Effects of Dietary β-Glucan on Serum Lipids and Performance Indices in Rats Fed a Diet Enriched with Cholesterol

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ABSTRACT

The aims of this study were to investigate effects of β-glucan on body weight gain, food intake, food conversion ratio and serum total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol) and triglyceride levels in female rats fed hypercholesterolemic diet. Female Sprague-Dawley rats (8-weeks-old) weighing 161.78±3.88 g were divided into three equal groups. Group 1 (control) was fed basal diet (2% liquid-vegetable oil, 0% cholesterol), group 2 was fed high-cholesterol diet (2% liquid-vegetable oil, 15% hydrogenated-oil and 1.5% cholesterol) and group 3 was fed high-cholesterol diet with 1% β-glucan. The trial period was 30 days. Blood samples were withdrawn on days 0 and 30. Also, all rats were weighed on same days. Serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride levels were detected with commercial kits by auto-analyzer. Body weight gain, food intake and food conversion ratio, and serum total cholesterol and LDL-cholesterol levels were significantly lower (P<0.05) in group 3 (the group fed fatty and added β-glucan) than in the other two groups. Serum HDL-cholesterol and triglyceride levels were not significant between all groups at the end of the study. β-glucan supplementation negatively affected food intake. However, β-glucan effectively lowered serum LDL-cholesterol and total cholesterol concentrations without affecting HDL-cholesterol and triglyceride levels. Therefore, β-glucan may decrease the cholesterol synthesizing ability of liver and the risk for atherosclerotic vascular disease.

INTRODUCTION

The leading cause of morbidity and mortality in many countries in the world is still cardiovascular disease (CVD), in spite of remarkable improvements in its prevention, diagnosis and therapy (Anderson et al., 1990). Hypercholesterolemia is caused by increased concentrations of low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C). The increase in VLDL causes the increase in triglycerides (TGs). High TG and greater LDL-C are predictors of increased CVD risk. High-density lipoprotein cholesterol (HDL-C) concentrations provide the opposite relationship, with increased blood concentrations of HDL-C predicting reduced risk (Chen et al., 2009). To lower serum LDL-C levels by making dietary changes is the well-established way to reduce the risk of developing CVD. 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 has become increasingly recognized (Theuwissen and Mensik, 2008). β-glucans are water-soluble fibers, which are found in a wide variety of product such as oats, barley, and yeast (Bell et al., 1999; Esghii and Akhundova, 2010). One of the richest sources of β-glucan is the cell wall of baker’s yeast Saccharomyces cerevisiae. Regardless of their source, all β-glucans are polysaccharides composed of glucose molecules (Theuwissen and Mensik, 2008). Natural (1,3)-β-D-glucans from yeast, grain and mushrooms are well-established biological response modifiers which represent highly conserved structural components of cell walls in yeast, fungi, seaweed or grain seeds (Borchers et al., 1999). Although the exact mechanism explaining the cholesterol-lowering effect of β-glucan is not known, the most likely explanation is that

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water soluble fibers lower the absorption of bile acids. As a result, hepatic conversion of cholesterol to bile acids increases, hepatic pools of free cholesterol decrease, and, to reach a new steady-state, endogenous cholesterol synthesis increases (Lia et al., 1997). Also, hepatic LDL-C receptors become up-regulated to re-establish hepatic cholesterol stores, which lead to decrease serum LDL-C concentrations (Reihner et al., 1990).

The cholesterol lowering effects of fibers are routinely associated with β-glucans. Due to the most of the attention has been focused on the relationship between glucan and cholesterol levels. In a study by Vetvicka and Vetvickova (2007), 8 weeks old female BALB/c mice were given diet supplemented with either glucan or cholesterol corresponding to the final daily doses 100 µg of glucan or 16 µg of cholesterol. The cholesterol-rich diet was followed by 40 days of feeding with yeast derived glucan-rich diet, and it was showed that glucan lowered the cholesterol levels in hypercholesterolemic mice. Kalra and Jood (2000) conducted an experiment in rats to investigate the effect of dietary barley β-glucan on cholesterol and lipoprotein fractions. Rats were fed three barley cultivars containing 6.23, 4.60 and 2.18% total β-glucan and 5.39, 2.06 and 1.08% soluble β-glucan, respectively. As a result, total and soluble β-glucan appeared to be strong predictors of the cholesterol-lowering in serum and liver of rats. Mursito et al. (2011) used β-glucans isolated of *Termittomyces eurrhizus* extracts in hypercholesterolemic rats for 4 weeks and 72 mg/kg b.wt β-glucan had proven lowering cholesterol level of rats equivalent with giving cholestyramine 72 mg/kg b.wt. In contrast, Bobek et al. (1997) reported that diet containing 5% beta-1, 3-D-glucan isolated from oyster mushroom did not affect neither cholesterol levels in serum and liver nor its distribution in lipoproteins. Delaney et al. (2003) reported that the addition of β-glucan concentrate (2, 4, or 8%) prepared from oats and barley to hypercholesterolemic diet containing 0.15% cholesterol resulted dose dependent decreases in plasma total and LDL cholesterol concentrations in hamsters. Also, liver cholesterol and aortic cholesterol ester concentrations were significantly reduced in hamsters consumed 8% β-glucan.

In this study, rats were selected because appears to offer a useful model for the study of human hyperlipoproteinemia and hyperlipidemia (Koletsky, 1977). The aim of this study was to investigate the effect of β-glucan on plasma lipids in hypercholesterolemic rats.

**MATERIALS AND METHODS**

This study was conducted at Istanbul University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Istanbul, Turkey.

Thirty white female, 8-weeks-old Sprague-Dawley rats weighing 161.78±3.88 g were used in the study. The rats, which were separated to three equal groups, each consisting of 10 rats, were housed individually in polypropylene cages kept in a controlled environment maintained at 20-22°C with a 12-hr light and dark cycle. Group 1 (control) was fed basal diet (2% liquid-vegetable oil, 0% cholesterol), group 2 was fed high-cholesterol (HC) diet (2% liquid-vegetable oil, 15% hydrogenated-oil and 1.5% cholesterol) and group 3 was fed HC-diet with 1% β-glucan. Yeast β-glucan was obtained from Immudyne (Houston, TX). The trial period was 30 days. Food and water were provided *ad libitum*. All diets were isonitrogenic (20% CP) and formulated in Istanbul University, Department of Animal Nutrition and Nutritional Diseases. Composition of diets was presented in Table 1.

Rats were weighed individually at the beginning of the study for initial body weights. On d 0 and 30, rats were fasted for 12 h and then weighed. Amounts of offered and refused foods were recorded daily and food consumption was determined at the end of the experiment. Data were recorded to determine body weight (BW), average daily gain (ADG), average daily food intake (ADFI) and gain: food ratio during the experiment.

Blood samples were collected on d 0 and 30 from tail vena of rats and centrifuged at 3000 rpm for 10 min. to obtain serum. Serum samples were stored in screw capped vials and kept in freezer at -80 ºC until further analysis.

Serum triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol levels were detected with a commercial kit (Accurex Biomedical Pvt. Ltd., Boisar, Thane, India) by automatic analyzer (Hitachi-704). All statistical analyses were performed by using software package program (SPSS for windows, Standard version 10.0, 1999; SPSS Inc., Headquarters, Chicago, IL, USA) package software. One way analysis of variance (ANOVA) was used for each experiment and mean differences were determined by Duncan’s multiple range tests. Results of performance and serum parameters are presented as means ± standard deviation.

**RESULTS**

Initial and final body weight, average daily weight gain, average daily food intake and food efficiencies for group 1, 2 and 3 were presented in Table 2. There were no significant differences between group 1 fed basal diet and group 2 fed high cholesterol (HC) diet for final body weight, average daily gain and average daily food intake (P>0.05). Group 3 fed HC-diet + β-glucan had lower values for these parameters than those of group 1 and 2 (P<0.05). Amount of food intake per g of weight gain (gain: food) were higher in group 2 than those of group 1 and 3 (P<0.05). β-glucan supplementation to HC-diet improved food efficiency of rats.

The levels of total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol determined in serum samples obtained from rats on d 0 and 30 were presented in Table 3. There were no significant differences between groups for serum total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol levels at the beginning of the experiment. On day 30, the lowest triglyceride level was determined in group 1 fed basal diet (P<0.05), and there were no difference between group 2 and 3 fed HC-diet and HC-diet + β-glucan, respectively. As expected, rats fed HC-diet had the highest total cholesterol and LDL-cholesterol (P<0.05), however, β-glucan supplementation effectively decreased the level of total cholesterol and LDL-cholesterol in rats fed HC-diet (P<0.05), and there were no significant difference between rats fed basal and β-glucan diet for LDL-cholesterol on day 30.
Although high cholesterol content in diets of group 2 and 3 caused relatively decrease in HDL-cholesterol in these groups on day 30, there were no significant differences among these groups for HDL-cholesterol. With respect to VLDL-cholesterol levels, rats fed basal and HC diet had lowest and highest VLDL-cholesterol level, respectively (P<0.05). When it was compared to rats fed HC-diet, β-glucan supplementation to HC-diet significantly lowered the level of VLDL-cholesterol on day 30 (P<0.05).

### Table 1: Composition of diets (%)

<table>
<thead>
<tr>
<th>Parameters/Days</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>161.43±9.12</td>
<td>160.73±18.62</td>
<td>163.24±9.12</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>233.95±17.09</td>
<td>243.05±23.88</td>
<td>211.92±23.45</td>
</tr>
<tr>
<td>Average daily gain, g d⁻¹</td>
<td>2.59±0.32</td>
<td>2.93±0.34</td>
<td>1.73±0.37</td>
</tr>
<tr>
<td>Average daily food intake, g d⁻¹</td>
<td>14.24±0.74</td>
<td>13.37±0.83</td>
<td>11.48±1.39</td>
</tr>
</tbody>
</table>

Means±SD within a row without a common superscript are different (P<0.05).

### Discussion

There were no significant differences between groups for initial body weight because of the study carried out under equal conditions with respect to body weight. β-glucan supplementation to HC-diet resulted in the decrease in final body weight, weight gain and food intake in rats (P<0.05). Many studies have shown beta-glucans reduced body weight (Artiss et al., 2006). Sánchez et al. (2008) reported that oat bran β-glucan at the concentration of 10% caused the lowering body weight in Zucker rats. In our study, weight reducing effect of β-glucan observed by at dose of 1% seems to agree with these reports. However, some researches also showed that β-glucan supplementation had no effect on performance parameters (Yokoyama et al., 1998; Kalra and Jood, 2000).

In this study, β-glucan supplementation to HC-diet decreased serum total cholesterol, LDL-cholesterol and VLDL-cholesterol concentrations except for triglyceride and HDL-cholesterol. There are many studies on the effect of β-glucan on blood cholesterol levels in several species. Kalra and Jood (2000) reported that three barley cultivars containing 6.23, 4.60, 2.18% total β-glucan and 5.39, 2.06, 1.08% soluble β-glucan decreased levels of total cholesterol and LDL-cholesterol and increased HDL-cholesterol in rats and both decreases was significantly correlated with β-glucan content. Also, Mursito et al. (2011) carried out a study in hypercholesterolemic rats by using -glucans isolated of *Termotomyces euryhiza* extracts and observed that 72 mg kg⁻¹ b.wt β-glucan had total cholesterol and LDL-cholesterol of rats equivalent with giving choleystyramine 72 mg kg⁻¹ b.wt. In a study with Swiss albino mice by Dhillon and Bhatia (2008), mice were injected single dose of 200 mg kg⁻¹ body weight cholesterol to induce hypercholesterolemic condition and mice were inoculated with a commercial β-glucan dose i.e. 20 mg kg⁻¹ body weight. As a result, total serum cholesterol levels reduced and HDL-cholesterol levels increased significantly in hypercholesterolemic mice by β-glucan inoculation. To investigate the effects of different type of β-glucans, Vetvicka and Vetvickova (2007) supplemented mice diet with either glucan or cholesterol at the daily dose of 100 µg and 16 µg, respectively. The cholesterol-rich diet was followed by 40 days of feeding with glucan-rich diet and results showed that during short time intervals all glucans lowered the cholesterol levels in hypercholesterolemic mice. Wilson et al. (2004) evaluated β-glucan preparations at a concentration of 8 g 100 g⁻¹ at the expense of cellulose in Syrian Golden F1/B hamsters fed hypercholesterolemic diet. Plasma total cholesterol and non-HDL-cholesterol concentrations decreased in hamsters fed β-glucan diets, whereas HDL-cholesterol did not differ. Also, in a study conducted by Wang et al. (1992) on broiler chickens with barley β-glucan, control group was fed only corn basal diet, experimental groups were fed diets containing barley or enzyme β-glucanase (1 g kg⁻¹) and barley. When compared to control group, experimental groups had significantly lower total cholesterol and LDL-cholesterol. In addition to animal studies, there are some human trials presenting notable results. For example, Nicolosi et al. (1999) carried out an experiment with 15 obese, hypercholesterolemic men. After a 3-week period on their usual diets, they consumed 15 g of yeast-derived β-glucan fiber was added to the diet for 8 wk. When compared with baseline, β-glucan fiber consumption significantly reduced serum total cholesterol by 6%. LDL-cholesterol concentrations declined significantly by 8% and HDL-cholesterol increased significantly with 9%. Similarly, Shimizu et al. (2008) also showed that 7.0 g β-glucan from barley per day for 12 weeks caused marked decrease in LDL-cholesterol.

The mechanism for β-glucan to lower LDL is considered to be mediated by bile acids binding property of β-glucans. Therefore β-glucans increase exclusion of bile acids (Lia et al., 1995), and this is in turn activates
cholesterol 7-hydroxylase and up-regulates LDL-receptor and thus increase the transport of LDL into hepatocytes and the conversion of cholesterol into bile acids (Nilsson et al., 2007). Sadiq et al. (2008) reported that the advantages of β-glucans are that they exhibit high viscosities at very low concentration (1%) and are stable with pH. When it was considered the similarities of cholesterol metabolism in poultries, humans and laboratory animals (Leveille et al., 1975), same mechanism may be valid for our study presenting similar results, which agree with prior experiments mentioned above, in the effect of 1% β-glucan on the reduction of blood cholesterol.

In conclusion, β-glucan supplementation to HC-diet resulted in the decrease in final body weight, weight gain and food intake in rats. Also, β-glucan supplementation decreased serum total cholesterol, LDL-cholesterol and VLDL-cholesterol significantly without any alteration in HDL-cholesterol. Weight reducing and hypocholesterolemic effects of β-glucan observed in this study may provide alternative approach with respect to reduce risk factors for obesity and CVD which has been associated with increased total cholesterol, LDL-cholesterol, VLDL-cholesterol, and decreased HDL-cholesterol.

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Bobek P, L Ozdin and L Kuniak, 1997. Effect of oyster mushroom and food intake in rats. Also, result in the decrease in final body weight, weight gain and food intake in rats. Also, β-glucan supplementation decreased serum total cholesterol, LDL-cholesterol and VLDL-cholesterol significantly without any alteration in HDL-cholesterol. Weight reducing and hypocholesterolemic effects of β-glucan observed in this study may provide alternative approach with respect to reduce risk factors for obesity and CVD which has been associated with increased total cholesterol, LDL-cholesterol, VLDL-cholesterol, and decreased HDL-cholesterol.

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