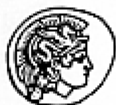

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# Evaluation of the toxicity of concentrated barley β-glucan in a 28-day feeding study in Wistar rats

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**Abstract**

β-Glucans are water-soluble cell-wall polysaccharides consisting of (1→3,1→4)-linked β-D-glucopyranosyl monomers that comprise a considerable proportion of soluble fiber from certain grains including oats and barley. Consumption of foods containing β-glucan or β-glucan-enriched fractions prepared from these grains lower serum cholesterol concentrations in humans and in animal models of hypercholesterolemia. The present study was conducted to evaluate the toxicity of β-glucan-enriched soluble fiber from barley in Wistar rats on dietary administration at concentrations of 0.7, 3.5 and 7% β-glucan for 28 days. There were no adverse effects on general condition and behavior, growth, feed and water consumption, feed conversion efficiency, red blood cell and clotting potential parameters, clinical chemistry values, and organ weights. Necropsy and histopathology findings revealed no treatment-related changes in any organ evaluated. A dose-dependent increase in full and empty cecum weight was observed. This is a common physiological response of rodents to high amounts of poorly digestible, fermentable carbohydrates, and was of no toxicological concern. The only finding of possible biological relevance was an increase in the number of circulating lymphocytes observed in males. However, the increase was not dose-dependent and was not observed in females. Results of this study demonstrated that consumption of concentrated barley β-glucan was not associated with any obvious signs of toxicity in Wistar rats even following consumption of large quantities.
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*Keywords:* β-Glucan; Barley; Soluble fiber; Lymphocytes

## 1. Introduction

Oats and barley have long been known to lower serum cholesterol concentrations in animal models of hypercholesterolemia (De Groot et al., 1963). Consumption of these cereal grains has also demonstrated cholesterol-lowering activity in humans (Judd and Truswell, 1981; Anderson et al., 1984; Newman et al., 1989a,b; Kestin et al., 1990; McIntosh et al., 1991; Ripsin et al., 1992; Lupton et al., 1994; Ikegami et al., 1996; Kahlon and Chow, 1997). However, consumption of relatively large amounts is required to produce clinically relevant reductions (Braaten et al., 1994). Therefore, many studies have been conducted to identify components present in these grains to which cholesterol-lowering activity could be attributable. Several substances have been identified that possess cholesterol-lowering activities (e.g. tocotrienols: Peterson and Qureshi, 1997; Qureshi et al., 1980, 1986). However, it is the bran fractions to which the cholesterol-lowering activity has most consistently been associated (Kirby et al., 1981; Anderson et al., 1984, 1990, 1991), more specifically, to the β-glucan component (Davidson et al., 1991; Braaten et al., 1994).

Relative to other cereal grains, oat bran contains high concentrations of soluble fiber of which a considerable proportion is β-glucan (>50%: Asp et al., 1992; Aman and Graham, 1987). The concentration of β-glucan in barley appears to be even higher than that of oats (Aman and Graham, 1987; Newman et al., 1992). Chemically, β-glucans are a heterogeneous group of water-soluble

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endospermic cell-wall polysaccharides consisting of (1→3,1→4)-β-D-linked glucose units (Wood et al., 1989). The β-glucan from barley and oats appears to be chemically indistinguishable (Jeraci and Lewis, 1989).

Concentrated preparations of oat β-glucan have demonstrated cholesterol-lowering activity in humans and animal models of hypercholesterolemia (Kirby et al., 1981; Davidson et al., 1991; Knuckles et al., 1992; Jonnalagadda et al., 1993; Kahlon et al., 1993; Braaten et al., 1994; Zhang et al., 1994; Yokoyama et al., 1998; Onning et al., 1999), as have similar products prepared from barley (Newman et al., 1989a,b; Knuckles et al., 1992; Wang et al., 1992; Kahlon et al., 1993; German et al., 1996; Maqueda de Guevara et al., 2000). A recent study comparing β-glucan-enriched fractions from oats and barley in hypercholesterolemic hamsters demonstrated that the cholesterol-lowering activity of β-glucan from oats and barley was nearly identical (B. Delaney et al., unpublished data, 2002).

These studies suggest that β-glucan-enriched soluble fiber from barley may be useful in controlling elevated serum cholesterol concentrations in humans and an interesting commercial product. Although barley itself and foods containing soluble fibers are not considered unsafe, little toxicological information is available regarding the safety following repeated exposure to concentrate enriched in β-glucan. In the present study, the toxicity of a water-extracted β-glucan-enriched soluble fiber from barley (Barley Betafiber) was evaluated in a 28-day feeding study in Wistar rats. This study was conducted in accordance with OECD Guideline for Testing Chemicals n. 407 (adopted July 27, 1995) and in compliance with the OECD Principles of Good Laboratory Practice.

## 2. Materials and methods

### 2.1. Preparation of β-glucan enriched soluble fiber concentrate

β-Glucan from hulless barley (Azhul variety) was extracted by a process similar to that of Aman and Hesselman (1985) but without the amyloglucosidase treatment step. The composition of the concentrated β-glucan product (Barley Betafiber, Cargill, Inc., Wayzata, MN) is presented in Table 1.

Table 1
Composition of β-glucan-enriched soluble fiber concentrate from barley (Barley Betafiber)[a]

| | Percent |
|---|---|
| Carbohydrates | 80.2 |
| (Total fiber) | (79.6) |
| (β-Glucan) | (64.0) |
| (Soluble fiber) | (60.3) |
| (Insoluble fiber) | (19.3) |
| Protein | 7.04 |
| Fat | 3.00 |
| Ash | 1.94 |
| Moisture | 7.82 |
| Total | 100.0 |

[a] Total, soluble, and insoluble dietary fiber were determined by AOAC method 991.43, protein concentration was determined by AOAC method 991.20, fat concentration was determined by AOAC method 933.05, and moisture was determined by AOAC method 926.08 (AOAC, 2000). The ash concentration was determined using Corn Refiners Association Standard Analytical Method A-4 (CRA, 1997). Carbohydrate concentration was determined by subtraction of the concentrations of protein fat, moisture, and ash from the total diet.

### 2.2. Analysis of β-glucan in barley concentrate and rat feed

β-Glucan in the barley concentrate was quantified enzymatically (McCleary, 1985) using a Megazyme β-glucan mixed linkage assay kit. β-Glucan-enriched soluble fiber samples were milled to pass through a 0.5-mm screen in an ultracentrifugal mill (Retsch Type ZM1) at 18,000 rpm and weighed 20-mg aliquots into 50-ml polypropylene tubes. Aqueous EtOH (0.1 ml, 50%, v/v) was added to the tubes followed by addition of 5.0 ml of sodium phosphate buffer (20 mM, pH 6.5) and the tubes mixed vigorously. The tubes were then incubated in boiling water for 2 min, mixed vigorously and incubated again in boiling water for 3 min. Tubes were then cooled to 50 °C, lichenase (0.2 ml, 10 U specific, endo-(1→3)(1→4)-β-D-glucan 4-glucanohydrolase; EC 3.2.1.73) was added, and the tubes were incubated at 50 °C for 1 h. The volume in each tube was then adjusted to 30 ml with deionised water, mixed and centrifuged at 1000 $g$ for 10 min. Aliquots (0.1 ml) of the supernatant were removed, placed into three glass test tubes and then 0.1 ml of acetate buffer (50 mM, pH 4.0) was added to the blank. To the other two tubes was added 0.1 ml of β-glucosidase (0.2 U; EC 3.2.1.21) in acetate buffer and the test tubes incubated at 50 °C for 15 min. The Megazyme glucose oxidase/peroxidase reagent was added to each tube (3.0 ml) and they were incubated at 50 °C for 20 min and absorbance measured at 510 nm. The concentration of β-glucan was determined using the following equation:

$$\beta\text{-}Glucan(\%w/w) = dE \times F \times 300 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

where: $dE$ = Absorbance (sample) − Absorbance (blank)
$F$ = 100 μg of glucose/absorbance of glucose standard (conversion factor from Absorbance to μg glucose).

300 = Volume correction (i.e. 0.1 ml taken from 30.0 ml)

$\frac{1}{1000}$ = Conversion from μg to mg

$\frac{100}{W}$ = Factor to express β-glucan content as a percentage ($W$ = Sample dry weight)

$\frac{162}{180}$ = Adjustment from free glucose to anhydro glucose (as occurs in β-glucan)

The concentration of β-glucan in rat feed was determined by extraction and quantified using the McCleary method (1985). Feed samples were dried at 105 °C for 1 h, after which 0.5 g of each blend was weighed into a 50-ml polypropylene conical test tube. One milliliter of EtOH (95%, v/v), 5.0 ml of sodium phosphate buffer (20 mM, pH 6.5) and 4.0 ml of deionised water were added to each sample and mixed vigorously. The samples were then incubated in a boiling water-bath for 2 min, mixed vigorously and incubated in a boiling water-bath for an additional 3 min, allowed to cool to 50 °C. Lichenase (0.2 ml, 10 U specific, endo-(1→3)(1→4)-β-D-glucan 4-glucanohydrolase; EC 3.2.1.73) was added to each tube followed by incubation at 50 °C for 1 h. The volume in each tube was then adjusted to 30 ml with deionised water. After mixing, 1 ml of each sample solution was filtered through an Acrodisc® syringe filter (0.45 μm) and 0.1-ml aliquots of each filtrate were carefully transferred into three glass test tubes (13 ×100 mm). To the first test tube, 0.1 ml of acetate buffer (50 mM, pH 4.0) was added and designated as the sample blank. To the other two test tubes, 0.1 ml of β-glucosidase (0.2 U; EC 3.2.1.21) in acetate buffer was added. All the samples were then incubated at 50 °C for 15 min. With each set of samples, a reagent blank and a glucose standard were prepared consisting of 0.1 ml deionised water and 0.1 ml sodium acetate buffer (50 mM, pH 4.0). The glucose standard consisted of 0.1 ml sodium acetate buffer (50 mM, pH 4.0) and 0.1 ml glucose standard (100 μg/0.1 ml). Three ml of the Megazyme™ glucose oxidase/peroxidase reagent was added to all tubes (blanks, samples and standards) followed by incubation at 50 °C for 20 min. The absorbance of the mixture was measured at 510 nm and the concentration of β-glucan in dried rat feed was determined using the equation described above.

### 2.3. Stability, homogeneity and content of β-glucan in rat feed

Following blending of the diets, samples of each dietary blend were covered in dry ice and shipped from TNO to Cargill. On arrival, small samples (0.5 g) of each diet were removed and the remainder stored in the dark at −20 °C. Of the samples removed for day 0, two samples (0.5 g) were weighed for extraction and quantitation of β-glucan. The remainder was left at room temperature (approximately 23 °C) with alternating light/dark cycles of approximately 12 h for 6 days prior to analysis. At weekly intervals, two samples of each feed blend were removed from the freezer, stored at room temperature for 6 days (corresponding to the days that new feed was administered to the animals) and β-glucan analysis was conducted as indicated above. Results were expressed as an adjusted value by subtracting the concentration detected in the starch control diet from the value obtained for the different samples containing the test material (Table 2). Additionally, the concentration of β-glucan was measured in five separate samples of each dietary blend, taken at different locations from the feed mixture to determine if the diets were blended homogeneously.

### 2.4. Animals and maintenance

Wistar outbred [Crl:(WI)WU BR] rats were obtained from Charles River Deutschland (Sulzfeld, Germany), and acclimatized to the laboratory conditions for 1 week. At the start of treatment, they were about 6 weeks old. On the starting day, the rats were allocated to four groups of five rats per sex, proportionate to body weight, using a computer-randomization program. Animals were identified by unique tattoos on the ears and housed individually in macrolon cages with stainless-steel grid covers and sterilized wood shavings (Woody Clean, Type 3/4) as bedding material. The experimental room was ventilated at 10 air exchanges per hour and maintained at a temperature of 22±3 °C with a relative humidity of 30–70%. Artificial lighting was sequenced at 12-h light/dark cycles. Feed (during the treatment period provided as a powder in stainless-steel cans) and drinking water (tap water provided in polypropylene bottles) were available ad lib. except for overnight fasting prior to necropsy. The welfare of the animals was maintained in accordance with the general principles governing the use of animals in toxicity experiments of the European Communities (Directive 86/609/EEC) and

Table 2
Content and stability of β-glucan in rat feed

| | Dietary concentration of barley β-glucan concentrate (in dried diet) | | | |
|---|---|---|---|---|
| | Control | 1.0% | 5.0% | 10.0% |
| Day 0 | 0.66 | 1.44 | 4.05 | 7.01 |
| Adjusted[a] | | 0.78 | 3.39 | 6.35 |
| Week 1 | 0.71 | 1.41 | 4.04 | 7.13 |
| Adjusted[a] | | 0.70 | 3.33 | 6.42 |
| Week 2 | 0.78 | 1.44 | 4.27 | 7.58 |
| Adjusted[a] | | 0.66 | 3.49 | 6.80 |
| Week 3 | 0.79 | 1.52 | 4.49 | 7.84 |
| Adjusted[a] | | 0.73 | 3.70 | 7.05 |
| Week 4 | 0.64 | 1.36 | 4.21 | 7.55 |
| Adjusted[a] | | 0.72 | 3.57 | 6.91 |

[a] Adjusted concentration is the amount of β-glucan measured in the control feed subtracted from the value obtained in the corresponding test diet sample for the same time point. Control diet was formulated with pregelatinized potato starch. Values presented represent the concentrations of β-glucan determined from two separate extractions of the same dietary blend. Target β-glucan concentrations were 0.7, 3.5 and 7.0%, corresponding to 1, 5 and 10% inclusion of the barley β-glucan concentrate in the experimental diets.

Dutch legislation (The Experiments on Animals Act, 1997). The research plan for this study was approved by the ethical review committee for animal experiments.

### 2.5. Exposure regimen

Prior to initiation of treatment, all animals were fed a cereal-based, closed-formula rodent diet (Rat and Mouse No.3 Breeding diet; RM3) from Special Diets Services (Witham, UK). From the start of the 4-week treatment period (day 0), animals were fed modified diets prepared from the RM3 diet. Animals in the control group were fed the modified RM3 diet in which 20% of the natural ingredient barley was replaced by 20% of a purified, digestible carbohydrate ingredient (pregelatinized potato starch; Paselli WA-4 from AVEBE, Foxhol, The Netherlands). Animals in the experimental groups were fed modified RM3 diets supplemented with barley β-glucan concentrate at 1% (low-dose), 5% (mid-dose) or 10% (high-dose), and 19, 15 or 10% pregelatinized potato starch, respectively. One batch of experimental diets was prepared shortly before the start of the treatment period and stored at −20 °C in quantities required for 1 week.

### 2.6. Observations

The rats were observed twice daily for abnormal clinical signs (general clinical observations). Neurobehavioral functioning was evaluated by weekly, detailed clinical observations made outside the home cage in a standard arena, and a functional observational battery and motor activity assessment conducted in the fourth week of exposure. The functional observational battery consisted of non-invasive, observational and interactive measures designed to assess the neurobehavioral and functional integrity of the rat, using measures taken from different functional domains including autonomic and neuromuscular function, sensorimotor reactivity, arousal and excitability. Spontaneous motor activity was assessed during a 30-min test period using an automated quantitative microprocessor-based video image analysis system (Ethovision, Noldus Information Technology b.v., The Netherlands).

Body weights were recorded on days 0, 7, 14, 21, 27 and 28. Food consumption was measured over successive periods of 7 days (weeks 1–3) and 6 days (week 4) by weighing the feeders. Food conversion efficiency was calculated as gram weight gain per gram food consumed. The intake of the test material per kg body weight per day was calculated from the feed intake and the body weight. Water consumption was measured by weighing the drinking bottles daily during 4 consecutive days in week 1.

On day 28, in such a sequence (stratified randomly) that the average time of killing was about the same for each group, the rats were killed by exsanguination from the abdominal aorta under $CO_2/O_2$ anesthesia. Routine hematology and clinical chemistry were conducted on all animals at the end of the exposure period. Following an overnight fast, animals were anesthetized with $CO_2/O_2$ and blood was drawn from the abdominal aorta into tubes with $K_2$-EDTA for hematology and heparin for clinical chemistry. Plasma was prepared by centrifugation. Hemoglobin, PCV, RBC, reticulocytes, thrombocytes and total WBC were determined with an ABX Pentra 120 Haematology Analyzer (ABX Diagnostics, France). The MCV, MCH and MCHC were calculated from hemoglobin, PCV and RBC. Prothrombin time was evaluated with the Normotest modified method for EDTA blood (Nyegaard and Co A/S, Oslo, Norway). Differential white blood cell counts were determined by microscopic examination of stained blood smears (Gorter and de Graaff, 1955). Plasma concentrations of glucose, ALP, ALAT, ASAT, GGT, total protein, albumin, urea, creatinine, total bilirubin, total cholesterol, triglycerides, phospholipids, $Ca^{2+}$, $Na^{+}$, $K^{+}$, $Cl^{-}$ and inorganic phosphate were determined using a Hitachi-911 Analyzer. The ratio of albumin to globulin was calculated by dividing the concentration of albumin by the difference in total protein and albumin concentrations.

Following sacrifice a thorough necropsy was performed on all animals. The following organs were weighed (paired organs together) after dissection: adrenals, brain, cecum (full and empty), epididymides, heart, kidneys, liver, ovaries, spleen, testes and thymus. The organ-to-body weight ratios (relative organ weights) were calculated from the absolute organ weights and the terminal body weight of the rats. Samples of the weighed organs and of the colon, gut associated lymphoid tissue including Peyer's patches, lymph nodes (axillary, mesenteric), lungs, mammary gland, peripheral nerve (sciatic), esophagus, parathyroids, pituitary, prostate, rectum, small intestines (duodenum, ileum, jejunum), spinal cord (three levels), sternum with bone marrow, stomach, thyroid, trachea with bronchi, urinary bladder, uterus, vagina and all gross lesions were preserved in a neutral aqueous phosphate buffered 4% solution of formaldehyde. Histopathologic analysis was conducted on 5-μ sections of paraffin-embedded tissues, stained with haematoxylin and eosin, of the preserved organs from all control and high-dose animals by light microscopy. Additionally, histopathologic analysis was conducted on the spleens from all experimental groups.

### 2.7. Statistics

Evaluation of body weights was conducted by one-way analysis of covariance (covariate: body weight on day 0) followed by Dunnett's multiple comparison tests.

Feed consumption and efficiency, water consumption, red blood cell parameters except for reticulocytes and WBC (total and absolute differential) counts, coagulation variables, clinical chemistry values, organ weights, continuous neurobehavioral parameters and motor activity were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Independent from ANOVA, the homogeneity of variances was tested by means of Bartlett's test (all parameters showed homogeneous variances). Reticulocytes, relative differential white blood cell counts and rank order neurobehavioral parameters were evaluated by Kruskal–Wallis non-parametric analysis of variance followed by Mann–Whitney U-tests. Categorical neurobehavioral data were evaluated by Pearson chi-square analysis. Evaluation of histopathological changes was conducted by Fisher's exact probability test. Statistical significance is indicated as *for $P$-values $\leqslant 0.05$ and **for $P$-values $\leqslant 0.01$.

## 3. Results

### *3.1. Analysis of β-glucan in rat feed*

The concentration of β-glucan was determined in the different feed samples blended with the indicated concentration of the (Barley Betafiber). Limited specificity of the analytic method was observed since control diets appeared to possess approximately 0.7% β-glucan (Table 2). This observation indicated either that a small amount of glucose was released by the enzymatic treatment during the analysis or that the dietary blend did contain a small amount of β-glucan. Following correction for the β-glucan detected in the control diets, the corresponding concentrations of β-glucan in the experimental diets were approximately 0.7, 3.5 and 7.0%, respectively in the low-, mid- and high-dose groups. The results were close to the target concentrations, indicating that the dietary blending process and the conditions under which the experimental diets were stored did not cause breakdown of the β-glucan (Table 2). A separate analysis to determine the concentration of β-glucan in multiple dietary blend samples demonstrated that β-glucan was blended homogeneously into the diet (data not shown).

### *3.2. Clinical signs and neurobehavioral observations*

All rats survived until scheduled necropsy. Daily general observations and neurobehavioral examinations revealed no treatment-related changes in the animals' appearance, general condition or behavior.

### *3.3. Body weight, feed and water consumption and β-glucan intake*

Compared to animals fed the starch control diet, there were no significant changes in body weight (Table 3), feed consumption (Table 4) except for two incidental decreases in the low-dose (0.7% β-glucan) group, or feed conversion efficiency (Table 5). Water consumption was comparable in all groups during the first week of exposure (data not shown); therefore, it was not monitored at subsequent time points. The overall mean intake of β-glucan in the low-, mid- and high-dose group was, respectively 0.55, 2.9 and 5.6 g/kg body weight/day.

Table 3
Body weights of rats consuming barley β-glucan concentrate for 28 days

| | Dietary concentration of barley β-glucan concentrate | | | |
|---|---|---|---|---|
| | Control | 1.0% | 5.0% | 10.0% |
| Males | | | | |
| Day 0 | 163.2±3.5 | 163.9±3.2 | 163.5±3.8 | 165.3±3.0 |
| Day 7 | 203.3±3.1 | 201.9±3.1 | 201.4±5.4 | 201.0±2.8 |
| Day 14 | 246.6±4.2 | 238.0±2.5 | 240.5±6.5 | 241.3±4.3 |
| Day 21 | 279.5±5.9 | 265.9±2.6 | 269.6±6.8 | 269.2±4.6 |
| Day 27 | 303.6±7.0 | 288.8±3.2 | 291.7±7.2 | 293.0±3.9 |
| Day 28 | 280.8±6.8 | 267.0±3.2 | 271.2±7.4 | 270.1±3.9 |
| Females | | | | |
| Day 0 | 133.4±2.5 | 135.4±2.7 | 133.7±2.0 | 135.2±2.7 |
| Day 7 | 148.4±3.5 | 145.3±3.8 | 147.8±2.0 | 150.7±2.9 |
| Day 14 | 165.9±3.1 | 158.9±2.9** | 161.9±1.5 | 165.2±3.2 |
| Day 21 | 178.5±4.0 | 170.8±4.5 | 175.2±1.4 | 177.3±4.9 |
| Day 27 | 191.8±5.3 | 182.3±5.8 | 186.0±2.6 | 187.2±5.2 |
| Day 28 | 177.3±4.2 | 167.1±4.9 | 170.2±2.0 | 169.5±3.9 |

Body weight means (g/rat+S.E.M.) of Wistar rats ($n=5$/sex/dose) consuming different dietary concentrations of barley soluble fiber enriched with β-glucan. **$P<0.01$ compared with control (0%) group by analysis of covariance followed by Dunnett's $t$-test.

Table 4
Feed consumed by rats exposed to barley β-glucan concentrate for 28 days

| | Dietary concentration of barley β-glucan concentrate | | | |
|---|---|---|---|---|
| | Control | 1.0% | 5.0% | 10.0% |
| Males | | | | |
| Day 7 | 18.0±0.5 | 17.6±0.3 | 17.3±0.8 | 16.9±0.3 |
| Day 14 | 20.4±0.5 | 19.0±0.3 | 19.8±0.7 | 19.0±0.5 |
| Day 21 | 20.7±0.7 | 19.0±0.3 | 20.1±0.6 | 19.3±0.5 |
| Day 27 | 19.8±0.6 | 17.4±0.2* | 18.6±0.7 | 18.5±0.6 |
| Females | | | | |
| Day 7 | 13.3±0.4 | 12.0±0.5 | 12.9±0.4 | 12.7±0.6 |
| Day 14 | 13.5±0.3 | 12.1±0.3* | 13.3±0.4 | 13.5±0.4 |
| Day 21 | 13.9±0.2 | 12.5±0.4 | 13.4±0.5 | 13.3±0.5 |
| Day 27 | 13.4±0.5 | 12.6±0.7 | 12.9±0.5 | 12.9±0.2 |

Feed consumption means (g/rat/day+S.E.M.) of Wistar rats ($n=5$/sex/dose) consuming different dietary concentrations of barley soluble fiber enriched with β-glucan. *$P<0.05$ compared with control (0%) group by ANOVA followed by Dunnett's $t$-test.

Table 5
Feed conversion efficiency of rats exposed to barley β-glucan concentrate for 28 days

| | Dietary concentration of barley β-glucan concentrate | | | |
|---|---|---|---|---|
| | Control | 1.0% | 5.0% | 10.0% |
| Males | | | | |
| Day 7 | 0.32±0.01 | 0.31±0.01 | 0.31±0.00 | 0.30±0.01 |
| Day 14 | 0.30±0.02 | 0.27±0.01 | 0.28±0.01 | 0.30±0.01 |
| Day 21 | 0.23±0.01 | 0.21±0.01 | 0.21±0.01 | 0.21±0.01 |
| Day 27 | 0.20±0.01 | 0.022±0.01 | 0.20±0.01 | 0.21±0.01 |
| Females | | | | |
| Day 7 | 0.16±0.01 | 0.12±0.01 | 0.16±0.01 | 0.17±0.02 |
| Day 14 | 0.18±0.01 | 0.16±0.03 | 0.15±0.02 | 0.15±0.01 |
| Day 21 | 0.13±0.01 | 0.13±0.02 | 0.14±0.01 | 0.13±0.02 |
| Day 27 | 0.16±0.02 | 0.15±0.02 | 0.14±0.02 | 0.13±0.01 |

Mean feed conversion efficiencies [(g weight gain)/(g feed consumed)±S.E.M.] of Wistar rats ($n$=5/sex/dose) consuming different dietary concentrations of barley β-glucan concentrate. Values were determined over a period of 7 days (days 7, 14 and 21) or 6 days (day 27). No statistically significant differences were observed between the β-glucan groups and control males or females.

### 3.4. Hematology and clinical chemistry

Historical values (means±S.D., range) are from untreated control groups [WBC ($n$=5/group) or clinical chemistry values ($n$=8/group)] from recent studies conducted at TNO Nutrition and Food Research with animals of the same strain and age as used in the present study.

Red blood cell and coagulation parameters did not show any statistically or biologically significant differences between rats fed the test substance and starch controls (Table 6). However, significant increases were noted in total WBC counts in low-dose males (12.2±0.8 × $10^9$ cells/l; 37% increase) and mid-dose males (14.1±0.9 × $10^9$ cells/l; 58% increase) compared with starch control males (8.9±0.7 × $10^9$ cells/l). Total WBC counts also increased in high-dose males (10.9±0.9 × $10^9$ cells/l; 22% increase) but the difference from controls was not statistically significant compared with control males (Table 7). The values observed in the males consuming the control diet were similar to those observed in historical control (untreated) male animals.[1] The increase in total WBC was attributable to an increase in the number of lymphocytes since no changes were noted in the numbers of other white blood cells (neutrophils, eosinophils, monocytes and basophils; Table 7). Compared with WBC counts in female control animals (11.7±1.0 × $10^9$ cells/l), a decrease was noted in high-dose females (8.1±0.6 × $10^9$ cells/l) but no changes were observed in the low- or mid-dose females. However, in this study, the WBC counts in the control female animals were higher than the values observed in historical control females of the same age.[2] Substitution of the control values observed in the current study with historical control values revealed the same bell-shaped dose–response curve as observed in the males.

Clinical chemistry values showed statistically significant changes in the plasma concentrations of total protein, albumin, urea, creatinine, calcium and chloride (Table 8). A significant decrease in total protein concentration was observed in mid- and high-dose females compared with controls but the values were within the range historically observed in control female animals.[3] These changes were accompanied by decreased concentrations of albumin in the mid- and high-dose female groups. Although significantly different from the control animals, the albumin values in both groups were well within those historically observed in control female animals.[4] There were no changes in the albumin to globulin ratio in either sex, or in the concentration of total protein or albumin in the males. Plasma urea concentrations were elevated in the mid- and high-dose groups, but the increase was only statistically significant in males. In both sexes, the urea levels in mid- and high-dose rats fell within the historical range, whereas concurrent control values were on the lower end of this range.[5] The increased plasma level of creatinine in low-dose males was considered a fortuitous finding because it was not confirmed at the higher dose levels. Plasma concentrations of electrolytes showed a decrease in calcium in mid- and high-dose females and an increase in chloride in males at all dose levels. These electrolytes were not significantly changed in the other sex, and the values remained within the range observed in historical controls.[6] Fasting glucose tended to be decreased, dose-dependently, in both sexes, but the differences from controls did not reach the level of statistical significance.

### 3.5. Organ weights and pathology

There was a dose-related increase in the weight of the full and empty cecum in mid- and high-dose rats of both sexes (Table 9). At the high-dose level, the differences from controls amounted to about 30% in females and

[1] Total WBC count in untreated males=9.3±2.2 × $10^9$ cells/l; range 6.3–11.6.

[2] Total WBC count in untreated females=7.2±1.4 × $10^9$ cells/l; range 5.0–8.3.

[3] Total plasma protein concentration in untreated females=63±3 g/l; range 58–66.

[4] Plasma albumin concentration in untreated females=42±2 g/l; range 38–45.

[5] Historic concentration of urea in untreated males (6.9±1.1 mmol/l; range 5.4–8.8) and females (7.6±1.3 mmol/l; range 5.9–9.8).

[6] Historic concentrations of calcium (2.85±0.23 mmol/l, range 2.49–3.22) and chloride (102±2 mmol/l, range 98–105) from untreated animals.

Table 6
Hematology values (RBC and coagulation parameters) from rats exposed to barley β-glucan concentrate for 28 days

| | Dietary concentration of barley β-glucan concentrate | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | RBC ($\times 10^{12}$/l) | HB (mmol/ll) | PCV (l/l) | MCV (fl) | MCH (fmol) | MCHC (mmol/l) | PT (s) | Reticulocytes ($\times 10^9$/l) | Thrombocytes ($1000 \times 10^9$/l) |
| Males | | | | | | | | | |
| Control | 8.04±0.09 | 10.3±0.0 | 0.454±0.002 | 56.5±0.7 | 1.28±0.01 | 22.6±0.1 | 39.8±0.5 | 42.0±1.0 | 1024±25 |
| 1.0% | 7.70±0.14 | 10.1±0.1 | 0.440±0.006 | 57.2±0.5 | 1.31±0.01 | 22.9±0.1 | 41.3±0.6 | 45.7±5.4 | 1020±29 |
| 5.0% | 8.02±0.26 | 10.2±0.2 | 0.443±0.007 | 55.3±1.1 | 1.27±0.02 | 23.0±0.1 | 39.6±0.7 | 40.3±1.6 | 954±30 |
| 10.0% | 8.03±0.23 | 10.3±0.3 | 0.448±0.013 | 55.8±0.6 | 1.28±0.02 | 22.9±0.2 | 41.2±1.2 | 43.2±1.4 | 1005±29 |
| Females | | | | | | | | | |
| Control | 7.43±0.15 | 10.0±0.2 | 0.419±0.010 | 56.4±0.9 | 1.34±0.02 | 23.8±0.2 | 32.5±0.7 | 39.2±3.0 | 958±39 |
| 1.0% | 7.45±0.15 | 9.9±0.2 | 0.416±0.009 | 55.8±0.6 | 1.33±0.02 | 23.9±0.2 | 34.4±0.4 | 35.0±1.3 | 885±24 |
| 5.0% | 7.26±0.07 | 9.8±0.1 | 0.405±0.006 | 55.8±0.9 | 1.35±0.02 | 24.2±0.3 | 34.1±0.7 | 35.8±1.6 | 928±36 |
| 10.0% | 7.46±0.16 | 9.7±0.2 | 0.403±0.009 | 54.0±0.2 | 1.30±0.00 | 24.1±0.1 | 33.7±0.7 | 32.6±2.2 | 919±28 |

Mean hematologic values (±S.E.M.) of Wistar rats ($n=5$/sex/dose) consuming different dietary concentrations of barley soluble fiber enriched with β-glucan. RBC, red blood cell concentration; HB, hemoglobin concentration; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PT, prothrombin time. Statistical analysis demonstrated no significant differences between the β-glucan groups and control males or females.

Table 7
Hematology values (total and differential white blood cell counts) from rats exposed to barley β-glucan concentrate for 28 days

| | Dietary concentration of barley β-glucan concentrate | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | WBC[a] | Lymphocytes[a] | (%)[b] | Neutrophils[a] | (%)[b] | Eosinophils[a] | (%)[b] | Monocytes[a] | (%)[b] |
| Males | | | | | | | | | |
| Control | 8.9±0.7 | 8.2±0.6 | (92.0±0.5) | 0.7±0.1 | (7.4±0.7) | 0.0±0.0 | (0.2±0.2) | 0.0±0.0 | (0.4±0.2) |
| 1.0% | 12.2±0.8* | 11.0±0.6 | (90.8±1.7) | 1.0±0.3 | (7.8±1.6) | 0.0±0.0 | (0.2±0.2) | 0.1±0.1 | (1.2±0.7) |
| 5.0% | 14.1±0.9** | 13.3±1.0** | (94.0±1.8) | 0.7±0.2 | (5.2±1.7) | 0.1±0.0 | (0.4±0.2) | 0.1±0.0 | (0.4±0.2) |
| 10.0% | 10.9±1.1 | 10.0±1.1 | (91.4±1.9) | 0.8±0.2 | (7.4±1.9) | 0.1±0.0 | (0.6±0.4) | 0.1±0.0 | (0.6±0.2) |
| Females | | | | | | | | | |
| Control | 11.7±1.0 | 11.3±0.9 | (96.8±0.7) | 0.2±0.1 | (1.8±0.6) | 0.0±0.0 | (0.2±0.2) | 0.1±0.1 | (1.2±0.6) |
| 1.0% | 9.0±0.9 | 8.6±0.9 | (95.0±1.1) | 0.3±0.1 | (3.6±0.9) | 0.1±0.0 | (0.8±0.2) | 0.1±0.0 | (0.6±0.2) |
| 5.0% | 11.8±0.9 | 11.2±0.8 | (95.4±1.4) | 0.5±0.1 | (3.8±1.0) | 0.1±0.0 | (0.4±0.2) | 0.1±0.1 | (0.4±0.4) |
| 10.0% | 8.1±0.6* | 7.7±0.6* | (95.2±1.2) | 0.3±0.1 | (4.0±0.8) | 0.0±0.0 | (0.2±0.2) | 0.0±0.0 | (0.6±0.4) |

Mean hematologic values (±S.E.M.) of Wistar rats ($n=5$/sex/dose) consuming different dietary concentrations of barley soluble fiber enriched with β-glucan. Values in parentheses indicate the percentage of the total WBC fraction. Mean basophil counts were also evaluated but values were 0.0 in all groups (data not shown). *$P<0.05$ and **$P<0.01$ by ANOVA followed by Dunnett's $t$-test.

[a] $\times 10^9$/l.

[b] Differential of total WBC count (% indicated in parentheses).

50% in males. At the low-dose level, there were no statistically significant differences in cecum weights, though the weight of the full cecum of males was nearly 20% higher than that of controls. The increased full cecum weight was due to increases in the weights of both the cecal contents and the empty cecum. The proportionate increases in the weights of the full and empty cecum and the cecal contents were similar. The statistically significant increase in relative spleen weight in low-dose males was neither confirmed at the higher dose levels nor accompanied by histopathological alterations and, therefore, not ascribed to treatment.

Gross examination at necropsy and microscopic examination revealed no changes attributable to the ingestion of the test substance (data not shown). The changes observed represent common findings in rats of the strain and age used, and occurred only incidentally or at comparable incidences in the treated group(s) and controls.

## 4. Discussion

Oats and barley lower serum cholesterol concentration in humans (Judd and Truswell, 1981; Anderson et al., 1984; Newman et al., 1989a,b; Kestin et al., 1990; McIntosh et al., 1991; Ripsin et al., 1992; Lupton et al., 1994; Ikegami et al., 1996; Kahlon and Chow, 1997). This activity is attributable to the soluble fiber fraction of these cereal grains, more specifically, to the β-glucan

Table 8
Clinical chemistry values from rats exposed to barley β-glucan concentrate for 28 days

| Dietary concentration of barley β-glucan concentrate | ALP (U/l) | ALAT (U/l) | ASAT (U/l) | GGT (U/l) | TP (g/l) | Alb (g/l) | Creat. (μmol/l) | Bili. (μmol/l) | Urea (mmol/l) | Chol. (mmol/l) | Glucose (mmol/l) | TG (mmol/l) | PL (mmol/l) | P (mmol/l) | Ca (mmol/l) | Cl (mmol/l) | K (mmol/l) | Na (mmol/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | | | | | | | | | | | | | | | | | | |
| Control | 174±8 | 46±3 | 56±1 | 0.0±0.0 | 69±1 | 44±1 | 20±1 | 1.2±0.2 | 5.7±0.2 | 1.61±0.06 | 9.84±0.66 | 1.01±0.05 | 1.66±0.09 | 3.85±0.20 | 3.11±0.05 | 98±1 | 5.8+0.1 | 145±2 |
| 1.0% | 143±6 | 44±1 | 60±2 | 0.3±0.2 | 66±2 | 42±1 | 24±1* | 1.4±0.1 | 6.3±0.2 | 1.40±0.04 | 7.98±7.93 | 0.85±0.11 | 1.51±0.04 | 3.59±0.19 | 3.06±0.05 | 101±1* | 5.7+0.2 | 145±1 |
| 5.0% | 170±13 | 46±4 | 62±5 | 0.1±0.1 | 66±0 | 43±0 | 21±1 | 1.8±0.4 | 6.8±0.3* | 1.79±0.12 | 7.93±0.34 | 0.95±0.10 | 1.76±0.08 | 3.46±0.18 | 3.03±0.03 | 101±0* | 6.1±0.1 | 145±1 |
| 10.0% | 159±7 | 48±2 | 62±3 | 0.0±0.0 | 65±1 | 42±1 | 23±1 | 1.5±0.2 | 6.9±0.3* | 1.58±0.06 | 8.08±0.67 | 0.71±0.13 | 1.65±0.09 | 3.85±0.42 | 3.00±0.06 | 102±1** | 5.8±0.3 | 144±1 |
| Females | | | | | | | | | | | | | | | | | | |
| Control | 94±6 | 35±2 | 59±3 | 0.0±0.0 | 66±1 | 45±1 | 20±1 | 0.8±0.1 | 5.2±0.2 | 2.33±0.14 | 7.18±0.86 | 0.77±0.05 | 2.34±0.10 | 3.15±0.12 | 2.98±0.02 | 101±1 | 5.7±0.1 | 144±1 |
| 1.0% | 94±7 | 37±3 | 65±3 | 0.0±0.0 | 64±1 | 44±1 | 23±1 | 0.8±0.2 | 5.3±0.2 | 2.39±0.21 | 6.96±0.19 | 0.64±0.03 | 2.36±0.14 | 3.23±0.19 | 2.92±0.04 | 103±1 | 6.0±0.2 | 144±1 |
| 5.0% | 86±5 | 39±3 | 65±4 | 0.0±0.0 | 62±1* | 42±1* | 22±0 | 0.9±0.2 | 5.8±0.3 | 2.45±0.10 | 6.04±0.44 | 0.76±0.12 | 2.34±0.07 | 3.03±0.12 | 2.83±0.03* | 105±0 | 5.7±0.2 | 143±0 |
| 10.0% | 101±8 | 41±2 | 70±4 | 0.0±0.0 | 61±1* | 42±1* | 22±1 | 1.0±0.3 | 6.0±0.3 | 2.29±0.14 | 5.85±0.49 | 0.62±0.06 | 2.25±0.08 | 3.20±0.13 | 2.82±0.04** | 103±1 | 5.7±0.1 | 143±0 |

ALP, alkaline phosphatase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma-glutamyl transferase; TP total protein; Alb, albumin, Creat., creatinine; Bili., bilirubin; Chol., cholesterol; TG, triglycerides; PL, phospholipids; P, inorganic phosphate.
Statistical significance relative to control indicated as *$P$ <0.05 and **$P$ <0.01 by ANOVA followed by Dunnett's $t$-test.

component (Davidson et al., 1991; Braaten et al., 1994). The β-glucan in oats and barley appears to be chemically similar (Jeraci and Lewis, 1989). Although preparations enriched in β-glucan from oats or barley lower serum cholesterol concentrations in animal models of hypercholesterolemia (Inglett, 1991; Knuckles et al., 1992; German et al., 1996; Kahlon and Chow, 1997; Rieckhoff et al., 1999; Maqueda de Guevara et al., 2000) they have not been evaluated for the potential to cause adverse effects.

In the present study (product name), a water-soluble β-glucan-enriched concentrate was prepared from Azhul barley and evaluated in Wistar rats exposed by incorporation of the concentrate into a cereal-based, natural ingredient diet. The feeding of the barley soluble fiber product enriched in β-glucan at dietary levels of 1, 5 and 10% (corresponding to 0.7, 3.5 and 7% β-glucan) to male and female Wistar rats for 4 weeks was not associated with adverse effects on general condition and behavior, growth, feed and water consumption, feed conversion efficiency, red blood cell and clotting potential parameters, clinical chemistry values, and organ weights, nor by abnormalities in necropsy and histopathology findings.

Not unexpectedly, the ingestion of barley soluble fiber resulted in cecal enlargement as reflected in the dose-related increase in the weight of the full and empty cecum. Cecal enlargement is commonly observed in rats fed high levels of fermentable carbohydrates that are either poorly digestible or slowly absorbed (Newberne et al., 1988). Because such substances are incompletely absorbed in the small intestines, they reach the large intestine where they are fermented by microflora. It is believed that fermentation products, including short-chain fatty acids, increase the osmotic load and attract water leading to distension and increased weight of both the large intestine itself and its contents. This phenomenon is considered of no toxicological concern (WHO, 1987).

Significant increases in total WBC counts were observed in males from the low- and mid-dose barley β-glucan concentrate groups. The WBC counts were also increased in males from the high-dose group; however, the increase was not statistically significant compared with controls. The increases in WBC count were attributable to the lymphocytes as no significant changes were noted in the numbers of other white blood cell types. Similar effects have been observed in laboratory animals consuming high concentrations of soluble fiber from other dietary sources such as rice (Takenaka and Itoyama, 1992). In contrast to males, WBC and lymphocyte counts in females were decreased in the high-dose group compared with controls ($P$ <0.05). However, in this study, the WBC counts in the concurrent female control group were considerably higher than those in historical controls. If the historic value for age- and

Table 9
Organ to body weight ratios from rats exposed to barley β-glucan concentrate for 28 days

| | Dietary concentration of barley β-glucan concentrate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adrenals | Kidneys | Thymus | Brain | Spleen | Heart | Liver | Cecum | | Testes |
| | | | | | | | | Full | Empty | |
| Males | | | | | | | | | | |
| Control | 0.189±0.006 | 6.94±0.22 | 2.08±0.11 | 6.51±0.15 | 1.87±0.04 | 3.54±0.08 | 32.2±0.6 | 14.0±1.2 | 3.1±0.1 | 10.84±0.42 |
| 1.0% | 0.211±0.008 | 6.44±0.12 | 2.28±0.14 | 6.65±0.13 | 2.30±0.12** | 3.75±0.11 | 31.4±0.8 | 16.5±2.1 | 3.3±0.2 | 11.70±0.27 |
| 5.0% | 0.194±0.007 | 6.79±0.25 | 2.25±0.10 | 6.52±0.13 | 2.13±0.05 | 3.50±0.07 | 31.0±0.5 | 17.9±1.3** | 3.8±0.1* | 11.16±0.47 |
| 10.0% | 0.190±0.007 | 6.65±0.06 | 2.08±0.08 | 6.63±0.05 | 2.10±0.04 | 4.26±0.56 | 32.4±0.9 | 20.5±1.0** | 4.7±0.2** | 11.00±0.50 |
| Females | | | | | | | | | | Ovaries |
| Control | 0.324±0.031 | 7.51±0.21 | 2.40±0.11 | 8.98±0.24 | 2.24±0.07 | 3.81±0.11 | 29.5±0.5 | 14.0±0.6 | 4.0±0.2 | 0.440±0.028 |
| 1.0% | 0.338±0.014 | 7.24±0.14 | 2.13±0.19 | 9.85±0.30 | 2.16±0.07 | 3.91±0.13 | 29.5±0.3 | 14.9±0.7 | 3.7±0.2 | 0.454±0.019 |
| 5.0% | 0.331±0.012 | 7.29±0.09 | 2.15±0.08 | 9.65±0.13 | 2.34±0.06 | 3.93±0.06 | 28.8±1.2 | 17.6±0.7** | 4.2±0.2 | 0.409±0.020 |
| 10.0% | 0.337±0.010 | 6.85±0.21 | 2.22±0.21 | 9.79±0.18 | 2.34±0.10 | 3.70±0.16 | 29.7±1.0 | 18.2±0.8** | 5.1±0.3** | 0.425±0.023 |

Mean relative organ weights in g/kg of body weight (±S.E.M.) of Wistar rats ($n=5$/sex/dose) consuming different dietary concentrations of barley soluble fiber enriched with β-glucan. Epididymus weight was also evaluated but values in treated males were not different from the values observed in the control group (data not shown). Statistical significance relative to control indicated as * $P<0.05$ and ** $P<0.01$ by ANOVA followed by Dunnett's $t$-test.

sex-matched controls was substituted for the values observed in the current study, a dose-dependent increase similar in trend and magnitude to that observed in male rats could also be implied for the females. These results suggest that β-glucan enriched soluble fiber from barley possesses immune modulating activity following oral exposure. Numerous studies have demonstrated immunologic activity of β-glucan from various sources including barley. Human macrophages appear to possess receptors for barley β-glucan (Czop and Austen, 1985) and immune stimulating activity has been reported following exposure to β-glucan (Czop and Kay, 1991; DiRenzo et al., 1991). The effects of β-glucan on immunity are commonly attributed to effects on macrophages (Seljelid et al., 1987; Suzuki et al., 1990) either directly or indirectly through modulation of growth factors and cytokines (Rasmussen et al., 1992). The characterized effects appear to be more directed at modulating macrophage activity rather than on proliferation (Bogwald et al., 1984; Rasmussen et al., 1987; Seljelid et al., 1987). Results from those studies are in agreement with the results of the current study in that the number of circulating monocytes and macrophages was not altered from control values in any β-glucan exposure group. Increased lymphokine production (IL-2, IL-4 and IFN-γ) has been reported following β-glucan exposure that could lead to proliferation of lymphocytes as was observed in the current study (Estrada et al., 1997).

Compared with concurrent controls, rats fed barley-soluble fiber showed slight changes in some clinical chemistry values (plasma levels of urea, total protein, albumin, chloride, calcium) but these were not regarded to represent adverse effects of the test substance for the following reasons. The increases in urea, total protein and albumin, possibly indicating renal or hepatic toxicity, were not accompanied by significant changes in other indicators of damage to the kidneys or liver. Moreover, all values in the test groups fell within the historical control range whereas several concurrent control values were at the lower (chloride, urea) or upper (total protein, albumin) end of this range. Finally, most of these finding were not confirmed in the opposite sex. Despite the ability of β-glucan-enriched preparations to reduce serum cholesterol, such an effect was not observed in the present study. This does not indicate a lack of effectiveness of the current preparation because this toxicity study was not designed to examine efficacy. For example, the diets were not formulated with supplemental cholesterol and cholic acid, both of which are necessary to elevate serum cholesterol in rats and thus to create a more sensitive rat model for detection of cholesterol lowering activity (Beynen et al., 1986; Abbey et al., 1993).

The current study demonstrated that daily consumption of large amounts of concentrated barley β-glucan was not associated with any obvious signs of toxicity in Wistar rats. The only finding of possible biological relevance was the increased number of circulating lymphocytes and even that effect did not appear to be dose-dependent and did not occur in the females. The highest dietary concentration of β-glucan from the enriched barley-soluble fiber administered in this study (approximately 7% β-glucan) corresponds with an overall intake of 5.6 g β-glucan/kg body weight/day. For a 60-kg adult, this corresponds to 336 g of β-glucan/day, which is more than 100-fold higher than the daily amount recommended for lowering serum cholesterol (i.e. 3 g/day). It is therefore concluded that consumption of barley-soluble fiber enriched in β-glucan is not likely to cause adverse effects under the conditions of intended use.

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