Comparison of the Cholesterol-Lowering Properties of Whole Barley, Oat Bran, and Wheat Red Dog in Chicks and Rats

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ABSTRACT

Ground meal of two barley cultivars, Waxbar (WXB) and Prowashonupana (PWSNP), was compared with oat bran (OB) and wheat red dog (WRD) for their cholesterol-lowering effects in chicks and rats. Total β-glucan, total dietary fiber (TDF), and soluble dietary fiber were 16.9, 33.4, and 18.1% for PWSNP; 7.9, 15.0, and 8.3% for WXB; 6.6, 16.6, and 6.9 for OB; and 2.6, 35.0, and 4.1 for WRD. In both chicks and rats, OB and WXB demonstrated a significant ($P < 0.05$) lowering effect on total and low-density lipoprotein (LDL) cholesterol levels, but high-density lipoprotein cholesterol was not decreased. Compared with controls, the WRD diet increased serum cholesterol in chicks and had no effect on rats. PWSNP did not lower cholesterol levels in the rat experiment but did in the chick experiment ($P < 0.05$). In rats, a high fiber level of WXB and OB (10% TDF) was more effective than a lower level in reducing total and LDL cholesterol. Soluble β-glucan appeared to be the strongest predictor of the serum cholesterol response in both species. Barley grain can offer a high-fiber, cholesterol-lowering alternative to OB. Further research is required to clarify inconsistencies in lipid responses among animals fed different barley cultivars.

Cereal grains, noted for their dietary fiber content, have an important place in the well-balanced diet. Differentiation of water-soluble and insoluble fiber components has helped elucidate the physiological effects of fiber (Schneeman 1989). The soluble fractions appear to exert a hypocholesterolemic effect in humans and animals (Anderson et al 1990), whereas the insoluble fiber fractions generally do not exhibit this effect (Anderson and Tietyen-Clark 1986). Among the cereal grains, oats (Chen et al 1981) and barley (Newman et al 1989) appear to be the most effective in lowering serum cholesterol. Both grains have a high proportion of soluble dietary fiber (SDF), particularly β-glucans. Klopfenstein (1988) reviewed the role of β-glucans in nutrition and health.

Individual barley cultivars vary widely in contents of total and soluble β-glucans (Anderson et al 1978, Åman and Graham 1987), and differences have been reported in their physiological effects. For example, when Fadel et al (1987) fed two barley cultivars with similar β-glucan content to chicks, one was highly effective in lowering serum cholesterol, whereas the other had no effect. When β-glucanase was added to the diets, no hypocholesterolemic effect was found.

This experiment was designed to test the hypocholesterolemic property of four cereal products: oat bran (OB), wheat red dog (WRD), and ground meal of two barley cultivars, Waxbar (WXB) and Prowashonupana (PWSNP). Both of the barley cultivars previously had demonstrated serum cholesterol lowering properties when fed to chicks (R. K. Newman and C. W. Newman, *unpublished data*). This study was undertaken as a cooperative venture at Montana State University, where the chick model was used, and at Kansas State University, where identical raw materials were fed to rats.

MATERIALS AND METHODS

Source Preparation of Grains

Two barley cultivars were obtained from Rocky Mountain Grains, Inc., Bozeman, MT. PWSNP, a high-protein, waxy, short-awned, hull-less barley, was developed by the late R. F. Eslick at Montana State University. WXB, a commercially released waxy barley cultivar, was developed by Western Plant Breeders, Bozeman, MT. WRD (a mixture of wheat bran, germ, and flour) and OB (SCM 350) were obtained from ConAgra Milling Co., Omaha, NE. The barleys also were ground at that facility.

Before incorporation into the test diets, all cereal products were ground through a 0.159-cm hammermill screen (Fitz Mill, The FitzPatrick Co., Elmhurst, IL) to produce similar particle size in the products.

Chemical and Physical Analysis

Analyses for moisture, protein (N × 6.25) and fat (ether extract) were performed using AACC (1983) methods 44-15A, 46-16, and 30-20, respectively. Total dietary fiber (TDF) was measured using a TDF assay kit (Sigma Chemical Co., St. Louis, MO) based on a modification of the method of Prosky et al (1988). Insoluble dietary fiber was determined using AACC (1983) methods 32-20 and 32-21, and SDF was calculated by difference. The method of Åman and Graham (1987) was used for total and soluble β-glucans at Montana State University. Relative viscosity was measured using an acid extract according to Aastrup (1979). Results of the chemical and physical analyses are shown in Table I.

Chick-Feeding Experiment

Day-old chicks were obtained from H & N Hatchery, Redmond, WA, and fed an 18% protein corn and soybean meal diet for seven days to allow for growth and adjustment. For an additional six days, chicks were fed the same diet with 0.5% added cholesterol to increase serum cholesterol levels. Four randomly chosen chicks were fasted for 12 hr, and blood was drawn to determine initial serum cholesterol levels.

Experimental chick diet formulations are shown in Table II. All diets were calculated to contain approximately 20% protein,

TABLE I
Chemical Composition[a] and Physical Measurements of Barley, Oat Bran, and Wheat Red Dog

	Fraction			
Component	PWSNP[b] Barley	Waxbar Barley	Oat Bran	Wheat Red Dog
Protein, %	21.6	18.3	23.5	25.1
Ether extract, %	3.9	1.8	6.6	5.1
Ash, %	2.2	2.0	3.0	4.1
Dietary fiber, %[c]				
Total	33.4	15.0	16.6	35.0
Soluble	18.1	8.3	6.9	4.1
β-Glucan,[d] %				
Total	16.9	7.9	6.6	1.3
Soluble	6.6	4.7	3.8	0
Particle size, μm	630.0	591.0	516.0	196.0
Viscosity, cP	2.47	7.33	7.32	1.38

[a] Dry matter basis.
[b] Prowashonupana.
[c] Prosky et al (1988).
[d] Åman and Graham (1987).

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7% fat, and 11% TDF. Three replicate groups of 10 chicks received each diet treatment. Chicks were wing-banded with individual numbers, and body weights were recorded initially and at the conclusion of the trial. Feed consumed by each group was recorded, and feed-to-gain ratio was calculated for each cage group at the conclusion of the experiment.

TABLE II
Percent Composition of Chick Diets

Component[a]	PWSNP[b] Barley	Waxbar Barley	Oat Bran	Wheat Red Dog	Corn Control
	Diet				
PWSNP[c] barley	35.0	...	...	...	...
Waxbar[c] barley	...	35.0	...	...	...
Oat bran[c]	...	...	35.0	...	...
Wheat red dog[c]	...	...	...	35.0	...
Ground corn	...	...	...	...	69.4
Casein[d]	13.5	15.4	13.5	14.4	14.2
Cornstarch[d]	41.3	33.1	35.4	40.6	0.5
Cellulose[e]	0.2	6.5	6.1	...	5.9
Oil[f]	4.2	4.2	4.2	4.2	4.2
Protein (analyzed, %)	21.0	20.9	20.8	19.8	20.6
Fat (analyzed, %)	7.1	6.5	7.5	6.8	7.9
Calculated values, %					
Total dietary fiber	11.9	11.8	11.9	12.3	12.0
Insoluble dietary fiber	5.6	8.9	9.5	10.9	10.0
Soluble dietary fiber	6.3	2.9	2.4	1.4	2.0
Total β-glucans	6.1	2.9	2.2	0.5	0
Soluble β-glucans	2.3	1.7	1.3	0	0

[a]In addition, all diets contained 2.8% dicalcium phosphate, 1.5% limestone, 0.50% sodium chloride, 0.275% vitamin mix (ICN Nutritional Biochemicals, Cleveland, OH), 0.125% DL-methionine, 0.10% antibiotic (Terramycin, 110 g/kg), and 0.50% cholesterol (ash-free, Sigma Chemical Co., St. Louis MO).
[b]Prowashonupana.
[c]All cereal fiber sources were supplied by ConAgra, Inc., Omaha, NE.
[d]Technical grade, Sigma Chemical Co.
[e]Alphacel, ICN Nutritional Biochemicals.
[f]Oil, one half Mazola corn oil and one half Crisco oil.

Chicks were fed experimental diets for 10 days, after which they were fasted for 12 hr and blood was drawn from the brachial vein. After chicks were sacrificed by cervical dislocation, their livers were removed, immersed in liquid nitrogen, and then freeze-dried. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were determined as previously described (Fadel et al 1987).

The data were statistically evaluated by animal with analysis of variance using the General Linear Models procedure of the Statistical Analysis System, and correlation analysis by group was performed using Pearson correlation coefficients (SAS 1985).

Rat-Feeding Experiment

Ten groups of 10 male Wistar rats (Charles River Breeding Laboratory, Wilmington, MA) were fed low- or high-fiber diets containing the same cereal fractions, similarly ground as those used for the chick-feeding study (Table III). All diets were formulated to contain approximately 20% protein and 6% fat.

Animals were individually housed in an environmentally controlled room (22°C, 12-hr light-dark cycle), and diets and water were provided ad libitum. Mean weight of animals in each group was 159.1 ± 4.6 g (standard deviation) initially, and means for all groups were statistically the same. Animals were weighed weekly, feed consumption recorded, and feed efficiencies determined.

After six weeks on experimental diets, rats were lightly ether-anesthetized, and fasting (12 hr) blood samples were taken by cardiac puncture. Blood was allowed to clot at room temperature and then centrifuged at 12,000 × g at 5°C for 15 min. All serum samples were analyzed in duplicate for total and HDL cholesterol and triglycerides using reagents from Sigma (kits 352, 352-3, and 339, respectively). Animals were sacrificed by placing them in an ether atmosphere, and livers were quickly removed, rinsed in cold tap water, blotted dry, weighed, and stored frozen. Lipids were extracted from chloroform-methanol homogenates of the livers (Klopfenstein and Clegg 1980). Aliquots of the extracts were dried under nitrogen, redissolved in absolute ethanol, and assayed for cholesterol (Rosenthal et al 1957).

Data were statistically evaluated by animal by analysis of

TABLE III
Percent Composition of Rat Diets

Component[a]	Cellulose Control	Low-Fiber Diet: PWSNP[b] Barley	Low-Fiber Diet: Waxbar Barley	Low-Fiber Diet: Oat Bran	Low-Fiber Diet: Wheat Red Dog	High-Fiber Diet: PWSNP Barley	High-Fiber Diet: Waxbar Barley	High-Fiber Diet: Oat Bran	High-Fiber Diet: Wheat Red Dog
PWSNP[c] barley	...	20.0	...	...	...	29.1	..	...	...
Waxbar[c] barley	...	...	20.0	...	...	...	60.6	...	...
Oat bran[c]	...	...	...	20.0	...	...	...	50.7	...
Wheat red dog[c]	...	...	...	...	20.0	...	...	...	26.9
Casein[d]	23.5	17.4	18.1	17.0	16.6	15.2	10.2	9.3	16.2
Starch[e]	47.0	40.3	39.2	41.2	41.3	33.7	7.2	20.0	35.1
Sucrose	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Cellulose[f]	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fat[g]	5.5	4.3	4.7	3.8	4.1	4.0	4.0	2.0	3.8
Protein (analyzed, %)	21.0	20.0	20.6	20.5	19.8	20.6	19.1	19.3	20.5
Fat (analyzed, %)	5.7	6.4	6.3	5.5	5.5	6.0	6.0	6.2	5.9
Calculated values, %									
Total dietary fiber	6.00	6.68	3.00	3.32	7.00	9.71	9.09	8.42	9.41
Insoluble dietary fiber	6.00	3.12	1.34	1.94	6.18	4.45	4.06	4.93	8.31
Soluble dietary fiber	0.00	3.56	1.66	1.38	0.82	5.26	5.03	3.49	1.10
Total β-glucans	0.00	3.37	1.58	1.31	0.26	4.92	4.80	3.32	0.34
Soluble β-glucans	0.00	1.28	0.94	0.77	0.00	1.93	2.84	1.95	0.00

[a]In addition, all diets contained 4% USP XVII mineral mix and 2% vitamin mix 2 (both from ICN Nutritional Biochemicals, Cleveland, OH) and 1% cholesterol (ash-free, Sigma Chemical Co., St. Louis, MO.)
[b]Prowashonupana.
[c]All cereal fiber sources were supplied by ConAgra, Inc., Omaha, NE.
[d]Technical grade, Sigma Chemical Co.
[e]Corn starch, Sigma Chemical Co.
[f]Alphacel, ICN Nutritional Biochemicals.
[g]Soybean oil (Food Club brand), Dillons, Inc., Manhattan, KS.

variance with Duncan's test, using the Statistical Analysis System 5.16. Correlation analysis (Pearson) by group was performed by the PROC CORR procedure (SAS 1985).

RESULTS

Protein in the test grains varied from 18.3% for WXB to 25.1% for WRD, and ether extract varied from 1.8% in WXB to 6.6% in OB (Table I).

Total dietary fiber levels were similar in PWSNP and WRD, both approximately 35%, whereas OB and WXB had similar lower levels, about 16% (Table I). Ratios of soluble to insoluble fiber, however, were extremely different between WRD and the other grains. The fiber of the two barleys was approximately 55% soluble and that of OB about 40%, whereas the WRD fiber had only 12% soluble fiber. The highest total β-glucan level was found in PWSNP barley, followed by WXB, then OB, with the lowest level in WRD. Percentage of soluble β-glucan also was highest in PWSNP barley. However, soluble β-glucans constituted a lower percentage of the total β-glucan in PWSNP (39%) than in WXB (59%) and OB (58%). WRD contained no soluble β-glucan.

Viscosity was notably higher for the OB and WXB barley (over 7 cP) than for PWSNP (2.47 cP) and WRD (1.38 cP) (Table I).

Chick-Feeding Study

Body and liver weights. The biological data for the chick feeding experiment are shown in Table IV. Chicks fed the WXB barley or OB had significantly lower ($P < 0.05$) body weights than those fed other diets. Chicks fed WRD and the corn-fed controls had the highest body weights, and those fed PWSNP were intermediate. Liver weights tended to be relative to body weight, and no differences were observed in liver weights as a percentage of body weights.

Serum and liver lipids. Chicks fed WXB barley or OB had significantly lower ($P < 0.05$) total and LDL serum cholesterol than those fed the other diets. PWSNP-fed chicks had lower ($P < 0.05$) total and LDL cholesterol than WRD-fed chicks or corn-fed controls, but not as low as those fed WXB or OB. HDL cholesterol levels were highest ($P < 0.05$) for WXB- or OB-fed chicks, with no differences among other treatments. HDL-total cholesterol ratios followed the same pattern: highest for WXB and OB and lowest for WRD and controls. Serum triglycerides were lowest in chicks fed WXB or OB compared with all others, and chicks fed PWSNP had intermediate values. Chicks fed PWSNP, WXB, or OB had significantly lower ($P < 0.05$) liver cholesterol than those fed WRD or corn, and those fed WXB or OB had lower values than chicks fed PWSNP barley.

Rat-Feeding Study

Weight gains and feed efficiency. Biological data for the rats are given in Table V. Although fiber contents of the diets varied, weight gains for all groups were statistically the same. Feed efficiencies, in general, were lower (better) for low-fiber diets, which is probably related to their higher energy content.

TABLE IV
Biological Data for Chicks[a] Fed Grain Diets

	Diet					
Parameter	PWSNP[b] Barley	Waxbar Barley	Oat Bran	Wheat Red Dog	Corn Control	SE[c]
Body weight gain, g	112.0 b[d]	93.0 c	92.0 c	124.0 a	122.0 a	3.46
Liver weight, g	4.5 a	3.7 b	3.9 b	5.2 a	4.9 a	0.11
Liver, % body weight	2.4 a	2.2 a	2.3 a	2.5 a	2.4 a	0.07
Serum lipids, mg/dl						
Total cholesterol	426.0 c	341.0 d	366.0 d	541.0 a	499.0 b	14.05
HDL[e] cholesterol	93.0 b	102.0 a	101.0 a	95.0 b	91.0 b	2.21
LDL[f] cholesterol	317.9 b	231.3 c	257.2 c	430.0 a	394.0 a	13.75
HDL-total cholesterol	0.218	2.999	0.276	0.176	0.182	
Serum triglycerides	57.0 b	37.0 c	38.0 c	81.0 a	70.0 ab	4.71
Liver cholesterol, mg/g	42.7 b	28.5 a	38.2 b	59.1 c	66.8 c	3.50

[a]Thirty chicks per treatment.
[b]Prowashonupana.
[c]Standard error of the means.
[d]Different letters in rows denote statistically significant differences at the $P < 0.05$ level.
[e]High-density lipoprotein.
[f]Low-density lipoprotein.

TABLE V
Biological Data of Rats[a] Fed Grain Diets or Cellulose

		Low-Fiber Diet				High-Fiber Diet				
Parameter	Cellulose Control	PWSNP[b] Barley	Waxbar Barley	Oat Bran	Wheat Red Dog	PWSNP Barley	Waxbar Barley	Oat Bran	Wheat Red Dog	SE[c]
Body weight gain, g	183.0 a[d]	218.0 a	213.0 a	182.0 a	190.0 a	206.0 a	211.0 a	189.0 a	189.0 a	8.31
Feed efficiency F/G[e]	4.50 c	3.76 a	3.89 ab	4.17 a–c	4.46 c	4.07 a–c	4.29 bc	4.22 a–c	4.41 bc	0.02
Serum lipids, mg/dl										
Total cholesterol	121.6 a	116.2 a	121.6 a	115.2 a	114.2 a	123.2 a	83.7 b	87.6 b	125.3 a	5.87
HDL[f] cholesterol	53.0	43.0	44.0	38.0	38.0	54.0	47.0	52.0	62.0	2.67
LDL[g] cholesterol	54.0	57.0	63.0	64.0	63.0	55.0	22.0	23.0	68.0	2.32
HDL-total cholesterol	0.432 b	0.369 b	0.364 b	0.330 b	0.329 b	0.439 b	0.558 a	0.558 a	0.415 b	0.03
Serum triglycerides	74.6 ab	83.7 ab	72.2 a–c	66.3 bc	66.7 bc	72.4 a–c	75.7 ab	65.9 c	85.6 a	9.42
Liver cholesterol, mg/g	5.33 ab	6.21 ab	4.91 a–c	3.54 c	5.30 ab	6.28 a	4.67 bc	4.70 bc	6.02 a–c	0.05

[a]Ten rats per treatment.
[b]Prowashonupana.
[c]Standard error of means.
[d]Different letters in columns denote statistically significant differences at the $P < 0.05$ level.
[e]Feed-to-gain ratio.
[f]High-density lipoprotein.
[g]Low-density lipoprotein.

Serum and liver lipids. At the lower dietary fiber level, rats fed OB, WXB, PWSNP, or WRD had similar serum total and HDL cholesterol concentrations that were not different from those in animals fed the cellulose control diet. However, animals fed the higher levels of OB and WXB had lower serum total cholesterol and higher HDL-total cholesterol ratios than those in any other group.

Rats fed diets containing OB and WXB barley also appeared to have lower concentrations of cholesterol in their livers than those fed the control diet, but the difference was only significant for animals fed OB at the lower concentration (Table V). As with serum cholesterol, liver cholesterol was not lower in rats fed PWSNP barley or WRD fractions.

DISCUSSION

Animal models are not entirely satisfactory for lipid metabolism studies because the classes of lipoprotein density vary with species (Chapman 1980). Extrapolation of results to humans should therefore be interpreted with caution. However, when responses in different experimental species are consistent relative to diet treatment, some general conclusions can be made.

In this study, in both species and at different locations, similar results in most parameters were obtained, supporting earlier reports on the hypocholesterolemic effect of SDF (Vahouny 1982). WXB barley, which closely compares to OB in fiber composition and viscosity, elicited a hypocholesterolemic response similar to that of the OB in both chicks and rats. PWSNP barley, on the other hand, lowered total and LDL cholesterol in the chick compared with WRD and corn controls, but not to the same degree as did WXB or OB. Furthermore, this barley did not produce a cholesterol-lowering response in the rat, despite its high β-glucan and soluble-fiber content. The differing results with PWSNP may be due to loss of β-glucan by endogenous β-glucanases. Addition of β-glucanase to chick diets disrupts the β-glucans to the extent of negating the hypocholesterolemic effect (Fadel et al 1987), and the possibility of similar action by endogenous β-glucanases because of grinding and/or exposure to moisture exists. When harvested barley is exposed to moisture by rainfall or other means, there is a loss in acid-extract viscosity presumably due to activity of β-glucanases (Aastrup 1979).

One theory of the hypocholesterolemic mechanism relates to modification of lipid absorption caused by increased viscosity created by soluble fiber (Vahouny et al 1981). Genetic differences of barley cultivars can cause considerable variations in extract viscosity (Fengler et al 1990). The molecular weight of β-glucans is also a possible factor of variation in viscosity and metabolic response (Bengtsson et al 1989) and should be investigated in future β-glucan studies.

The viscosity of the PWSNP barley preparation used in this study was considerably lower than that of preparations of WXB barley or OB, which could be a key factor in the corresponding cholesterol responses. The WRD preparation had the lowest viscosity and elicited no hypocholesterolemic effect. Extract viscosity might therefore be considered as one predictor of the hypocholesterolemic property of barley and other cereal grains.

TABLE VI
Pearson Correlation Coefficients for Dietary Fiber Components and Rat and Chick Serum Cholesterol

Fiber Component	Total Serum Cholesterol
Total dietary fiber	
Chick[a,b]	...
Rat[c]	−0.298
Soluble dietary fiber	
Chick	−0.427
Rat	−0.499
Total β-glucan	
Chick	−0.513
Rat	−0.519
Soluble β-glucan	
Chick	−0.758**[d]
Rat	−0.718*

[a] Chick diets were formulated to contain the same amounts of total dietary fiber, so correlation with total dietary fiber for those diets was not performed.
[b] Thirty chicks per treatment.
[c] Ten rats per treatment.
[d] Significant at $P < 0.01$ and 0.05 for ** and *, respectively.

CONCLUSIONS

WXB barley and OB demonstrated a cholesterol-lowering effect in chicks and rats when about 10% TDF was provided in the diet. In the rat study, total β-glucan and, especially, soluble β-glucan appeared to be stronger predictors of serum total cholesterol than TDF or SDF. In the chick study, total serum cholesterol was significantly correlated with soluble β-glucan (Table VI). Barley cultivars with high soluble β-glucan and SDF content are good possibilities for healthful ingredients in food products, but consideration should be given to cultivar, chemical composition, and viscosity to assure presence of active fiber components.

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