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Research Article

The Effect of Orally Administrated β-glucan and Dietary Restriction on Faecal Microflora in Rats [1]

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Abstract

In this study we aimed to evaluate the effect of orally administered β-glucan and/or dietary restriction on *Lactobacillus* spp., coliforms, *Enterobacteriaceae* and enterococci counts in rat faeces. For this aim, rats were divided into three experimental groups: i) first group animals received normal diet for 6 months and administrated orally with β-glucan (20 mg/kg for bodyweigth) over the last 14 day of experiment, ii) second group was dietary restricted animals for 6 months and receiving β-glucan as those of first group animals, iii) last group was the control group rats receiving only *ad libitum* feed. Compared to control group, numeric increase was observed in the number of coliforms, *Enterobacriaceae* and lactobacillus counts in first group but this was not statistically important. The increase in coliforms and *Enterobacteriaceae* counts was nearly 2 log while this was 1 log for lactobacillus counts. Interestingly, dietary restriction + β-glucan administration had no significant influence on the increase of defined bacterial groups. The results of the present study showed that orally administration of the β-glucan, widely used as prebiotic, has the potential to modify faeces microbiota in rat model.

Keywords: *β-glucan, Probiotic, Prebiotic, Lactobacillus*

Oral Yolla Uygulanan β-glukan ve Diyet Kısıtlamasının Ratların Fekal Mikroflorasına Etkisi

Özet

Bu çalışmanın amacı oral yolla verilen β-glukan ve/veya diyet kısıtlamasının rat feçesindeki *Lactobacillus* spp., koliform, *Enterobacteriaceae* ve enterokok sayıları üzerine etkisini ortaya koymaktır. Bu amaçla ratlar üç deneysel gruba ayrıldı: i) birinci grup 6 ay süresince normal diyetle beslenen ve son 14 gün oral yolla β-glukan (20 mg/kg, canlı ağırlığa) verilen hayvanlar ii) ikinci grup 6 ay boyunca diyet kısıtlaması uygulanan ve ilk grupla aynı şekilde β-glukan verilen hayvanlar iii) yalnızca *ad libitum* beslenen kontrol grubu ratlardan oluşmaktadır. Kontrol grubu ile karşılaştırıldığında, ilk grupta koliform, *Enterobacteriaceae* ve laktobasil sayılarında artış gözlendi, fakat bu artış istatistiksel olarak önemli değildi. Koliform ve *Enterobacteriaceae* sayılarındaki artış yaklaşık 2 log iken, laktobasil sayılarında bu artış 1 log idi. İlginç olarak diyet kısıtlaması ile birlikte β-glukan uygulaması adı geçen bakterilerin artışında önemli bir etki oluşturmadı. Mevcut çalışmanın sonuçları, prebiyotik olarak geniş kullanım alanı bulunan β-glukan'ın oral yolla takviyesinin rat modelinde dışkıdaki mikroorganizmaları modifiye etme potansiyeline sahip olduğunu gösterdi.

Anahtar sözcükler: *β-glukan, Probiotik, Prebiotik, Laktobasil*

INTRODUCTION

Prebiotic is defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" [1,2]. Today prebiotics have been widely used to enhance health in the human life [3]. Additionally they benefits on growth and performance of animals [4]. Probiotics and prebiotics are mostly consumed for strenghtening of friendly microflora of gut [5]. β-glucan, which is a novel prebiotic, has many health benefits. Some of these effects are immunomodulation, increasing the defence system to invading pathogens, anti-tumor activity, lowering the blood cholesterol level and beneficially affect to gut health [4,6]. Because of this effects, β-glucan

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is considered as biological response changers and regard from the pharmaceutical and functional food industries [7]. In addition to these positive properties β-glucan has prebiotic effect by improving the growth of lactobacilli in gut microflora [8] and is one of the most studied prebiotics [9]. Degradation of β-glucan by microbiota resuted with some fermentation products such as short chain fatty acids (SCFA) which are important for the development of the colon microflora. Presence of SCFA reduce the colonic pH and increases the bacterial cultivation [10]. Production of SCFA also prevents growth of potential pathogens [11]. Many researchers reported that β-glucan has positive effects on probiotic bacteria such as lactobacilli and bifidobacteria in gut microflora by animal experiments [12-14]. But there is not enough research about impact of β-glucan to intestinal microflora such as *Lactobacillus*, coliform, *Enterobacteriaceae*, enterococci.

The composition of the gastrointestinal microrganisms is intensively influenced by several factors that include the main gut flora at birth, host genetics, immunological factors, antimicrobial consumption and dietary effects. Change the intakes of the carbonhydrates, proteins and fats can significantly modify the composition of the microflora [15].

Dietary restriction (DR) that underfeeding without malnutrition, extends rats life-span in comparison with *ad libitum* feeding [16,17]. DR is the only experimental orientation that has been presented to delay aging, decrease deseases, health risks and the occurence and proceeding of tumors [16]. DR delays onset of various age-related neurodegenerative diseases such as Alzheimer's disease and insuline resistance. DR is also ameliorate oxidative stres-releated impairment in tissues [17]. Altough these possitive effects there is not enough research about impact of dietary restriction to intestinal microflora.

The aim of this study was to evaluate the in vivo prebiotic potential of β-glucan and the efficiacy of long term dietary restriction supported with β-glucan on rat faecal microbiota such as *Lactobacillus*, coliform, *Enterobacteriaceae*, enterococci.

MATERIAL and METHODS

Animals and Management

Male, 3-months old Sprague-Dawley rats were used in this study. Rats were purchased from Experimental Animals Breeding and Research Centre of Uludag University. Thirty Sprague-Dawley rats were housed under controlled humidity (50-60%) and temperature (20-23°C) with a 12 h light-dark cycle. Rats had free access to tap water, and fed with a laboratory chow diet. The composition of the diet was as follows: protein 18% (min), lipid 2.5% (min), fiber 4% (max), ash 5.5% (max), nitrogen free extract 57.0% (max), metabolic energy 2650 kcal/kg (min), water 13% (max) plus various amino acids, minerals and vitamins (data obtained from the supplier). This study was performed after approval of the Local Ethical Committee of Animal Experiments of Uludag University with decision number 2012-14/05.

Study Design

All rats were divided to 3 groups and each group consisted of 10 rats. All animals were cared during 6 months. First and third (control) groups were given tap water and standart laboratory chow diet with *ad libitum*. Also, first group was orally administreted by β-glucan. Second group rats was applied by DR (monday, wednesday and friday mornings) (the food hoppers were placed the following morning as an every-other-day feeding schedule) and tap water ad libitum [16,17]. During last 14 days of 6 months housing, daily β-glucan intake in first and second groups were 20 mg/kg dose by intragastric way.

Microbiological Analyses

Rat faecal samples were collected from cage litters at 1st, 7th, and 14th days under sterile conditions and stored at -20°C for microbiological analysis until the last required sample is collected. Approximately 5 g of each faecal sample were homogenised in 45 ml sterile peptone water (0.1%) solution using a stomacher for at least two minutes. Decimal dilutions were made in sterile pepton water (0,1%) and plated in duplicate on the selective agars. Coliforms were isolated on Violet Red Bile agar (CM0107, Oxoid, UK) with overnight (20-24 h) incubation at 35-37°C. For enterococci the culture was grown on Slanetz and Bartley medium (CM0377, Oxoid, UK) at 35-37°C for 48 h. *Enterobactericeae* count was performed by Violet Red Bile Glucose agar (CM0485, Oxoid, UK) and incubated 20-24 h at 35-37°C [18]. *Lactobacillus* spp. was enumerated on de Man Rogosa Sharpe medium (CM0361, Oxoid, UK) and plates were incubated under anaerobic conditions at 35-37°C for 72 h [19].

Statistical Analyses

Statistical analyses were performed by SPPS 20 programme by Kruskal-Wallis test. When differances among the groups were significant, Mann-Whitney test was used. For interpreting results $P<0.05$ significance level was used.

RESULTS

The results related to the counts of coliforms, *enterobactericeae, enterococci* and *Lactobacillus* spp. according to groups are summarized in *Table 1*. Increase in coliform counts was almost 2 logs in the first group, while this increase was sligtly lower in other groups with a change of 1 log. But there was no statistically significant difference ($P>0.05$) among the groups. Only the first group animals exhibited changes in term of *Enterobactericeae* counts which were increased 2 log in 2 weeks period. In

***Table 1.** Lactobacillus spp., Coliform bacteria, Enterobacteriaceae and Enterococci counts (log_{10} cfu/g) according to groups and sampling days*
***Tablo 1.** Gruplara ve örnekleme günlerine göre Lactobacillus spp., koliform bakteri, Enterobacteriaceae ve enterokok sayıları (log_{10} kob/g)*

Microorganism	Day 1			Day 7			Day 14		
	First (β-glucan)	Second (DR+ β-glucan)	Third (Control)	First (β-glucan)	Second (DR+ β-glucan)	Third (Control)	First (β-glucan)	Second (DR+ β-glucan)	Third (Control)
	Mean±SEM			Mean±SEM			Mean±SEM		
Lactobacillus spp.	8.67±0.07*	9.02±0.17*	8.92±0.15*	8.53±0.07	9.02±0.16	8.64±0.05	9.04±0.40	9.11±0.10	8.66±0.09
Coliform	4.62±0.13	5.13±0.59	4.27±0.28	5.11±0.27	5.17±0.31	5.01±0.38	6.37±0.37	5.36±0.42	5.01±0.30
Enterobacteriaceae	4.80±0.09	5.27±0.60	4.97±0.14	5.18±0.22	5.20±0.27	5.06±0.42	6.35±0.51	5.44±0.45	4.77±0.39
Enterococci	5.77±0.23	5.81±0.48	5.57±0.42	6.08±0.14	5.83±0.12	5.75±0.07	6.26±0.22	5.34±0.23	6.07±0.38

** Values sharing the same symbol in the same row are not significantly (P>0.05) different among groups*

other two (control and DR + β-glucan) groups, the counts of *Enterobacteriaceae* did not change. Although the higher counts of enterococci in first and third groups were detected, the faecal samples of the second group rats were not assigned any change for enterococci counts after 14 days period. For *Enterobacteriaceae* and enterococci counts, the differences among groups were not statistically significant (P>0.05).

Lactobacillus spp. population in first group were similar at 1st and 7th days. But on 14th day the counts of these bacteria were observed a 1 log increase. On the other hand the increase was not statistically significant (P>0.05). On 1st, 7th and 14th days, *Lactobacillus* spp. counts in second and third groups were not seen any difference.

DISCUSSION

This study was carried out to determine the effect on faecal microrganism counts of the feeding by β-glucan. The addition of β-glucan in the diet of rats did not significantly increased (P>0.05) the numbers of coliform, enterococci, *Enterobacteriaceae*, enterococci and *Lactobacillus* spp. in the faecal samples (1, 7 and 14 days after feeding).

In the present study, coliform counts increased at least one log at first (β-glucan) and third groups. On the other hand, Turunen et al.[10] reported that feacal coliform populations were reduced in the human faeces with β-glucan feed on 30th days. We found that feed with β-glucan caused a 2 log increase in *Enterobacteriaceae* counts. A similar result was reported by Murphy et al.[20] who suggested that oat based β-glucan rised *Enterobacteriaceae* populations 2 log in the porcine gastrointestinal tract. In second (DR+ β-glucan) and third (control) groups, *Enterobacteriaceae* counts didn't change on 14th day. Enterococci populations at first and third group rats increased in the end of the feed. In another study investigated of the effect on faecal microflora of β-glucan, Lowry et al.[21] reported that β-glucan as feed addictive significantly reduced the incidence of *Salmonella enterica* serovar *Enteritidis* organ invasion in immature chickens. In this study, cause of the increase in the number of coliforms and enterococcus bacteria in the group treated with β-glucan, may be due to lack of the dose of β-glucan or administration time.

In only β-glucan feed rats, *Lactobacillus* spp. populations showed an increasing at 1 log level. There are reports showing that a β-glucan was able to increase *Lactobacillus* spp. in rat faeces [14,22]. Again, Reiilly et al.[12] and Murphy et al.[20] assigned that β-glucan raised lactobacillus counts in porcine gastrointestinal tract. Also, a study conducted by Mitsou et al.[23] and Kuda et al.[13] exhibited that β-glucan significantly increased probiotic bacteria in human and rat faeces, respectively. Dietary restriction supplemented with β-glucan (second group) did not effect *Lactobacillus* spp. populations in the our study. This result could depends on the β-glucans efficacy inhibited by long time dietary restriction treatment. Therfore could not observed any increase in *Lactobacillus* spp. in second group rats. In our knowledge, the results of the present study represent the first data on the effect of dietary restriction + β-glucan on some selected faecal microorganisms in rats. On the other hand, several studies indicated that the addition of different dietary supplements such as sorbitol, heparin or heparosan at the diet increased *Lactobacillus* spp. population in gut microbiota [9,24].

In conclusion, we found that addition of prebiotic matter β-glucan has the potential to change at rat faeces microflora, and to increase, despite not a statistically significant, both probiotic lactobacillus and other microorgamisms including coliform, *Enterobacteriaceae* and enterococci populations. In the future we aim to find the exact β-glucan dose which can be used to reduce the colonization of pathogenic bacteria and to increase probiotic bacteria populations as lactobacillus in gastrointestinal tract of animals.

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