

## EFFECT OF YEAST AND BOTANICAL $\beta$ -GLUCAN ON SERUM LIPID PROFILE AND CECUM PROBIOTIC BACTERIA USING RATS FED CHOLESTEROL DIET

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The purpose of this study was to examine the effect of yeast  $\beta$ -glucan, as compared to  $\beta$ -glucan from a botanical source, on blood lipids and intestinal probiotic bacteria in hypercholesterolemic male Wistar rats. The barley, isolated barley-derived  $\beta$ -glucan, and isolated yeast-derived  $\beta$ -glucan were used in cholesterol AIN-93 diets (~3 g  $\beta$ -glucan/100 g diet) of the rats.

After an 8-week feeding trial, the barley, barley-derived  $\beta$ -glucan and yeast-derived  $\beta$ -glucan diets fed to rats caused significant ( $p < 0.05$ ) reductions in the levels of total cholesterol (11%, 15%, 9%, respectively) and LDL-cholesterol (24%, 33%, 20%, respectively) in serum as compared to the control group. Among the test diets, the barley-derived  $\beta$ -glucan diet had the greatest cholesterol-lowering effect. There was a significant increase in the HDL-cholesterol/total cholesterol ratio in the serum of all test diets-fed rats ( $p < 0.05$ ) and a significant decrease in the LDL-cholesterol/HDL-cholesterol ratio in the serum of barley and barley-derived  $\beta$ -glucan-fed rats ( $p < 0.05$ ), as compared to the control group. The number of Lactobacilli was significantly higher ( $p < 0.05$ ) in comparison to the control group for the barley and barley-derived  $\beta$ -glucan groups (11.8 and 12.6  $\log_{10}$  counts/g wet fresh caecum, respectively). The same trend was also found for Bifidobacteria (12.3 and 13.1  $\log_{10}$  counts/g wet fresh caecum, respectively).

The botanical and yeast glucans worked in the same manner, leading to a conclusion that the cholesterol-lowering potency of  $\beta$ -glucan is not dependent on its source. On the other hand, the probiotic activity of  $\beta$ -glucan does depend on the source.

### INTRODUCTION

Cardiovascular disease (CVD) is a severe public health concern. A number of studies indicate that the risk of heart attacks in hypercholesterolemic individuals can be significantly reduced by lowering their serum cholesterol. One well-established way to reduce the risk of developing CVD is to lower serum LDL-C levels by making dietary changes. In addition to reducing saturated fat and cholesterol intake, and increasing *cis* unsaturated fat intake, the importance of other dietary approaches, such as increasing the intake of water-soluble dietary fibers as functional food ingredients has become increasingly recognized [Spiller, 2001].

$\beta$ -Glucan is a water-soluble fiber found in cereals, oats and barley in particular, as well as in yeast. Content of cereals  $\beta$ -glucan ranges from 1% in wheat grains, to 3–7% in oats, and 5–11% in barley. Thus, barley grains are acknowledged as its rich source [Kalra & Jood, 2001]. Functional foods enriched with  $\beta$ -glucan from mainly oats are widely available on the market to decrease serum LDL-C [Braaten *et al.*, 1994]. In fact, one of the richest sources of  $\beta$ -glucan is the cell wall of baker's yeast *Saccharomyces cerevisiae*. The cell walls are an ideal raw material for the manufacture of  $\beta$ -glucan. They are inexpensive and show a sufficiently high content of glucan, which accounts for more than 50% of dry weight [Magnelli *et al.*, 2002]. In addition, functional food ingredient from yeast

glucan could benefit the yeast industry by receiving an additional source of income and eliminating the costs of waste disposal.

Regardless of its source,  $\beta$ -glucan is a polysaccharide composed of glucose molecules. In yeast, the glucose molecules are joined by  $\beta$ -(1→3, 1→6)-glycosidic bonds, and in oats and barley by  $\beta$ -(1→3, 1→4)-glycosidic bonds. Thus,  $\beta$ -glucan from these sources is composed of branched chains [Newman *et al.*, 1992]. Barley glucan disperses in water with difficulty, because it has a high viscosity and produces gel-like lumps even at a low concentration. On the other hand, yeast-derived  $\beta$ -glucan is more palatable than the barley-derived  $\beta$ -glucan, and unlike the latter  $\beta$ -glucan is tasteless, odorless, colorless, water insoluble, and therefore non-gelling, even after heating and cooling. The product is heat stable (121°C for 30 min), pH stable (between 2 and 12), and shear stable, because most of the  $\beta$ -1-3 linkages are insoluble, unlike the soluble  $\beta$ -1-4 linkages found in barley. This attribute is of particular importance because the yeast-derived product can readily mix with liquids without gelling or forming an unpalatable viscous mass. The yeast-derived product when added to liquids has a creamy mouthfeel [Kalra & Jood, 2000; Dijkgraaf *et al.*, 2002, Papageorgiou *et al.*, 2005].

The branched  $\beta$ -glucan from barley and oat fiber has been shown to improve lipid profiles. The cholesterol-lowering ability was first ascribed to oats but more recently to barley.

No significant difference was observed between the two botanical sources, leading to a conclusion that the cholesterol-lowering potency of  $\beta$ -glucan is not dependent on its botanical source [Kalra & Jood, 2001]. Botanical glucans, derived from barley and oat, are fermented by the intestinal microflora *in vivo* and *in vitro*, resulting in the activation of probiotic bacteria [Newman *et al.*, 1992]. The purpose of this study was to examine the effect of isolated yeast-derived branched- $\beta$ -glucan fiber ( $\beta$ -(1 $\rightarrow$ 3, 1 $\rightarrow$ 6) glycosidic bonds) on blood lipids and intestinal probiotic bacteria *in vivo* in hypercholesterolemic rats in comparison to  $\beta$ -glucan from botanical sources ( $\beta$ -(1 $\rightarrow$ 3, 1 $\rightarrow$ 4) glycosidic bonds).

## MATERIALS AND METHODS

### Chemicals and analytical methods

The content of  $\beta$ -glucan ( $\beta$ -(1 $\rightarrow$ 3, 1 $\rightarrow$ 4)-glycosidic bonds) in barley and barley isolate was determined enzymatically using a Megazyme kit (Megazyme International, Bray, Ireland.). The content of (1 $\rightarrow$ 3, 1 $\rightarrow$ 6)- $\beta$ -D-glucan in the cell wall fractions was determined enzymatically by means of the commercial Yeast Beta Glucan Assay Kit (Megazyme International, Bray, Ireland). The total starch content was analysed enzymatically using a Megazyme kit (Megazyme International, Bray, Ireland.) after its extraction with dimethyl sulfoxide. Dietary fiber (total, insoluble, and soluble fraction) was analysed by the enzymatic-gravimetric AOAC method [Prosky *et al.*, 1988].

The concentrations of total cholesterol (TC, Cat No. CH 200), high-density lipoprotein (HDL-C) cholesterol precipitate (Cat No. CH 203) and triglyceride (TG, Cat No. TR 1575) were determined by the enzymatic endpoint method according to the instruction of the Kit's manufacturer (Randox Laboratories, Antrim, UK). Serum levels of low-density lipoprotein cholesterol (LDL-C) and serum very low-density lipoprotein cholesterol (VLDL-C) were calculated by using the Friedewald equation:  $([LDL-C] = [TC-C] - [HDL-C] - \{[TG]/2.2\})$  [Friedewald *et al.*, 1972].

The endogenous populations of colonic probiotic bacteria (Lactobacilli and Bifidobacteria) were counted. Hundred fold serial dilutions were performed in a pre-reduced Ringer solution containing 0.5% of cystein. Petri dishes of various media were inoculated and incubated for 72 h at 37°C in anaerobic atmosphere using Gen Kits in an Oxoid jar. Bacteria were detected on selective media as follows: deMan, Rogosa, Sharpe (MRS) medium (Oxoid, Hampshire, United Kingdom) for Lactobacilli and Tryptone Phytone Yeast (TPY) medium (Oxoid) for Bifidobacteria. After incubation, the colonies were counted. Bacterial counts were expressed as  $\log_{10}$  colony forming units ( $\log_{10}$  CFU/g) of fresh caecum digesta sample, with detection limit of 3.30  $\log_{10}$  CFU/g [Ezz El-Arab *et al.*, 2006].

### Rat feeding experiments and diets

Male Sprague – Dawley rats weighing  $75 \pm 5$  g were purchased from the Animal House Colony at the National Research Center, Cairo, Egypt. The rats were maintained at 22°C in a room with a 12-h light:dark cycle and given free access to diets and water at all times. All rats were fed a non-

purified commercial diet for 3 days, an AIN-93 diet [Reeves *et al.*, 1993] for the next 2 weeks as an adaptation period (Table 1), followed by a cholesterol AIN-93 diet (0.1% cholesterol) for 2 months, to induce hypercholesterolemia. The hypercholesterolemic rats were divided randomly into four groups of 7 rats each and fed the experimental diets (Table 1) for another 8 weeks. Rats from the control group were fed a cholesterol AIN-93 diet with only cellulose as the source of dietary fiber (control diet). Rats from the barley group were fed a cholesterol AIN-93 diet supplemented with barley, which contained  $\beta$ -glucan and other forms of dietary fibers (barley diet). Rats from the isolated barley-derived  $\beta$ -glucan group were fed a cholesterol AIN-93 diet supplemented with isolated barley  $\beta$ -glucan (30 g/kg diet) as a source of branched  $\beta$ -(1 $\rightarrow$ 3, 1 $\rightarrow$ 4)-glycosidic bond (isolated barley-derived  $\beta$ -glucan diet). In turn, rats from the isolated yeast derived  $\beta$ -glucan group were fed a cholesterol AIN-93 diet supplemented with isolated yeast  $\beta$ -glucan (30 g/kg diet) as a source of  $\beta$ -(1 $\rightarrow$ 3, 1 $\rightarrow$ 6)-glycosidic bonds (isolated yeast-derived  $\beta$ -glucan diet), (Table 1). All diets were prepared one time and kept in a refrigerator to avoid batch variation. The protocol of this study was approved by Animal House Ethic Committee, National Research Center, Egypt.

At the end of experiments, the rats were deprived of diets for 12 h of overnight fasting, and then anesthetized by ether [Sahu *et al.*, 2005]. The abdomen was opened longitudinally and blood samples were collected by cardiac puncture into tubes. Blood samples were centrifuged at 4°C and the serum was separated and stored at -40°C until analysis. The collected samples of caecum digesta were immediately (within 30 min) placed in anaerobic jars and kept at -40°C until analyses of caecum probiotic bacteria count [Ezz El-Arab *et al.*, 2006].

### Samples preparation

Barley (*Hordeum vulgare* L.) was purchased from the Agriculture Research Center, Ministry of Agriculture. The grains were cleaned, boiled, dried and powdered in a Cyclotec mill to pass through a 60-mesh sieve (60 mesh per inch) and then stored in air-tight polyethylene bottles for further chemical analysis. Barley analysed content of  $\beta$ -glucan, starch, protein and fat were 3.89, 65.9, 9.7, and 1 g/100 g, respectively. Barley-derived  $\beta$ -glucan was isolated according to the method of Pappageorgiou *et al.* [2005], resulting in  $90.55 \pm 0.43$   $\beta$ -glucan% of the dry weight. Yeast-derived  $\beta$ -glucan was isolated according to methods of Freimund *et al.* [2003] and Magnelli *et al.* [2002], resulting in  $87.39 \pm 0.24$   $\beta$ -glucan% of the dry weight.

### Statistical analysis

All data were expressed as mean  $\pm$  standard error of the mean (SEM) and analyzed by one-way analysis of variance ANOVA using the SAS statistical program (SAS version 6.11, SAS Institute, Cary, NC). When significant, Duncan's Multiple Range test was used to compare difference between means. The level of significance was set at  $p < 0.05$ . Counts of caecum probiotic bacteria were transformed to logarithms for statistical analysis.

## RESULTS AND DISCUSSION

### Effects on serum lipid profile

The content of barley  $\beta$ -glucan, the most important dietary fiber, is associated with advantageous physiological effects. Therefore, we used barley (which contains  $\beta$ -glucan in addition to dietary fiber component present in the whole grain barley) and  $\beta$ -glucan from botanical and yeast sources. In contrast to the control diet, the barley diet consisted of 771 g/kg of barley. Therefore, it contained more total dietary fiber but 3% of  $\beta$ -glucan as well as fewer cornstarch in addition to reduction in kcal/kg diet than the control diet (Table 1). Furthermore, 20 g/kg of cellulose, a low fermentable dietary fiber, was present in barley-derived  $\beta$ -glucan and yeast-derived  $\beta$ -glucan diets. The presence of 3% barley-derived  $\beta$ -glucan and yeast-derived  $\beta$ -glucan resulted in no significant changes in kcal/kg diet in comparison to the control diet (Table 1). All diets were well accepted by the rats. There were no treatment-related changes in the appearance nor behavior of the rats during the experiment. All rats remained healthy during the experimental period.

In this study, a new source of dietary fiber from yeast (yeast-derived  $\beta$ -glucan) was evaluated in hypercholester-

olemic rats. As evident from Table 2, after 8-week feeding trial, the barley, barley-derived  $\beta$ -glucan and yeast-derived  $\beta$ -glucan diets fed to rats caused significant ( $p < 0.05$ ) reductions in the levels of TC (11%, 15%, 9%, respectively). Among the test groups, TC was the lowest in the barley-derived  $\beta$ -glucan group ( $4.27 \pm 0.10$  mmol/L) followed by the barley group and the yeast-derived  $\beta$ -glucan group ( $4.45 \pm 0.15$  and  $4.55 \pm 0.09$  mmol/L, respectively), which differed significantly from the control group ( $4.99 \pm 0.09$  mmol/L). Four mechanisms have been proposed to explain how botanical  $\beta$ -glucan from barley lowers serum cholesterol. First, it has been postulated that soluble fiber binds to bile acids in the intestinal lumen, which results in a reduced bile acid pool back to the liver. This binding action stimulates the production of more bile acids derived from cholesterol that is either made endogenously or captured from the circulation. Secondly, soluble fibers are fermented in the large bowel by colonic bacteria. This action results in the production of the short-chain fatty acids (SCFAs) — acetate, propionate, and butyrate. These SCFAs are absorbed through the portal vein, inhibiting hepatic cholesterol synthesis by limiting the action of HMG-CoA reductase (the rate-limiting enzyme required for cholesterol biosynthesis) or by increasing catabolism of LDL-C. Thirdly, soluble fiber may delay gastric emptying, thereby reducing post-prandial serum insulin concentrations. This action reduces hepatic cholesterol production through mediation of HMG-CoA reductase. Fourthly, botanical soluble fiber may interfere with the absorption of dietary fat, including cholesterol, by increasing intestinal viscosity. The increased viscosity causes the digesta to hold on extra water, which slows its movement. For the yeast-derived glucan, some of the proposed mechanisms by which botanical  $\beta$ -glucan affects serum lipids are similar to those of the yeast-derived  $\beta$ -glucans. There appears to be some binding of the yeast-derived  $\beta$ -glucan to the bile acids in the small bowel [Glore *et al.*, 1994; Cummings & Macfarlane, 1997; Bell *et al.*, 1999].

In 1972, Friedewald *et al.* published a landmark report describing a formula to estimate LDL-C as an alternative to tedious ultra centrifugation. Because VLDL-C carries most of the circulating TGs, VLDL-C can be estimated reasonably well from measured TGs divided by 5 for mg/dL units or by 2.2 for mmol/L units. LDL-C is then calculated as TC minus HDL-C minus estimated VLDL-C. The bibliography available regarding the effect of dietary and non-dietary factors on serum cholesterol includes several publications that calculate LDL-C levels in rats and other animal models by the Friedewald formula. The authors of these publications thus assume that the serum TG/VLDL-C ration in animals is identical to that of humans and that the Friedewald formula can be used to rapidly calculate LDL-C values in hypercholesterolemic animals (*e.g.* rats) [Kalra & Jood, 2000]. On the other hand, Sanchez-Muniz & Bastida [2008] demonstrated that the Friedewald formula generally did not accurately estimate VLDL-C levels in hypercholesterolemic rats and therefore cannot be used to find LDL-C levels either. Moreover, in the hypercholesterolemic rats who did present  $\beta$ -VLDL-C, the results clearly show that the Friedewald formula overestimates LDL-C levels. In their original publication, Sanchez-Muniz & Bastida [2008] collected data belonging to five

TABLE 1. Composition of diets fed to rats for 8 weeks.

Ingredients (g/kg diet)	Dietary treatments				
	AIN-93 <sup>a</sup>	Control <sup>b</sup>	Barley <sup>c</sup>	Barley glucan <sup>d</sup>	Yeast glucan <sup>e</sup>
Starch	620.70	608.70	71.34	608.70	608.70
Casein	140	140	65.19	140	140
Sucrose	100	100	0	100	100
Soybean oil	40	40	32.29	40	40
Cellulose	50	50	0	20	20
Salt mix.*	35	35	35	35	35
Vitamin mix.*	10	10	10	10	10
Choline chloride	1.8	1.8	1.8	1.8	1.8
L-cystine	2.5	2.5	1.16	2.5	2.5
Tert-butylhydroquinone	0.008	0.008	0.008	0.008	0.008
Cholesterol	0	10	10	10	10
Cholic acid	0	2	2	2	2
Barley	0	0	771.21	0	0
Barley $\beta$ -glucan	0	0	0	30	0
Yeast $\beta$ -glucan	0	0	0	0	30
% of energy:					
from CHO	75.61	73.54	69.56	73.54	73.54
from fat	9.44	11.67	13.50	11.67	11.67
from protein	14.95	14.79	16.94	14.79	14.79

\* Mineral and vitamin mixtures of the diet were based on the AIN-93M formulation [Reeves *et al.*, 1993].

<sup>a</sup> AIN-93 diet [Reeves *et al.*, 1993] for adaptation period, <sup>b</sup> Cholesterol (0.1% cholesterol) AIN-93 diet (control diet), <sup>c</sup> Cholesterol AIN-93 diet supplemented with barley (Barley diet), <sup>d</sup> Cholesterol AIN-93 diet supplemented with isolated barley-derived  $\beta$ -glucan (Barley-derived  $\beta$ -glucan diet), <sup>e</sup> Cholesterol AIN-93 diet supplemented with isolated yeast-derived  $\beta$ -glucan (yeast-derived  $\beta$ -glucan diet).

TABLE 2. Effects of the barley, barley-derived  $\beta$ -glucan, and yeast-derived  $\beta$ -glucan containing diets on serum lipid profile of rats.

Groups	Measurements (mmol/L)						
	Cholesterol	TG	HDL-C	LDL-C	VLDL-C	LDL-C / HDL-C	HDL-C / TC
Control *	4.99 <sup>a</sup> ±0.09	2.05±0.09	1.49±0.09	2.64 <sup>a</sup> ±0.06	0.93±0.04	1.80 <sup>a</sup> ±0.19	0.29 <sup>a</sup> ±0.01
Barley **	4.45 <sup>b</sup> ±0.15	1.93±0.13	1.64±0.09	2.00 <sup>b</sup> ±0.05	0.88±0.06	1.24 <sup>b</sup> ±0.07	0.36 <sup>b</sup> ±0.01
Barley glucan ***	4.27 <sup>c</sup> ±0.10	1.87±0.10	1.69±0.10	1.77 <sup>c</sup> ±0.06	0.85±0.04	1.07 <sup>c</sup> ±0.12	0.39 <sup>c</sup> ±0.02
Yeast glucan ****	4.55 <sup>d</sup> ±0.09	1.94±0.09	1.61±0.08	2.12 <sup>d</sup> ±0.04	0.88±0.04	1.35 <sup>a</sup> ±0.09	0.35 <sup>d</sup> ±0.01

Data are mean±SE. Values in the same column that do not share the same superscript letter are significantly different (t-test,  $p < 0.05$ ).

\* Rats fed cholesterol (0.1% cholesterol) AIN-93 diet (control group), \*\* Rats fed cholesterol AIN-93 diet supplemented with barley (Barley group), \*\*\* Rats fed cholesterol AIN-93 diet supplemented with isolated derived-barley  $\beta$ -glucan (Barley-glucan group), \*\*\*\* Rats fed cholesterol AIN-93 diet supplemented with isolated yeast-derived  $\beta$ -glucan (Yeast glucan group).

different studies (two of them are unpublished data). In this five different studies, the rats ( $n = 54, 80\text{--}300$  g) were fed various diets, that affected TC levels and the lipoprotein profile to different degrees, as well as use was made of various hypercholesterolemic agents and various different experimental designs. In fact, this finding has not been standardized in large populations and increases cholesterol assay costs [Sahu *et al.*, 2005; Can *et al.*, 2009]. In addition, the measurement of LDL-C by ultracentrifugation, direct methods, is time-consuming (e.g. at least 48 h when assayed with the method of Havel *et al.* [1995], at least 24 h with that of Redgrave *et al.* [1975], and at least 7 h with that of Tersptra *et al.* [1981]).

Significantly ( $p < 0.05$ ) lower levels were also found for LDL-C ( $2.0 \pm 0.05, 1.77 \pm 0.06$  and  $2.12 \pm 0.04$  mmol/L, respectively) in the serum with reduction percentage of 24%, 33%, 20%, respectively, for rats fed barley, barley-derived  $\beta$ -glucan and yeast-derived  $\beta$ -glucan diets in comparison to the control group. Kalra & Jood [2000] also obtained similar results in a healthy rat study, in which total and LDL-C were reduced by 39% and 61%, respectively, both decreases being significantly correlated with barely  $\beta$ -glucan content. Animal studies suggested that molecular weight (MW) is an important determinant of the lipid lowering properties of oat fiber [Tietzen *et al.*, 1990]. Beer *et al.* [1997] also observed that the MW of  $\beta$ -glucan extracted from oats and barley with NaOH was lower than that extracted with hot water. Knuckles *et al.* [1997] also demonstrated that sequential extractions resulted in a decrease in the molecular weight of the  $\beta$ -glucan in the extract. However, the temperature used for sequential water extractions has also been shown to affect MW, the ratio of (1→4) to (1→3) linkages, and the amount of cellulosic regions on the  $\beta$ -glucan chain [Storsley, 2003]. In other form, water-solubility and molecular weight of  $\beta$ -glucan may also influence its hypocholesterolemic effect. Indeed, it has been postulated that the viscosity of  $\beta$ -glucan in the intestinal tract, which is positively related to its solubility in water and molecular weight, is an important determinant of its LDL-C lowering effects. Highly water-soluble  $\beta$ -glucan, with moderate to high molecular weight, may reduce serum LDL-C levels better than  $\beta$ -glucan with a low water-solubility and low molecular weight. This difference in effect is explained by the assumption that a higher intestinal viscosity lowers the reabsorption of bile acids, leading to an increased excretion of bile acids. Increased bile acid excretion promotes bile acid synthesis from cholesterol, which will increase LDL-C uptake in the liver.

The yeast  $\beta$ -glucan used in this study also had a low solubility and a low viscosity. Thus, it is not apparent whether the high amount of  $\beta$ -glucan or its low viscosity contributed to the increases in HDL-C concentrations because we and Beer *et al.* [1997] used materials with both properties.

Our results display no significant changes in the serum TG and HDL-C levels of rats fed on barley, barley-derived  $\beta$ -glucan, and yeast-derived  $\beta$ -glucan diets. Klopfenstein & Hosoney [1987] found no significant changes in the serum HDL-C levels of rats fed on  $\beta$ -glucan-enriched breads. Other workers reported that barley diets caused no significant change in serum TG in human subjects [Kahlon & Chow, 1997]. It was also reported that the amount of dietary fiber showed no effects on plasma TG in some studies with normal and hypercholesterolemic subjects [Behall *et al.*, 1997]. An increase in TG and HDL-C levels (21% and 34%, respectively) was observed when a high concentration of barley  $\beta$ -glucan (6.23 g%) was used in healthy rats [Kalra & Jood, 2000].

In the present study, there was a significant ( $p < 0.05$ ) increase in the HDL-C/TC ratio in rats fed barely, barley-derived  $\beta$ -glucan and yeast-derived  $\beta$ -glucan diets. The HDL-C / TC ratio was found to be the highest (0.39) for the barley-derived  $\beta$ -glucan group followed by the barley group (0.36), and the yeast-derived  $\beta$ -glucan group (0.35) in comparison to the control group (0.29), which indicates a decrease in the risk of coronary disease. The use of dietary fibers rich in  $\beta$ -glucan from oats has a similar effect on serum lipids [Newman *et al.*, 1992; Braaten *et al.*, 1994]. A high content of LDL-cholesterol is considered as being "bad cholesterol" and as a risk factor for coronary heart disease. As evident from Table 2, the barley and barley-derived  $\beta$ -glucan fed to rats caused a significant ( $p < 0.05$ ) decrease in the LDL-C/HDL-C ratio (1.24 and 1.07, respectively) in comparison to the control group. Among the test diets, the barley-derived  $\beta$ -glucan diet evoked the lowest LDL-C/HDL-C ratio. Kalra & Jood [2000] also obtained similar results in a healthy rat study, in which the HDL-C/TC ratio was increased and the LDL-C/HDL-C ratio was reduced, both being significantly correlated with barley  $\beta$ -glucan content.

#### Effects on caecum probiotic bacteria

Effects of dietary fiber on the intestinal microflora depend on both the type and structure of the fibers and their concentration. Stimulation of beneficial bacteria and inhibition of the growth of harmful bacteria were reported for inulin and oligofructose [Hopewell *et al.*, 1993].

TABLE 3. Lactobacilli and Bifidobacteria in fresh caecum content of the rats at the end of experiment of consuming control, barley or  $\beta$ -glucan samples containing diets.

Groups	Probiotic bacteria count ( $\log_{10}$ CFU/g fresh material)	
	Lactobacilli	Bifidobacteria
Control*	9.92 <sup>a</sup> $\pm$ 0.31	8.83 <sup>a</sup> $\pm$ 0.18
Barley**	11.78 <sup>b</sup> $\pm$ 0.41	12.25 <sup>b</sup> $\pm$ 0.27
Barley glucan***	12.63 <sup>c</sup> $\pm$ 0.36	13.13 <sup>c</sup> $\pm$ 0.23
Yeast glucan****	10.25 <sup>a</sup> $\pm$ 0.31	9.51 <sup>a</sup> $\pm$ 0.22

Data are mean  $\pm$  SE. Values in the same column that do not share the same superscript letter are significantly different (t-test,  $p < 0.05$ ).

\* Rats fed cholesterol (0.1% cholesterol) AIN-93 diet (control group), \*\* Rats fed cholesterol AIN-93 diet supplemented with barley (Barley group), \*\*\* Rats fed cholesterol AIN-93 diet supplemented with isolated barley-derived  $\beta$ -glucan (Barley glucan group), \*\*\*\* Rats fed cholesterol AIN-93 diet supplemented with isolated yeast-derived  $\beta$ -glucan (Yeast glucan group).

$\beta$ -(1 $\rightarrow$ 4, 1 $\rightarrow$ 3) glucans are components of plant cell walls, especially of those of the endosperm of cereals such as barley.  $\beta$ -(1 $\rightarrow$ 4, 1 $\rightarrow$ 3) glucans are not hydrolyzed in the small bowels of monogastric animals and thus pass to the large bowel, where they become substrates for bacterial fermentation [Walter *et al.*, 2005]. In our experiments, the number of caecum Lactobacilli was significantly ( $p < 0.05$ ) higher in the barley and barley-derived  $\beta$ -glucan groups (11.8 and 12.6  $\log_{10}$  counts/g wet fresh caecum, respectively) in comparison to the control group. The same trend was also found for Bifidobacteria (12.3 and 13.1  $\log_{10}$  counts/g wet fresh caecum, respectively), (Table 3). Like other soluble dietary fibers, barley-derived  $\beta$ -glucan is rapidly fermented by the intestinal microflora *in vivo* [Crittenden *et al.*, 2002; Newman *et al.*, 1992]. In addition, this is probably due to the production of extracellular  $\beta$ -glucanases (lichenases).  $\beta$ -Glucanases derived and are purified from gut microbes and catalyze the hydrolysis of  $\beta$ -(1 $\rightarrow$ 4, 1 $\rightarrow$ 3) glucans. Doubtless some of the hydrolysis products, of various degrees of polymerization, become available as fermentable substrates to other members of the community [Walter *et al.*, 2005].

## CONCLUSION

The botanical (barley, barley-derived  $\beta$ -glucan) and yeast (yeast-derived  $\beta$ -glucan)  $\beta$ -glucan containing diets have been shown to exert a cholesterol and LDL-C lowering effect in hypercholesterolemic rats. Therefore, the botanical and yeast glucans work in the same manner, leading to a conclusion that the cholesterol-lowering potency of  $\beta$ -glucan is not dependent on its source. On the other hand, the caecum probiotic activity of  $\beta$ -glucan does depend on its source, as the botanical (barley, barley-derived  $\beta$ -glucan)  $\beta$ -glucan containing diets have probiotic activity.

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## REFERENCES

- Beer M.U., Wood P.J., Weisz J., Molecular weight distribution, and (1 $\rightarrow$ 3) (1 $\rightarrow$ 4)- $\beta$ -D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chem.*, 1997, 74, 476–480.
- Behall, K.M., Schofield, D.J., Hallfrisch, J., Effect of beta glucan level in oat fibre extracts on blood lipids in men and women. *J. Amer. Coll. Nutr.*, 1997, 16, 46–51.
- Bell S., Goldman V.M., Bistrrian B.R., Arnold A.H., Ostroff G., Forse R.A., Effect of  $\beta$ -glucan from oats and yeast on serum lipids. *Crit. Rev. Food Sci. Nutr.*, 1999, 39, 189–202.
- Braaten J.T., Wood P.J., Scott F.W., Wolynetz M.S., Lowe M.K., Bradley-White P., Collins M.W., Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.*, 1994, 48, 465–474.
- Can M., Acikgoz S., Mungan G., Ugurbas E., Ankarali H., Sumbuloglu V., Demirtas S., Karaca L., Is direct method of low-density lipoprotein cholesterol measurement appropriate for targeting lipid lowering therapy? *Int. J. Cardiol.*, 2009, Jan 7.
- Crittenden R., Karppinen S., Ojanen S., Tenkanen M., Fagerstrom R., Matto J., Saarela M., Mätiila-Sandholm T., Poutanen, K., *In vitro* fermentation of cereal dietary carbohydrates by probiotic and intestinal bacteria. *J. Sci. Food Agr.*, 2002, 82, 781–789.
- Cummings J.H., Macfarlane G.T., Colonic microflora: nutrition and health. *Nutrition*, 1997, 13, 476–478.
- Dijkgraaf G.J.P., Li H., Bussey H., Cell- $\beta$ -glucans of *Saccharomyces cerevisiae*, 2002. *in: Polysaccharides*, vol. 6 (eds. E.J. Vandamme, S. De Baets, A. Steinbuchel). Weinheim, Germany, pp. 179–213.
- Ezz El-Arab A.M., Girgis S.M., Hegazy E.M., Abd El-Khalek A.B., Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. *J. Complem. Altern. Med.*, 2006, 6, 6.
- Freimund S., Sauter, M., Kappeli, O., Dutler H., A new non-degrading isolation process for 1,3- $\beta$ -D-glucan of high purity from baker's yeast *Saccharomyces cerevisiae*. *Carbohydr. Polym.*, 2003, 54, 159–171.
- Friedewald W.T., Levy R.I., Fredrickson D.S., Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 1972, 18, 499–502.
- Glore S.R., Van Treeck D., Knehans A.W., *et al.*, Soluble fiber and serum lipids. A literature review. *J. Am. Diet. Assoc.*, 1994, 94, 425–436.
- Havel R.J., Eder H.A., Bragdon J.H., The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*, 1955, 34, 1345–1353.
- Hopewell R., Yeater R., Ullrich I., Soluble fiber: Effect on carbohydrate and lipid metabolism. *Prog. Food Nutr. Sci.*, 1993, 17, 159–182.
- Kahlon T.S., Chow F.I., Knuckles B.E., Chiu M.M., Cholesterol lowering effects in hamsters of  $\beta$ -glucan-enriched barley fraction, de-hulled whole barley, bran, and oat bran and their combinations. *Cereal Chem.*, 1993, 70, 435–440.
- Kalra S., Jood S., Effect of dietary barley beta-glucan on cholesterol and lipoprotein fractions in rats. *J. Cereal Sci.*, 2001, 31, 141–145.

17. Klopfenstein, C.F., Hosney, R.C. Cholesterol lowering effect of  $\beta$ -glucan enriched bread. *Nutr. Rep. Int.*, 1987, 36, 1091–1098.
18. Knuckles B.E., Yokoyama W.H., Chiu M.M., Molecular characterization of barley  $\beta$ -glucan by size exclusion chromatography with multiple angle laser light scattering and other detectors. *Cereal Chem.*, 1997, 74, 599–604.
19. Magnelli P., Cipollo J.F., Abeijon C., A refined method for the determination of *Saccharomyces cerevisiae* cell wall composition and  $\beta$ -(1 $\rightarrow$ 6)-glucan fine structure. *Anal. Biochem.*, 2002, 301, 136–150.
20. Newman R.K., Klopfenstein C.F., Newman C.W., Gu-Peritno N., Hofer P.J., Composition of the cholesterol lowering properties of whole barley, oat bran, wheat red Atherodog in chicks and rats. *Cereal Chem.*, 1992, 69, 240–244.
21. Papageorgiou M., Lakhara N., Lazaridou A., Biliaderis C.G., Izydorczyk M.S., Water extractable (1 $\rightarrow$ 3,1 $\rightarrow$ 4)- $\beta$ -D-glucans from barley and oats: An intervarietal study on their structural features and rheological behavior. *J. Cereal Sci.*, 2005, 42, 213–224.
22. Prosky L., Asp N-G., Furda I., De Vries J.W., Schweizer T.F., Determination of insoluble, soluble and total dietary fiber in foods, and foods products: Interlaboratory study. *J. Assoc. Off. Anal. Chem.*, 1988, 71, 1017–1023.
23. Redgrave T.G., Roberts D.C., West C.E., Separation of plasma lipoproteins by density gradient ultracentrifugation. *Anal. Biochem.*, 1975, 65, 42–49.
24. Reeves P.G., Nielsen F.H., Fahey G.C., AIN-93 purified rodent diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN- rodent diet. *J. Nutr.*, 1993, 123, 1939–1951.
25. Sahu S., Chawla R., Uppal B., Comparison of two methods of estimation of low density lipoprotein cholesterol, the direct versus Friedewald estimation. *Indian J. Clin. Bioch.*, 2005, 20, 2, 54–61.
26. Sanchez-Muniz F.J., Bastida S., Do not use the Friedewald formula to calculate LDL-cholesterol in hypercholesterolemic rats. *Eur. J. Lipid Sci. Technol.*, 2008, 110, 295–301.
27. Spiller G.A., Dietary Fiber in Human Nutrition. 2001, in: *CRS Handbook of Dietary Fiber in Human Nutrition*. CRC Press Inc., Boca Raton, Florida, pp. 15–18.
28. Storsley J.M., Izydorczyk M.S., You S., Biliaderis C.G., Rossnagel B., Structure and physicochemical properties of  $\beta$ -glucans and arabinoxylans isolated from hull-less barley. *Food Hydrocoll.*, 2003, 17, 831–844.
29. Terpstra A.H., Woodward C.J., Sanchez-Muniz F.J., Improved techniques for the separation of serum lipoproteins by density ultracentrifugation: Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. *Anal. Biochem.*, 1981, 111, 149–157.
30. Tietyen J.L., Nevins D.J., Schneeman B.O., Characterization of the hypercholesterolemic potential of oat bran. *FASEB J.*, 1990, 4, A527 (abst).
31. Walter J., Mangold M., Tannock G.W., Construction, analysis, and  $\beta$ -glucanase screening of a bacterial artificial chromosome library from the large-bowel microbiota of mice. *Appl. Environ. Microb.*, 2005, 71, 2347–2354.

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