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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A thesis submitted in partial fulfillment
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Master of Science

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Home Economics

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 1990

APPROVAL

of a thesis submitted by

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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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ACKNOWLEDGEMENTS

I wish to express genuine appreciation to Dr. Rosemary K. Newman, my graduate advisor and major professor, for her patience, encouragement, and professional scholarship; to my graduate committee members, Dr. Jacquelynn O'Palka for encouraging me to persevere through undergraduate and graduate school, and Dr. C. Walter Newman for his concern, guidance, and ongoing support; and to Dr. Margaret Briggs for her confidence in me.

I am most grateful to the Minority Biomedical Research Support project for providing financial support for my education, and particularly to Dr. David Young who provided moral support. I also want to thank my fellow MBRS students for their friendships and understanding. I wish to recognize Petrea Hofer for her expertise; Gayle Watts and Jill Abbot for their contributions in completing my laboratory work and for their empathy; and my co-graduate students, Linji, Ted, Alan, and Qi, and Dr. Adam Fengler for helping with sacrificing chicks, obtaining bloods and livers, and statistical analysis of data. I appreciate the help from the Animal Resource Center crew for care of the chicks, and Dr. Nancy Roth at the Nutrition Center for analyzing samples and data analysis.

Finally, thanks to my family in Colorado for their prayers, love, pride and hope. I am especially grateful to my ex-husband, Jimmy, for his love, confidence, understanding, support and encouragement.

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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.

<u>Cholesterol</u>

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.

<u>Human studies</u>. 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 lipoproteins.

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.

<u>Tocotrienols</u>

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]						
Cornmeal	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).