affected plasma cholesterol. The results indicated those fed high fat diets with high monounsaturates and cholesterol decreased their serum lipids more than those fed high fat diets with high saturates.

Recent findings by Qureshi et al. (1986) indicated that palm oil is an excellent source of tocotrienols, which may have importance to this study as these compounds inhibit the synthesis of cholesterol in the liver.

Liquid diets of 40% fat with high palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1) were tested on humans for their effects on plasma lipoproteins (Bonanome & Grundy, 1988). When subjects were on both the stearic and oleic acid diets, they had decreased cholesterol, making stearic acid as effective as oleic acid. Total serum cholesterol was lowered when the men were fed stearic compared to palmitic acid. The researchers suggested stearic acid may be converted to oleic acid. Palmitic acid does not exhibit the same effect on cholesterol, indicating different saturated fats have different cholesterolemic potential.

Grundy et al. (1986) compared diets of varying saturated-to-monounsaturated-to-polyunsaturated ratios (S:M:P). The diets consisted of a high polyunsaturated (PUFA) diet 10:13:17 (40% fat), AHA Phase I 10:10:10 (30% fat), and AHA Phase III 6.7:6.7:6.7 (20% fat). High PUFA diets did not lower cholesterol as much as the AHA I and AHA III diets, which were not

significantly different. AHA Phase I was more acceptable to subjects accustomed to the normal 40% fat diet of North Americans.

Research has established some dietary fats contribute to development of CHD, while others inhibit the chances of acquiring CHD.

Cholesterol

Much research has been directed toward understanding the role cholesterol plays in hypercholesterolemia. Eggs, because of their high cholesterol content of 213 mg/large egg, have also been implicated as hypercholesterolemic foods, and are often used in studies to determine their effects on serum lipoprotein levels.

Human studies. Effects of cholesterol-fat diets on normolipidemic adult subjects were studied by Tan et al. (1980). Diets were isocaloric and contained equal percentages of protein, fat, and carbohydrate. A diet high in cholesterol (1021 mg/dl) with a low P:S (.14) increased human serum cholesterol, HDL-cholesterol, and LDL-cholesterol more than a lower cholesterol diet (98 mg/dl) with high P:S (1.6). In a crossover study by Chenoweth et al. (1981), healthy men were fed 45% fat and P:S 3:5 diets versus 35% fat and P:S 1:0 diets plus either two eggs or a cholesterol-free egg substitute. The results showed a significant decrease in total serum cholesterol for subjects on the lower fat-egg substitute diet. There was no difference attributed to P:S ratio.

Vorster et al. (1987) fed African subjects a high cholesterol-low fat diet with five eggs daily. The control group was fed a lower cholesterol-low fat diet with two eggs. In the subjects on the high cholesterol egg diet, serum cholesterol and LDL-cholesterol were slightly higher than in the control group, although HDL-cholesterol was not adversely affected. The study indicated that both low fat diets maintained lowered cholesterol and LDL-cholesterol, but HDL-cholesterol was also lowered.

In a study by Edington et al. (1987), hypercholesterolemic volunteer men and women were fed 26% fat with P:S .8, and normocholesterolemic males were fed 35% fat with P:S .6. They were also fed two eggs or seven eggs weekly. The results indicated that dietary cholesterol from eggs did not increase serum cholesterol, and a high fat diet increased total serum cholesterol and LDL-cholesterol. The authors suggested that a low SAFA diet is usually low in total fat and there is no need to decrease egg consumption if the national guidelines are followed.

The effects of corn oil and lard with differing cholesterol levels (egg yolk) on total serum cholesterol of healthy women were studied by Zanni et al. (1987). Dietary cholesterol increased total serum cholesterol with or without lard. Dietary SAFA and cholesterol both increased total cholesterol and LDL-cholesterol. The Zanni et al. study did not separate the effects of dietary SAFA and cholesterol.

Animal studies. Griminger and Fisher (1986) studied the severity of aortic lesions in Leghorn chicks. Quantitative measurements of the atherosclerotic lesions indicated that chicks fed cholesterol oxides in powdered eggs had smaller lesions than chicks fed cholesterol in fresh eggs. However, serum and liver cholesterol levels were higher in chicks fed powdered eggs and fresh eggs than in chicks fed a cholesterol-free diet. Atherosclerotic lesions were more apparent in chicks fed fresh eggs, but serum lipids were similarly elevated in both groups.

Conflicting results concerning the effects of egg cholesterol on serum lipids indicate a need for further research in this area.

Fiber

Nutritional factors are complex, and dietary fats and cholesterol are only two possible contributing factors. Dietary fiber is another food component to be considered when studying nutritional effects on serum lipids.

Dietary fiber has undergone a series of updated definitions and may be defined either physically, chemically, or botanically (Spiller, 1986). Dietary fiber (DF) is generally defined physically and botanically as those components of plant material that are resistant to digestion by the enzymes of the human gastrointestinal tract and are therefore not fully digested, or as the residue derived from plant cell walls that is resistant to hydrolysis by human alimentary enzymes (Trowell, 1972). The chemical definition proposed by Southgate (1977) is nonstarch polysaccharides plus lignin.

There are two general components of DF: water-soluble and water-insoluble. Water-soluble fractions include polysaccharide gum, mucilage, pectins, and some hemicelluloses. Water-insoluble fractions have little water holding ability and include celluloses and lignin (Spiller, 1986).

Human studies. Dietary treatments, of which fiber was one variable, have been studied for their hypocholesterolemic effects. Trowell (1972) reviewed early studies of the '50s, '60s, and early '70s. He found that tropical Africans ate more high fiber foods than Westerners. The indigenous Africans had a much lower incidence of CHD than people who ate a more refined carbohydrate diet. Research studies on the diets of vegetarian monks and American Seventh Day Adventists were also reviewed; it was noted that their diets were higher in fiber and lower in SAFAs and they had lower serum cholesterol levels and less ischemic heart disease than modern non-vegetarians (Trowell, 1972).

Kies (1985) studied human subjects who were fed ordinary food plus insoluble fiber from psyllium seed fiber, cellulose, rice bran, corn bran, and wheat bran, or no fiber. He ranked the water holding capacity of the fiber and found responder subjects who ate diets with fiber of lower water holding capacity experienced no serum cholesterol lowering effect; those fed psyllium with more water holding capacity showed a decrease in cholesterol. Story and Thomas (1980) suggested soluble dietary fiber (SDF) binds to bile acid and prevents reabsorption of cholesterol or cholesterol precursors in the

intestine, resulting in lower blood total cholesterol and increased fecal fats.

In an experiment conducted by Anderson et al. (1984), hypercholesterolemic men were fed diets with beans containing 16 g SDF, oat bran containing 17 g SDF, and a control diet containing 6 g SDF daily. They determined that oat bran and bean fiber, foods rich in soluble fibers, decreased total serum cholesterol and LDL-cholesterol, and had no effect on HDL-cholesterol.

Animal studies. In a study by Chen et al. (1981), oat bran selectively decreased serum total cholesterol in rats while it increased HDL-cholesterol. This effect appeared to be related to the SDF properties. Oat gum, oat bran, and pectin lowered total serum cholesterol in rats while dietary cholesterol raised total serum cholesterol.

The effects of DF on serum and liver lipids in rats were studied by Kritchevsky et al. (1988). The rats were fed different fiber diets, including cellulose, alfalfa, pectin, guar gum, Metamucil (psyllium), mixed fiber, and no fiber. The outcome indicated serum cholesterol increased with alfalfa and decreased with cellulose, guar gum, and psyllium. Liver cholesterol increased with alfalfa, cellulose, pectin, guar gum, Metamucil, and even with no fiber. The researchers noted the patterns were inconsistent.

A study was undertaken by Welch et al. (1988) to examine the effects of oat bran on chick total plasma cholesterol. Oat bran, oat bran fractions (oil, insoluble fiber, protein, gum, and soluble residues), and a control diet based

on the Standard Reference Purified Diet for chicks (NRC, 1977) were fed to determine which component affects cholesterol levels. Oat bran, gum, and protein lowered total cholesterol, with oat bran and gum affecting cholesterol the most.

Human Interactions. Many animals consume varied diets, and some nutrition research was designed to study the interactions of various food components. In a study by Williams et al. (1986), three-day diet records of sedentary healthy men with cholesterol levels below 300 mg/dl were analyzed for fat, protein, carbohydrate, and fiber intake. Blood was taken after a 12-hour fast. Men with diets high in PUFA had lower total cholesterol and LDL-cholesterol levels. The effect of total dietary fat was not studied, but PUFA intake lowered lipoproteins. Dietary cholesterol had no effect on LDL-cholesterol or total cholesterol. SDF was not available in food composition tables, so it was not evaluated. Animal protein eaters had higher total cholesterol than plant protein eaters, indicating that protein source makes a difference. Williams et al. suggested that diets high in PUFAs and plant protein decreased risk of CHD by lowering atherogenic lipo-proteins.

Healthy adults were fed American Heart Association (AHA) fat modified diets, < 30% fat and polyunsaturated to monounsaturated to saturated ratio (P:M:S) 1:1:1, with or without 250 mg cholesterol and with or without oats. Addition of 60 g/d oats to an AHA fat modified diet enhanced human serum

lipid response by lowering cholesterol 5.6 to 6.5 mg/dl, while the AHA fat modified diet with no cholesterol decreased total serum cholesterol by 1.2 mg/dl (Van Horn et al., 1986).

The effects of fiber, cholesterol, lipid, and protein on serum and liver lipids in rats were studied by Stewart et al. (1987). The variables were combined into sets of three, and response-surface regression analysis examined more than two variables. A carbohydrate-lipid-protein combination affected lipoproteins more than any combination with dietary fiber. Again, the results were conflicting, confirming the need for additional research. Since data on soluble fiber are not readily available to researchers at this time, there exists a need for further analysis of the fiber.

Barley Dietary Fiber

Barley (*Hordeum Vulgare* L.) is rich in fiber which has been studied for its effects on plasma lipids, yet the dietary influences of barley are complex and may be a result of several components.

Human studies. Volunteer normocholesterolemic males who were fed 42 g DF/d experienced a greater decrease when the DF source was a waxy hulless barley grown in Arizona (AH), but levels actually increased when fed a wheat control (Newman et al., 1989a). In another study by Newman et al. (1989b), AH barley and commercial oats were ground and made into muffins, flatbread, and cereal fed daily as a part of the regular diet of men and women with average initial total cholesterol 248-256 mg/dl and

LDL-cholesterol 157-173 mg/dl. The results indicated that there were no significant differences between the total cholesterol and LDL-cholesterol lowering properties of barley and oats; both decreased total plasma cholesterol by 12 mg/dl, and LDL-cholesterol levels decreased 24 and 11 mg/dl for barley and oats, respectively.

Animal studies. Fadel et al. (1987) fed broiler chicks waxy hulless and non-waxy hulless barley and a cornmeal control diet to determine their effects on serum lipids and excreta. The researchers reported barley had a cholesterol lowering effect, with one waxy hulless barley exhibiting lower total blood cholesterol and LDL-cholesterol levels than the other barley when compared to corn. Excreta fats were higher in chicks fed waxy hulless barley.

β-Glucans

The effect of DF on hyperlipidemia is a complicated issue. DF needs to be divided into more specific elements if the effects are to be fully understood.

Soluble β-glucans are mixed-linked B-(1-3), (1-4)-D-glucopyranosyl units with a ratio of 1:2.5. They are found mainly in the endosperm cell wall of barley and oats and can be extracted from barley with water for 2 h at 38°C (Åman & Graham, 1987a). β-glucans are similar to cellulose but with mixed linkages, and are viscous, water soluble fibers (Spiller, 1986). According to a review by Newman et al. (1989b), (1-3) linkages in the β-glucan chain cause

peculiarly shaped molecules; therefore, the compounds have unique physio-chemical properties, including water solubility and viscosity.

In a study by Klopfenstein and Hoseney (1987), β-glucans were extracted from oats, barley, wheat, and sorghum and mixed into white bread with 7% and 13% added β-glucan. Diets containing a control bread without added β-glucan and the β-glucan enriched bread were fed to 60 rats to determine their effects on serum lipoproteins. In general, serum and liver total cholesterol decreased in those fed the β-glucan bread, but when the control diet was substituted, serum and liver cholesterol levels increased. The outcome demonstrated that β-glucan may be used in bread or other foods as a means of controlling cholesterol.

Tocotrienols

In addition to fiber, barley contains another factor which may contribute to the cholesterol lowering effect. Qureshi et al. (1986) demonstrated that cholesterol synthesis by HMG CoA reductase, the rate-limiting enzyme for cholesterol synthesis, may be inhibited by a fat soluble component in barley. Chick and rat livers were incubated with 10 isolated barley fractions. Livers were assayed enzymatically, and two fractions were quite active in cholesterol suppression *in vitro.* The two were fed to chicks in screening trials and exhibited decreased HMG CoA reductase activity. The results indicated a decrease in total serum cholesterol and LDL-cholesterol, and an increase in fatty acid synthesis. The active fractions were identified by high resolution

mass spectral analysis as d-a-tocotrienol, a compound which appears to inhibit cholesterogenesis.

Tocotrienols are but another contributing factor to the puzzle of how cereal grain factors influence serum lipid levels.

Animal Models

Hypercholesterolemia in Chicks

Blood cholesterol is affected by heredity, age, nutrition, activity, and species. Animal models are needed whose lipoprotein responses to diet are similar to humans. Experimentally induced hypo- and hypercholesterolemia make the chick a valuable test model (Sturkie, 1976).

Screening models were developed by Newman et al. (1988). Total cholesterol in chicks was elevated and barley products were fed to determine their cholesterol lowering potential. The researchers determined from the response of chicks they are good screening models for testing the potential of hypocholesterolemic foods.

In an experiment by Chandler et al. (1979), rats exhibited resistance to hypercholesterolemia, while chicks exhibited susceptibility to hypercholesterolemia when diets were supplemented with 1% cholesterol. When selecting a model for evaluation of hypercholesterolemic effects, an applicable model is essential for success.

Summary

In conclusion, CHD due to atherosclerosis may best be managed by controlling total blood cholesterol, HDL-cholesterol, and LDL-cholesterol levels. Dietary management of these blood components has been investigated on man and animal models. The focus of this literature has been the effects of dietary cholesterol, dietary fat (including type and amount), and dietary fiber (especially SDF) on serum lipoproteins in chick models.

CHAPTER 3

MATERIALS AND METHODS

Experimental Design

The experiments designed and conducted for this study are described below.

Experiment 1: Forty Leghorn chicks were fed four diets containing four dietary lipids (butter, corn oil, lard, or olive oil) at 10% of the diet, with cornmeal as a grain source and soybean meal as a protein supplement. This screening pilot trial was intended to examine the effect of fat source on total serum cholesterol in the chick.

Experiment 2: Sixty-four broiler chicks were fed eight diets containing four dietary lipids (corn oil, egg yolk, palm oil, or lard) at 4.7% of the diet, and either cornmeal or barley as grain sources with soybean meal as a protein supplement. This pilot trial studied the effects of fat sources and grain sources on total serum cholesterol in the chick.

Experiment 3: One hundred and four broiler chicks were fed 13 diets containing five dietary lipids (palm oil, butter, beef suet, corn oil, or egg yolk) at 10% or 15% of the diet. Varying levels of fat were used to determine the best level to produce cholesterolemic responses in the chick.

Cholesterol was added to duplicate corn oil diets at each fat level in order to test the need for dietary cholesterol as a hypercholesterolemic agent in addition to dietary fat. An additional corn oil diet at 4.7% was used as a reference to earlier tests. The grain in all of these diets was cornmeal with soybean meal as a protein supplement.

Experiments 4a and 4b: Two sets of 144 broiler chicks were fed in Experiments 4a and 4b. Five dietary lipids (palm oil, egg yolk, or corn oil in Experiment 4a, and tallow in beef, butter, or corn oil in Experiment 4b) were fed at 10% of the diet. Cholesterol was added to all diets, except the egg yolk diet, to equal the cholesterol in the egg yolk diets. Duplicate diets were prepared using either wheat or barley as the grain source. Cholesterol was used in all the diets to determine if fat or grain had an effect on lipid metabolism.

Feed consumption and weight gain were recorded, and feed/gain ratios were computed. Blood samples were drawn and plasma lipids were measured. Fecal samples were collected and analyzed for ether extract (crude fat) content and dry matter. At the conclusion, livers were taken and analyzed for cholesterol. The data were examined for changes in levels affected by lipids, fiber, and lipid x fiber interaction.

The first three experiments were designed as pilot studies. Experiment 4 was designed using the chick model to evaluate the effects of barley or wheat and various fats on plasma lipids.

Chicks

In the first experiment, Leghorn cockerel chicks were used. In the next three experiments, newly hatched male broiler chicks were ordered from Fors Farms, Inc., Puyallup, Washington. All chicks were pre-fed a starter diet (Table 1) for two to six days. Chicks were wingbanded with consecutive numbers.

To get a representative sample of chicks, body weights were taken on day 1 of each study; weights were listed from heaviest to lightest and outliers were eliminated. The remaining stratified chicks were assigned to the dietary treatment cages. Body weights were taken again at the conclusion of the studies. Chicks were fed *ad libitum* and consumption per cage was recorded daily. Dead chicks were immediately removed from cages and recorded. Temperatures of cages were kept at the optimal level for growing chicks. (Refer to Table 2 for chicks used in each experiment.)

Experimental Diets

Diet Analysis

Dietary components were analyzed using cited methods. The dehydrated egg yolk, Hard Red Spring wheat, and Arizona Hulless barley were analyzed for ether extract (crude fat) by the Folch ether extraction method (Folch et al., 1957). Protein content in egg yolk, wheat, barley, soybean protein isolate,

Table 1. Composition of pretest diets[a] fed to chicks.

Ingredients	EXPERIMENT			
	1	2	3	4
	<---------- Percent (%) ---------->			
Cornmeal[b]	62.73 (9.0)*	47.53 (9.0)	47.86 (8.8)	68.77 (9.0)
Soybean meal[b]	27.27 (45.3)	42.45 (44.1)	42.12 (44.6)	
Soy protein isolate[c]				20.23 (83.1)
Soy/corn oils[d]	4.69	4.69	4.69	
Corn oil[d]				4.70
Supplement[f]	5.31	5.31	5.31	6.28
Antioxidant[e]		.02	.02	.02

[a]Experiment 1 contained 18% protein and 4.69% added fat. Experiments 2 and 3 contained 23% protein and 4.69% added fat. Experiment 4 contained 23% protein and 4.7% added fat.

[b]Cornmeal and soybean meal were obtained from a local feedmill.

[c]Soy protein isolate was purchased from ICN Biochemicals, Cleveland, Ohio.

[d]Mazola corn oil and Crisco soybean oil were purchased locally from Albertson's, Inc.

[e]Antioxidant (ethoxyquin) was purchased from ConAgra/West Feeds, Billings, Montana.

[f]For supplement, refer to Table 4.

*Numbers in parentheses equal percent protein in ingredient.

Table 2. Type and number of chicks in each experiment.

Description	EXPERIMENT				
	1	2	3	4a	4b
Chick type	Leghorn	Broiler	Broiler	Broiler	Broiler
Treatment no.	4	8	13	6	6
Reps/treatment	1	1	1	4	4
Chick/cage	10	8	8	6	6
Chick total	40	64	104	144	144

and casein were analyzed by the Kjeldahl method (AOAC, 1980, sec. 7.015). DF was measured enzymatically by the Prosky method (Prosky et al., 1988) on wheat, barley, and soybean protein isolate. The cholesterol content of the dehydrated egg yolk was analyzed by the Folch method (Folch et al., 1957). Values for cholesterol in butter and beef tallow were taken from the USDA handbook, *Composition of Foods: Dairy and Egg Products* (Posati & Orr, 1976). (Refer to Table 3 for analyzed values.)

Diet Formulations

All diets were formulated according to the National Research Council requirements (NRC, 1977) for day-old to three-week-old chicks (Tables 4 through 7).

Chemical Analysis

Blood Analysis

Blood samples (3 ml) were drawn via wing vein in all trials after a 10-hour fast at d 1 and again at the conclusion of the trials. Blood was drawn into Vacutainer tubes containing EDTA. Samples were analyzed for total cholesterol, HDL-cholesterol, and triglycerides (TG). In Experiments 1, 2, and 3, serum lipids were analyzed at the Montana State University Veterinary Research Laboratory using the precipitation and enzymatic and calorimetric method of Allain and Poon (1974) with Baker Centrifichem instruments. Plasma lipids rather than serum lipids were analyzed in Experiment 4.

Table 3. Protein, total dietary fiber, crude fat, and cholesterol contained in feed ingredients.

INGREDIENTS	EXPER 1	EXPER 2	EXPER 3			EXPER 4			
	Protein	Protein	Protein	Crude Fat	Cholesterol	Protein	Crude Fat	Cholesterol	Total Dietary Fiber
	<---------- Percent (%) ---------->								
Barley		12.6				12.60	3.3		19.03
Wheat						16.25	1.8		11.43
Soybean meal	45.6	44.1	44.6[a]						
Commeal	9.0	9.0	8.8[a]						
Casein		84.6							
Soy protein isolate						83.40			3.79
Egg yolk		32.1	32.1	47.5	4.05	32.36	47.4	4.76	
Tallow								10.90[b]	
Butter								21.90[b]	

[a]Experiment 3, diets with 15% corn oil and 4.69% soy/corn oils, contained 43.7% protein in soybean meal.
[b]Cholesterol in tallow and butter values from USDA Handbook (Posati & Orr, 1979).

Table 4. Composition of supplements in chick diets.

Ingredients	EXPERIMENT	
	1, 2, and 3	4
	<---------- Percent (%) ---------->	
Dicalcium phosphate	2.800	2.800
Limestone	1.500	1.500
Salt	.500	.500
Vitamin premix[a]	.275	
Vitamin diet fortification[b]		1.000
Trace mineral mix[c]		.150
Choline chloride[d]		.200
DL-methionine[d]	.125	.125
Biotin	.01 mg	.01 mg
Antibiotic	.100	

[a]Vitamin premix -- Furnishes the following per g/mixture: vitamin A, 4000 USP U; vitamin D-3, 1200 ICU; vitamin E, 4 IU; vitamin B-12, .004 mg; riboflavin, 2.4 mg; niacin, 12 mg; d-pantothenic acid, 3.6 mg; choline chloride, 200 mg; menadione SBC, 1.6 mg; folic acid, .12 mg; thiamine, .4 mg; pyridoxine, .4 mg. The following are listed per 5.5 pounds of mixture: manganese, 3%; iron, 1.67%; copper, .160%; biotin, 100 mg; iodine, .05%; zinc, 2.0%; selenium, 272.4 mg.

[b]Vitamin diet fortification -- Furnishes the following per kg/mixture: vitamin A acetate (500,000 IU/g), 1.8; vitamin D concentrate (850,000 IU/g), .125; alpha tocopherol (250 IU/g), 22.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 4.25; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; thiamine hydrochloride, 1.0; calcium pantothenate, 3.0. Purchased from ICN Biochemicals, Cleveland, Ohio.

[c]Trace mineral mix -- Furnishes the following percentage per mixture: zinc, 20.0; iron, 10.0; manganese, 5.5; copper, 1.0; iodine, 0.15; selenium, 0.02.

[d]Choline chloride and methionine were purchased from Sigma Chemical Co., St. Louis, Missouri.

Table 5. Composition of diets[a] fed to chicks in Experiments 1 and 2.

INGREDIENTS	EXPERIMENT 1				EXPERIMENT 2							
	Butter	Corn Oil	Lard	Olive Oil	Barley/ Corn Oil	Cornmeal/ Corn Oil	Barley/ Egg Yolk	Cornmeal/ Egg Yolk	Barley/ Palm Oil	Cornmeal/ Palm Oil	Barley/ Lard	Cornmeal/ Lard
	<---------- Percent (%) ---------->											
Barley[b]					60.00		60.00		60.00		60.00	
Soybean meal[b]	28.36	28.36	28.36	28.36	24.52	42.45	22.20	35.10	24.52	42.45	24.52	42.45
Cornmeal[b]	56.33	56.33	56.33	56.33		47.53		50.22		47.53		47.53
Casein[c]					5.46		3.12		5.46		5.46	
Olive oil[d]				10.00								
Corn oil[d]		10.00			4.69	4.69						
Lard[d]			10.00								4.69	4.69
Butter[d]	10.00											
Palm oil[e]									4.69	4.69		
Egg yolk[f]							9.35	9.35				
Supplement[g]	5.31	5.31	5.31	5.31	5.31	5.31	5.31	5.31	5.31	5.31	5.31	5.31
Antioxidant[h]					.02	.02	.02	.02	.02	.02	.02	.02

[a]Experiment 1 diets contained 18% protein and 10% fat; Experiment 2 diets contained 23% protein and 4.69% fat.
[b]Barley, soybean meal, and cornmeal were obtained from a local feedmill.
[c]Casein was purchased from ICN Biochemicals, Cleveland, Ohio.
[d]Bertolli olive oil, Mazola corn oil, Armour lard, and Darigold butter were purchased locally from Albertson's, Inc.
[e]Palm oil was obtained from Palm Company Refinery, Portland, Oregon.
[f]Egg yolk, Type Y1-FF, was purchased from Henningsen Foods, Inc., Omaha, Nebraska.
[g]For supplement, refer to Table 4.
[h]Antioxidant (ethoxyquin) was purchased from ConAgra/West Feeds, Billings, Montana.

Table 6. Composition of diets[a] fed to chicks in Experiment 3.

INGREDIENTS	Palm Oil		Butter		Suet		Corn Oil				Egg Yolk		Soy/Corn Oils
	10	15	10	15	10	15	10	15	10 + Chol.	15 + Chol.	10	15	4.69
	<---------- Percent (%) ---------->												
Soybean meal[b]	43.43	44.66	43.43	44.66	43.43	44.66	43.43	44.66	40.17	46.03	27.34	20.42	42.45
Cornmeal[b]	41.25	35.02	41.25	35.02	41.25	35.02	41.25	35.02	43.66	32.37	46.29	42.68	47.54
Supplement[c]	5.30	5.30	5.30	5.30	5.30	5.30	5.30	5.30	5.30	5.30	5.30	5.30	5.30
Palm oil[d]	10.00	15.00											
Corn oil[e]							10.00	15.00	10.00	15.00			
Butter[e]			10.00	15.00									
Soy/corn oils[e]													4.69
Suet[f]					10.00	15.00							
Egg yolk[g]											21.05	31.58	
Cholesterol[h]									.85	1.28			
Antioxidant[i]	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02

[a]Diets contained 23% protein and 4.69%, 10%, or 15% fat.
[b]Soybean meal and cornmeal were obtained from a local feedmill.
[c]For supplement, refer to Table 4.
[d]Palm oil was obtained from Palm Company Refinery, Portland, Oregon.
[e]Mazola corn oil, Darigold butter, and Crisco soybean oil were purchased locally from Albertson's, Inc.
[f]Suet was purchased from Albertson's, Inc.
[g]Egg yolk, Type Y1-FF, was purchased from Henningsen Foods, Inc., Omaha, Nebraska.
[h]Cholesterol was obtained from Sigma Chemical Co., St. Louis, Missouri.
[i]Antioxidant (ethoxyquin) was purchased from ConAgra/West Feeds, Billings, Montana.

Table 7. Composition of diets[a] fed to chicks in Experiments 4a and 4b.

INGREDIENTS	EXPERIMENT 4a				EXPERIMENT 4b							
	Barley/ Palm Oil	Wheat/ Palm Oil	Barley/ Egg Yolk	Wheat/ Egg Yolk	Barley/ Corn Oil	Wheat/ Corn Oil	Barley/ Butter	Wheat/ Butter	Barley/ Tallow	Wheat/ Tallow	Barley/ Corn Oil	Wheat/ Corn Oil
	<---------- Percent (%) ---------->											
Barley[b]	60.00		60.00		60.00		60.00		60.00		60.00	
Wheat[b]		60.00		60.00		60.00		60.00		60.00		60.00
Soy protein isolate[c]	18.51	15.89	10.32	7.49	18.51	15.89	18.51	15.89	18.51	15.89	18.51	15.89
Palm oil[d]	10.00	10.00										
Egg yolk[e]			21.10	21.10								
Corn oil[f]					10.00	10.00					10.00	10.00
Tallow[g]									10.00	10.00		
Butter[f]							10.00	10.00				
Cholesterol[h]	.99	.99			.99	.99	.97	.97	.98	.98	.99	.99
Supplement[i]	6.28	6.28	6.28	6.28	6.28	6.28	6.28	6.28	6.28	6.28	6.28	6.28
Cornstarch[f]	4.13	2.10	1.87		4.13	2.10	4.15	2.12	4.14	2.11	4.13	2.10
Cellulose[c]		4.56		4.56		4.56		4.56		4.56		4.56
Antioxidant[j]	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02
Lysine[h]		.07	.28	.42		.07		.07		.07		.07
Methionine[h]	.07	.09	.13	.13	.07	.09	.07	.09	.07	.09	.07	.09

[a]Diets contained 23% protein, 10% fat, 11.42% total dietary fiber, 0.99% cholesterol, 1.2% lysine, and 0.5% methionine.
[b]Barley and wheat were obtained from a local feedmill.
[c]Soy protein isolate and cellulose (alphacel) were purchased from ICN Biochemicals, Cleveland, Ohio.
[d]Palm oil was obtained from Palm Company Refinery, Portland, Oregon.
[e]Egg yolk, Type Y1-FF, was purchased from Henningsen Foods, Inc., Omaha, Nebraska.
[f]Mazola corn oil, Darigold butter, and cornstarch were purchased locally from Albertson's, Inc.
[g]Tallow was rendered from suet purchased at Albertson's, Inc.
[h]Cholesterol, lysine, and methionine were purchased from Sigma Chemical Co., St. Louis, Missouri.
[i]For supplement, refer to Table 4.
[j]Antioxidant (ethoxyquin) was purchased from ConAgra/West Feeds, Billings, Montana.

For Experiment 4, blood was centrifuged, supernatant was decanted, and plasma was analyzed for lipoproteins at the Montana State University Nutrition Research Laboratory using the Eastman Kodak Ektachem DT60 Analyzer. Warnick's method (Warnick et al., 1983) was used for HDL analysis, and the Tietz method (Tietz, 1987) was used for TG analysis. LDL-cholesterol was calculated by using Friedwald's equation (Friedwald et al., 1972):

LDL Cholesterol = Total Cholesterol - HDL Cholesterol - TG/5.

Ratios of LDL/HDL were computed since LDL/HDL is the proportion of cholesterol transported in LDL-cholesterol versus HDL-cholesterol, and is an important indicator of risk for CHD.

Excreta Analysis

Feces were collected on plastic coated paper after an 18-hour period on d 8 and d 15 of Experiment 2. The pan tare, pan tare + fresh feces, pan tare + dried feces, and difference between fresh and dried feces weights were recorded. The material was dried six days at 58°C in an Electric Hotpack Company dryer. Dried feces were ground and ether extract (crude fat) was obtained by an acid extraction method ("Determination of Crude Oils and Fats," 1971). Ether extraction was completed on a Soxtec System HT 1043 extraction unit and samples were dried in a Precision Thelco Model 18 dryer. Dried samples were weighed and ether extracts were calculated.

Feces were dried and percentage ether extract on a dry matter basis was obtained.

Liver Analysis

At the conclusion of Experiments 4a and 4b, livers were analyzed for cholesterol by the following method. Chicks were sacrificed on d 18 and livers were weighed, sealed in plastic bags, and frozen immediately in liquid nitrogen. Lipids were extracted using the Folch method (Folch et al., 1957) as modified by Bligh and Dyer (1959). Samples were thawed and homogenized in phosphate buffered saline (PBS). Chloroform/methanol and water were added and samples were mixed and centrifuged. The top layer was aspirated and the remaining liquid was dried under nitrogen. The dried lipids were reconstituted in isopropanol and mixed. Samples, standards, and a blank were mixed according to the modified sequence in Table 8.

The samples were allowed to stand 18 minutes and optical density (OD) was read on a Bausch and Lomb Spectronic 710 at 500 nm. OD values were calculated and converted to cholesterol concentrations using a standard curve. Values of less than 20% of standard error were acceptable; samples with values above 20% of standard error were rerun.

Table 8. Sequence for preparing reconstituted lipids for cholesterol analysis.

Description	Blank	Standard	Sample
Sample			100 ul
PBS[a]	200 ul		
Cholesterol Calibrator[b]		180 ul 20 ul	100 ul
PBS Cholesterol Calibrator		160 ul 40 ul	
PBS Cholesterol Calibrator		100 ul 100 ul	
PBS Cholesterol Calibrator		0 ul 200 ul	
Cholesterol Reagent[c]	2 ml	2 ml each	2 ml

[a]PBS: Phosphate buffered saline (Speck, 1976).
[b]Cholesterol calibrator: Sigma Diagnostics, Catalog No. C-0534.
[c]Cholesterol reagent: Sigma Diagnostics, Catalog No. 352-50.

Statistical Analysis

Data were analyzed by the SAS General Linear Model procedure; when effects were significant ($P < 0.05$), means were separated by least squares means PDIFF (SAS, 1988). Least squares means (LSM) were used to obtain the best unbiased estimate, as there were missing chicks and cells in some experiments due to death unrelated to the experimental design.

CHAPTER 4

RESULTS AND DISCUSSION

Experiment 1

Blood Analysis

Mean total serum cholesterol values are presented in Table 9. The mean results indicated that there were higher ($P < 0.05$) cholesterol levels for chicks fed 10% butter (200.6 mg/dl) and olive oil (196.2 mg/dl) than those fed corn oil (145.9 mg/dl) or lard (166.9 mg/dl).

Table 9. Mean total serum cholesterol of chicks* fed diets with various fat sources (Experiment 1).

	Butter	Corn Oil	Lard	Olive Oil
	<---------- mg/dl ---------->			
Cholesterol	200.6[a]	145.9[b]	166.9[b]	196.2[a]

*There were 10 chicks per treatment at the beginning of the experiment.

[ab]Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

This pilot study evaluated a comparison of means to determine the effects of fats without added dietary cholesterol on serum total cholesterol. As reported in the literature, higher total cholesterol levels resulted from butter, a highly saturated fat, while chicks fed corn oil, a polyunsaturated oil,

had lower levels. Chicks fed lard were intermediate, while those fed olive oil had total cholesterol levels similar to those fed butter.

Experiment 2

Blood Analysis

Table 10 shows that the egg yolk with cornmeal diet resulted in chicks with higher ($P < 0.05$) total serum cholesterol and LDL-cholesterol than those fed all other diets, except LDL-cholesterol from corn oil or palm oil with cornmeal. There were no differences between the other treatments. Serum TGs were higher in all other chicks than those fed egg yolk with cornmeal, although the differences were not significant. In all diets there were no differences in serum HDL-cholesterol levels. Total serum cholesterol and LDL-cholesterol were lower ($P < 0.05$) in chicks fed the egg yolk and barley diet compared to egg yolk with cornmeal.

It was determined from this experiment that 4.7% fat, the normal chick requirement, without cholesterol in the diet was too low to increase serum cholesterol sufficiently to demonstrate the effects of dietary fiber. The differences observed on the egg yolk diet were probably due to cholesterol in the fat sources. Therefore, it was determined that a higher level of fat was required, and that if egg yolk were used as one treatment, the other fats should have cholesterol added to equalize them with egg yolk fat.

Table 10. Mean serum lipoproteins of chicks* fed barley or cornmeal and various fats (Experiment 2).

	Corn Oil		Egg Yolk		Palm Oil		Lard	
	Barley	Corn Meal	Barley	Corn Meal	Barley	Corn Meal	Barley	Corn Meal
	<---------- mg/dl ---------->							
Cholest.	152.5^{b}	157.3^{b}	146.0^{b}	196.4^{a}	137.0^{b}	139.0^{b}	151.8^{b}	151.0^{b}
TG	25.9^{ab}	22.9^{ab}	33.9^{ab}	17.4^{b}	33.8^{ab}	18.6^{ab}	28.5^{ab}	20.0^{ab}
HDL-C	99.3^{a}	103.4^{a}	96.0^{a}	112.9^{a}	97.6^{a}	96.3^{a}	98.5^{a}	113.9^{a}
LDL-C	48.0^{b}	49.1^{ab}	43.1^{b}	79.9^{a}	33.4^{b}	38.9^{b}	47.4^{b}	33.0^{b}

*There were eight chicks in each treatment at the beginning of the study.

^{abcd}Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

Experiment 3

Blood Analysis

The serum lipid data from Experiment 3 are presented in Table 11. The highest ($P < 0.05$) total serum cholesterol values were from chicks fed 15% egg yolk and corn oil plus cholesterol, at 250.9 and 236.8 mg/dl, respectively. There were no significant differences in total serum cholesterol between the 10% and 15% levels for butter, palm oil, and suet. The lowest serum cholesterol levels were from chicks fed 10% and 15% corn oil without cholesterol and 4.7% soy and corn oil, and none of these values were significant.

The objectives of this experiment were to determine whether 10% or 15% fat was optimum to produce hypercholesterolemia, and whether there would

Table 11. Serum lipids of chicks* fed cornmeal and various fats at different levels with cholesterol added to selected diets (Experiment 3).

INGREDIENTS	Palm Oil		Butter		Suet		Corn Oil				Egg Yolk		Soy/Corn Oils
	10	15	10	15	10	15	10	15	10 + Chol.	15 + Chol.	10	15	4.7
	<---------- mg/dl ---------->												
Cholesterol	143.9^{cd}	167.7^{c}	166.5^{c}	169.0^{c}	146.0^{cd}	146.8^{cd}	125.8^{d}	133.4^{d}	206.9^{b}	236.8^{a}	203.1^{b}	250.9^{a}	131.7^{d}
Triglycerides	55.7^{ab}	53.3^{abc}	51.6^{abc}	55.8^{ab}	56.3^{a}	56.0^{ab}	52.2^{abc}	46.9^{cd}	38.1^{e}	41.2^{d}	47.5^{bcd}	50.6^{abc}	53.7^{abc}

*There were eight chicks in each treatment at the beginning of the study.

^{abcde}Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

be an advantage in including cholesterol in the diets. It was determined from the results that a 10% fat level was adequate, and judging from the corn oil treatments, added cholesterol increased the hypercholesterolemic effect. It was therefore decided that in the succeeding trial, cholesterol would be added to diets to equalize all fat sources with the cholesterol level of the egg yolk diets. The 4.7% soy/corn oil diet was included as a control, since the previous experiment provided that level.

Experiments 4a and 4b

Feed Consumption, Body Weight Gain, and Feed/Gain Ratio

Responses of chicks in Experiments 4a and 4b are detailed in Table 12. There were no differences in feed consumption in either experiment except that chicks fed wheat with palm oil consumed less ($P < 0.05$) feed that those fed barley with palm oil in Experiment 4a.

All chicks fed wheat diets gained more weight than their respective barley fed counterparts, although differences were not significant for the egg yolk diets. In Experiment 4a, chicks fed corn oil with wheat gained the most weight, followed by palm oil with wheat, and then corn oil with barley. There were no significant differences in weight gain in Experiment 4a between wheat and barley fed chicks except for those fed palm oil ($P < 0.05$). In Experiment 4b, all barley fed chicks gained less ($P < 0.05$) weight compared to wheat fed chicks and gain differences between barley or wheat fed chicks were not different, regardless of source of fat.

Table 12. Feed consumption, body weight gain, and feed/gain ratio of chicks* fed barley or wheat and various fat sources (Experiments 4a and 4b).

EXPERIMENT 4a	Palm Oil		Egg Yolk		Corn Oil		SEM
	Barley	Wheat	Barley	Wheat	Barley	Wheat	
Food consumption (g)	4304.8[a]	2946.3[c]	3944.1[ab]	4285.0[a]	3692.3[abc]	3388.5[bc]	132.8
Body weight gain (g)	1595.6[c]	2207.8[ab]	1783.2[bc]	1872.3[bc]	2010.7[abc]	2401.2[a]	78.2
Feed/Gain	2.71[a]	1.33[d]	2.27[b]	2.31[b]	1.80[c]	1.41[d]	.11

EXPERIMENT 4b	Butter		Tallow		Corn Oil		SEM
	Barley	Wheat	Barley	Wheat	Barley	Wheat	
Feed consumption (g)	3229.3[a]	3952.3[a]	3543.3[a]	3844.5[a]	3243.5[a]	3790.5[a]	110.5
Body weight gain (g)	1416.0[b]	2667.5[a]	1605.0[b]	2525.4[a]	1718.3[b]	2799.0[a]	127.1
Feed/Gain	2.30[a]	1.48[c]	2.21[a]	1.52[c]	1.90[b]	1.36[c]	.08

*There were 24 chicks in each treatment at the beginning of the study.

[abcd]Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

Considering the feed-to-gain ratios, lower ($P < 0.05$) values indicating better nutrient utilization were seen in all wheat fed chicks except for those fed egg yolk. This is not surprising, considering literature reports of poor chick performance when fed barley (Newman et al., 1987). Corn oil and palm oil with wheat produced the lowest feed/gain ratios, while the highest feed/gain ratios were produced by palm oil and butter with barley.

The egg yolk diets were different from the others, in that there was no difference between wheat and barley diets in feed consumption, body weight gain, or feed/gain. It is known that egg yolk protein is more biologically available and the chicks were probably better nourished. However, the total weight gains for egg yolk-wheat fed chicks were the lowest of all wheat fed chicks.

The trend indicated by the results was that chicks fed barley ate more and gained less, while chicks fed wheat diets were more efficient. Chicks fed both barley and wheat with egg yolk had gummy beaks and feed had to be removed from their beaks every three hours during the last two weeks of the experiment. Inability to eat regularly due to gummy beaks may explain the lower weight gain in chicks fed egg yolk. SDF in barley has water holding ability and may cause water and fiber to form a gummy mixture, particularly in combination with egg. It has been postulated that the soluble fiber may also form a viscous solution in the intestine, resulting in wetter feces. A viscous digesta solution can also interfere with fat absorption, resulting in

undigested energy; therefore, barley fed chicks would have lower utilization of all nutrients and poor feed efficiency.

Plasma Lipids

Mean plasma lipid values for Experiments 4a and 4b are shown in Table 13. In every instance, barley fed chicks had lower ($P < 0.05$) plasma cholesterol and LDL-cholesterol compared with those fed wheat. The highest ($P < 0.05$) total cholesterol and LDL-cholesterol levels by far were seen in chicks fed palm oil with wheat (438.1 and 352.4 mg/dl, respectively). Other wheat fed chicks had total cholesterol levels ranging from 233.7 to 267.9 mg/dl and LDL-cholesterol from 123.0 to 184.2 mg/dl.

HDL-cholesterol levels were higher for all egg yolk fed chicks than for barley fed chicks given palm oil and corn oil (Experiment 4a), but there was no difference between barley and wheat for other fat sources. There were no differences in TGs between chicks fed barley or wheat with any fat source. LDL/HDL ratios were higher for all wheat fed chicks, ranging from 1.0 to 4.6, whereas chicks fed barley had much lower and more desirable values, from 0.2 to 0.8 ($P < 0.05$).

Corn oil was fed in both Experiments 4a and 4b, in order to provide a reference point between the two experiments. Results were similar, although all cholesterol values were slightly higher in Experiment 4a, while TG levels were slightly lower than those observed in Experiment 4b. Figures 1 through 3 provide visual comparisons of the differences in serum lipids between chicks fed barley and wheat.

Table 13. Plasma lipid values of chicks* fed barley or wheat and various fat sources (Experiments 4a and 4b).

EXPERIMENT 4a	Palm Oil		Egg Yolk		Corn Oil		SEM
	Barley	Wheat	Barley	Wheat	Barley	Wheat	
	<---------- mg/dl ---------->						
Cholesterol	119.8^{c}	438.1^{a}	156.0^{c}	248.6^{b}	133.3^{c}	261.3^{b}	11.5
Triglycerides	35.3^{a}	40.8^{a}	33.6^{a}	35.2^{a}	26.3^{b}	24.4^{b}	1.1
HDL-C	91.9^{b}	77.6^{c}	111.7^{a}	118.5^{a}	91.2^{b}	72.2^{c}	2.0
LDL-C	22.2^{d}	352.4^{a}	38.2^{d}	123.0^{c}	37.2^{d}	184.2^{b}	11.9
LDL/HDL	0.2^{b}	4.6^{a}	0.3^{b}	1.0^{a}	0.4^{b}	2.6^{a}	0.2

EXPERIMENT 4b	Butter		Tallow		Corn Oil		SEM
	Barley	Wheat	Barley	Wheat	Barley	Wheat	
	<---------- mg/dl ---------->						
Cholesterol	126.6^{c}	244.8^{ab}	121.1^{c}	267.9^{a}	131.3^{c}	233.7^{b}	6.8
Triglycerides	48.1^{abc}	56.6^{a}	49.4^{ab}	41.4^{bc}	40.4^{bc}	37.6^{c}	1.8
HDL-C	83.1^{a}	85.4^{a}	77.3^{abc}	80.1^{ab}	68.2^{c}	69.2^{bc}	1.8
LDL-C	34.4^{c}	148.1^{b}	34.2^{c}	179.5^{a}	55.1^{c}	156.9^{ab}	7.0
LDL/HDL	0.4^{c}	1.8^{b}	0.5^{c}	2.5^{a}	0.9^{c}	2.6^{a}	0.1

*There were 24 chicks in each treatment at the beginning of the study.

^{abcd}Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

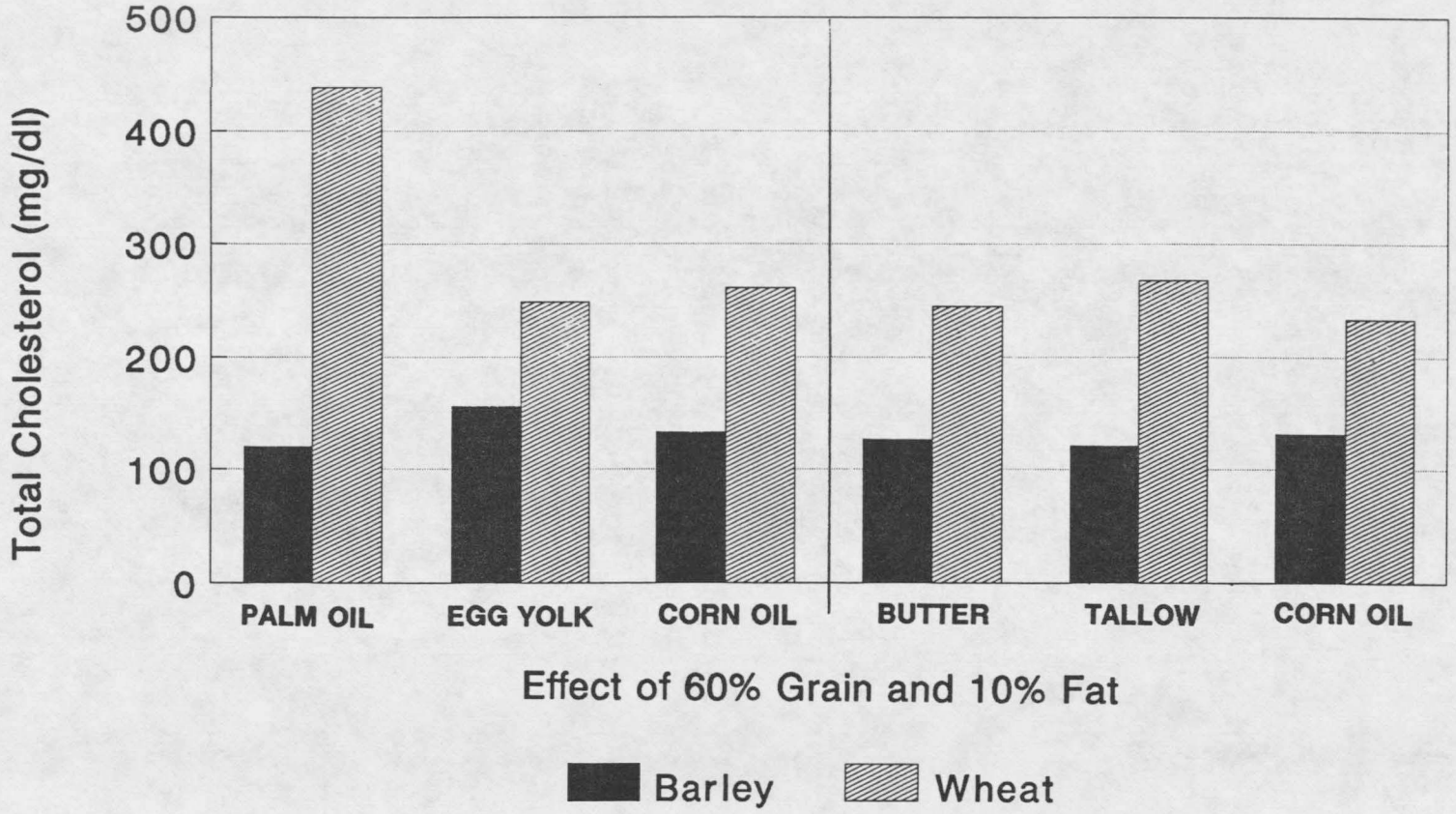

Figure 1. Total plasma cholesterol of chicks fed barley or wheat and palm oil, egg yolk, butter, tallow, or corn oil (Experiments 4a and 4b).

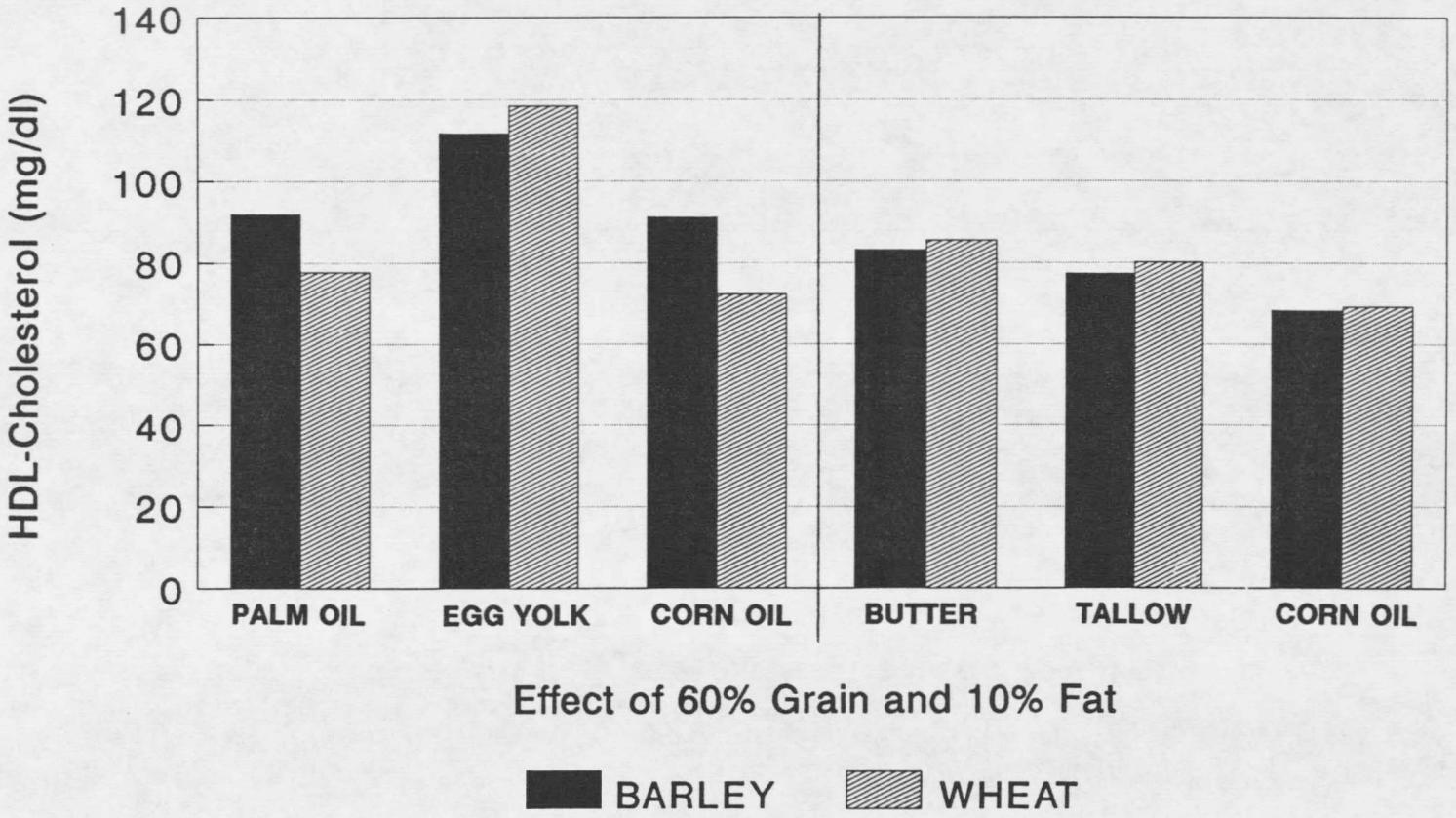

Figure 2. HDL-cholesterol of chicks fed barley or wheat and palm oil, egg yolk, butter, tallow, or corn oil (Experiments 4a and 4b).

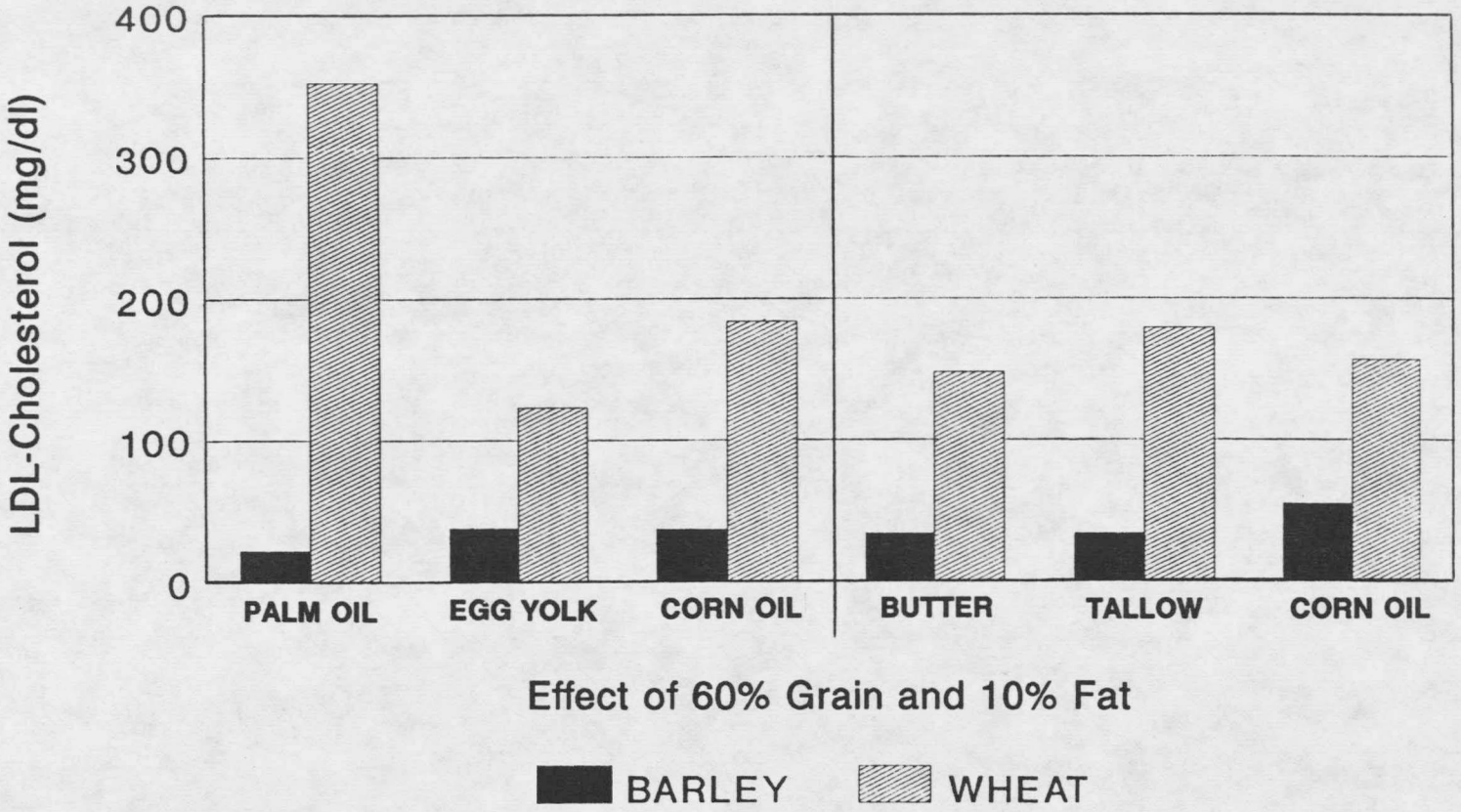

Figure 3. LDL-cholesterol of chicks fed barley or wheat and palm oil, egg yolk, butter, tallow, or corn oil (Experiments 4a and 4b).

Liver Measurements and Analysis

Results of liver measurements and analysis for Experiments 4a and 4b are shown in Table 14. Liver weights were greater ($P < 0.05$) for all chicks fed wheat compared to barley. Considering that body weights were also greater for wheat fed chicks, however, it is more meaningful to compare liver weight as a percent of body weight. In Experiment 4a, there were no significant differences between grain treatments; in Experiment 4b, there were small but nevertheless significant differences, with the smaller ratio from chicks fed wheat diets. Liver weights for all treatments varied from 2.5% to 3.2% of total body weight.

The liver cholesterol percentage was higher ($P < 0.0001$) for all wheat fed chicks (8.8 to 33.4 mg/g) compared to those fed barley (2.6 to 4.7 mg/g) except for the comparison between wheat-butter and barley-corn oil diets, which were different at a probability of $P < .03$. The differences were dramatic in most cases, such as for palm oil, where over a ten-fold difference was observed. Those data correspond closely to plasma cholesterol values. The lowest liver cholesterol values for wheat fed chicks occurred where butter and tallow were fed (8.8 and 11.5 mg/g, respectively). This may be due to the specific fatty acid/cholesterol composition of these two fats. Butter has 53% SAFA excluding stearic acid and tallow has 32% SAFA excluding stearic acid; both contain more stearic acid (9.8 and 18.9 mg/100 g, respectively) than either corn oil with 11% SAFA excluding stearic acid or palm oil with 47%

Table 14. Liver weight, liver weight as percent of body weight, and liver cholesterol of chicks* fed barley or wheat and various fat sources (Experiments 4a and 4b).

EXPERIMENT 4a	Palm Oil		Egg Yolk		Corn Oil		SEM
	Barley	Wheat	Barley	Wheat	Barley	Wheat	
Liver weight (g)	9.1^{d}	13.3^{ab}	10.1^{cd}	12.0^{b}	10.4^{c}	13.5^{a}	0.2
Liver weight/body weight %	2.7^{bc}	2.8^{abc}	3.0^{ab}	3.2^{a}	2.5^{c}	2.6^{bc}	0.1
Cholesterol (mg chol/g tissue)	2.8^{c}	33.4^{a}	3.7^{c}	27.5^{ab}	4.7^{c}	25.8^{b}	1.5

EXPERIMENT 4b	Butter		Tallow		Corn Oil		SEM
	Barley	Wheat	Barley	Wheat	Barley	Wheat	
Liver weight (g)	10.0^{b}	13.9^{a}	9.4^{b}	13.9^{a}	10.3^{b}	14.3^{a}	0.2
Liver weight/body weight %	3.0^{a}	2.7^{b}	2.9^{a}	2.7^{b}	2.9^{a}	2.6^{b}	0.0
Cholesterol (mg chol/g tissue)	2.6^{c}	8.8^{b}	2.9^{c}	11.5^{b}	4.6^{c}	19.6^{a}	0.7

*There were 24 chicks in each treatment at the beginning of the study.

^{abcd}Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

SAFA excluding stearic acid (1.8 and 4.3 mg/100 g, respectively). Stearic acid may have been converted to oleic acid and the saturation may not have affected cholesterol as much as would be expected of such a highly saturated fat. The dietary cholesterol values were higher for butter and tallow than the other fats, but cholesterol was added to all the diets to equal the amount of cholesterol in the egg yolk diets. Therefore, dietary cholesterol in butter and tallow may not have affected liver cholesterol values any more than the other fats.

Excreta Analysis

The analytical composition of chick excreta is presented in Table 15. In every case, except for butter in Experiment 4b on day 15, ether extract was greater ($P < 0.05$) for excreta from barley fed chicks compared to those fed wheat. This indicates that dietary lipid is being poorly digested when barley is fed, carrying total fat, possibly including dietary cholesterol, into the feces. This confirms work reported by Fadel et al. (1987), in which barley fed chicks excreted more fat than corn fed chicks. The effect appears to be consistent, and helps to explain the poor growth rate of barley fed chicks.

The dry matter percentage of excreta was lower in all cases for barley fed chicks on both observations. To consider this phenomena conversely, barley fed chicks had wetter feces when collected. This confirms the often quoted expression, "wet, sticky droppings," used in the literature related to barley fed chicks. Considering the high fat composition, one may relate the stickiness to high fat more so than to high moisture.

Table 15. Excreta dry matter and ether extract (crude fat) of chicks fed barley or wheat and various fat sources (Experiments 4a and 4b).

EXPERIMENT 4a		Butter		Tallow		Corn Oil		SEM
		Barley	Wheat	Barley	Wheat	Barley	Wheat	
Excreta Fats : % EE dmb	d 8	14.6^{a}	9.1^{b}	9.2^{b}	8.2^{c}	11.7^{a}	6.2^{c}	1.9
	d 15	16.3^{a}	7.9^{bc}	10.8^{b}	7.0^{c}	10.2^{b}	6.8^{c}	2.1
Excreta Dry Matter : %	d 8	50.0^{d}	61.4^{bc}	50.1^{d}	70.3^{a}	58.2^{c}	66.3^{ab}	0.6
	d 15	37.9^{c}	59.4^{a}	43.6^{b}	59.0^{a}	42.3^{bc}	60.1^{a}	0.8

EXPERIMENT 4b		Palm Oil		Egg Yolk		Corn Oil		SEM
		Barley	Wheat	Barley	Wheat	Barley	Wheat	
Excreta Fats : % EE dmb	d 8	13.4^{b}	17.4^{a}	18.5^{ab}	9.2^{cd}	12.7^{bc}	7.4^{d}	1.6
	d 15	11.4^{bc}	10.2^{c}	15.8^{a}	12.7^{b}	10.9^{bc}	7.0^{d}	1.9
Excreta Dry Matter : %	d 8	41.9^{b}	52.4^{a}	46.7^{ab}	54.9^{a}	44.8^{ab}	52.5^{a}	0.8
	d 15	38.4^{e}	50.0^{bc}	40.3^{de}	59.8^{a}	46.7^{cd}	57.5^{ab}	0.6

^{abcde}Values within a horizontal row with different superscripts are significantly different ($P < 0.05$).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

According to the literature, elevated total cholesterol, LDL-cholesterol, and LDL/HDL, and lower HDL-cholesterol are convincing predictors of atherosclerosis and CHD risk in humans. There is an increased risk of CHD proportional to elevated total cholesterol and LDL-cholesterol in the population at risk. LDL-cholesterol transports cholesterol to the tissues to be used for membrane synthesis or steroids, but it also has an affinity for artery walls, thereby causing arteriosclerosis. Elevated HDL-cholesterol levels are desirable since HDL-cholesterol catabolizes very low density lipoproteins (VLDL), rich in TG and cholesterol, and carries the TG and cholesterol to the liver for excretion. Lower LDL/HDL indicates less risk for CHD since there is less LDL-cholesterol being transported than HDL-cholesterol and less likelihood of plaque formation inside artery walls (Kris-Etherton et al., 1988).

It can be implied that barley reduces risk for CHD in humans, as indicated by lower total cholesterol and LDL-cholesterol levels found in a study by Newman et al. (1989). When barley or wheat products were consumed by male volunteer subjects, serum and total LDL-cholesterol were significantly lowered when barley products were consumed, and subjects

consuming wheat had significant increases in total serum cholesterol. The same trend was observed in the chick experiments of this study. The difference in results between human monogastric subjects and chicks was that the volunteers maintained their weight during the study, indicating adult humans may not experience massive malabsorption the way growing chicks do. However,the dietary proportion of the cereal grain fiber was much higher for the chicks.

These experiments showed chicks fed barley diets had lower total cholesterol and LDL-cholesterol levels, lower liver cholesterol values, more excreta fats, and higher feed-to-gain ratios. Since barley contains SDF, lipoprotein levels were decreased because lipids were sequestered and excreted. It is possible that barley fed chicks experienced lipid malabsorption, which could contribute to lowered cholesterol and blood lipids.

Lipoprotein metabolism is a complex interaction of genetics, diet, gender, age, activity, and hormones. The studies reported here examined certain dietary factors that affected lipid metabolism. In Experiment 4a, grains, fats, and grain x fat interactions significantly influenced total plasma cholesterol, HDL-cholesterol, and LDL-cholesterol. In Experiment 4b, grains and fats influenced the plasma lipids; however, the grain x fat interaction was not significant.

An explanation for the serum cholesterol reduction from barley consumption may be that dietary fat, cholesterol, and SDF bind together in the

intestine into a viscous complex. The viscosity may tend to interfere with lipoprotein micelle formations and absorption of fat and cholesterol. Consequently, cholesterol in the blood and liver would be reduced. The chicks that were fed barley experienced less weight gain and had smaller livers than those fed wheat. Energy in the form of fat calories was excreted, producing wet, sticky droppings with low dry matter. It is possible that bile acids are also bound by fiber in the intestine, taking them out of the metabolic cycle. Available cholesterol may be converted to bile acids to replace those excreted. As chicks ate barley, there was more SDF in the diet, and thus more bile acids, fats, and water were possibly excreted, removing even more cholesterol for synthesis of bile acids which emulsify fat. The continued cycle would result in lower total cholesterol, LDL-cholesterol, and liver cholesterol. The SDF components of barley, particularly the β-glucans, were likely responsible for many of the physiological effects noted.

Recommendations for a prevention, normalization, or maintenance diet for humans should include a variety of food sources which contain soluble and insoluble dietary fiber. Beans, oats, fruits, and vegetable sources are recommended to provide a fiber intake of 35-45 gm/day (Spiller, 1986). Since barley is a source of soluble dietary fiber and exhibits hypocholesterolemic effects similar to oats, the author of this study suggests barley be included as a source of dietary fiber.

The American Heart Association (AHA) recommends that fat not exceed 30% of calories and that the S:M:P be 10:10:10; AHA also recommends that cholesterol consumption be limited to 300 mg/day or less. In Experiments 3, 4a, and 4b, butter and tallow fed chicks exhibited the highest total cholesterol levels, while corn oil treatments had the lowest values. These results confirm previous research which indicated that SAFAs are hypercholesterolemic and PUFAs are hypocholesterolemic.

Barley and palm oil both contain tocotrienols, which are hypocholesterolemic. SDF and tocotrienols in barley and tocotrienols in palm oil may be synergistic, resulting in the dramatic differences between palm oil fed chicks on barley and wheat diets in Experiment 4a. The author of this research suggests the combination of barley and palm oil be further studied to determine their effect of human lipoproteins.

Further, the author recommends daily fiber intake include a source of SDF, such as barley, to lower total cholesterol, LDL-cholesterol, and LDL/HDL, and thereby decrease the risk of atherosclerosis, the leading cause of coronary heart disease.

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APPENDIX

Table 16. Summary table of statistical tests for serum analyses (Experiments 1, 2, and 3).

EXPERIMENT 1		TOTAL CHOLESTEROL		
Source	DF	Mean Square	F-Value	Pr > F
Model	3	6647.27	32.37	0.0001
Oil	3	6647.27	32.37	0.0001
Error	36	205.38		
Total	39			

EXPERIMENT 2		TOTAL CHOLESTEROL			TRIGLYCERIDES			HDL-CHOLESTEROL			LDL-CHOLESTEROL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	8	25898.83	33.94	0.0001	2983.46	12.62	0.0001	338.43	0.55	0.8158	21325.61	34.77	0.0001
Oil	8	25898.83	33.94	0.0001	2983.46	12.62	0.0001	338.43	0.55	0.8158	21325.61	34.77	0.0001
Error	59	763.08			236.47			618.10			613.27		
Total	67												

EXPERIMENT 3		TOTAL CHOLESTEROL			TRIGLYCERIDES		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	13	12775.74	18.00	0.0001	243.33	4.99	0.0001
Oil	13	12775.74	18.00	0.0001	243.33	4.99	0.0001
Error	94	709.74			48.80		
Total	107						

Table 17. Summary table of statistical tests for feed consumption, body weight gain, and feed/gain analyses (Experiments 4a and 4b).

EXPERIMENT 4a		FEED CONSUMPTION			BODY WEIGHT GAIN			FEED/GAIN		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	990555.95	4.25	0.0109	331698.84	3.93	0.0149	1.11	18.60	0.0001
Grain	1	1102826.53	4.73	0.0441	752962.75	8.93	0.0083	1.98	33.30	0.0001
Oil	2	757415.33	3.25	0.0639	317884.79	3.77	0.0442	0.90	15.06	0.0002
Grain x Oil	2	1345229.83	5.77	0.0123	128624.19	1.53	0.2459	0.95	15.98	0.0001
Error	17	233285.05			84323.30			0.06		
Total	22									

EXPERIMENT 4b		FEED CONSUMPTION			BODY WEIGHT GAIN			FEED/GAIN		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	390333.74	1.47	0.2492	1477718.97	16.86	0.0001	0.64	42.12	0.0001
Grain	1	1645884.38	6.19	0.0229	7052395.75	80.45	0.0001	2.80	183.48	0.0001
Oil	2	63144.79	0.24	0.7912	113300.84	1.29	0.2989	0.17	10.97	0.0008
Grain x Oil	2	89747.38	0.34	0.7181	54798.70	0.63	0.5464	0.04	2.59	0.1025
Error	18	266108.96			87657.18			0.02		
Total	23									

Table 18. Summary table of statistical tests for plasma analyses (Experiments 4a and 4b).

EXPERIMENT 4a		TOTAL CHOLESTEROL			TRIGLYCERIDES			HDL-CHOLESTEROL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	24064.48	56.26	0.0001	706.12	5.33	0.0002	6913.75	30.00	0.0001
Grain	1	984340.17	230.12	0.0001	92.89	0.70	0.4039	2371.46	10.29	0.0017
Oil	2	78537.47	18.36	0.0001	1707.19	12.90	0.0001	14920.53	64.74	0.0001
Grain x Oil	2	139486.10	32.61	0.0001	130.82	0.99	0.3753	2094.41	9.09	0.0002
Error	120	4277.50			132.39			230.47		
Total	125									

EXPERIMENT 4b		TOTAL CHOLESTEROL			TRIGLYCERIDES			HDL-CHOLESTEROL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	104609.75	41.29	0.0001	1148.49	2.96	0.0146	1161.54	2.77	0.0207
Grain	1	508869.99	200.83	0.0001	21.21	0.05	0.8156	138.01	0.33	0.5674
Oil	2	1771.15	0.70	0.4989	2003.57	5.16	0.0070	2811.95	6.70	0.0017
Grain x Oil	2	5842.27	2.31	0.1038	793.01	2.04	0.1339	8.89	0.02	0.9790
Error	130	2533.82			388.32			419.74		
Total	135									

Table 18--Continued.

EXPERIMENT 4a		LDL-CHOLESTEROL			LDL/HDL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	291903.18	60.50	0.0001	61.40	55.14	0.0001
Grain	1	1070424.36	238.19	0.0001	197.21	177.09	0.0001
Oil	2	116215.64	25.86	0.0001	32.71	29.37	0.0001
Grain x Oil	2	155696.63	34.65	0.0001	36.39	32.68	0.0001
Error	120	4493.94			1.11		
Total	125						

EXPERIMENT 4b		LDL-CHOLESTEROL			LDL/HDL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	101417.49	33.94	0.0001	23.06	22.89	0.0001
Grain	1	490829.07	164.27	0.0001	103.36	102.63	0.0001
Oil	2	3440.51	1.15	0.3194	4.55	4.52	0.0127
Grain x Oil	2	5793.78	1.94	0.1480	1.55	1.54	0.2175
Error	130	5987.86			1.01		
Total	135						

Table 19. Summary table of statistical tests for liver analyses (Experiments 4a and 4b).

EXPERIMENT 4a		LIVER WEIGHT			LIVER WT/BODY WT %			LIVER CHOLESTEROL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	69.96	16.09	0.0001	1.39	2.91	0.0162	4143.02	35.39	0.0001
Grain	1	305.37	70.23	0.0001	0.72	1.49	0.2239	20347.88	173.80	0.0001
Oil	2	10.07	2.32	0.1029	3.11	6.50	0.0021	102.11	0.87	0.4206
Grain x Oil	2	13.93	3.20	0.0440	0.02	0.03	0.9670	247.65	2.12	0.1249
Error	124	3.35			0.48			117.08		
Total	129									

EXPERIMENT 4b		LIVER WEIGHT			LIVER WT/BODY WT %			LIVER CHOLESTEROL		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	118.47	31.22	0.0001	0.61	8.04	0.0001	970.38	34.53	0.0001
Grain	1	575.67	151.70	0.0001	2.72	35.96	0.0001	3352.97	119.33	0.0001
Oil	2	4.87	1.28	0.2808	0.11	1.47	0.2340	500.44	17.81	0.0001
Grain x Oil	2	1.25	0.33	0.7205	0.08	1.10	0.3374	232.88	8.29	0.0004
Error	130	3.79			0.08			28.10		
Total	135									

Table 20. Summary table of statistical tests for excreta analyses (Experiments 4a and 4b).

EXPERIMENT 4a		EXCRETA EE d 8			EXCRETA EE d 15			EXCRETA DM d 8			EXCRETA DM d 15		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	33.88	22.56	0.0001	50.42	11.54	0.0001	277.91	14.05	0.0001	397.56	37.24	0.0001
Grain	1	90.69	60.39	0.0001	153.70	35.17	0.0001	1000.45	50.59	0.0001	1895.50	177.55	0.0001
Oil	2	21.33	14.20	0.0002	28.31	6.48	0.0081	82.14	4.15	0.0340	15.79	1.48	0.2557
Grain x Oil	2	13.12	8.73	0.0025	13.90	3.18	0.0670	78.09	3.95	0.0390	17.06	1.60	0.2313
Error	17	1.50			4.37			19.78			10.68		
Total	22												

EXPERIMENT 4b		EXCRETA EE d 8			EXCRETA EE d 15			EXCRETA DM d 8			EXCRETA DM d 15		
Source	DF	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F	Mean Square	F-Value	Pr > F
Model	5	52.11	7.67	0.0005	34.31	13.13	0.0001	105.25	2.25	0.0936	305.18	10.52	0.0001
Grain	1	167.48	24.64	0.0001	46.20	17.69	0.0005	464.64	9.93	0.0055	1166.22	40.21	0.0001
Oil	2	41.59	6.12	0.0094	58.61	22.43	0.0001	26.22	0.56	0.5806	133.20	4.59	0.0245
Grain x Oil	2	4.95	0.73	0.4963	4.06	1.55	0.2383	4.58	0.10	0.9073	46.64	1.61	0.2277
Error	18	6.80			2.61			46.79			522.01		
Total	23												