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Production of impure prebiotic galacto-oligosaccharides and their effect on calcium, magnesium, iron and zinc absorption in Sprague-Dawley rats

Kaleemullah Maawia[a], Sanaullah Iqbal[a,*], Tahir Rasool Qamar[a], Pakeeza Rafiq[a], Azmat ullah[a], Mansur-ud-Din Ahmad[b]

[a] Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Out Fall Road, Civil Lines, Lahore, Pakistan
[b] Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Out Fall Road, Civil Lines, Lahore, Pakistan

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ABSTRACT

Prebiotic galacto-oligosaccharides (GOS) are important "functional foods" of current scenario and used for various health benefits including improved mineral absorption. In the present study, it was hypothesized that novel GOS mixture, produced through transgalactosylation, with significant amount of mono and disaccharides may enhance mineral absorption in Sprague-Dawley rats. The non-purified GOS having β-(1→6) and β-(1→3) glycosidic linkage, were evaluated for apparent absorption of calcium, magnesium, iron and zinc. The rats were divided into two main groups (n = 12 per group, 6 male/6 female) fed on control and GOS (5 g/100 g) diet. The feces were collected after 7 days interval for 28 days. The weight gain, feed and water intake were statistically similar ($p < 0.05$) in both groups irrespective of gender. Similarly, the absorption of minerals was statistically not different in both genders during whole study. The GOS diet significantly ($p < 0.05$) improved absorption of Ca (34.55–39.93%), Mg (51.22–58.05%) and Fe (31.58–39.21%) as compared to control diet at the end of study. However, no impact on Zn absorption was observed during the whole study. It can be inferred that the use of non-purified GOS for 3–4 weeks may enhance Ca, Mg and Fe absorption.

1. Introduction

Although the role of diet in human health has always beyond any doubt, however from the last few decades the nutritionists are focusing attention to improve community health through dietary interventions. Several foods and their bio-active components have been evaluated for their impact on human health [1]. In this regard, functional foods "which exhibit some specific benefits in addition to providing nutrients" have received special attention. One such example is that of "prebiotics" which are non-digestible oligosaccharides, stimulate the growth of beneficial bacteria and ultimately improve the host's health. Only few oligosaccharides fulfill the criteria of prebiotics including galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and lactulose [2]. It has been reported that prebiotics have several functions including improvement in immune system [3], modulation in gastrointestinal microflora [4], relief in inflammatory bowel diseases [5], prevention in cancers [6], lipid metabolism [7], reduction in diarrhea [8] and effect on mineral absorption [9]. The mechanism of enhanced mineral absorption by prebiotics is their selective fermentation by beneficial bacteria in the colon and production of short chain fatty acids that result in reduction in luminal pH and thus creating favorable acidic environment for mineral absorption [10]. Recently, it has been reported that prebiotics affect the regulation of factors as divalent metal transporters responsible for mineral absorption [11].

A number of studies evaluated the effect of different prebiotics with wide range of compositions including various glycosidic linkages, degree of polymerizations and inclusion level in diet ranging from very low i.e. 2–3 g/100 g of diet, to very high i.e. 15 g/100 g of diet to observe effect on mineral absorption. These studies have either used purified prebiotics [12], or mixture of sugars, however most of them have not mentioned the composition of prebiotics. The outcome of these studies end up in conflicting

* Corresponding author.
E-mail address: sanaullah.iqbal@uvas.edu.pk (S. Iqbal).

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results from very poor to excellent role of prebiotics in mineral absorption.

The prebiotic GOS are produced by transgalactosylation of lactose catalyzed by β-galactosidase (β-gal) from different sources. Depending upon the process conditions various amounts of monosaccharides i.e. glucose, galactose and untrasgalactosylated lactose are produced in prebiotic mixture [13]. It has been reported that maximum GOS production in the sugars mixture is ranging from 12 to 50% [14] with an average of ~30%. The purity of commercially available GOS and FOS is from 55 to 99% after expensive and laborious purifications [14,15].

Micro-nutrient deficiencies are one of the major nutritional issues prevailing across the globe. Deficiency of minerals like calcium, iron and zinc severely affect the human health by reducing healthy life-span through serious complications like osteoporosis or sometimes creating life-threatening problems like reduced hemoglobin in pregnancy and iron deficiency anemia. These problems are even grave in developing countries. In the current scenario it is imperative to explore natural or minimally processed functional foods that have the capability to boost mineral absorption so that the problem of "hidden hunger" could be addressed. In the present study, cost effective, non-purified GOS mixture was produced through bio conversion of lactose followed by its evaluation for mineral absorption i.e. Calcium (Ca), Magnesium (Mg), Iron (Fe) and Zinc (Zn) in Sprague-Dawley rats.

2. Materials and methods

2.1. Materials and reagents

All chemicals and reagents were purchased form Merck (Darmstadt, Germany) unless otherwise stated. The Isopropyl β-D-thiogalactopyranoside (IPTG), *ortho*-nitrophenyl β-D-galactopyranoside (*o*NPG) and ampicillin were procured from Sigma-Aldrich (GmbH, Germany) and LB from Lab M (Lancashire, UK).

2.2. Production of β-gal and prebiotic GOS

The *Escherichia coli* BL21 (DE3) containing β-gal gene from *Lactobacillus sakei* Lb790 was prepared in our earlier study [16] in the Food Biotechnology Lab. University of Natural Resources and Life Sciences, Vienna Austria and shifted to current working place. The enzyme, β-gal was produced by following the procedure explained by [16] with minor modifications. The specified strain was grown in LB broth at 37 °C and induced by 0.1 mM IPTG at 0.3–0.4 of OD_{600}. After induction the culture was grown at 25 °C for 18 h. The cells were centrifuged at 5000 rpm for 10 min, washed and re-suspended in 50 mM sodium dihydrogen phosphate (NaPP) buffer, pH 6.5 and disrupted by sonication. The crude extract was obtained after centrifugation at 13,000 rpm for 15 min. The activity of β-gal was determined using *o*NPG and lactose assays [17].

The crude cell extract was used for the production of prebiotic GOS through transgalactosylation of lactose (600 mM) prepared in 50 mM NaPP buffer, pH 6.5 at 37 °C using 300 rpm. The reaction was stopped by heating mixture at ~90 °C for 5 min and samples were stored at −20 °C for further analysis [16], [17]. The Megazyme assay kits (Wicklow, Ireland) were used to analyze glucose (GOPOD assay kit, K-GLUC), galactose and lactose (Lactose/Galactose assay kit, K-LACGAR) in the transgalactosylated mixture by following standard protocol given in the manual and GOS were calculated by subtraction method as given below:

GOS = Initial conc. of lactose − (Glucose + Galactose + Untransgalactosylated lactose)

The final GOS mixture was also analyzed by thin layer chromatography (TLC) [17] and high performance liquid chromatography – refractive index (HPLC-RI) [18].

2.3. Animals

Laboratory-reared, seven weeks old Sprague-Dawley rats (n = 24) with average weight 160 ± 10 g were purchased from Institute of Health, Islamabad-Pakistan and delivered to working place for further experiments. Animals were housed in bottom wired cages in temperature and humidity controlled rooms with 12 h' light-dark cycles. All procedures and protocols were approved by and performed in compliance with Institutional "Animal Care and Use Committee" (ACUC) following University of Minnesota, USA rules and regulations.

2.4. Diet formulation

Twenty four 7-weeks old rats were fed on standard diet formulated according to a previous study [12] containing 5% mineral-vitamin mixture supplied by Syman Pharmaceuticals (pvt.) Ltd. Lahore, Pakistan, for a period of one week to obtain base line for the experiment. After acclimatization period, rats were divided into 2 main groups (A, B) having 12 in each with further division to male (06) and female (06) with almost similar mean body weights. The Group A was fed on control diet and group B on GOS diet. The experimental diet was prepared by replacing 5% of corn starch from basal diet with prebiotic GOS mixture containing mono-and di-saccharides. All diet ingredients were mixed thoroughly and newly produced prebiotic mixtures (liquid) were used for kneading of components followed by pallet formation. The pallets were dried at 70 °C for 24 h. Throughout the experiment all groups were given free access to deionized water and the diet. Feed and water intake was measured daily and animal weights were recorded on weekly basis.

2.5. Sample collection and analysis

One day before of sample collection, all rats of one group were shifted to previously washed and dried cages separately and feces samples were collected from each rat. After that all rats of one group were combined again. Samples were collected for a period of 28 days with time interval of 7 days. Feces were dried at 105 °C for 24 h and milled. Wet digestion of all samples were done using the method given by Hill and others [19]. The final feed, water and collected samples were analyzed for Ca, Mg, Fe and Zn through atomic absorption spectrophotometer (Perkin Elmer, AAnalyst 400, Massachusetts, USA). Apparent absorption of minerals was calculated by the following equation;

$$\text{Apparent absorption (\%)} = \frac{\text{Ingestion of mineral} - \text{excretion of that material in feces}}{\text{Total ingestion}} \times 100$$

2.6. Statistical analysis

Descriptive statistics was worked out and data was analyzed by analysis of variance (ANOVA) with repeated measures using SPSS software (ver. 18). When there was an interaction between gender, treatments and time interval, Post hoc comparison was performed using Duncan Multiple Range test (DMRt). Differences were considered significant at $P < 0.05$.