Effect of oat betaglucan and probiotic yoghurt containing *Bifidobacterium lactis* and *Lactobacilus acidophilus* in wistar rats fed on a cholesterol-enriched diet

Fatemeh Zahra Shirzad
College of Food Science and Technology, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran.
fatemeh.shirzad@srbiau.ac.ir

Mohamad Reza Ehsani
College of Food Science and Technology, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran.

Faramarz khodaiyan
Department of Food Science, Engineering and Technology, University of Tehran, Karaj, Iran.
khodaiyan@ut.ac.ir

ABSTRACT
The effect of oat betaglucan and non-fat yoghurt supplemented with *B.animalis subsp.lactis* and *L.acidophilus* on plasma lipids was determined in rats fed on a cholesterol-enriched diet. The groups fed on a cholesterol-enriched diet supplemented with oat betaglucan and probiotic yoghurt had significantly lower levels of plasma total cholesterol and low-density lipoprotein (LDL) cholesterol than the positive control group (without supplementation). Probiotic yoghurt supplemented with oat betaglucan was more effective in the lowering of plasma cholesterol levels than yoghurt .
Keywords: betaglucan, cholesterol ,probiotic yoghurt, rat

Introduction

A major risk factor related to coronary heart diseases is hypercholesterolaemic. Fermented dairy products have been suggested as dietary supplements due to their hypocholesterolaemic influence(El-gawad, El-sayed, Hafez, El-zeini, & Saleh, 2005). Betaglucan is a soluble dietary fiber that has been derived from the barley, oat, yeast and some of the bacteria, algae and fungi. betaglucan is the polysaccharide with bonds $(4 \leftarrow 1), (3 \leftarrow 1)$ of the beta-D-glucan (Lazaridou et al., 2014).Great interest of betaglucans with health benifit properties has been studied.Betaglucan exhibited biological activities including anti- inflammatory, antitumor anti-tumor and provide help for lowering blood cholesterol (Zhu, Du, & Xu, 2016).Some *in-vivo* studies have shown that products containing probiotics have wide range of health benefits that contain prevention of cardiovascular diseases(Ogunremi, Sanni, & Agrawal, 2015). In addition, studies determined the effect of oat flour were used as a substrate of symbiotic yoghurt feeding on mice showed significantly decreased cholesterol level (Mahrous, El-kholy, & Elsanhoty, 2014). The objective of this reaserch was to study the effects of oat betaglucan and probiotic yoghurt containing *Bifidobacterium lactis* and *Lactobacilus acidophilus* in rats fed on a cholesterol-enriched diet on the levels of plasma lipids and triglycerides.

Materials and Methods

Strains and substratetes

Freezed –dried culture (DVS type) ABY-3 contaning a mixture of *B.animalis subsp.lactis* and *L.acidophilus* by co- culturing *streptococcus thermophiles* and *Lactobacilus bulgaricus* , was purchased from CHR Hansen co., (Denmark).

Oat beta-glucan (purity 35%)was purchased from Promoat (Sewden) SMP (Skim milk powder) was obtain from Pegah co., (Tehran , Iran) .

Probiotic Yogurt making Method

First, non-fat skimmed milk of the Tehran Pegah factory were reconstituted with the SMP (Skimmed Milk powder) to the 12% w/v. After that, heat process was performed at $80-85^0C$ for 15-20 min. The starter culture with the amount of 0 0.4% (v/v) were inoculated in accordance with the plan and it incubated at three temperatures of 43^0C. After achieving the samples pH to the 4.5-4.7, the transportation to the refrigerator is performed.

Animal feeding experiments

Twenty-four male wistar rats with an average weight 200 ± 20g were obtained from Pasteur Institute of Iran. All rats were housed in cages floors in a room at 21 ± 2 °C and 60 ± 5% relative humidity. All animals were fed on a standard diet and water for one week. Afterwards this adaptation period, the rats were divided randomly into 4 experimental groups. One group received a standard diet (cholesterol-free diet) throughout the experimental period of 8 weeks and served as a negative control group(C) . The other 3 groups were fed on the standard diet with cholesterol added at a level of 1% (w/w) (cholesterol-enriched diet) to create hypercholesterolaemic rats. One of the 4 groups, which served as a positive control group, was

fed only on a cholesterol-enriched diet for the 8week period(CCH). The other 2 groups were fed for 8 weeks on a cholesterol-enriched diet supplemented with one of the following products: CHY,CHYB. The experiment was carried out for 8 weeks by oral gavages; dose level 10^8cfu/mL. The experimental diets groups are showed in Table 1.

Table 1 Experimental groups of rats and diets used in the trials

Diet treatment	Code
Cholesterol-free diet	C
Cholesterol-enriched diet	CCH
CCH+ yoghurt	CHY
CCH+ yoghurt+betaglucan	CHYB

The rats were allowed free access to experimental diet and water, and their body weights were monitored. The experiments were conducted according to the ethical norms Guidelines.
At the end of the 8week experimental period, the rats were fasted for 12 hours before blood collection. blood samples were collected under diethyl ether anesthesia. The samples were collected in tubes, with EDTA as an anti-coagulating agent. The tubes were centrifuged at 3000rpm for 15 min to obtain the plasma, which was kept frozen until analysis. The rats were sacrificed and the liver, heart and kidney and were excised immediately and weighed. The liver was washed with ice-cold saline solution (0.9%, w/v, NaCl) and stored until analysed.

Determination of plasma lipid Total plasma cholesterol, high-density lipoprotein

(HDL) and triglycerides were measurd by using the enzymatic colourimetric method according to Trinder (1969).

Statistical analysis

Data are presented as means and standard deviation .The significance differences between groups and treatments were analysed using the full factorial exprements by Statistical Analysis Systems (SAS)release 9.1 (SAS Institute, Inc., Cary, NC, USA) to determine the biological examination data .The mean values were compared with using the least significant difference (LSD) at 95% confidence level.

Results and discussion

Total cholesterol

The effect of the experimental diets on the levels of plasma lipids in rats are presented in Table 2. The total cholesterol was significantly reduced from 120mg/100mL in the positive control to a mean value of 61mg/100mL in CHYB group and to a mean value of 74mg/100mL in CHYgroup. This decrease corresponds to a 49.17% and 38.34% reduction, respectively. In contrast, there was significant difference in the total cholesterol detected between CCH, CHY, CHYB and negative control groups (Table 2).

Table 2. Levels of plasma lipids in rats fed on experimental diets for 8 weeks

Diet treatment	Total cholesterol mg/100mL	LDL cholesterol mg/100mL	HDL cholesterol mg/100mL 100mL mg/	Triglycerides mg/100mL
C	64 ± 0.81^{a}	59 ± 0.46^{a}	23 ± 0.74^{d}	160.2 ± 1.29^{a}
CCH	$120 \pm 0.0.58^{b}$	69 ± 0.42^{b}	20 ± 0.78^{d}	128 ± 1.10^{b}
CHY	74 ± 0.78^{c}	$18 \pm 0.0.52^{c}$	22 ± 0.88^{d}	147 ± 0.91^{c}
CHYB	61 ± 0.57^{d}	13 ± 0.55^{d}	20.6 ± 0.81^{d}	111 ± 0.81^{d}

Mean values(±SD) within the same column presenting different superscripts are significantly different P>0.05

While the cholesterol-enriched diet supplemented with CHYBwas more efficient than the diet supplemented with CHY for decreasing total cholesterol. However, themechanism of this effect could not been explained definitely. There are two hypotheses explain that mechanism. First one is that bacteria may bind with cholesterol directly into the cell membrane. Second one is, bile salt hydrolysis enzymes deconjugatethe bile salts which are more likely to be exerted influenc on rose cholesterol breakdown(Mahrous et al., 2014; Prakash & Jones, 2005; Taranto, Medici, & Perdigon, 2000)

HDL-cholesterol and LDL-cholesterol

It can be seen that Table 2, there was no significant difference in the HDL- cholesterol level between the negative control group and other experimental groups at the end of the 8week experimental period. These findings are in agreement with those reported that the addition of the probiotic to the diet had no effect on the HDL- cholesterol level(El-gawad et al., 2005; Yang et al., 2014).

Triglyceride

It is significant changes from the result in Table2 that the CHYB diets were more effective in loweringa triglycerides than the CHY diets. These results are in associate with other researches(El-gawad et al., 2005; Ogunremi et al., 2015) who suggested that the yoghurt diets supplemented with B. bifidum lowered cholesterol in rat .

Conclusion

This study illustrated that probitic-based substrate containing *B.animalis subsp.lactis* and *L.acidophilus* plus oat betaglucan positively influenced serum lipid levels in groups of rats fed cholesterol-containing diets.Enreched diet cosist of oat betaglucan and probiotic yoghurt could improve serum lipid levels, particularly to reduce the risk of cardiovascular diseases. In addition, clinical studies are required to confirm these effects in humans.

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