



Influences of oil and soluble fiber of barley grain on plasma cholesterol concentrations in chicks and hamsters
by Linji Wang

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Crop and Soil Science
Montana State University
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Abstract:

β -Glucans and α -tocotrienol (α -T3) from barley grain are hypocholesterolemic in animal models. To further identify the hypocholesterolemic components in barley, whole grain, oil, and soluble fiber were tested for their hypocholesterolemic effects. Barley cultivars were analyzed for comparative oil and α -T3 concentration and for the influence of environment and processing methods. From seven barley cultivars, the average oil and vitamin E (tocotrienols plus tocopherols) concentration were 22 g/kg and 63 mg/kg, respectively. α -T3 accounted for 52% (33 mg/kg) of vitamin E. Variation due to cultivar differences was significant in these traits. The cultivar differences were mainly due to the difference of hulled versus hull-less cultivars with the hull-less cultivars being higher in the concentrations of oil, vitamin E and α -T3 with lighter kernel weights. Pearling was the more effective processing method compared to milling to concentrate oil, vitamin E and α -T3. A pearling flour contained approximately 3-fold greater oil, vitamin E and α -T3 than whole barley grain. Barley oil was not hypocholesterolemic in Leghorn chicks fed no cholesterol and in hamsters fed with cholesterol (3 g/kg diet), however, barley oil was hypocholesterolemic in a feeding trial with broiler chicks fed cholesterol (5 g/kg diet). Barley soluble fiber was hypocholesterolemic in hamsters. Chicks fed barley diets with a high β -glucan concentration had a higher small intestinal viscosity. The viscosities were negatively correlated with plasma total cholesterol and low density lipoprotein cholesterol levels, lipid and protein digestibility and body weight gain, but positively correlated with excreta lipid concentration. Small intestinal viscosity may mediate the effects of barley soluble fiber by reducing the absorption of dietary nutrients including cholesterol, triacylglycerol and protein.

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APPROVAL

of a thesis submitted by

Linji Wang

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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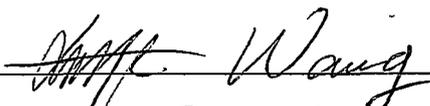
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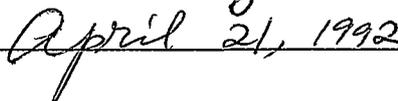
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ABSTRACT

β -Glucans and α -tocotrienol (α -T3) from barley grain are hypocholesterolemic in animal models. To further identify the hypocholesterolemic components in barley, whole grain, oil, and soluble fiber were tested for their hypocholesterolemic effects. Barley cultivars were analyzed for comparative oil and α -T3 concentration and for the influence of environment and processing methods. From seven barley cultivars, the average oil and vitamin E (tocotrienols plus tocopherols) concentration were 22 g/kg and 63 mg/kg, respectively. α -T3 accounted for 52% (33 mg/kg) of vitamin E. Variation due to cultivar differences was significant in these traits. The cultivar differences were mainly due to the difference of hulled versus hull-less cultivars with the hull-less cultivars being higher in the concentrations of oil, vitamin E and α -T3 with lighter kernel weights. Pearling was the more effective processing method compared to milling to concentrate oil, vitamin E and α -T3. A pearling flour contained approximately 3-fold greater oil, vitamin E and α -T3 than whole barley grain. Barley oil was not hypocholesterolemic in Leghorn chicks fed no cholesterol and in hamsters fed with cholesterol (3 g/kg diet), however, barley oil was hypocholesterolemic in a feeding trial with broiler chicks fed cholesterol (5 g/kg diet). Barley soluble fiber was hypocholesterolemic in hamsters. Chicks fed barley diets with a high β -glucan concentration had a higher small intestinal viscosity. The viscosities were negatively correlated with plasma total cholesterol and low density lipoprotein cholesterol levels, lipid and protein digestibility and body weight gain, but positively correlated with excreta lipid concentration. Small intestinal viscosity may mediate the effects of barley soluble fiber by reducing the absorption of dietary nutrients including cholesterol, triacylglycerol and protein.

CHAPTER 1

INTRODUCTION

Cholesterol Metabolism

Cholesterol is an essential compound for growth and physiological function of the human body. Approximately one half of the molecules in erythrocyte plasma membranes is cholesterol and most of the cells in the human body are capable of synthesizing cholesterol. Synthesis of cholesterol occurs in the endoplasmic reticulum prior to transport to cell membranes. Cholesterol reduces ion permeability and activity of some enzymes in cell membranes by interaction with phospholipids and membrane proteins, and inhibits the membrane phase change from liquid crystalline to the gel crystalline state. Cholesterol is also a precursor of many metabolically active molecules including bile acids, steroid hormones and vitamin D.

Total body cholesterol comes from two sources, endogenous biosynthesis and exogenous dietary foods. The main site of cholesterol biosynthesis is the liver which produces half to two thirds of total body cholesterol. Other sites are the skin and gut (Mayes, 1988). Animal fat, butter and eggs are the most cholesterol-rich food ingredients, while plant foods contain very little cholesterol. After a meal, dietary cholesterol is mixed with other lipids into oil droplets in the stomach and the oil droplets

become micelles as they are mixed with bile salts and pancreatic lipase in the small intestine. Cholesterol is absorbed in the jejunum by passive diffusion and from 80% to 90% of the cholesterol will be esterified with free fatty acids within the enterocytes (Mayes, 1988). The cholesterol esters and the free cholesterol either absorbed or newly synthesized by enterocytes are then incorporated into chylomicrons within the enterocytes and released. The chylomicrons are transported via the lymphatic system and enter the blood circulation through the thoracic duct. In the circulation, cholesterol in the chylomicrons is removed by the liver and peripheral tissues with LDL receptors anchored in cell membranes (Goldstein and Brown, 1984). Cholesterol, either absorbed from the diet or synthesized, is packed with lipoproteins in liver cells and excreted by the cells as very low density lipoprotein (VLDL) into blood circulation. As VLDL particles move along in the blood stream they lose some components, particularly triacylglycerol, and the particles become smaller in size and more dense. The remnants of VLDL are low density lipoproteins (LDL). These lipoproteins are the major cholesterol carriers in the circulation, accounting for 70% of total plasma cholesterol in the human body. It is thought that LDL is the most atherogenic carrier of cholesterol. Low density lipoprotein cholesterol is removed from blood by both liver and peripheral tissues by LDL receptors. High density lipoprotein (HDL) is synthesized and secreted from both intestine and liver (Mayes, 1988). Circulating HDL in the blood has the higher protein and lower lipid composition which confers on HDL a higher density than other lipoprotein fractions (Fielding and Fielding, 1985). Apolipoprotein A-I found in HDL is an activator of lecithin:cholesterol acyltransferase (LCAT) which catalyzes the reaction from free

cholesterol to cholesterol ester. This is believed to create a flow of free cholesterol from VLDL, LDL and epithelium of blood vessels to HDL and cholesterol ester from HDL to VLDL and LDL. More free cholesterol is removed away from blood circulation by HDL when VLDL and LDL are taken up by liver and peripheral tissues. Uptake of HDL is mainly by the liver. The cholesterol absorbed is used as a precursor for biosynthesis of bile acids in the liver cells. By carrying out a reversed cholesterol transport, HDL levels in the plasma are negatively correlated with the incidence of coronary heart disease. Plasma cholesterol concentrations are indicators of body cholesterol supply since all available cholesterol for the body is transported in the blood.

The main excretion route of body cholesterol is through fecal loss of bile acids and free cholesterol which are later oxidized by gut bacteria to fecal steroids. Human bile contains 6.1 mmol/L free cholesterol (Cheillan et al., 1989). Small amounts of cholesterol are lost by excretion through the skin (Bhattacharyya et al., 1972) and urine (Vela and Acevedo, 1969).

The human body has an elaborate mechanism for maintaining a sufficient cholesterol level in the blood for normal growth of children and maintenance of adults. Cholesterol input may be greater than its output in children because cholesterol is used to build an increasing number of cells. However in adults, cell number appears to be constant, with cholesterol input balanced with output. An average adult was reported to excrete 1,100 mg cholesterol through fecal loss to counterbalance 850 mg cholesterol from biosynthesis and 250 mg from absorption of dietary cholesterol every day (Zubay, 1988).

Cholesterol biosynthesis is recognized as the center of regulation in cholesterol

homeostasis. Cholesterol is synthesized from acetyl-CoA in the mevalonate pathway with 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase being a rate-limiting enzyme. The activity of HMG-CoA reductase is inhibited by cholesterol intake level and reduction of dietary cholesterol lessens its inhibitory effect (Goldstein and Brown, 1984; Sabine, 1977). Increase in bile acid synthesis as the result of increased bile acid excretion also activates HMG-CoA reductase (Goldfarb and Potit, 1972).

Hypercholesterolemia and Diseases

Oversupply of cholesterol in the body in susceptible individuals may cause atherosclerosis. Atherosclerosis is related to coronary heart diseases (CHD) and brain stroke. The coronary arteries of patients suffering from CHD are blocked by plaque which is composed of cholesterol, cholesterol esters, other lipids, LDL lipoprotein and some dead cells. The deposit of plaque is assumed to start with the damage of artery wall by high blood pressure, bacterial infection or other unknown causes, followed by aggregation of smooth muscle cells and monocytes in the inflamed areas and uptake of LDL lipoprotein by the cells in these areas.

Hypercholesterolemia is one major risk factor of CHD. Based on clinical studies, plasma total cholesterol higher than 6.72 mmol/L (260 mg/dL), LDL cholesterol higher than 4.14 mmol/L (160 mg/dL) and HDL cholesterol lower than 0.91 mmol/L (35 mg/dL) are considered high risk factors of CHD (Anderson et al., 1987). Among lipoprotein fractions, HDL cholesterol levels are negatively correlated with the incidence of CHD (Anderson et al., 1987). Familial hypercholesterolemia (HF) is a genetic disease

of deficient or missing LDL receptors. In these patients LDL cholesterol cannot be removed by peripheral or liver cells and blood cholesterol concentration can be as high as 25.8 mmol/L (1000 mg/dl). Severe CHD development at the age of 4 to 6 years has been observed in these patients (Marinetti, 1990).

Dietary Prevention of Hypercholesterolemia

Cholesterol and saturated fats are hypercholesterolemic dietary components. In a seven country survey, Keys (1970) found that the proportions of saturated fat and cholesterol in the diets are positively correlated with plasma cholesterol levels and CHD incidence in humans. The main dietary sources of saturated fat are animal meat and dairy products which are also generally rich in cholesterol. Polyunsaturated oils (Keys, 1988; Keys et al., 1965) and monounsaturated oils (Grundy, 1986; Grundy et al., 1988) were reported to lower plasma cholesterol levels compared to saturated fats. Monounsaturated oils are more favorable to health due to their stability in lipid oxidation which is believed to be related to carcinogenesis.

Some soluble dietary fibers (SDF) have hypocholesterolemic effects (Topping, 1991). Soluble dietary fiber is a mixture of water soluble organic polymers including plant gums, algal polysaccharides, microbial polysaccharides and cellulose derivatives (Sandford and Baird, 1983) not hydrolyzed by human enzymes. Common SDF in human diets are plant gums from fruits, cereal grains and vegetables. Pectin, guar gum and oat gum are three plant gums found in plant cell walls. Pectin (Durrington et al., 1976) and guar gum (Miettinen and Tarpila, 1989; Superko et al., 1988) have hypocholesterolemic

effects. Pectin lowered plasma LDL cholesterol concentration by 18% (Durrington et al., 1976) and increased fecal excretion of bile acids (Stasse-Worthington et al., 1980). Oat gum containing 80% β -glucans, was responsible for the hypocholesterolemic effects of oat bran (Wood et al., 1989).

Other plant hypocholesterolemic components are saponins, plant proteins and nonsaponifiable lipids. Saponins are sterols or triterpene glycosides found in a variety of plant species including soybean, chick peas, peanuts, garden peas and spinach (Oakenfull, 1981). Saponins were found to inhibit cholesterol absorption in the small intestine (Oakenfull et al, 1984; Sidhu and Oakenfull, 1986). Plant proteins have also been reported to have hypocholesterolemic effects (Beynen et al., 1989; Sautier et al. 1979; Sirtori et al. 1979), however with many controversial results (Mol et al., 1982; Munoz et al., 1979). Additionally, nonsaponifiable lipid components such as oryzanol in rice oil (Seetharamaiah and Chandrasekhara, 1988) and tocotrienols in palm oil (Qureshi et al., 1991) were reported to be hypocholesterolemic.

Based on these findings, adults in North America have been advised to modify their diet composition by reducing their intake of animal fats, eggs and high-fat dairy products and increasing the intake of plant fiber from the current 11-23 g/d to 20-35 g/d (Life Sciences Research Office, 1987). Hypercholesterolemia can be reversed by treatments with either diets or diets plus drugs (Virkkunen, 1985), thereby reducing the risk of CHD. The general goal of dietary therapy is to reduce elevated levels of plasma cholesterol while maintaining a nutritionally adequate diet. Two diets have been developed by National Cholesterol Education Program (The Expert Panel, 1988) for this

purpose. The Step One Diet (total fat < 30% of total calories, saturated fatty acids < 10% of total calories, and cholesterol < 300 mg/d) is tried first. If it is not effective, the Step Two Diet (total fat < 30% of total calories, saturated fatty acids < 7% of total calories and cholesterol < 200mg/d) is initiated (The Expert Panel, 1988). Dietary therapy regimes are suggested before drug therapy is initiated.

Barley consumption has been shown to have a hypocholesterolemic effect in humans (Newman et al., 1989; McIntosh et al., 1991). Soluble β -glucans were found to be one of the responsible components for this effect (Oakenfull et al., 1992; Klopfenstein and Hosney, 1987). Soluble β -glucans and arabinoxylans are polysaccharides found in the cell walls of barley grains and are the major components of the SDF. Barley grain contains about 40 to 110 g/kg β -glucans with one third of it being water soluble and 44 to 78 g/kg arabinoxylans with 20% being water soluble (Ahluwalia and Fry, 1986). Likewise, soluble fiber from barley grain may also be hypocholesterolemic, but this needs to be verified experimentally.

Oil from brewers' grain has been shown to have hypocholesterolemic effects in chickens (Burger et al., 1984), swine (Qureshi et al., 1987) and humans (Robinson and Lupton, 1990). α -Tocotrienol of barley oil was found to lower serum cholesterol levels by inhibiting HMG-CoA reductase activity in the liver of chicks (Qureshi et al., 1986). Barley grains have relatively high concentrations of α -tocotrienol, although its total oil concentration is lower than that of most cereal grains.

Anitschkow (1913), a Russian scientist, fed rabbits diets high in cholesterol and induced atheromas very similar to arterial plaque found in humans. Since then numerous

cholesterol feeding studies have been done with other laboratory animals including chickens (Chandler et al., 1979), hamsters (Singhal et al. 1983), rats, mice, guinea pigs (Behr et al., 1963), dogs (Mahley et al., 1974), calves (Wigger et al., 1973), pigs (Link et al., 1972) and monkeys (Malinow et al., 1972). Hypercholesterolemia and arterial plaques were induced in all species except that rats are recalcitrant (Singhal et al., 1984). Chicks and hamsters have been popular animal models in the studies of lipid metabolism. In both species, arterial plaques can be induced and HMG-CoA reductase activity is inhibited by cholesterol feeding. The liver is the main site of cholesterol biosynthesis, and plasma cholesterol concentrations of 100 to 200 mg/dl are reached, close to that of humans. In addition, hamsters consume relatively less diet and chicks are easier for handling than other laboratory animals such as pigs and rats.

The objectives of this study were (1) to characterize the influence of cultivar, environment and processing on oil, α -tocotrienol and tocopherol concentrations in barley grain, (2) to determine fatty acid, tocopherol and tocotrienol concentrations of barley oil, (3) to examine the possible hypocholesterolemic effects of oil and soluble fiber of barley grain and their interaction, and (4) to characterize the modes of action by which barley oil and soluble fiber affect plasma cholesterol levels.

CHAPTER 2

VITAMIN E AND OIL CONCENTRATION OF SEVEN BARLEY CULTIVARS GROWN IN THREE DIFFERENT ENVIRONMENTS

Introduction

Barley oil concentration varies among barley cultivars and environments (Fedak and de la Roche, 1977) with an average of 2.1% (Newman and McGuire, 1985). Prowashonupana, a high-protein, high-lysine mutant of Compana (CI 5438) and the high-lysine mutant of Bomi, Risø 1508 were reported to contain 7.0% and 5.3% oil, respectively (Åman and Newman, 1986; Bhatti and Rosnagel, 1980). Both of these cultivars have shrunken endosperms and therefore low starch levels, which accounts in part for the higher oil concentration.

Vitamin E, a group of oil soluble compounds composed of tocopherols and tocotrienols, is essential for human health. Vitamin E acts as an antioxidant, preventing damage to cell membranes by free radicals. Rats fed a vitamin E deficient diet were found to have surface blebbing of the femoral artery endothelium (Hubel et al., 1989). According to Qureshi et al. (1986) α -tocotrienol inhibits the activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase in the liver of chicks and therefore has a hypocholesterolemic function. Qureshi et al. (1989) reported that in contrast to α -

T3, α -tocopherol stimulates HMG-CoA reductase activity.

Barley grain has a higher α -tocotrienol concentration (Barnes, 1983), but lower vitamin E and oil concentration (Newman and McGuire, 1985) than most cereal grains. The objective of this study was to examine vitamin E and oil concentration of seven barley cultivars grown in three environments.

Materials and Methods

Barley Cultivars and Growing Environments

The seven barley cultivars investigated in this study were WPB 501, Clark (CI 15857), Betzes (CI 6398), Nubet (CI 16559), Franubet, Wafranubet and Waxbar. WPB 501 is a six-rowed, hulled spring feed barley released by Western Plant Breeders (WPB) (811 Timberline Drive, Bozeman, MT 59715). Clark and Betzes are two-rowed hulled malting cultivars. Nubet is a hull-less cultivar selected from a backcross of Betzes x Sermo (Hockett, 1981). Franubet, a chemically induced mutant selected from Nubet has "fractured" starch granules (Chung 1982). Wafranubet is a waxy hull-less selection from a backcross of waxy Betzes on Franubet (R. F. Eslick, Personal Communication). Waxbar is a two-rowed, hull-less, short-awned cultivar selected from Washonupana crosses to Hector (CI 15514), released by WPB. These cultivars were planted in Scottsdale and Marana, AZ in November 1989 and harvested in May, 1990. The same set of cultivars was planted in Bozeman, MT in early June and harvested in September, 1990. Table 1 contains a description of these three environments.

Table 1. Description of the three growing environments

Parameter	Location		
	Scottsdale	Marana	Bozeman
Longitude	111°55' W	111°13' W	111°03' W
Latitude	33°30' N	32°27' N	45°40' N
Elevation (m)	368	597	1482
Planting date	11/14/89	11/14/89	6/1/90
Harvesting date	5/11/90	5/14/90	9/13/90
Length of daylight (h) ^a	12	12	15
Temperature (°C) ^a	13	13	18
Precipitation (cm) ^b	3.1	4.2	3.5
N fertilizer (kg/ha)	308	193	193
Irrigation ^c	Yes	Yes	No

^a Average values over the time from planting to harvesting.

^b Total precipitation over the time from planting to harvesting.

^c Equal to 0.9-1.2 cm precipitation.

Chemical Analysis

Barley grain was first ground in a laboratory mill (Laboratory Construction Co. Kansas City) producing particle sizes smaller than 2 mm, and then reground in a cyclone sample mill (Udy Corporation, Fort Collins, Colorado) through a 0.5 mm screen. Oil content was determined by ether extraction (AOAC, 1980). Oil was extracted from ground barley with hexane, and total tocotrienols and tocopherols were extracted from the oil using a modification of the method of Piironen, et al. (1984). Barley oil (200 mg) was weighed into a brown glass Erlenmeyer flask, mixed with 50 mg pyrogalllic acid, 4 ml of H₂O, 4 ml KOH (50% w/v) and 40 ml ethanol (absolute, HPLC grade) and refluxed under nitrogen for 45 min. After being cooled in water for 20 min, the mixture

was extracted with hexane and washed with distilled water four times. Water was removed by filtration of the extract through granular anhydrous sodium sulfate. Hexane was removed by vacuum rotary evaporation. Standard α -, β -, γ - and δ -tocopherol were purchased from Em Science (480 Democrat Road, Gibbstown NJ). A barley oil with known concentration of tocotrienols was obtained from General Mills (Minneapolis, MN 55427). Tocotrienols and tocopherols were quantified by HPLC with a silica column (250 mm x 4.6 mm) using a fluorescence detector at ex 290 nm and em 320 nm. Hexane with 0.5% 2-propanol was eluted through the column at 1 ml/min flow rate.

Statistical Analysis

Data were analyzed by analysis of variance and Pearson's correlation using the General Linear Models Procedure (SAS, 1985).

Results

Concentration of vitamin E including tocopherols and tocotrienols of barley grain was 63 mg/kg on the average with a range from 48 mg/kg in WBP 501 to 92 mg/kg in Wafranubet (Table 2). Variation in vitamin E concentration was significant ($P < 0.05$) among cultivars but not environments (Table 3). Oil concentration of these barley cultivars averaged 22 g/kg with a range from 16 g/kg in Betzes to 31 g/kg in Wafranubet (Table 2). Cultivar variation in oil concentration was significant ($P < 0.01$) but not by environment (Table 3).

Barley kernel weight was 35 mg (dry matter basis) on the average with a range from 31 mg in Franubet to 39 mg in WBP 501. Both cultivar and environment affected kernel

Table 2. Mean values of vitamin E, oil concentration and kernel weight of seven barley cultivars averaged over environments^{a,b}

Cultivar	Ear type ^c	Vitamin E ^d	Oil	Kernel wt
		mg/kg	g/kg	mg ^e
WBP-501	6RH	48±5 a	19±5 a	39±5 d
Clark	2RH	50±16 ab	19±11 a	38±4 cd
Betzes	2RH	53±16 ab	16±8 a	37±2 bc
Nubet	2RHL	72±14 bc	23±3 ab	34±4 ab
Franubet	2RHL	59±23 ab	20±8 ab	31±4 a
Wafranubet	2RHL	92±19 c	31±3 c	32±3 a
Waxbar	2RHL	68±7 ab	26±3 bc	35±5 abc
Mean	--	63	22	35
Hulled vs hull-less ^f	--	265.4**	83.7**	61.3**

^a Mean±SD, n=3.

^b Values in each column sharing a common letter are not different at P<0.05.

^c R: row; H: hulled; HL: hull-less.

^d Including tocotrienols and tocopherols.

^e Dry matter basis.

^f Contrast; ** P<0.01.

Table 3. Mean values of vitamin E, oil concentration and kernel weight of barleys grown in three environments averaged over seven cultivars^a

Environment	Vitamin E	Oil	Kernel Weight
	mg/kg	g/kg	mg ^b
Scottsdale	52±13	21±6	35±3 a
Marana	69±14	25±4	39±3 b
Bozeman	69±26	20±7	33±4 a

^a Mean±SD, n=7.

^b Values in each column sharing a common letter are not different at P<0.05.

weight ($P < 0.05$) (Table 2).

Contrast of hulled versus hull-less cultivars was significant ($P < 0.01$) in vitamin E, oil and kernel weight (Table 2), with the hull-less cultivars containing more oil and vitamin E than the hulled cultivars, but with lighter kernel weight.

α -Tocotrienol (α -T3) concentration in barley grain averaged 33 mg/kg, with a range from 23 mg/kg in Clark to 53 mg/kg in Wafranubet (Table 4). α -Tocotrienol accounted for 52% of vitamin E. γ -Tocotrienol (γ -T3) was the second largest tocotrienol fraction (7 mg/kg), slightly smaller than α -tocopherol (α -T) (11 mg/kg) and γ -tocopherol (γ -T) (11 mg/kg). Total tocotrienols accounted for 64% of total vitamin E on the average in these barley cultivars. Cultivar variation was significant ($P < 0.05$) in tocotrienols and tocopherols except α -T. Contrast of hulled versus hull-less cultivars was significant ($P < 0.05$) in α -T3, γ -T3 and γ -T concentration. The hull-less cultivars were higher in these compounds than the hulled types. Environmental variation was not significant in these fractions except for γ -T (Table 5). Kernel weight was negatively correlated with α -tocotrienol concentration of barley oil ($r = -0.46$, $P = 0.03$) but not with α -T3, total vitamin E and oil concentration of barley grain ($P > 0.05$) (Table 6). Values of the correlations between kernel weight and kernel α -T3, vitamin E and oil concentration of barley grain were affected by environments with a tendency for higher correlation in barleys grown in Bozeman and Marana, compared to those grown in Scottsdale.

Table 4. Mean values of tocotrienol (T3) and tocopherol (T) concentration of seven barley cultivars averaged over three environments^{a,b}

Cultivar	α -T3	γ -T3	δ -T3	α -T	β -T	γ -T	δ -T
	mg/kg						
WBP 501	25±4 a	4±1 a	0.3±0.1 a	12±1	0.3±0.1 a	8±1 a	0.3±0.1 a
Clark	23±8 a	5±2 ab	0.8±0.4 cd	10±3	0.6±0.2 abc	10±1 ab	0.3±0.1 a
Betzes	25±8 a	6±2 abc	0.7±0.3 abc	10±2	0.5±0.1 ab	11±4 abc	0.2±0.1 a
Nubet	34±6 ab	8±2 bcd	1.1±0.2 d	12±2	0.6±0.1 abc	16±4 c	0.4±0.3 ab
Franubet	29±10 ab	8±4 cd	0.7±0.3 bcd	8±4	0.4±0.1 a	12±5 abc	0.3±0.0 a
Wafranubet	53±10 c	11±3 d	0.7±0.2 bcd	12±3	0.7±0.1 bc	13±4 bc	0.6±0.3 b
Waxbar	39±3 b	6±1 abc	0.4±0.1 ab	12±3	0.8±0.1 c	9±3 ab	0.2±0.1 a
Mean	33	7	1	11	0.6	11	0.3
Mean % of Vitamin E ^c	52	11	1	17	0.9	18	0.5
Hulled vs Hull-less	175.7**	42.6**	1.6	---	1.6	2.4*	0.6

^a Average values, n=3.

^b Values in each column sharing a common letter are not different at p<0.05.

^c Vitamin E including tocotrienols and tocopherols.

* P<0.05 and ** P<0.01.

Table 5. Mean values of tocotrienol (T3) and tocopherol (T) concentration of barleys grown in three environments averaged over barley cultivars^a

Environment	α -T3	γ -T3	δ -T3	α -T	β -T	γ -T ^b	δ -T
				mg/kg			
Scottsdale	28±9	5±2	0.5±0.3	10±3	0.5±0.2	8±3 b	0.2±0.1
Marana	37±11	8±3	0.8±0.3	11±2	0.6±0.1	12±2 b	0.3±0.1
Bozeman	34±15	8±4	0.7±0.3	12±3	0.6±0.2	13±5 a	0.5±0.3

^a Mean±SD, n=7.

^b Values in each column sharing a common letter are not different at P<0.05.

Table 6. Correlations of α -tocotrienol, vitamin E and oil concentration with kernel weight in seven barley cultivars grown in three environments

Kernel weight vs	Correlation (r)			
	Scottsdale n=7	Marana n=7	Bozeman n=7	Three locations n=21
α -T3 ^a	-0.52	-0.59	-0.61	- 0.46**
α -T3 ^b	-0.09	-0.63	-0.74*	-0.14
Vitamin E ^c	0.11	-0.66	-0.74*	-0.34
Oil	-0.05	-0.56	-0.73*	-0.15

^a α -Tocotrienol concentration of barley oil.

^b α -Tocotrienol concentration of barley grain.

^c Tocopherols plus tocotrienols.

* P=0.06 and ** P=0.03.

Discussion

Cultivar variations in vitamin E, α -tocotrienol and oil concentration of barley grain are partially due to hull type difference. Hull-less cultivars generally have higher concentrations of these components than hulled cultivars. The hull on hulled barley accounts for 9 to 13% of the kernel weight (Briggs, 1978), thus diluting the concentration of these components.

Environments affected barley kernel weight rather than vitamin E, α -T3 or oil concentration. Barley grown in Scottsdale was excessively fertilized with N which caused lodging and thus reduced kernel weight. Low kernel weight in Bozeman may be caused by high average temperature, long day length and low available moisture as the result of late planting. In both environments, low kernel weight implied less energy

storage as indicated by low oil concentration in these barley. The variation in vitamin E and α -T3 affected by environment was smaller than those due to cultivar even though the environmental conditions were radically different.

Increase of oil concentration in plump barley kernels has been reported mainly due to the increase in triglycerides and phospholipids (De Main and Ververne, 1988). The negative correlation between kernel weight and α -T3 concentration of barley oil suggests that α -T3 is an active molecule of direct metabolic or structural functions within cells instead of serving as a storage nutrient.

α -Tocotrienol and vitamin E concentration of barley grain were reported to be from 13.0 mg/kg to 30.5 mg/kg (Barnes, 1983; Työppönen and Hakkarainen), and from 35.5 mg/kg to 80.6 mg/kg (Barnes, 1983; Työppönen and Hakkarainen, 1985), respectively. The discrepancy in α -T3 and vitamin E concentration may be partially due to differences in barley samples. Barley samples differ depending on cultivars, storage and processing. A maximum difference in vitamin E and α -T3 concentration between two cultivars in our seven cultivars was about 100%. Reduction of tocotrienol and tocopherol concentration in cereal grain due to length of storage was reported (Työppönen and Hakkarainen, 1985). Vitamin E concentration decreased at a rate of 5 mg/kg per week when barley flour was stored at room temperature and heat also reduced vitamin E concentration. Milling and pearling processes produced barley fractions with different α -T3, vitamin E and oil concentration. A pearling flour accounting for 20% of kernel weight had nearly three fold greater α -T3, vitamin E and oil concentration than whole barley grain (Wang et al., 1991). Without knowing about the source and history of barley samples, it is very

difficult to make any meaningful comparisons of the values from different laboratories. In our study, the possible damage caused by storage and grinding was minimized. Barley grain was harvested and stored for about one month before grinding. Grain was ground using two mills, as mentioned early in this report, which prevented the flour from being exposed to high temperature. Procedures used in preparing barley samples in this study may partially explain why concentration of α -T3 and total vitamin E reported are higher than those from Barnes (1983) and close to those from Työppönen and Hakkarainen (1985).

Conclusion

Average vitamin E, α -tocotrienol and oil concentration of barley grain in the seven cultivars were 63 mg/kg with a range from 48 mg/kg to 92 mg/kg, 33 mg/kg with a range from 23 mg/kg to 53 mg/kg, and 22 g/kg with a range from 16 g/kg to 31 g/kg, respectively. α -Tocotrienol accounted for 52% of total vitamin E which included tocotrienols and tocopherols. Cultivars were the main source of variation in α -tocotrienol, vitamin E and oil concentration. These cultivar variations were partially due to hull type differences among the seven cultivars. Hull-less cultivars generally had higher concentrations of α -tocotrienol, vitamin E and oil and lower kernel weight than hulled cultivars. The negative correlation between kernel weight and α -tocotrienol concentration of barley oil in these cultivars suggests that α -tocotrienol has an active metabolic or structural function in cells instead of being a storage nutrient.

CHAPTER 3

ENRICHMENT OF VITAMIN E AND OIL BY MILLING AND PEARLING BARLEY

Introduction

Cereal grains are the major sources of vitamin E for the human diet. α -Tocopherol, the largest component of the vitamin E complex in most cereals is a natural antioxidant and is reported to reduce the risk of ischemic heart disease, cataracts and to enhance the immune function (Gaby and Machlin, 1991). α -Tocotrienol, an isomer of α -tocopherol and a component of the vitamin E complex, has been shown to reduce plasma cholesterol in experimental animals (Qureshi et al., 1986). The tocopherols and tocotrienols are extracted with the oil of cereals.

Barley, as one of the most ancient cereal grains in the world, is now gaining renewed interest for food use due to its hypocholesterolemic property and other desirable nutritional and functional characteristics (Newman and Newman, 1991). Barley grain contains about 21 g/kg oil (Newman and McGuire, 1985) and the vitamin E (tocopherol and tocotrienol) concentration has been reported to range from 30.5 mg/kg (Barnes, 1983) to 80.6 mg/kg (Työppönen and Hakkarainen, 1985). The oil and total vitamin E levels in barley grain are lower, but the concentration of tocotrienols in barley grain is

higher than most other grains (Barnes, 1983).

Milling and common pearling are processing methods for cereal grain and pearling has been used to produce fractions of the barley kernel that are high in oil (Pomeranz and Chung, 1983). The objective of this study was to characterize the influence of pearling and milling on the concentrations of oil, tocopherols and tocotrienols in barley.

Materials and Methods

Milling and Pearling

Waxbar and Azhul, two- and six-rowed waxy hull-less barley cultivars, respectively, were selected for fractionation by milling and pearling because of their potential use for human food. Azhul was developed by R. T. Ramage, USDA-ARS, Department of Plant Sciences, University of Arizona, Tucson AZ and Waxbar was bred by Western Plant Breeders, Bozeman MT. These cultivars were grown during the winter of 1989 at the Arizona Agricultural Experiment Station, Marana AZ and harvested in May 1990. The grains were tempered to 10% moisture for 12 h prior to milling through a MIAG multomat 8-roller experimental mill with a feed rate of 900 g/min at the USDA Western Wheat Quality Laboratory, Washington State University, Pullman WA. Mill streams were individually collected with the 1st break flour through the 4th middling fractions combined, mixed and designated as flour. The six milling fractions obtained were flour, 5th middling, red dog, reduction shorts, break shorts and bran. Pearling fractions were produced with a laboratory model barley pearler (Model 6K572A, Dayton Electric Mfg., Co., Chicago, IL). The whole barley grain, pearling and milling fractions were sampled

and finely ground through a 0.5 mm screen with a cyclone sample mill (Udy Corporation, Fort Collins Co.) prior to analysis.

Chemical Analysis

Oil and protein (N x 6.25) content of all the barley materials were determined (AOAC 1980). Oil to be analyzed for tocopherols and tocotrienols was extracted with hexane (0.5 kg sample/liter hexane) for 1 h with constant stirring at 27°C. The extract was filtered through fiberglass filter paper. The extraction was repeated and the two extracts combined. Hexane was evaporated under vacuum at 45°C. The oil was stored at -20°C under nitrogen until analyzed. Oil was extracted from ground whole grain and pearling fractions immediately after processing. Oil extractions of the milling fractions were done after they were stored at room temperature for about 2 months. Free tocopherols and tocotrienols were extracted and determined as described in the previous chapter.

Chemical analytical data of whole grain, milling and pearling fractions were statistically analyzed using one way analysis of variance (SAS, 1985).

Results

Milling fraction yields were different ($P < 0.05$), with a range from 1% in 5th middling to 39% in bran (Table 7). Greater cultivar variations were found in flour and bran which were the larger fractions compared to the other four fractions. However, cultivar effect was not significant. The two pearling fractions were pearling flour and pearled grain with 20% and 80% of total weight respectively, in both barley cultivars.

Table 7. Pearling and milling fraction yield of two waxy hull-less barleys

Fraction	Yield (% of Total)		
	Waxbar	Azhul	Mean
<u>Milling Fraction</u>			
Flour	41.0	19.6	30.3
5th Middling	1.5	0.9	1.2
Red dog	10.6	7.2	8.9
Reduction shorts	4.0	2.6	3.3
Breakshorts	15.4	18.7	17.1
Bran	27.5	51.0	39.3
<u>Pearling Fraction</u>			
Pearling flour	20.0	20.0	---
Pearled grain	80.0	80.0	---

Azhul had much harder texture than Waxbar so that the pearling time was 25 sec for Azhul and 19 sec for Waxbar. Seven vitamin E fractions including α -, γ - and δ -tocotrienol (T3) and α -, β -, γ - and δ -tocopherol (T) were detected in the barley grain, its milling and pearling fractions (Table 8). α -T3, γ -T3, α -T and β -T concentration varied ($p < 0.05$) between whole grain, the milling and pearling fractions, but no significant differences were found in the concentrations of δ -T3, γ -T and δ -T in barley grains and their fractions.

Pearling flour was found to have the highest concentration of tocotrienols and tocopherols of all barley fractions and barley grain (Table 8). The pearling flour had 116 mg/kg α -T3 and 28 mg/kg γ -T3, 35 mg/kg α -T and 2.0 mg/kg β -T which were 2.7, 3.5, 4.4 and 5.0 times greater respectively, than that in whole grain. Among the milling fractions, the reduction shorts and red dog were the highest ($p < 0.05$) in α -T3

Table 8. Tocotriol (T3) and tocopherol (T) concentration in whole grain, milling and pearling fractions of two waxy hull-less barleys^a

	α -T3	γ -T3	δ -T3	α -T	β -T	γ -T	δ -T
Item	-----mg/kg ^b -----						
<u>Milling Fraction</u>							
Flour	21±8ab	3±1a	0.5±0.4	9±3abc	0.8±0.4b	8±4	0.2±0.1
5th middling	33±10bc	6±2a	0.7±0.5	12±7c	1.7±0.6c	13±7	0.8±0.7
Red dog	42±2cd	9±2a	1.1±0.6	11±1bc	0.8±0.0b	13±3	0.4±0.3
Reduction shorts	50±4d	11±1a	1.3±0.7	3±1ab	0.4±0.2ab	13±1	0.2±0.1
Break shorts	28±0ab	5±0a	0.7±0.4	6±1abc	0.4±0.1ab	14±10	0.1±0.1
Bran	29±1ab	6±0a	0.7±0.6	3±0ab	0.3±1ab	8±2	0.1±0.1
<u>Pearling Fraction</u>							
Pearling flour	116±3e	28±10b	2.1±1.0	35±9d	2.0±0.3c	21±5	0.8±0.2
Pearled grain	18±3a	2±0a	0.4±0.1	1±1a	0.1±0.0a	5±0	0.1±0.0
<u>Whole grain</u>	43±4cd	8±2a	1.0±0.7	8±0abc	0.4±0.1ab	10±2	0.4±0.3

^a Means±SEM of cultivar Azhul and Waxbar with two determinations for each cultivar.

^b Values in each column sharing a common letter are not different at P<0.05.

concentration, while 5th middling was the highest ($p < 0.05$) in α - and β -T concentration. Vitamin E (tocopherols and tocotrienols) concentration of the pearling flour was 205.3 mg/kg, which was 2.9 fold greater than that of whole grain (Table 9).

Concentration of α -T3 in vitamin E of the pearled grain did not differ from that of reduction shorts, bran, pearling flour and whole grain but was higher than the other fractions (Table 9).

Oil concentration of the milling and pearling fractions varied from 9.7 g/kg in the pearled grain to 81.5 g/kg in the pearling flour (Table 9). Oil concentration of pearling flour was 2.9 times greater ($p < 0.05$) than that of whole grain. The 5th middling had the highest ($p < 0.05$) oil concentration of all milling fractions except red dog, which was 1.6 fold of that in the whole grain. Pearled grain contained the least oil of all samples compared except bran.

Protein concentration of pearling flour, whole grain and pearled grain were 195, 119 and 101 g/kg, respectively (Table 9). Since embryo and aleurone layers are the tissues of barley grain richest in protein, it appears that the pearling flour may be composed of seed coat, embryo and aleurone layers.

Distribution of oil, vitamin E and α -T3, expressed as percent of the total of each component contained in the six milling fractions and in two pearling fractions is listed in Table 10. Bran was highest of milling fractions in α -T3 but similar in vitamin E to flour and red dog. The bran also contained the highest proportion of oil compared to 5th middling, reduction shorts and break shorts even though it was not different from either flour or red dog. Between two pearling fractions, the pearling flour contained the

Table 9. Concentration of protein, oil, vitamin E and tocotrienols in vitamin E of whole grain, milling and pearling fractions of two waxy hull-less barleys^{ab}

	Protein g/kg	Oil g/kg	Vitamin E ^c mg/kg	α -T3 ----- g/kg of vitamin E ^d -----	γ -T3	δ -T3
<u>Milling Fraction</u>						
Flour	95±2a	24±5 bc	43±17 ab	490±7 ab	100±28	10±5
5th middling	123±3d	45±6 e	77±31 b	430±14 a	80±7	10±3
Red dog	113±6cd	38±2 de	77±8 b	540±42 abc	120±7	10±7
Reduction shorts	108±5bc	30±1 cd	79±3 b	640±35 cd	140±0	20±10
Break shorts	105±7bc	22±2 bc	54±12 ab	530±106 abc	110±21	10±6
Bran	115±6cd	19±1 ab	47±4 ab	620±35 cd	130±7	20±11
<u>Pearling Fraction</u>						
Pearling Flour	195±4e	82±9 f	205±31 c	570±71 bcd	140±35	10±6
Pearled grain	101±2ab	10±3 a	28±5 a	660±14 d	100±7	20±6
<u>Whole grain</u>	119±2d	28±4 bc	71±10 b	610±28 cd	110±14	10±8

^a Means±SEM of cultivar Azhul and Waxbar with two determinations for each cultivar.

^b Values in each column sharing a common letters are not different at P<0.05.

^c Vitamin E including tocotrienols and tocopherols detected in barley samples.

^d Tocotrienol concentration in vitamin E, respectively.

Table 10. Distribution of α -tocotrienol, vitamin E (tocopherols and tocotrienols) and oil in milling and pearling fractions of two waxy hull-less barleys^{ab}

	α -Tocotrienol	Vitamin E	Oil
	----- % of total ^c -----		
<u>Milling Fraction</u>			
Flour	21 \pm 4 c	23 \pm 2 cd	31 \pm 7 bc
5th middling	1 \pm 0 a	3 \pm 0 a	2 \pm 0 a
Red dog	14 \pm 4 abc	22 \pm 5 cd	15 \pm 2 abc
Reduction shorts	6 \pm 2 ab	7 \pm 3 ab	9 \pm 5 a
Break shorts	17 \pm 1 bc	17 \pm 3 bc	12 \pm 7 ab
Bran	41 \pm 9 d	28 \pm 6 d	32 \pm 9 c
<u>Pearling Fraction</u>			
Pearling flour	61 \pm 3 e	93 \pm 3 e	80 \pm 6 d
Pearled grain	39 \pm 3 d	7 \pm 3 ab	20 \pm 6 abc

^a Means \pm SEM of cultivar Azhul and Waxbar.

^b Values in each column sharing a common letter are not different at $P < 0.05$.

^c Ratio of a component in each barley fraction to that in all fractions of the same type of processing.

larger proportion of vitamin E, α -T3 and oil, which were 93%, 61% and 80% of the total, respectively.

Discussion

Differences between barley cultivars in milling yields have been reported (Kent 1983). In the present study, flour yield of Azhul was only half that of Waxbar. Lower flour and higher bran yield in Azhul may be due to the hard texture of the outer grain surface which contributes to the bran fraction.

Pearling was the more effective way of processing barley grain to concentrate vitamin

E and oil than was the milling method used in this study. The pearling flour fraction (20% kernel weight) contained the highest concentrations of vitamin E including Ts and T3s, and oil of all samples compared. Abrasive pearling has been used for many years to produce pearl barley for food and pet feed products in which the pearling flour is a by-product. In this study, the pearling flour had 2.7 to 4.4 times greater α -T3, α -T, total vitamin E and oil concentration than that of barley grain, respectively, which suggests possible use of this by-product as a nutrient-rich, health-promoting food ingredient.

Bioavailability of vitamin E is affected by many factors. Since vitamin E is enclosed in the cells of grain particles, the bioavailability of vitamin E of barley pearling flour could be less than that of a pure compound. Bioavailability of α -T3 as a cholesterol-lowering agent has not been reported. However, biopotency of vitamin E in barley, the ratio of vitamin E recovered in the liver to the amount of dietary vitamin E intake, was reported to be 37% (Hakkarainen et al 1984). Some other factors reducing bioavailability of vitamin E in food may be storage, processing (Piironen et al 1988) and cooking. One concern is shelf life of the pearling flour and possible deterioration of oil and oil-soluble components, resulting in lowered bioavailability as well as rancidity. Työppönen and Hakkarainen (1985) reported a rate of 5% reduction of vitamin E per week in milled barley when it was exposed to normal room temperature and light. Further investigation of bioavailability, shelf life and possible treatments to stabilize these components is suggested.

Conclusion

Milling and pearling processing of two waxy hull-less barleys produced significant differences in the concentration of oil, vitamin E, α -tocotrienol, β -tocotrienol, α -tocopherol and β -tocopherol in nine barley fractions including six milling fractions, two pearling fractions and ground barley grain. The concentration differences between the two pearling fractions were much greater than those between milling fractions. The pearling flour fraction accounting for 20% of grain weight, had the highest α -T3 (116 mg/kg), α -T (35 mg/kg), vitamin E (205 mg/kg), and oil (82 g/kg) concentrations which were 2.7, 2.9, 4.4, and 2.9 fold, respectively, of those in whole barley grain. These data suggest that pearling is a more effective processing method than milling to increase vitamin E and oil in a barley fraction.

CHAPTER 4

TOCOTRIENOL AND FATTY ACID COMPOSITION OF BARLEY OIL
AND EFFECTS ON LIPID METABOLISMIntroduction

The concentration of cholesterol in blood is regulated by absorption of dietary cholesterol, synthesis of bile acids from cholesterol, and cholesterol synthesis activity in the liver. Diets containing ground barley have been shown to have a hypocholesterolemic effect in chicks and humans (Newman et al., 1989). β -Glucans have been shown to be responsible for some of the hypocholesterolemic effect of barley (Fadel et al., 1987). Three mechanisms have been postulated for this effect: (1) reduced absorption of dietary lipids including cholesterol, (2) reduced reabsorption of bile acids and (3) production of volatile fatty acids in the large intestine which are reabsorbed and act as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the liver (McIntosh and Oakenfull, 1990). α -Tocotrienol extracted from barley flour was identified as a hypocholesterolemic component for chicks (Qureshi et al., 1986). This compound was shown to inhibit HMG-CoA reductase activity in the liver, thus reducing *in vivo* cholesterol synthesis.

In an effort to find barley cultivars with greater hypocholesterolemic activity, major

components of the grain are being studied in this laboratory. In this study the concentration of tocotrienols, tocopherols and fatty acids in barley oil are reported and the hypocholesterolemic activity of oil obtained from a waxy hull-less barley is demonstrated.

Materials and Methods

Barley and Chemical Analysis

Prowashonupana (PWSNP) used in this study is a waxy, short-awned, hull-less barley cultivar derived from Washonupana by chemically induced mutation using diethyl sulfate (R. F. Eslick, personal communication). Washonupana was produced by crossing Wapana, a waxy isolate of Compana (CI 5438) with a short-awn, hull-less isolate (Shonupana) of the same cultivar (Fox, 1981). PWSNP was selected for study because it contains 7% oil (Åman and Newman, 1986) which is much higher than the average level reported in barley (Newman and McGuire, 1985). PWSNP barley was ground and sieved through a 1 mm screen. The oil of the ground barley was extracted with hexane (0.5 kg/L) using constant stirring at 27°C for 2 h. The extract was collected by vacuum filtration. The extraction procedure was repeated twice. Extracts were combined and evaporated at 45°C in a vacuum rotary evaporator to remove hexane. Mazola corn oil (CPC International Inc. Winston-Salem, NC) and Parkay margarine (Kraft Inc., Glenview, IL) partially hydrogenated from soybean oil were purchased from a local food market.

The fatty acid composition of the extracted barley oil and the two commercial

vegetable fats were determined by gas chromatography of fatty acid methylesters on a capillary column of DB-FFAP, 30m x 0.25mm (Bannon et al., 1982). Tocotrienols and tocopherols were determined by HPLC on a silica column (25 cm x 4.6 mm). The mobile phase was 0.5% 2-propanol in hexane and the flow rate was 1 ml/min. The sample volume was 10 μ l. Peaks were detected with a fluorescence detector at ex 290 nm and em 320 nm (Piironen et al., 1984).

Chicks and Diets

Broiler chicks were fed cornmeal-based diets supplemented with soybean protein isolate, vitamins, minerals and lipids (Table 11) to meet NRC recommendations (NRC, 1984). Twelve four-day-old male broiler chicks (Fors Farm, Puyallup, WA) were fed a corn diet containing 0.5% cholesterol (Diet 1) for 10 d to elevate the plasma cholesterol level. The chicks were then randomly divided into three diet groups and fed three test diets. The three test diets contained either 10% barley oil (Diet 2), 10% corn oil (Diet 3), or 10% margarine (Diet 4). Diet 4 was used as a control diet. Chicks were fed test diets ad libitum with four chicks per diet for another 10 d. Body weights were recorded and blood samples taken from the brachial veins after a 10 h fast on d 14 and d 24. Concentrations of total plasma cholesterol and plasma high density lipoprotein cholesterol were determined for blood samples using a DT 60 Kodak analyzer. Plasma low density lipoprotein cholesterol concentration was calculated as described by Friedwald et al. (1972).

Table 11. Composition of chick diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Corn meal	68.91	62.44	62.44	62.44
Soybean protein ¹	20.09	20.79	20.79	20.79
Supplement ²	6.28	6.28	6.28	6.28
Crisco oil ³	4.22	----	----	----
Cholesterol	0.50	0.50	0.50	0.50
Barley oil	----	10	----	----
Corn oil ⁴	----	----	10	----
Margarine ⁵	----	----	----	10

¹ Composition of soybean protein isolate (%) produced by ICN Biochemicals: protein (92.0), moisture (6.0), ash (4.0), fat (0.8), fiber (0.25), calcium (0.15), phosphorus (0.8) sodium (1.3), potassium (0.05).

² Supplement contents (%): dicalcium phosphate (2.80), limestone (1.50), vitamin fortification (1.00), salt (0.50), trace mineral mix (0.15), choline chloride (0.20), DL-methionine (0.125) and biotin (0.01).

³ Proctor and Gamble Co, Cincinnati, OH; 100% soybean oil.

⁴ CPC International Inc., Winston-Salem, NC.

⁵ Kraft Inc., Glenview, IL; soybean oil saturated by hydrogenization.

This experiment was approved by Montana State University's Animal Care Committee.

Statistical Analysis

Body weight gains from d 14 to d 24 and plasma lipid concentrations were analyzed by analysis of variance using MSUSTAT (Lund, 1987).

Results

Compared with margarine, barley oil and corn oil contained more than 3 fold

polyunsaturated fatty acids and about half the oleic acid (Table 12). Barley oil was found to have 127 g/kg saturated fatty acids which was about 1.5 fold of that in corn oil or margarine. Content of ω -3 linolenic acid was highest in barley oil which was the only

Table 12. Fatty acid composition of barley oil, corn oil and margarine

Fatty acid ²	Barley oil	Corn oil g/kg ¹	Margarine
16:0	120 \pm 8	56 \pm 5	59 \pm 2
18:0	6.9 \pm 0.6	9.2 \pm 0.4	27 \pm 3
18:1	91 \pm 1	128 \pm 6	228 \pm 10
18:2	237 \pm 11	290 \pm 15	74.0 \pm 0.6
18:3	16.0 \pm 0.9	5.1 \pm 0.4	6.9 \pm 0.1
20:0	Trace	1.8 \pm 0.2	Trace
Total	470.9	490.1	394.9
SFA ³	127	67	86
PUFA ⁴	253	295	81
TUFA ⁵	343	423	308

¹ Mean \pm standard error of two determinations.

² Palmitate, stearate, oleate, linoleate, ω -3 Linolenate and arachidate, respectively.

³ Saturated fatty acids.

⁴ Polyunsaturated fatty acids.

⁵ Total unsaturated fatty acids.

isomer of linolenic acid detected in these vegetable fats. Barley oil had the highest α -tocotrienol content and total content of the tocopherol family including tocopherols and tocotrienols, of the three vegetable fats (Table 13). α -Tocotrienol comprised 52% of the tocopherol family in barley oil and only 4% of that in corn oil. In corn oil and margarine, γ -tocopherol was the most abundant fraction. α -Tocotrienol and γ -

tocotrienol content of the barley oil were 24 and 17 fold greater, respectively, than those in corn oil. Tocotrienols were not detectable in the margarine. Content of tocopherols in barley oil was 49% and 34% of that in corn oil and margarine, respectively.

Table 13. Tocopherol (T) and tocotrienol (T3) concentration of barley oil, corn oil and margarine

Fraction	(mg/kg) ¹		
	Barley oil	Corn oil	Margarine
α -T	142 \pm 21	116 \pm 4	77 \pm 9
β -T	6 \pm 0	8 \pm 1	11 \pm 0
γ -T	104 \pm 8	375 \pm 35	536 \pm 57
δ -T	1 \pm 0	13 \pm 1	128 \pm 5
α -T3	558 \pm 22	23 \pm 0	nd ²
β -T3	nd	nd	nd
γ -T3	85 \pm 7	5 \pm 1	nd
δ -T3	5 \pm 0	nd	nd
Total	901	540	752
α -T3 (% of total)	62%	4%	nd
γ -T3 (% of total)	9%	1%	nd

¹ Mean \pm standard error of two determinations.

² nd: not detected.

Initial body weights of the chicks in three diet groups on d 14 were not different ($P > 0.1$) (Table 14). Chicks fed the diet containing 10% barley oil gained 16% and 17% more ($P < 0.05$) weight than those fed either corn oil or margarine diets, respectively. No difference was found in weight gain between corn oil and margarine diet groups. Total plasma cholesterol concentration of the chicks fed barley oil was 34%

lower ($P < 0.05$) than those fed margarine (Table 14). Plasma low density lipoprotein cholesterol concentration of the barley oil group was 53% and 59% lower ($P < 0.05$) than those of the corn oil and the margarine groups, respectively. Plasma high density lipoprotein cholesterol concentration and triacylglycerol concentration of the barley oil group were not different ($P > 0.05$) from those of the margarine, but 120% and 300% higher ($P < 0.05$), respectively, than those of the corn oil group.

Discussion

Differences in the plasma cholesterol levels of the chicks fed in this study can be attributed to differences in diet composition. The test diets were formulated quantitatively to be equal in metabolic energy, total dietary fiber and protein. The type of vegetable oils was the only component that differed between the test diets. That vegetable oil shows hypocholesterolemic effect compared with animal fat may be partially due to its low cholesterol content. Margarine made from soybean oil was used as the control oil because it has no cholesterol.

Barley oil prevented the elevation of blood cholesterol concentration in the chicks compared to margarine. The chicks fed barley oil maintained their total and LDL cholesterol concentrations to the values that were similar to prefeed levels (Table 14). Corn oil has been previously reported to reduce total and LDL cholesterol concentrations in rats when compared to coconut fat (Beynen et al., 1987; Mendis et al., 1989). The high content of unsaturated fatty acids in the corn oil was believed to be the hypocholesterolemic factor. In this study, barley oil showed a greater

Table 14. Weight gain and plasma lipid concentrations of chicks fed barley oil, corn oil and margarine

Diet group	Body weight	Weight gain	Plasma cholesterol (mmol/L)			Plasma triacylglycerol
	(g) ¹	(g) ²	Total	LDL	HDL	(mmol/L)
Prefeed ³	----	----	4.2±0.3	1.3±0.2	2.9±0.2	0.4±0.0
Barley oil	260±5	387±20b	4.4±0.3a	1.7±0.3a	2.8±0.1b	0.6±0.2b
Corn oil	248±8	334±9a	5.9±0.4ab	3.6±0.5b	2.3±0.1a	0.2±0.2a
Margarine	264±2	330±17a	6.8±1.3b	4.1±1.0b	2.7±0.1b	0.5±0.1ab
Ratio of barley oil group over:						
Corn oil	-----	1.2	0.7	0.5	1.2	3.0
Margarine	-----	1.2	0.6	0.4	1.0	1.2

¹ day 14.

² From day 14 to day 24.

³ Data collected on d 14 before placing chicks on test diets (n = 12).

^{a,b,c} Values in each column sharing a common letter are not different (p < 0.05; n=4).

hypocholesterolemic effect than the corn oil, although the corn oil had the highest level of unsaturated fatty acids. This suggests that the unsaturated fatty acid level was not the only hypocholesterolemic factor in barley oil. The other effect of barley oil was that it suppressed HDL cholesterol concentrations less than corn oil in the chicks. Corn oil has been reported to reduce HDL cholesterol levels (Mendis et al., 1989) and this study confirmed that effect. Barley oil suppressed the elevation of LDL cholesterol but not HDL cholesterol levels compared to the corn oil in chicks. Diets reducing total and LDL cholesterol concentrations without decreasing HDL cholesterol concentrations is considered to be highly desirable for the prevention of coronary heart disease in humans (Grundy, 1987).

The mechanism by which barley oil lowers cholesterol is different from that of β -glucans, a fiber component of barley. Chicks fed a barley diet with high β -glucans showed a lower total and LDL cholesterol levels, a lower weight gain, and increased fecal fat excretion, and supplementation of β -glucanase to the diet reversed this effect (Fadel et al., 1987). In a separate study, chicks fed barley had lower protein digestibility than those fed a barley diet supplemented with β -glucanase (Hesselman and Aman, 1986). Reduced digestion and absorption of lipids and protein could cause a reduction in weight gain. However, chicks fed barley oil showed a greater weight gain yet lower total and LDL cholesterol concentrations. This result suggested that the digestibility and absorption functions were normal in chicks fed a diet supplemented with barley oil.

Tocotrienols and polyunsaturated fatty acids may be two groups of compounds responsible for the suppression of plasma cholesterol concentration. Qureshi, et al.

(1986) reported that broiler chicks fed a diet with 10 ppm α -tocotrienol for 21 days had significant decrease in the concentration of serum total and low density lipoprotein cholesterol. A 56 ppm α -tocotrienol was calculated in the barley oil diet in this study, which might have caused a substantial reduction in the activity of HMG-CoA reductase and thus suppression of blood total and LDL cholesterol concentrations. γ - and δ -tocotrienol are also reported to be hypocholesterolemic compounds (Weber et al., 1990). Inhibition of HMG-CoA reductase activity could cause accumulation of acetate and thereby an increased synthesis of triglycerides. The higher triglyceride concentration in the plasma of chicks fed barley oil may indicate greater reduction in activity of HMG-CoA reductase. Polyunsaturated fatty acids were believed to have a hypocholesterolemic effect by increasing fecal sterol excretion and inhibiting cholesterol synthesis in the liver (Choi et al., 1989). Polyunsaturated fatty acids may be another oil component responsible for the suppression of plasma cholesterol levels in chicks fed the barley oil diet.

Conclusion

Oil extracted from a waxy barley contained 558 mg/kg α -tocotrienol, and 253 g/kg polyunsaturated fatty acids. Commercial corn oil, Mazola, contained 23 mg/kg α -tocotrienol and 295 g/kg polyunsaturated fatty acids. Margarine of soybean oil contained no tocotrienols and 81 g/kg polyunsaturated fatty acids. A feeding trial with male broiler chicks fed corn based diets with either 10% barley oil, 10% corn oil or 10% margarine showed that barley oil suppressed the elevation of plasma total and low density

cholesterol concentrations compared to margarine, and suppressed plasma low density cholesterol but not high density cholesterol concentrations compared to corn oil. Both α -tocotrienol and polyunsaturated fatty acids may be responsible for the suppressive effect of barley oil on plasma cholesterol concentrations in these chicks.

CHAPTER 5

INFLUENCE OF BARLEY OIL ON PLASMA LIPID CONCENTRATION IN A
LEGHORN CHICK MODELIntroduction

Barley oil of brewers' grain has been reported to lower serum cholesterol in chicks (Burger et al., 1984) and pigs (Qureshi et al., 1987). α -Tocotrienol is an oil-soluble component which was reported to lower serum cholesterol by inhibiting the activity of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis in chicks. Therefore, α -tocotrienol may be responsible for the hypocholesterolemic property of barley oil. Barley grain has a higher α -tocotrienol level than most other cereal grains (Barnes, 1983) although the oil concentration of barley grain is lower (Newman and McGuire, 1985). Whether or not barley grain oil and α -tocotrienol as a component of the oil have hypocholesterolemic property, is a question yet to be resolved.

HMG-CoA reductase has been reported to be the center of regulation for cholesterol homeostasis. Dietary cholesterol intake inhibits HMG-CoA reductase activity (Ramirez et al., 1984) and excessive excretion of bile acids synthesized from cholesterol activates HMG-CoA reductase (Goldfarb and Potit, 1972). Reduction of cholesterol in the diet

produced a reduction of the inhibitory effect of dietary cholesterol on HMG-CoA reductase (Sabine, 1977).

The sources of body cholesterol are biosynthesis and absorption of dietary cholesterol. A combination of a low cholesterol, low saturated fat diet with drugs that inhibit HMG-CoA reductase activity is considered an effective therapy to treat hypercholesterolemia in humans (The Expert Panel, 1988).

The objective of this study was to examine the influence of barley oil on plasma cholesterol concentration. The white Leghorn chick model was used since this animal was utilized in brewers' grain oil (Burger et al., 1984) and α -tocotrienol studies (Qureshi et al., 1986).

Materials and Methods

Feeding Trial and Sample Collection

Fifty-two one-day old male white Leghorn chicks were fed a corn-soybean meal diet (control diet) for seven days. At that time, ten chicks were killed and blood samples taken by heart puncture for determination of initial values. The remaining forty-two chicks were randomly assigned to two prefeeding groups with Group 1 (24 chicks) fed the control diet and Group 2 (18 chicks) fed a similar corn-soybean diet with supplemental cholesterol (15 g/kg) (Table 15). These prefeed diets were fed for seven days, when six chicks from each group were killed and blood samples taken by heart puncture for determination of prefeeding values. The remaining 18 chicks from Group 1 were further randomly divided into three groups of six each and fed the control,

margarine and barley oil diets, respectively. The remaining chicks from Group 2 were randomly divided into two groups of six each and fed the margarine diet or the barley oil diet. The chicks were fed these diets for 21 d and then killed and blood samples taken from brachial veins. Chicks were housed two per cage during 21 d feeding trial. Body weight was recorded at the beginning and end of the 21 d trial and feed consumption was recorded weekly. Chicks were fed ad libitum throughout the trial. Blood samples were taken after a 12 h overnight fast.

This experiment was approved by Montana State University's Animal Care Committee.

Chick diet Preparation

Corn and Prowashonupana barley grain were ground with a feed mill at the MSU Animal Science Department one week prior to use. Oil was extracted from ground corn and barley by soaking the flour in hexane (2kg/L) for one hour with frequently stirring at 27 °C followed by filtration through a glassfiber filter. The extraction was repeated three times. The corn meal above the filter was collected and dried in the air as defatted corn. Barley oil was obtained by evaporating hexane from the mixture of barley oil and hexane using a vacuum rotary evaporator at 45°C. The barley oil was then nitrogen blanketed and stored at 4°C until use. A soybean oil margarine (Kraft Inc., Glenview, IL) was purchased from a local food market. The water concentration of the margarine was determined by drying it in 110°C oven overnight. Immediately before diet preparation, 1.6 g ethoxyquin was added and mixed with 1 kg lipids. Table 15 contains diet composition.

Table 15. Diet composition¹

Ingredient	Prefeeding 1 (Control)	Prefeeding 2	Margarine	Barley oil
			g/kg	
Corn	514.10	514.10	----	----
Defatted corn	----	----	405.90	405.90
Soybean meal ²	316.30	316.30	----	----
Alphacel ³	78.75	78.75	256.65	256.65
Supplement ⁴	63.85	63.85	63.85	63.85
Corn oil ⁵	27.00	12.00	----	----
Cholesterol	----	15.00	----	----
Casein	----	----	173.60	173.60
Margarine ⁶	----	----	100.00	----
Barley oil	----	----	----	100.00

¹ All diets were isoprotein (18 g/kg) and isoenergy (2900 ME Kcal/kg).

² Solvent extracted.

³ ICN Biochemicals Division, ICN Biochemicals Inc., Cleveland OH 44218.

⁴ Supplement content (%): dicalcium phosphate (2.80), limestone (1.50), vitamin fortification (1.00), salt (0.50), trace mineral mix (0.15), choline chloride (0.20), DL-methionine (0.125) and biotin (0.01).

⁵ CPC International Inc., Winston-Salem, NC.

⁶ Kraft Inc., Glenview, IL; Soybean oil saturated by hydrogenization.

Chemical and Statistical Analysis

Plasma lipids, tocotrienol and tocopherol were determined as previously described (Wang et al., 1992a). Data were analyzed by analysis of variance and Pearson's correlation with MSUSTAT (Lund, 1987).

Results

Barley oil had 800 mg/kg α -tocotrienol which accounted for 62% of total

concentration of vitamin E (tocotrienols and tocopherols). γ -Tocotrienol was the second largest tocotrienol fraction (190 mg/kg) in the barley oil (Table 16). Tocotrienols were not detected in margarine.

Table 16. Tocotrienol (T3) and tocopherol (T) concentration of barley oil and margarine¹

Fraction	Barley Oil mg/kg	Margarine mg/kg
α -T	181 \pm 7	127 \pm 1
β -T	7 \pm 1	13 \pm 1
γ -T	105 \pm 2	597 \pm 14
δ -T	3 \pm 1	160 \pm 3
α -T3	800 \pm 25	nd ²
β -T3	nd	nd
γ -T3	190 \pm 5	nd
δ -T3	20 \pm 1	nd
Total	1306	897
α -T3 (% of total)	62%	nd

¹ Mean \pm SEM of three determinations.

² not detected.

After seven days prefeeding, body weight, plasma total cholesterol and LDL cholesterol concentrations of the chicks fed the cholesterol diet were higher ($P < 0.05$) than those fed the cholesterol free diet, but the concentrations of plasma HDL cholesterol and triacylglycerol concentrations were not different (Table 17). When compared to the initial levels of the chicks, those fed the cholesterol diet had increased ($P < 0.05$) body weight, total cholesterol and LDL cholesterol. They were not different in plasma

Table 17. Plasma lipid concentration of chicks fed prefeeding diets with or without cholesterol¹

Treatment	Plasma cholesterol mmol/L			Plasma TAG ⁵ mmol/L	Body weight g
	Total	LDL	HDL		
Initial ²	4.7±0.2a	0.0±0.4a	4.9±0.4b	1.5±0.2	62±1a
Prefeeding 1 ³	3.9±0.3a	0.1±0.4a	3.6±0.3a	0.9±0.1	101±3b
Prefeeding 2 ⁴	6.5±0.6b	3.6±0.6b	2.4±0.1a	1.0±0.1	117±3c

¹ Values in each column sharing a common letter are not different at $P < 0.05$.

² Seven-day-old chicks, $n=10$.

³ Control diet; 14 days old chicks, $n=6$.

⁴ Control diet with cholesterol (15 g/kg); 14 days old chicks, $n=6$.

⁵ Triacylglycerol.

triacylglycerol levels, but HDL cholesterol levels decreased.

After 21 days of feeding the test diets, plasma LDL cholesterol concentration was lower ($P < 0.05$) in chicks fed prefeeding 1 followed by the barley oil diet (barley oil 1), prefeeding 2 followed by the margarine diet (margarine 2) and prefeeding 2 followed by the barley oil diet (barley 2) than the control group (Table 18). Chicks fed prefeeding 1 followed by the margarine diet (margarine 1) had a similar plasma LDL cholesterol concentration to the control. Chicks fed margarine 2 and barley oil 2 had lower ($P < 0.05$) LDL cholesterol levels than the chicks fed margarine 1 but not those fed barley oil 1. No treatment difference in levels of plasma total cholesterol, HDL cholesterol and triacylglycerol was observed.

No difference in body weight was observed between chicks fed barley oil 1, margarine 2 and barley oil 2 but they were lower ($P < 0.05$) than the control. Chicks fed margarine 1 had lower ($P < 0.05$) body weight than the control but higher ($P < 0.05$) body weight than the other three treatment groups.

Table 18. Plasma lipid concentration and growth performance of Leghorn chicks fed barley oil diets with two prefeeding conditions^{1,2}

Treatment ⁶	Plasma Cholesterol mmol/L			Plasma TAG ³	Body wt ⁴	ADI ⁵	Feed/Gain
	Total	LDL	HDL	mmol/L	g	g	g/g
Control	3.2±0.1	0.7±0.1c	2.2±0.1	0.6±0.1	315±11c	57±2c	5.3±0.1a
Margarine 1	3.4±0.2	0.5±0.1bc	2.6±0.1	0.6±0.1	249±19b	48±3bc	6.7±0.5ab
Barley oil 1	3.3±0.2	0.4±0.1ab	2.6±0.2	0.6±0.1	189±16a	32±3a	8.3±0.9b
Margarine 2	3.2±0.1	0.2±0.1a	2.7±0.2	0.7±0.1	198±22a	34±8ab	8.4±1.7b
Barley oil 2	3.0±0.2	0.2±0.1a	2.6±0.2	0.6±0.1	189±37a	33±5a	8.9±1.3b

¹ Mean±SEM, n=6.

² Values in each column sharing a common letter are not different at P<0.05.

³ Triacylglycerol.

⁴ Final body weight.

⁵ Average daily intake.

⁶ Margarine 1 and margarine 2 were margarine diets following prefeeding diets 1 and 2; barley oil 1 and barley oil 2 were barley oil diets following prefeeding diets 1 and 2, respectively.

There were differences in average daily intake (ADI) and feed/gain ratio (FG) between the chicks fed barley oil 1, margarine 2 and barley oil 2. ADIs of those fed barley oil 1, margarine 2 and barley oil 2 were lower ($P < 0.05$) and FGs of these treatment groups were higher ($P < 0.05$) than control (Table 18). ADI of the chicks fed margarine 1 was neither different from the control nor those on margarine 2, but higher than barley oil diet groups. FG of the chicks on margarine 1 was not different from the other treatment groups.

Body weight of the chicks was correlated with plasma total cholesterol ($r = 0.64$, $P < 0.001$) and LDL cholesterol concentrations ($r = 0.60$, $P < 0.05$) after the chicks on prefeeding diets and with plasma LDL cholesterol ($r = 0.57$, $P < 0.001$) and HDL cholesterol ($r = -0.59$, $P < 0.001$) at the end of the test (Table 19). No correlation between body weight and plasma triacylglycerol concentration was observed.

Discussion

The barley oil diet had a hypocholesterolemic effect on plasma LDL cholesterol concentration compared to the corn-soybean meal control in white Leghorn chicks fed cholesterol-free diets. The reduction in plasma LDL cholesterol concentration in chicks fed barley oil diet may have been caused by detrimental components in the oil which interfere with metabolism and lead to growth retardation. Cholesterol is a component of cell membranes, and a precursor of bile acids, steroid hormones and vitamin D. Consequently, chicks fed a cholesterol-containing prefeeding diet grew faster than those fed the cholesterol-free diet. Growth retardation due to deficiency of cholesterol was

Table 19. Correlations between body weight and plasma lipid concentrations in leghorn chicks

Correlation with	Body weight		
	Initial (n=10)	Prefeeding (n=12)	Test trial (n=30)
Cholesterol			
Total	-0.40	0.64*	-0.23
LDL	0.42	0.60*	0.57***
HDL	-0.28	-0.40	-0.59****
Triacylglycerol	-0.27	0.29	-0.26

*, ***, **** were $P < 0.05$, $P < 0.001$, $P < 0.0001$, respectively.

more severe in chicks as dietary cholesterol concentration dropped from 15 g/kg to zero in the 21-day feeding trial.

Correlations between body weight and plasma cholesterol concentration varied depending on dietary cholesterol levels. When cholesterol was fed, these correlations became significant.

Barley oil had no hypocholesterolemic effect on white Leghorn chicks fed cholesterol-free diets. This result disagreed with a previous report (Wang et al., 1992), in which young broiler chicks were fed a barley oil diet with 5 g/kg cholesterol for 10 days and barley oil was hypocholesterolemic. In the present study young Leghorn chicks were fed a cholesterol free barley oil diet for 21 days and the barley oil was not hypocholesterolemic. A possible explanation was an interaction between dietary cholesterol and barley oil, in which barley oil may have reduced the absorption of dietary cholesterol. Dietary cholesterol can affect hypocholesterolemic responses to dietary treatments. Pectin has been shown to lower plasma cholesterol concentration in rats fed

diets containing 2 to 10 g/kg cholesterol (Kelly and Tsai, 1987; Chang and Johnson, 1976), but have no effect in rats fed pectin diets with no cholesterol (Schneeman et al., 1984; Kelly and Tsai, 1987). A similar phenomenon appears in studies with α -tocotrienol where a diet containing 0.01 g/kg α -tocotrienol combined with cholesterol derived from meat scrap (5 g/kg diet) reduced serum cholesterol concentrations in chicks. However, a diet with 5 g/kg α -tocotrienol and 5 g/kg supplemental cholesterol was not hypocholesterolemic in rats (Hirahara, 1987).

α -Tocotrienol as a component of barley oil appears to have no hypocholesterolemic effect in Leghorn chicks fed cholesterol-free diets. Even though great differences existed in α -tocotrienol concentrations, plasma cholesterol levels were not different between barley oil and margarine fed chicks. The chicks were fed cholesterol free diets in order to keep HMG-CoA reductase sensitive to the possible inhibition of α -tocotrienol. Dietary α -tocotrienol at a concentration of 10 ppm was reported to be effective in lowering cholesterol in white Leghorn chicks estimated by using non-fasting blood samples (Qureshi et al., 1986). The calculated α -tocotrienol concentration of the barley oil diet of the present study was 80 ppm, with no hypocholesterolemic effect observed in fasting blood samples. Differences in plasma cholesterol levels between fasting and non-fasting blood samples were observed in Mongolian gerbils (DiFrancesco et al., 1990). If a great difference in plasma lipid level existed between the fasting and non-fasting status in these young Leghorn chicks, the lack of hypocholesterolemic effect from α -tocotrienol may be partially explained. In addition, since α -tocotrienol was fed to chicks as a component

of barley oil, other oil components having counteracting effects may need to be considered.

Conclusion

Barley oil was hypocholesterolemic in cholesterol fed chicks. Oil extracted from barley grain contained 800 mg/kg α -tocotrienol and had no hypocholesterolemic effect in Leghorn chicks fed cholesterol-free diets compared to margarine made from soybean oil.

CHAPTER 6
PLASMA LIPID CONCENTRATIONS OF HAMSTERS FED DIFFERENT LEVELS
OF BARLEY SOLUBLE FIBER AND OIL

Introduction

Barley has a hypocholesterolemic effect in humans (Newman et al., 1989; McIntosh et al., 1991). A barley component responsible for some of the lowering of cholesterol in animals is β -glucans (Fadel et al., 1987; Klopfenstein and Hosney, 1987). β -Glucans and arabinoxylans are two major fiber components in barley grain, with concentrations 20-100 g/kg (Newman and Newman, 1992) and 44-78 g/kg (Henry, 1988), respectively. β -glucans are divided into water soluble and insoluble fractions with a ratio of soluble/insoluble about 1:2. Arabinoxylans in barley grain are mostly water insoluble with only about 20% extractable in water at 100°C (Ahluwalia and Fry, 1986). A soluble fiber concentration was reported to be 44.5-78.5 g/kg in hull-less barley (Newman and Newman, 1991) where water soluble β -glucans and water soluble arabinoxylans would be the main components. Some soluble dietary fibers such as pectin and oat gum have been reported to lower plasma cholesterol (Fernandez et al., 1990; Wood et al., 1989). Soluble fiber from barley grain may have similar properties.

Barley oil as a petroleum ether extracted fraction of brewers' grain has been reported

to have a hypocholesterolemic effect in chicks (Burger et al., 1984) and pigs (Qureshi et al., 1987). α -Tocotrienol, an isomer of α -tocopherol, has been reported to be hypocholesterolemic and functions by inhibiting the activity of HMG-CoA reductase in the liver of chicks (Qureshi et al., 1986). Barley oil may also have a hypocholesterolemic property because it has a high α -tocotrienol concentration.

Hamsters have become a popular animal model in studies of lipid metabolism because they may have similar cholesterol metabolism to that of humans (Singhal et al., 1983). Similarities are mainly in anatomical structure for lipid digestion and absorption, sites of cholesterol biosynthesis, plasma total cholesterol concentration (Behr et al. 1963) and feedback inhibition of HMG-CoA reductase activity by dietary cholesterol (Singhal et al., 1983).

The objective of this study was to examine the possible hypocholesterolemic effects of barley soluble fiber and oil and their interaction in hamsters as a test animal.

Materials and Methods

Diet Preparation and the Test Diets

Azhul, a waxy hull-less barley, was ground in a laboratory mill (Meadows Mill Company, North Wilkesboro, NC) with particle size 20 mesh. Defatted ground barley was obtained by extracting the oil with hexane (1 kg/2 L) at 45 °C for 1 h with the extraction repeated three times. The extract was filtered through a glass fiber filter and vacuum evaporated to recover barley oil. Oil was nitrogen blanketed and stored at 4°C until use. De-soluble-fibered barley was produced by incubating ground barley with an

enzyme mixture composed of 4000 unit/ml β -glucanase and 2000 unit/mL of arabinoxylanase (Finnfeeds International Ltd, Surrey, UK) at pH 4.7 for 10 hour in a dialysis bag and drying at 60 °C. Defatted barley which was incubated with enzymes and then dried was termed defatted and de-soluble-fibered barley.

Six test diets are listed in Table 20. All diets were balanced to be iso-nitrogenous and to have the same concentration of oil (97 g/kg), cholesterol (3 g/kg) and insoluble fiber(110 g/kg). Table 21 contains some analytical data of these diets and the barley materials used.

Feeding Trial and Sample Collection

Golden Syrian hamsters (SASCO Inc., P. O. Box 66 DTS, Omaha, NE 68101) aged 21-days were fed the control diet composed of corn starch, casein with peanut oil (97 g/kg) and cholesterol (3 g/kg) for 10 d and then were randomly assigned to five diet treatments with 15 animals each. Hamsters were individually housed and fed diets ad libitum for 21 d. Feed consumption was recorded weekly and body weights recorded at the beginning and end of the test period. Hamsters were anesthetized with CO₂ and blood samples (3 mL) taken by heart puncture on day 21 after a 12 h overnight fast. Fecal samples were collected on d 19 and d 20, freeze dried, ground and stored at -20°C until analysis.

This experiment was approved by Montana State University's Animal Care Committee.

Table 20. Diet composition

Ingredient	Diet (g/kg) ¹					
	Corn starch	Barley oil	Ground barley ¹			
			Whole	-SF	-Oil	-SF-Oil
Barley	----	----	60.00	----	----	----
Barley-SF	----	----	----	60.00	----	----
Barley-Oil	----	----	----	----	60.00	----
Barley-SF-Oil	----	----	----	----	----	60.00
Corn starch ²	54.19	54.19	15.15	9.89	14.28	8.50
Casein	19.97	19.97	11.46	11.46	11.46	11.47
Cellulose ²	11.06	11.06	----	5.68	----	6.01
Peanut oil (PO) ³	9.70	----	8.26	8.08	8.89	8.87
Barley oil (BO)	----	9.70	----	----	----	----
Lysine	----	----	0.12	0.14	0.11	0.12
Arginine	0.08	0.08	0.01	0.03	0.01	0.01
Cholesterol	3.00	3.00	3.00	3.00	3.00	3.00
Choline bitartrate	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin mix ²	10.00	10.00	10.00	10.00	10.00	10.00
Mineral mix ²	35.00	35.00	35.00	35.00	35.00	35.00

¹ -SF: de-soluble-fibered, -Oil: defatted and -SF-Oil: defatted and de-soluble-fibered, respectively.

² ICN Biochemical Inc., Cleveland OH 44128.

³ Product of Planters Lifesavers Company, Winston-Salem NC 27102 USA.

Chemical and Statistical Analysis

Test diets were analyzed for content of soluble and insoluble dietary fiber (Prosky et al., 1988), protein (Nx6.25), lipid ether extract and ash (AOAC, 1980). Plasma total, HDL cholesterol and triglyceride concentration were determined using a Kodak DT 60 blood analyzer. LDL cholesterol concentration was calculated (Friedwald et al., 1972), fecal lipid content was analyzed (Anonymous, 1971), and viscosity of the diets was determined (Greenberg and Whitmore, 1974).

Data were analyzed by one way analysis of variance and multiple comparison among means using MSUSTAT (Lund, 1987).

Results

All six diets had similar protein, lipid and total fiber concentration, but different soluble fiber, insoluble fiber, barley oil concentration (Table 21). Soluble fiber concentration in the barley diet and defatted barley diet were 47 g/kg and 44 g/kg, respectively and the concentration of soluble fiber was reduced to 3 to 6 g/kg in the other four diets. Barley oil concentration was 8 g/kg in the defatted barley diet and defatted and de-soluble fiber barley diets, and was increased to 14 g/kg in the de-soluble fiber barley and doubled to 16 g/kg in the barley diet. The barley oil diet had 97 g/kg barley oil. Viscosity measurements of the barley diet and defatted barley diet were higher ($P < 0.05$) than other diets (Table 23). Peanut oil in the control diet contained no tocotrienols, whereas barley oil contained 1691 mg/kg α -tocotrienol, 231 mg/kg γ -tocotrienol and 51 mg/kg δ -tocotrienol (Table 22).

Table 21. Analytical data of barley and diets¹

	Protein g/kg	Lipids g/kg	Ash g/kg	Fiber (g/kg)			Barley oil g/kg
				Soluble	Insoluble	Total	
<u>Ground barley</u>							
Whole	128	24	30	87	97	184	24
-SF	132	27	22	10	79	89	27
-Oil	4	14	28	87	96	183	14
-SF-Oil	127	14	21	8	79	87	14
<u>Diet²</u>							
Corn starch	189	103	32	6	104	110	0
Barley oil	191	99	29	3	102	105	97
Barley	186	104	41	47	58	105	14
Barley-SF	189	105	46	6	102	108	16
Barley-Oil	188	98	41	44	61	105	8
Barley-SF-Oil	187	101	46	6	105	111	8

¹ Mean of two determinations.

² -SF: de-soluble-fibered, -Oil: defatted, -SF-Oil: de-soluble-fibered and defatted.

Table 22. Tocotrienol (T3) and tocopherol (T) concentrations of barley and peanut oil¹

Fraction	Barley oil	Peanut oil
	----- mg/kg -----	
α -T3	1691 \pm 85	nd ²
β -T3	nd	nd
γ -T3	231 \pm 15	nd
δ -T3	51 \pm 3	nd
α -T	385 \pm 18	227 \pm 4
β -T	20 \pm 2	8 \pm 0
γ -T	312 \pm 18	160 \pm 3
δ -T	7 \pm 1	15 \pm 0

¹ Mean \pm SD of three determinations.

² not detected.

Plasma total cholesterol concentration was different ($P < 0.0001$) between treatments (Table 23). Hamsters fed the barley diet and the defatted barley diet had lower ($P < 0.05$) total cholesterol levels than the control while hamsters fed barley oil diet and other diets were not different from the control. Treatments differed significantly ($P < 0.0001$) in plasma LDL cholesterol concentration (Table 23). Hamsters fed the barley diet had lower ($P < 0.05$) LDL cholesterol concentration than the control. Hamsters fed barley oil diet had higher ($P < 0.05$) LDL cholesterol levels than the control. LDL cholesterol levels of the other treatments were not different from the control. Fecal lipid concentration of the hamsters fed the barley oil diet and the four barley diets were higher ($P < 0.05$) than the control, with those fed the barley diet and defatted barley diet being the highest.

Viscosity of the barley diet was the highest ($P < 0.05$) of all diets with that of defatted

Table 23. Plasma lipid concentration of hamsters fed barley diets with different fiber and oil levels¹

Diet Group ³	Plasma cholesterol (mmol/L)			Plasma TAG ² mmol/L	Diet Viscosity Centipoise	Fecal lipids g/kg
	Total	LDL	HDL			
Corn starch	7.94±0.31 b	1.34±0.26 b	4.96±0.13	3.43±0.55	1.0±0.0a	15±1 a
Barley oil	8.09±0.23 b	2.04±0.28 c	4.53±0.13	3.28±0.37	1.0±0.0a	40±1 c
Barley	6.78±0.15 a	0.52±0.16 a	4.71±0.16	3.45±0.37	1.8±0.0c	63±3 e
Barley-SF	7.81±0.21 b	0.96±0.16 ab	5.02±0.16	3.98±0.42	1.0±0.0a	31±1 b
Barley-Oil	7.09±0.15 a	0.78±0.21 ab	4.60±0.18	3.71±0.34	1.5±0.0b	58±2 d
Barley-SF-Oil	7.86±0.18 b	1.42±0.26 bc	4.73±0.18	3.70±0.33	1.0±0.0a	30±1 b

¹ Mean±SEM, n=15 and values in each column sharing a common letter are not different at p<0.05.

² Triacylglycerol.

³ Barley-SF: de-soluble-fibered barley diet, Barley-Oil: defatted barley diet, Barley-SF-Oil: de-soluble-fibered and defatted barley.

Table 24. Growth performance of hamsters fed barley diets with different fiber and oil levels¹

Diet Group ⁴	Body wt ²	ADI ³	Feed/gain
	g	g	
Control	115±2.7a	8.5±0.22ab	9.5±1.06b
Barley oil	114±2.7a	8.1±0.22a	12.2±1.06c
Barley	129±2.9b	9.1±0.24bc	5.9±1.13a
Barley-SF	128±2.9b	9.3±0.24bc	6.6±1.13ab
Barley-Oil	130±2.9b	9.3±0.24bc	5.9±1.13a
Barley-SF-Oil	129±2.9b	9.5±0.24c	6.3±1.13ab

¹ Mean±SEM, n=15 and values in each column sharing a common letter are not different at p<0.05.

² Final body weight of the hamsters with 97±1 g as initial group weight.

³ Average daily intake.

⁴ -SF: de-soluble-fibered, -Oil: defatted, -SF-Oil: de-soluble-fibered and defatted.

barley diet being higher than the remaining four diets (Table 23).

Differences (P<0.001) between treatments were found in final body weight, average daily intake and feed/gain ratio (Table 24). Average daily intake of hamsters fed the diet with defatted and de-soluble fibered barley diet were higher (P<0.05) than the control while barley oil group and other barley diet treatments did not differ in ADI from the control. Feed/Gain ratio of hamsters fed the barley oil diet was higher (P<0.05) than the control while the hamsters fed the barley diet and the defatted barley diet had lower (P<0.05) FG than the control. Final body weight of the barley oil group was similar to the control while other barley diet groups had higher (P<0.05) final body weight than the control.

Discussion

Soluble fiber was the component responsible for the hypocholesterolemic effects of barley grain. When this fiber concentration was reduced more than seven fold from the barley diet and the defatted barley diet to the de-soluble fibered barley diet and the de-soluble fibered and defatted barley diet, barley in the latter two diets lost its hypocholesterolemic activity. This phenomenon was clearly demonstrated in plasma total cholesterol concentration. These results confirmed other studies that soluble fiber has hypocholesterolemic effects. However, barley soluble fiber had no effect on HDL cholesterol and triacylglycerol concentrations in the hamsters.

Woodward et al. (1983) reported that the aqueous solution of barley soluble β -glucans was viscous. High intestinal viscosity was reported in chickens fed barley (Burnett, 1966) and rats fed some other soluble dietary fibers (Ikegami et al., 1990). High viscosity in small intestinal contents may cause reduction in plasma total cholesterol concentration by reducing lipid absorption and increasing fecal lipid excretion. In this study, high viscosity in the barley diet and the defatted barley diet fed to the hamsters associated with high fecal lipid concentrations appear to support the above hypothesis.

β -Glucans were responsible for hypocholesterolemic effect of barley diets in hamsters. Supplementation of β -glucanase to barley diets and fed to rats (Mori, 1990) have been reported to have no effect on plasma cholesterol concentration while positive results have been observed in chicken models with β -glucanase supplemented to barley diets (Fadel et al., 1987; Newman et al., 1991). A possible explanation is the inactivation of β -

glucanase by the low pH condition in the stomach of rats. The optimum pH value for a bacterial source of β -glucanase is 4.6 (McCleary and Glennie-Holmes, 1985). Rats have stomachs where the pH value is about 1.5. When β -glucanase reaches the stomach it is probably inactivated, thus cannot degrade the β -glucans. Chickens have a crop which temporarily stores food where the pH is 4 to 6 (Champ et al., 1981). In that environment, the enzyme may extensively degrade the β -glucans before entering the small intestine. Hamsters have a similar gastrointestinal structure as rats and swine, however with proventriculus fermentation. In this study, the pre-treatment of ground barley with β -glucanase and arabinoxylanase, prior to feeding to the hamsters eliminated the hypocholesterolemic activity of the barley diet. Since the enzyme mixture contained both β -glucanase and arabinoxylanase, the possible function of arabinoxylans in lowering plasma cholesterol should not be excluded.

Barley oil had no hypocholesterolemic effect in hamsters in the present study. The barley oil diet contained barley oil six fold greater than that of the barley diet in order to demonstrate a hypocholesterolemic effect, but even at this level, no effect was observed in spite of the high α -tocotrienol concentration. We concluded that barley oil had no hypocholesterolemic property compared to peanut oil under these experimental conditions. However, our results have been modified by many factors such as animal model, types of control lipids, dietary cholesterol levels and even length of fast before taking blood samples. Additional research in this area is warranted.

High FG in the hamsters fed barley oil diet indicates that barley oil contained factors detrimental to utilization of nutrients in the diet and thus caused a higher lipid fecal

excretion than the control.

Conclusion

Soluble fiber containing β -glucans and arabinoxylans was the component responsible for the hypocholesterolemic effect of barley grain in the hamsters. Barley oil either as an integrated part of the grain or as a supplementation fed to hamsters had no effect in lowering plasma cholesterol. No interactive effect between barley soluble fiber and oil on plasma cholesterol and triacylglycerol concentrations was observed in this study. α -Tocotrienol fed to the hamsters as part of barley oil showed no effect on plasma cholesterol concentration.

CHAPTER 7

BARLEY DIETS INCREASE INTESTINAL VISCOSITY AND DEPRESS PLASMA
CHOLESTEROL IN CHICKSIntroduction

The hypocholesterolemic effect of soluble dietary fiber is well established (Miettinen and Tarpila, 1989; Davidson et al., 1991). Guar gum, a soluble fiber source has been reported to bind lipids (Gallaher and Schneeman, 1986), and to delay and reduce the absorption of cholesterol and lipids in rats (Ebihara and Schneeman, 1989; Ikeda et al., 1989). A high viscosity of the intestinal content of rats fed guar gum, gumxanthan (Ikegami et al., 1990) and methylcellulose (Topping et al., 1988) has been found. Viscosity may be a physical condition mediating the physiological effects attributed to soluble fiber. Experimental data confirming this hypothesis is rather limited.

β -Glucans are homopolysaccharides composed of glucopyranosyl units with (1 \rightarrow 3) and (1 \rightarrow 4) linkages in a ratio of approximately 1:2.5 (Staudte et al., 1983). Based on their extractability in water, β -glucans may be divided into soluble and insoluble fractions. An aqueous solution of β -glucans extracted from barley flour is viscous with the degree of viscosity attributed to molecular weight and concentration of β -glucans (Bengtsson et al., 1990). Monogastric animals including humans and birds cannot synthesize β -

glucanase. The amount of β -glucanase derived from barley grain and bacteria in the gastrointestinal tract is insufficient to completely hydrolyze β -glucans (Champ et al., 1981). Thus, β -glucans in barley diets are suspected of creating a viscous environment in the chick digestive tract (Fadel et al., 1987), causing poor absorption of dietary nutrients and reduction of growth rate. Viscosity in the digestive tract may also play a vital role in the hypocholesterolemic function of barley. Rats fed guar gum excreted more fecal steroids and bile acids than rats fed insoluble fiber (Miettinen, 1987). Increased excretion of steroids and bile acids may reduce blood cholesterol by drawing on the cholesterol pool to synthesize replacement biliary compounds.

Cholesterol metabolism of chickens is similar to humans in that liver is the major site of cholesterol synthesis (Leveille et al., 1975; Shrago et al., 1971), that 3-hydroxy-3-methylglutaryl-CoA reductase is the rate limiting enzyme, and that there is feedback inhibition by dietary cholesterol (Ramirez et al., 1984). Both species have gallbladders to store bile acids synthesized in the liver cells from cholesterol. Similarity has also been reported in morphology of LDL particles and chemical composition of HDL between chickens and humans (Chapman, 1980). Roosters are similar to humans in the lack of very low density lipoprotein (VLDL) (Fried et al., 1968) with most triacylglycerol of plasma being found in VLDL (Chapman, 1980). In addition, barley diets are reported to have hypocholesterolemic effects in humans and male broiler chicks (Newman et al., 1991; 1989). For these reasons male broiler chickens were used as the animal model in this study.

The objectives of this study were to determine the viscosity of the small intestinal

content of broiler chicks fed corn or barley diets supplemented with and without β -glucanase and the relationships of viscosity to plasma cholesterol concentration and growth performance.

Materials and Methods

Feeding Trial

Three test diets were prepared: a corn-soybean meal diet (corn) and two barley diets with (barley+ENZ) or without supplemental β -glucanase (barley). The activity of the feed grade β -glucanase (Enzyme Development Div., Bidde Sawyer Corp., 2 Penn Plaza, NY) was 200 units/g and was added at a level of 1 g/kg diet. These diets contained cholesterol (4 g/kg) and chromic oxide (2.5 g/kg) as an indigestible indicator for calculation of apparent digestibility of lipids and protein. The corn was obtained from a local feed mill and the barley was Azhul, a six-rowed waxy hull-less cultivar. Barley was ground and autoclaved at 120°C, 1.5 kg/cm² for 40 min to destroy the activity of endogenous β -glucanase and dried before mixing with other diet ingredients (Table 25). All diets were balanced to be iso-caloric and iso-nitrogenous.

Ninety six one-day-old male broiler chicks (Fors Farm, Puyallup WA) were fed the corn-soybean diet (less the chromic oxide) with 4 g/kg cholesterol for 14 d to increase body weights and blood cholesterol levels. The chicks were then randomly allotted to one of the three test diets, corn, barley+ENZ or the barley diet, with 4 replicates of 8 chicks each. The chicks in each replicate were housed in battery brooder cages (0.5 m x 1.0 m x 0.3 m) and fed ad libitum with free access to water.

Table 25. Diet composition¹

Ingredients	Diet Treatment		
	Corn	Barley+ENZ ²	Barley
		<i>g/kg</i>	
Corn	452.3	----	----
Soybean meal	426.4	----	----
Ground barley	----	698.3	698.3
Soybean protein ³	----	179.4	179.4
Supplement ⁴	62.8	62.8	62.8
Corn oil ⁵	50.0	50.0	50.0
β -Glucanase	----	1.0	----
Casein	----	----	1.0
Chromic oxide	2.5	2.5	2.5
Methionine	2.0	2.0	2.0
Cholesterol	4.0	4.0	4.0

¹ All diets are calculated to be iso-caloric (3310 Kcal/kg) and iso-nitrogenous (37 g/kg).

² Barley+ENZ is a barley diet with 0.1% β -glucanase.

³ Protein content: 92 % (dry matter basis); ICN Biochemicals, Cleveland OH.

⁴ Contribution to the diets (g/kg diet): $\text{Ca}_3(\text{PO}_4)_2$, 28; CaCO_3 , 15; vitamin diet fortification mixture (ICN Biochemicals, Cleveland OH.), 10; trace mineral premix (ConAgra-Westfeeds, Billings, MT; content: Zn, 20%; Fe, 10%; Mg, 5.5%; Cu, 1%; I, 0.15%; and Se, 0.02%), 1.5; choline chloride, 2.0; DL-methionine, 1.25; biotin, 0.1. Composition of the vitamin diet fortification mixture (g/kg of the mixture): vitamin A acetate, 1.8; vitamin D concentrate, 0.125; α -tocopherol, 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; biotin, 0.020; folic acid, 0.090; vitamin B-12, 0.00135.

⁵ Mazola, CPC International Inc., Englewood Cliffs, NJ 07632.

This research was approved by Montana State University's Animal Care Committee.

Sample Collection

Chicks were weighed before and after they were fed the test diets for eight d. Feed

consumption was recorded for each cage on a daily basis. One mL blood sample was collected from the brachial vein of each chick after a 12 h fast on d 9. The chicks continued to be fed the diets. Excreta samples were taken on d 12 for 1 h from all cages. The chicks were killed by carbon dioxide inhalation on d 13 and d 14. To reduce variation in individual feed ingestion and composition of fresh digesta in the gastrointestinal tract, the following regime was devised. The chicks were fasted overnight, then allowed free access to the diets for 1 h followed by a 2 h fast prior to killing. The digestive tract was removed and the whole digesta from the small intestine and large intestine (colon and cecum) were collected separately. Samples from each of these segments were pooled from 4 chicks. The digesta and excreta samples were weighed and frozen in liquid nitrogen upon collection. The samples were then lyophilized and stored at -20°C for later analysis. A portion of the fresh digesta from the small intestine was retained for viscosity measurements.

Viscosity and Cholesterol Determination

Approximately 0.5 g fresh small intestinal digesta was taken from each of four chicks of the same cage to obtain a pooled sample of 2.0 g. The 2.0 g of fresh digesta was diluted with 18 mL cold (3°C) HCl-KCl buffer (0.2 M, pH 1.5), vigorously mixed by use of a vortex blender for 30 sec and allowed to precipitate for 1 min. The top 16 mL solution was then transferred into the UL adaptor of a synchro-lectric viscometer (Brookfield Engineering Laboratories, Inc. Stoughton MA). The viscometer was calibrated by viscosity standard purchased from the manufacturer at 73.4/sec shear rate, 20°C and the same condition was used in sample determination.

Viscosity of diets and excreta was determined as described by Greenberg and Whitmore (1974). Excreta samples were first autoclaved at 120°C, 1.5 kg/cm² for 40 min prior to viscosity determination.

Concentration of plasma total and HDL cholesterol and triacylglycerol of the blood samples were determined using a DT 60 Kodak blood analyzer (Eastman Kodak Company, 255 East Avenue, Rochester NY). Plasma LDL cholesterol concentration was calculated as described by Friedwald et al. (1972).

Chemical Analyses

Test diets, excreta and digesta were analyzed for soluble and insoluble β -glucans (McCleary and Glennie-Holmes 1985). The diets and excreta were also analyzed for protein (Kjeldahl N x 6.25), fat (AOAC 1980), dietary fiber (Prosky et al. 1988) and chromium content (Fenton and Fenton 1979). Apparent protein and fat digestibilities were calculated using an indicator method (Church and Pond 1982).

All data were analyzed by analysis of variance using the General Linear Models procedure and the treatment means were compared using multiple comparison (SAS 1985). Pearson's correlation coefficients were calculated for digesta viscosity and excreta lipids with plasma cholesterol, rate of growth, protein and lipid digestibility (SAS 1985).

Results

Concentration of dietary fiber and β -glucans in the diets are listed in Table 26. The barley diet had much higher viscosity (3.1 cp) than the control diet (1.0 cp). Plasma

Table 26. Dietary fiber, β -glucan concentration and viscosity of the chick diets¹

Diet	Dietary Fiber (g/kg)		β -Glucans (g/kg)		Viscosity cP ³
	Soluble	Total	Soluble	Total	
Corn	15.3	129.6	0.2	0.5	1.0
Barley+ENZ ³	75.2	142.2	19.4	67.5	---
Barley	80.0	153.3	17.2	69.7	3.1

¹ Means of two replicate samples.

² Centipoise.

³ Barley diet with supplemental β -glucanase.

total cholesterol and LDL cholesterol concentrations were different ($P < 0.05$) among diet treatments (Table 27). Chicks fed the corn diet had highest total (4.34 mmol/L) and LDL cholesterol (2.04 mmol/L) levels. Chicks fed the barley diet without β -glucanase (barley) showed the lowest total cholesterol (3.15 mmol/L) and LDL cholesterol (0.67 mmol/L) levels. Chicks fed the barley+ENZ diet had higher ($P < 0.05$) total and LDL cholesterol compared to those fed the barley diet, but were lower ($P < 0.05$) in these parameters than those of the chicks fed the corn control diet. Plasma HDL cholesterol concentration was higher ($P < 0.05$) in chicks fed the Barley+ENZ than chicks fed the corn diet. Triacylglycerol concentrations were not different among treatments.

Viscosity of small intestinal digesta was significantly different ($P < 0.01$) among diet treatments, with the lowest in the chicks fed corn diet and highest in chicks fed the barley diet (Table 28). Supplementation of β -glucanase lowered ($P < 0.01$) viscosity of the intestinal digesta in chicks fed barley. Excreta was more ($P < 0.05$) viscous in chicks fed the barley diet compared to chicks fed corn, and those fed Barley+ENZ were

Table 27. Plasma lipid concentrations of chicks fed corn and barley diets with and without supplemental β -glucanase

Plasma lipids	Diet Treatment ^{1,2}		
	Corn	Barley+ENZ ³	Barley
Total cholesterol mmol/L	4.34±0.16c	3.72±0.08b	3.15±0.08a
LDL cholesterol mmol/L	2.04±0.13c	1.06±0.05b	0.67±0.05a
HDL cholesterol mmol/L	2.02±0.10a	2.33±0.05b	2.07±0.10ab
Triacylglycerol mmol/L	0.65±0.03	0.79±0.02	0.83±0.03

¹ Means±SEM, n=32.

² Values in each row sharing a common letter are not different at p<0.05.

³ Barley diet with supplemental β -glucanase.

Table 28. Growth performance, apparent digestibility of protein and lipids, viscosity of small intestinal digesta and excreta of chicks fed corn and barley diets with and without supplemental β -glucanase

Item	Diet treatment ¹		
	Corn	Barley+ENZ ³	Barley
Average daily gain g	19.1±0.7c	14.1±1.2b	8.6±0.9a
Average daily intake g	34.1±0.9	31.3±0.8	29.2±2.5
Feed/gain	1.8±0.0a	2.2±0.1a	3.4±0.2b
Protein digestibility %	53.1±0.5c	48.3±1.9ab	44.4±2.2a
Lipid digestibility %	92.9±0.3c	88.9±0.7b	76.1±1.1a
Intestinal viscosity cP ²	1.7±0.1a	2.0±0.1b	2.4±0.1c
Excreta viscosity cP ²	1.0±0.1a	1.3±0.1ab	1.6±0.2b
Excreta lipids g/kg	13.6±0.6a	21.4±1.3b	33.1±1.0c

¹ Means±SEM, n=8 per diet for viscosity and n=4 per diet for other items. Values in each row sharing a common letters are not different at p<0.01.

² Centipoise.

³ Barley diet with supplemental β -glucanase.

intermediate (Table 28). Viscosity of small intestinal digesta and excreta lipid content were positively correlated ($P < 0.0001$). Both were negatively correlated ($P < 0.01$) with average daily gain, digestibility of protein and lipids, plasma total and LDL cholesterol concentration (Table 29).

Table 29. Correlations of viscosity of small intestinal digesta and excreta lipid content with average daily gain, plasma cholesterol concentration and digestibility of protein and lipids of chicks fed corn and barley diets with and without supplemental β -glucanase

	Correlation Coefficients ¹	
	Viscosity cP ²	Excreta lipids g/kg dry excreta
Plasma total cholesterol	-0.89	-0.92
Plasma LDL cholesterol	-0.93	-0.86
Plasma HDL cholesterol	0.21	-0.04
Average daily gain	-0.91	-0.93
Protein digestibility	-0.83	-0.82
Lipid digestibility	-0.89	-0.98
Excreta lipids, g/kg dry excreta	0.91	---

¹ N=12, all values are significant at $P < 0.01$ except for correlations with plasma HDL cholesterol.

² Centipoise.

Initial chick body weight averaged 313 ± 2 g for replication groups. The average daily body weight gain (ADG) of chicks differed ($p < 0.01$) between diet treatments (Table 28). Chicks fed the corn diet had highest ($P < 0.01$) ADG, those fed the barley diet had the lowest ADG, and those fed Barley+ENZ were intermediate. Addition of β -glucanase to the barley diet produced higher ($P < 0.01$) ADG, from 8.6 g/d to 14.1 g/d. Faster

gains thus improved the feed/gain ratio from 3.4 to 2.2 since feed intakes were not different ($P=0.08$). Apparent digestibilities of protein and lipids were lowest ($P < 0.01$) in chicks fed the barley diet (Table 28). Supplementation of β -glucanase improved ($P < 0.01$) apparent digestibility of lipids and the same trend has been seen in protein digestibility. Insoluble β -glucan concentration of small intestinal digesta was lower ($P < 0.05$) in the chicks fed Barley+ENZ than that of chicks fed the barley diet (Table 30). Differences were not significant in the concentration of insoluble β -glucans in large intestinal digesta or excreta between the two barley diet treatments. The soluble β -glucan concentration of small and large intestinal digesta and excreta was higher ($P < 0.05$) in chicks fed Barley+ENZ compared to those fed the barley diet. Both soluble and insoluble β -glucan concentrations of small and large intestinal digesta and excreta from chicks fed the corn diet were very low compared to those of the chicks fed barley diets.

Discussion

Chicks fed viscous barley diets had more viscous small intestinal contents. β -Glucans in the barley diets were responsible for the high viscosity in the chick small intestine. Since β -glucans only accounted for about one fourth of the soluble fiber in the barley diets, other soluble fiber components such as arabinoxylans may also contribute to the viscosity. However, it can be assumed that β -glucanase added to the barley diets cleaved the molecule sufficiently to affect small intestinal viscosity. β -Glucanase as a diet ingredient has been shown to retain partial activity in the digestive tracts of chicks, with activity decreasing as ingesta passes down the digestive tract (Edney et al. 1986).

Table 30. Soluble and insoluble β -glucan concentration in small and large intestinal digesta and excreta of chicks fed corn and barley diets with and without supplemental β -glucanase

Diet group	β -Glucan Concentration ¹					
	Insoluble			Soluble		
	Small intestine	Large intestine	Excreta	Small intestine	Large intestine	Excreta
	g/kg dry matter					
Corn	1±0a	1±0a	1±0a	5±0a	1±0a	1±0a
Barley+ENZ ²	84±3b	44±5b	77±2b	64±3c	31±4b	58±3c
Barley	101±2c	56±9b	87±5b	23±3b	8±2a	30±3b

¹ Values were means±SEM, n=4 and values in each column sharing a common letter are not different at p<0.05.

² Barley diet with supplemental β -glucanase.

Low concentration of insoluble β -glucans in small intestinal digesta of chicks fed Barley+ENZ in this study may reflect the hydrolytic activity of β -glucanase in this area of the digestive tract. The high soluble β -glucan concentration in the small intestinal digesta of Barley+ENZ treatment compared to that of the barley diet treatment may reflect the presence of oligosaccharides derived from insoluble β -glucans as well as from other polysaccharides. The procedure of β -glucan determination used is based on the measurement of resulting glucose and cannot distinguish the oligosaccharides of one source from another.

Small intestinal viscosity appears to mediate the physiological effects of barley diets in the reduction of plasma cholesterol levels and growth performance in the chicks. The negative correlations between viscosity of small intestinal digesta and digestibility of lipids and protein indicated that a viscous environment in the small intestine reduced absorption of lipids, protein and possibly other dietary nutrients. Viscosity may act as a barrier preventing the contact of digestive enzymes with their substrates, thickening of the unstirred layer of the mucosa, and prevention of formation of micelles required for absorption of lipids.

Determination of viscosity in the small intestinal contents has been subjected to low accuracy because of great variation in the amount of feed intake and consequently volume of total fresh digesta among individual chicks. In this study, the fast-feed-fast regime was successful in reducing variation in feed intake and consequently viscosity measurements as indicated by the small standard error of the mean. Equal amounts of digesta were used and diluted with equal volumes of buffer to give the same

concentration of solution, where fresh digesta was considered as solute. Another main source of error in viscosity determination was the activity of β -glucanase in the sample. Results of viscosity determination in this study agreed with that of an earlier report that chicks fed barley diet without β -glucanase had more viscous intestinal content than that of chicks fed barley diet with the enzyme (Burnett 1966).

Conclusion

Chicks fed barley diets had a lower growth rate and a lower plasma cholesterol concentration associated with higher intestinal viscosity than chicks fed a corn diet. The depressed growth appears to be a result of poor digestibility of dietary nutrients, such as lipids and protein. Degradation of barley β -glucans by β -glucanase appears to be responsible for the reduction of small intestinal viscosity. These data suggest that the viscosity created by β -glucans in the small intestine reduced digestibility of protein and lipids, and this may be one mechanism by which barley diets cause low growth rate and reduced plasma cholesterol concentrations in broiler chicks.

CHAPTER 8

SUMMARY

Barley grain of nine cultivars contained an average of 22 g/kg of oil, ranging from 16 to 31 g/kg. Total vitamin E concentration was 63 mg/kg, with a range of 48 to 92 mg/kg. The vitamin E components, α -, γ - and δ -tocotrienol, α -, β -, γ - and δ -tocopherol, were detected in barley oil. α -Tocotrienol accounted for 52% of total vitamin E (tocotrienols plus tocopherols). α -Tocotrienol (α -T3) concentration in barley grain averaged 33 mg/kg with a range of 23 to 53 mg/kg.

Cultivar variations in vitamin E, oil and α -T3 concentration were significant ($P < 0.02$), but environmental differences were not significant in the concentration of these nutrients. Cultivar variations were caused mainly by differences between hulled and hull-less cultivars. Hull-less barley had a higher concentration of oil, vitamin E and α -T3, with lower kernel weight.

No correlation was found between kernel weight and the concentration of vitamin E, oil and α -T3 of barley grain. A negative correlation ($r = -0.46$, $P = 0.03$) between kernel weight and α -T3 concentration of barley oil suggests that α -T3 has an active metabolic or structural function within cells instead of being a storage nutrient.

Pearling and milling processes concentrated oil, vitamin E and α -T3 in some process

fractions, with pearling being more effective than milling. Concentration of oil, vitamin E and vitamin E components including tocotrienols and tocopherols were compared between six milling fractions, two pearling fractions and whole barley grain in Azhul and Waxbar cultivars. The highest oil concentrations were 82 g/kg and 45 g/kg in pearling and milling fractions respectively. The highest total vitamin E concentrations were 205 mg/kg and 79 mg/kg in pearling and milling fractions and the highest α -T3 concentrations were 116 mg/kg and 50 mg/kg in pearling and milling fractions, respectively. The concentration of oil, vitamin E and α -T3 of whole grain were 28 g/kg, 71 mg/kg, and 43 mg/kg, respectively. A pearling flour fraction, accounting for 20% of kernel weight, contained 80% of the oil, 93% of total vitamin E and 61% of the α -T3 of barley grain, respectively. The concentrations of oil, vitamin E and α -T3 in this pearling flour fraction were 2.9, 2.9 and 2.7 times greater than those of whole barley grain. This pearling flour may have practical application in the food industry for its high vitamin E and α -T3 concentration. Based on the above results, both selection of cultivars and pearling of barley grain were effective in increasing concentrations of oil, vitamin E and α -tocotrienol in barley.

Composition of fatty acids (% of total) in the oil of barley cultivar Prowashonupana were palmitate (25.5%), stearate (1.5%), oleate (19.4%), linoleate (50.4%) and linolenate (3.4%). Data from nine barley cultivars revealed the presence of small amounts of myristic acid (0.3%) and ω -9-eicosenoic acid (0.7%) in barley oil (Wang et al., 1992b). Concentrations of fatty acids in the oil of Prowashonupana barley were 120 g/kg, 7 g/kg, 91 g/kg, 237 g/kg and 16 g/kg in palmitate, stearate, oleate, linoleate and

linolenate, respectively. α -Linolenate (ω -3-linolenate) was the predominate isomer, with a trace amount of γ -linolenate (ω -6-linolenate) detected.

The effects of barley oil on plasma cholesterol of chicks were inconsistent in two studies. Barley oil was hypocholesterolemic in male broiler chicks fed cholesterol containing diets, but not hypocholesterolemic in another study with male white Leghorn chicks fed cholesterol-free barley oil diets. Ten-day old male broiler chicks were fed a barley oil diet for 10 days with cholesterol (5 g/kg diet). Barley oil was hypocholesterolemic compared to a margarine diet. In another study, fourteen-day old male white Leghorn chicks were fed a cholesterol-free barley oil diet for 21 days. No effect of barley oil in lowering plasma cholesterol concentration was observed. The lack of agreement in results with barley oil may be caused by the interaction of dietary cholesterol with barley oil in which barley oil may reduce the absorption of cholesterol in the broiler chicks.

Barley oil had no hypocholesterolemic effect in hamsters fed a cholesterol containing diet when compared to peanut oil, which contained no tocotrienols. Lack of hypocholesterolemic effect of barley oil in the hamsters may suggest a species difference. Barley oil extracted from brewers' grain has been found to be hypocholesterolemic in chicks and humans (Robinson and Lupton, 1990; Burger et al., 1984). However, oil from barley grain showed lack of a clear cut hypocholesterolemic effect in chicks in the present study and no hypocholesterolemic effect in hamsters. α -Tocotrienol from brewers' grain was reported to have a hypocholesterolemic effect by inhibiting cholesterol biosynthesis in the liver (Qureshi et al., 1986). However, α -tocotrienol was

not hypocholesterolemic when fed to white Leghorn chicks and hamsters as an integral part of barley oil.

Soluble fiber of barley grain was hypocholesterolemic in hamsters. Total plasma cholesterol and LDL cholesterol concentrations of the hamsters fed high soluble fiber diets were lower ($P < 0.05$) than those fed low soluble fiber diets. β -Glucans consist of both soluble and insoluble fractions while soluble β -glucans are the major component of barley soluble fiber. β -Glucans are hypocholesterolemic in male broiler chicks. Chicks fed barley diets had lower plasma cholesterol concentrations than those fed a corn based diet. When the barley diet was supplemented with β -glucanase and fed to male broiler chicks, the hypocholesterolemic effect was partially lost. In these chicks, soluble β -glucans may be the component responsible for lowering cholesterol, as has been shown in studies with rats (Oakenfull et al., 1992; Klopfenstein and Hosoney, 1987).

Intestinal viscosity may mediate the effects of barley diets in lowering plasma cholesterol and growth retardation in chicks. Increased small intestinal viscosity was associated with low plasma cholesterol, low growth rate and low lipid digestibility in chicks fed a barley diet compared to those fed a barley diet with β -glucanase. High viscosity in the small intestine was negatively correlated with plasma total cholesterol and LDL cholesterol levels, average body weight gain, digestibility of lipids and protein and excreta lipid concentration. High viscosity in the small intestine may inhibit absorption of dietary lipids including cholesterol and some other nutrients.

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