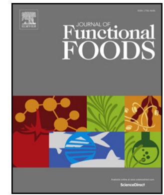
Journal of Functional Foods 49 (2018) 214–223

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff



Effect of milk gel acidity and β-glucan structure on fermentation processes in the caecum and bioavailability of mineral compounds in growing rats

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ARTICLE INFO

Keywords:
Beta-glucane
Yoghurt
Mineral
Osteoporosis
Mikrobiome
SCFA

ABSTRACT

A food product matrix and functional additives used to manufacture it are one of the key factors influencing the fermentation processes in the intestine and the bioavailability of minerals. The aim of this study was to analyse the effect of acidified and non-acidified milk gels with highly-purified (1-3)-, (1-3)(1-4)-, and (1-3)(1-6)-β-glucan on: gastrointestinal tract parameters, intestinal metabolic activity, and bioavailability of minerals in growing rats. Concentrations of ammonia, short-chain volatile fatty acids as well as bacterial enzyme activity, blood biochemistry, and bioavailability of Ca, Zn, Mn and Fe were significantly affected by β-glucan structure. The availability of minerals was higher upon the use of branched β-glucans. Milk gel acidity significantly influenced body weight gain, activity of faecal bacterial enzymes, blood levels of triglycerides and glucose, and bioavailability of all investigated mineral compounds. The use of branched β-glucans resulted in the most and least intense fermentation processes in the caecum, respectively.

1. Introduction

High contents of minerals in food products do not ensure their complete utilization (bioavailability) by a human or animal body. Their absorption from dairy products is significantly affected by acidity, protein and fat contents and potential presence of functional additives in these food products (Cámara-Martos & Amaro-López, 2002; Roohani, Hurrell, Kelishadi, & Schulin, 2013; Chiavaroli, Mirrahimi, Sievenpiper, Jenkins, & Darling, 2015).

One of the functional additives that might influence minerals bioavailability from intestines is β-glucans. Owing to their high molecular weight, they are fine thickening agents compared to other polysaccharide thickeners like inulin or guar gum. The use of a low concentration (2 g/L) of β-glucans results in development of a pseudoelastic, viscous gel (Kasprzak, Lærke, & Knudsen, 2012) whose viscosity depends on the structure arrangement of a polymer chain (Stone, 2009). Depending on glycosidic bonds localization, β-glucans may develop a structure of a single chain or a triple helix stabilized with hydrogen bridges in solutions. This is especially important considering that the structure of β-glucans as well as methods of heat and mechanical treatment affects the physicochemical properties of gels (Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Survase, Saudagar, Bajaj, & Singhal, 2007). The physicochemical properties of β-glucans, their biological activity in particular, depend on the sources they are isolated from and on their structure. For instance, β-glucans isolated from bacteria are composed of a simple chain consisting of β-D-glucopyranose units coupled only with (1 → 3) glycosidic bonds. On the other hand, β-glucans isolated from oats are linear polysaccharides of D-glucopyranosyl radicals, connected by two bonds: β-(1 → 4) and β-(1 → 3). Those connections appear in a predetermined sequence – each β-(1 → 3) bond is frequently followed by two or three β-(1 → 4)-glycosidic bonds. In turn, β-glucans isolated from fungi include a primary straight-chain composed of β-D-glucopyranosyl units. Those units are linked by (1 → 3) bonds with randomly distributed branches composed of three (1 → 6) linked β-D-glucopyranose units (Stone, 2009).

In the food production process, the raw materials undergo numerous individual operations, including thermal treatment. Mechanical and thermal processes in food production result in the development of a gel with different rheological properties compared to an aqueous standard solution. Physiological functions of β-glucan depend mainly on its ability to form gels and its characteristic properties in the small intestine (Hu, Zhao, Zhao, & Zheng, 2014; Wilczak et al., 2015). Previous pilot studies and investigations conducted by other authors (Banchathanakij & Suphantharika, 2009) showed that gels formed with the use of (1-3)-β-glucan, in comparison to (1-3)(1-4)-and (1-3)(1-4)-β-glucan, were characterized by higher stiffness due to an increase in the

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https://doi.org/10.1016/j.jff.2018.07.063
Received 25 March 2018; Received in revised form 18 July 2018; Accepted 25 July 2018
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number of triple helical structures. In consequence, they probably disintegrated very little in the first sections of the gastrointestinal tract and passed through the gut practically intact for the longest time. Enzymatic hydrolysis of (1-3) – bonds may occur only at the end of the gastrointestinal tract (Coudray, Tressol, Gueux, & Rayssiguier, 2003) and depends on the presence of specific enzymes synthesized by bacteria. Unlike (1-3)-β-glucan, the (1-3)-(1-4)-and (1-3)-(1-4)-β-glucan produce gels with a lesser rigidity but higher viscosity, which increases with the increase of chain length (Banchathanakij and Suphantharika, 2009). Increased viscosity of intestinal digesta decreases the rate of digestion and absorption of nutrients, which can have different effects on weight control (Kasprzak et al., 2012), and additionally influences the metabolic activity of bacteria (Stewart, Derek, & Joanne, 2008).

Molecules of β-glucans are resistant to the action of digestive enzymes (Lam & Cheung, 2013). Bacteria which colonise the colon may use them as sources of carbon, which results in a change of the enteric microbiome. The metabolic activity of this microbiome results in the synthesis of short-chain fatty acids (Ruppin, Bar-Meir, Soergel, Wood, & Schmitt, 1980; Wong, de Souza, Kendall, Emam, & Jenkins, 2006; Heimann, Nyman, Pålbrink, Lindkvist-Petersson, & Degerman, 2016) and a reduced pH value of intestinal digesta. This in turn contributes to increased ionization of mineral compounds and trace elements, and thereby to their increased bioavailability (Coudray et al., 2003). The increased acidity and the presence of the polysaccharide result also in: modification of intestinal mucosa structure and intestinal barrier function, intensification of passive diffusion and active transport, and enhanced expression of genes responsible for the synthesis of mineral transporters/ membrane proteins, i.e.: calbindin-D9k (calcium), DMT-1 and ferroportin (iron) or ZnT1 (zinc). Membrane proteins stimulate the para- and *trans*-cellular absorption and increase the low permeability of membranes, thereby facilitating ionic transfer across lipid layers (Schweigel & Martens, 2000; Tako et al., 2008; Lobo, Filho, Alvares, Cocato, & Colli, 2009).

Scientific literature provides numerous evidences for the health-promoting effects of polysaccharides, including β-glucans (Brennan & Tudorica, 2003; Wilczak et al., 2015). Several scientific studies have focused mainly on the rheological properties of beta-glucan in aqueous standard solutions and on viscoelasticity changes under different temperature conditions and acidity. In turn, little data are available on the effect of oat-derived β-glucans on the bioavailability of mineral compounds. Considering that functional ingredients of food products can influence the absorption of mineral compounds from the intestines, the use of β-glucans is an interesting approach in this respect. The comparative analysis of findings reported by different authors is, practically, impossible due to the use of preparations with diverse structures and concentrations, and additionally in different doses. Finally, many works lack information on the effect of the food matrix on the bioavailability of minerals and trace elements.

Therefore, the aim of this study was to determine the effect of supplementing standard diets for growing rats with milk gels, containing high-purity preparations of β-glucans of different structures on: their gastrointestinal tract parameters, activity of faecal enzymes, fermentation activity of intestinal microbiota, and bioavailability of calcium, magnesium, manganese, zinc, iron, phosphorus, and potassium.

2. Material and methods

2.1. Animals

Animals were selected and handled during the experiment in accordance with standard regulations. The experiment was conducted as a normal model with 64 male growing Crl:WI (Han) Wistar rats aged 7 weeks and weighing 224 ± 6.41 g at the beginning of the study.

Table 1
Composition of control (C) and experimental (E1-3) diet fed to rats.

Ingredient	C	E1	E2	E3
Ingrediens as diet compounds (%)				
Casein[1]	14.8	14.8	14.8	14.8
DL-methionine	0.2	0.2	0.2	0.2
Cellulose	8.0	8.0	8.0	8.0
Choline chloride	0.2	0.2	0.2	0.2
Rapeseed oil	8.0	8.0	8.0	8.0
Cholesterol	0.3	0.3	0.3	0.3
Mineral mix[2]	3.5	3.5	3.5	3.5
Vitamin mix[3]	1.0	1.0	1.0	1.0
Maize starch[4]	64.0	64.0	64.0	64.0
Other diet compounds (g)				
Milk gel[5]	2.0	2.0	2.0	2.0

[1] Casein preparation: crude protein 89.7%, crude fat 0.3%, ash 2.0%, and water 8.0%.

[2] AIN-93G-MX according (Reeves, 1997), per kg mix: 357 g calcium carbonate anhydrous (40.04% Ca), 196 g potassium phosphate monobasic (22.76% P, 28.73% K), 70.78 g potassium citrate, tripotassium monohydrate (36.16% K), 74 g sodium chloride (39.34% Na, 60.66% Cl), 46.6 g potassium sulfate (44.87% K, 18.39% S), 24 g magnesium oxide (60.32% Mg), 6.06 g ferric citrate (16.5% Fe), 1.65 g zinc carbonate (52.14% Zn), 1.45 g sodium *meta*-silicate·9H_2O (9.88% Si), 0.63 g manganous carbonate (47.79% Mn), 0.3 g cupric carbonate (57.47% Cu), 0.275 g chromium potassium sulfate·12H_2O (10.42% Cr), 81.5 mg boric acid (17.5% B), 63.5 mg sodium fluoride (45.24% F), 31.8 mg nickel carbonate (45% Ni), 17.4 mg lithium chloride (16.38% Li), 10.25 mg sodium selenate anhydrous (41.79% Se), 10 mg potassium iodate (59.3% I), 7.95 mg ammonium paramolybdate·4H_2O (54.34% Mo), 6.6 mg ammonium vanadate (43.55% V), 221.026 g powdered sucrose.

[3] AIN-93G-VM (Reeves, 1997), g/kg mix: 3.0 nicotinic acid, 1.6 Ca pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamin-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B-12 (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-*rac*-α-tocopheryl acetate, 500 IU/g), 0.8 vitamin A (all-*trans*-retinyl palmitate, 500000 IU/g), 0.25 vitamin D-3 (cholecalciferol, 400000 IU/g), 0.075 vitamin K-1 (phylloquinone), 974.655 powdered sucrose.

[4] Maize starch preparation: crude protein 0.6%, crude fat 0.9%, ash 0.2%, total dietary fibre 0%, and water 8.8%.

[5] Milk gel: average dry matter (ash, lactose and β-glucane in experimental milk gel) 14.09%, fat 3.2%, protein 3.7%.

2.2. Composition of experimental diets

Diet composition (Table 1) was based on AIN-1993 recommendations (Reeves, 1997). The control group and experimental groups were fed with a casein diet containing: maize starch – 64%, casein – 14.8%, rapeseed oil and cellulose – 8% each, mineral mix – 3.5% (AIN-93G-MX), vitamin mix – 1% (AIN-93G-VM), cholesterol – 0.3%, choline chloride – 0.2%, DL-methionine – 0.2%, and calculated ingredients: crude protein – 13.5%. The control (C) diet was supplemented with 2 g of non-acidified milk or acidified milk without the addition of β-glucans. The experimental diets (E1-E3) were supplemented with 2 g of acidified or non-acidified milk gel with the addition of high-purity (85%) preparations of β-glucans of different structures. According to the EFSA guidelines, the total daily intake of β-glucan at 3.0–4.0 g/day only has been shown to promote health. Therefore, to maintain the suggested level of β-glucan supplementation, based on the average weight of rats, the dose of acidified or non-acidified milk gel was fixed at 2 g. Considering β-glucan content in the product, the amount of 2 g corresponds to the dose suggested for human individuals weighing 65 kg.

2.3. Acidified milk gel and non-acidified milk gel production

Acidified milk gel and non-acidified milk gels were produced under laboratory conditions according to a patent application P. 418827 using: 1% of high-purity (1-3)-β-glucan with an average molecular weight (Mw) of 6.8×10^5 g/mol (Sigma-Aldrich, Poland), (1-3)(1-4)-β-

glucan with an average Mw of 9.4×10^5 g/mol (Beta-Bio, Poland), and (1-3)(1-6)-β-glucan with an average Mw of 3.5×10^5 g/mol (Cargill, Poland). The acidified milk gel was produced by acidifying milk with FD-DVS YC-X11 Yo-Flex starter culture (Chr. Hansen, Poland), and the non-acidified milk gel was obtained from fresh milk.

2.4. Experimental protocol

Rats of a similar body weight were randomly selected to 8 groups of 8 rats each. There were 2 control groups fed only a standard diet with the addition of an acidified or non-acidified milk gel, without β-glucan; and 2 groups fed only a standard diet with the addition of an acidified or non-acidified milk gel, with (1-3)-β-glucan; and 2 groups fed only a standard diet with the addition of an acidified or non-acidified milk gel, with (1-3)(1-4)-β-glucan; and 2 groups fed only a standard diet with the addition of an acidified or non-acidified milk gel, with (1-3)(1-6)-β-glucan. The rats were housed individually in metabolic cages in a controlled environment with a 12-h light-dark cycle, at a temperature of 22 ± 1 °C, relative humidity of 50 ± 5%, and 20 air changes/h. They had free access to water and feed. The food and water intake of individual rats were monitored daily throughout the study. Faeces and urine were collected after 0, 3, 10, 21 and 28 days from all rats kept in balance cages (Tecniplast Spa, Buguggiate, Italy).

2.5. Sampling procedures

At the end of the experiment, the rats were anaesthetised with sodium pentobarbital according to the recommendations for euthanasia of experimental animals (50 mg/kg body weight). After laparotomy, blood samples were drawn from *caudal vena cava*, then the small intestine, colon, liver, heart, kidneys, brain and lungs were removed and weighed. Caecal and colonic walls were flushed clean with ice-cold physiological saline, blotted on filter paper and weighed for tissue mass. The pH of the small intestine, caecum and colon digesta was measured immediately (ca. 10 min), directly in the segments (with model 301 pH meter; Hanna Instruments, Vila do Conde, Portugal). Samples of fresh caecal digesta were used for an immediate determination of ammonia content, dry matter, bacterial enzyme activity, and SCFA content, as described below.

2.6. Analytical procedures

2.6.1. Ammonia and dry matter (DM) content

In fresh caecal digesta, ammonia was extracted, trapped in a solution of boric acid in the Conway's dishes, and determined by direct titration with sulphuric acid. The dry matter (DM) content of caecal digesta was determined at 105 °C for 4 h to a constant mass not differentiating between subsequent measurements of ± 0.001 g.

2.6.2. Short chain fatty acids (SCFAs)

Caecal digesta samples were subjected to SCFA analysis using a gas chromatograph (Agilent, USA) with a flame ionization detector (FID) according to a previously described method by Krupa-Kozak, Markiewicz, Lamparski, and Juśkiewicz (2017). Briefly, the samples (0.2 g) were mixed with 0.2 mL of formic acid, diluted with deionised water and centrifuged at 7211*g* for 10 min. The supernatant was loaded onto a capillary column (SGE BP21, 30 m × 0.53 mm) using an on-column injector. The initial oven temperature was 85 °C and was raised to 180 °C by 8 °C/min and held for 3 min. The temperatures of flame ionization detector and the injection port were 180 and 85 °C, respectively. The sample volume for GC analysis was 1 µL. The caecal SCFA pool was calculated as the concentration of SCFA in the caecum (µmol/g) multiplied by the weight of caecal digesta (g), and was expressed in µmol per 100 g of body weight. The concentrations of caecal putrefactive SCFAs (PSCFAs) were calculated as the sum of isobutyric, isovaleric and valeric acids.

2.6.3. Activity of bacterial enzymes

The activity of bacterial enzyme activity was measured by the rate of *p*- or *o*-nitrophenol release from their nitrophenylglucosides according to the method described by Krupa-Kozak et al. (2017). The activity of α- and β-glucosidase, α- and β-galactosidase, and β-glucuronidase was expressed in µmol of the product synthesized per min (unit) per gram of digesta in a fresh caecal sample.

2.6.4. Plasma biochemistry

Heparinised blood was centrifuged at 380*g* and 4 °C for 15 min. Plasma was separated and transferred to Eppendorf tubes and stored at −70 °C until analysed. Plasma concentrations of total cholesterol, triglycerides, and glucose; and activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) as well as urinal urea and creatinine concentrations were determined using a biochemical analyser (Horiba, Pentra C200, Tokyo, Japan) according to the manufacturer's protocol.

2.6.5. Sample digestion and mineral concentration

Sample digestion and analytical procedures were conducted in a clean room (US Federal Standard 209 A Class 10 000). Faecal samples were dried at 105 °C for 4 h. After cooling, the dry faecal samples were homogenised in a mortar. Approximately 0.5 ± 0.0001 g of caecum, 5 ± 0.05 mL of urine, 0.5 ± 0.0001 g of blood, 2 ± 0.0001 g of a standard diet or milk gel, 6 mL of 65% suprapur nitric acid (Merck, Darmstadt, Germany), and 2 mL of 30% hydrogen peroxide (Merck, Darmstadt, Germany) were added into PTF vessels. The samples were mineralised using a Microwave System (MARS 5, CEM) until a colourless solution has been obtained. They were cooled and transferred to a volumetric flask containing 25 mL of ultra-pure deionised water. Concentrations of Ca, Mg, K, Fe, Mn, and Zn in the samples were determined by means of inductively coupled plasma optical emissions spectroscopy (ICP-OES, VISTA – MPX, Varian, UK). Phosphorus concentration was analysed with spectrometry according to Polish Standard PN-ISO 6491:2000, in the reaction of orthophosphates with ammonium molybdate and ammonium vanadate in acid environment. Method parameters were determined in the method validation process on reference materials: SBF-4 Soybean flour, BL SRM 1577b (Bovine liver), and CRM 422 (Cod muscle).

2.6.6. Analysis of minerals bioavailability

The bioavailability of Ca, P, Mg, K, Fe, Mn, and Zn, denoted by coefficients of apparent absorption and retention, was analysed in a nutritional experiment. The content of minerals in diets, as well as in faeces and urine collected in the balance period was determined by inductively coupled plasma optical emissions spectroscopy. Results were expressed in relative (%) values. The coefficient of apparent bioavailability was calculated as the difference between the intake of minerals and their quantity excreted with faeces and urine, and expressed in relative (%) values.

2.7. Statistical analysis

All experimental protocols applied in the study were approved by the Institutional Laboratory Animal Care and Use Committee (Olsztyn, Poland, Permit Number: 42/2015) which authorised the use of the current number of animals to protect animal life. The Institutional Laboratory Animal Care and Use Committee indicated that reliable scientific results could be reached (considering the preliminary animal studies conducted at Institute of Animal Reproduction and Food Research of Polish Academy of Sciences and the provided assumptions for statistical power analysis) with this number of animals, and this study adhered to the Directive of the European Parliament and of the Council on Animal Care Standards and to findings reported by Festing and Altman (2002). The experiment was conducted with 64 male rats. Eight experimental groups were formed, each including 8 rats with the

Table 2
Body weight (BW) gain; food intake, and gastrointestinal tract parameters in rats fed with the control and the experimental diets during 28 days.

Diets							
	CONTROL	(1-3)-β-glucan	(1-3)(1-4)-β-glucan	(1-3)(1-6)-β-glucan	acidity effect	β-glucan effect	β-glucan × acidity
Acidity milk gel							
Daily BW gain (g/animal)	3.93 ± 0.2^{a}	4.33 ± 0.5^{b}	4.51 ± 0.4^{b}	4.44 ± 0.2^{b}	< 0.001	0.053	0.002
Daily food intake (g/animal)	23.33 ± 1.0	23.37 ± 0.9	23.54 ± 0.6	23.29 ± 0.6	0.326	0.609	0.460
Caecum parameters							
pH of digesta	7.13 ± 0.0^{ab}	6.99 ± 0.2^{a}	7.19 ± 0.1^{bA}	7.14 ± 0.1^{ab}	0.885	0.045	0.013
Weight of cecum (g)	0.18 ± 0.0^{ab}	0.19 ± 0.0^{bA}	0.18 ± 0.0^{abA}	0.17 ± 0.0^{a}	0.002	0.218	0.139
Ammonia (mg/g digesta)	0.28 ± 0.0	0.26 ± 0.1	0.27 ± 0.0	0.29 ± 0.0^{A}	< 0.001	0.026	0.020
Weight of digesta (g/100 g BW)	0.48 ± 0.1^{aA}	0.45 ± 0.1^{a}	0.33 ± 0.1^{b}	0.58 ± 0.1^{cA}	0.002	< 0.001	< 0.001
Dry Matter % of digesta	25.29 ± 1.5^{aA}	24.50 ± 2.6^{a}	27.28 ± 1.2^{b}	26.26 ± 1.5^{ab}	0.333	0.007	0.091
Weight of organs (g)							
Heart	0.27 ± 0.0^{A}	0.27 ± 0.0	0.26 ± 0.0	0.26 ± 0.0^{A}	0.329	0.242	0.001
Kidneys	0.57 ± 0.0	0.60 ± 0.0	0.57 ± 0.0^{A}	0.60 ± 0.0^{A}	0.170	0.021	0.155
Liver	3.21 ± 0.1^{ab}	3.20 ± 0.2^{ab}	3.13 ± 0.1^{aA}	3.34 ± 0.1^{b}	0.112	0.005	0.199
Brain	0.55 ± 0.0	0.55 ± 0.0	0.54 ± 0.0	0.55 ± 0.0	0.200	0.896	0.848
Lungs	0.36 ± 0.0	0.37 ± 0.0	0.36 ± 0.0	0.37 ± 0.0	0.182	0.994	0.810
Non-acidity milk gel							
Daily BW gain (g/animal)	4.13 ± 0.2^{ab}	3.92 ± 0.2^{a}	4.11 ± 0.2^{ab}	3.86 ± 0.2^{a}			
Daily food intake (g/animal)	23.46 ± 1.2	22.68 ± 0.9	23.15 ± 0.8	23.39 ± 0.7			
Caecum parameters							
pH of digesta	7.26 ± 0.2^{b}	7.09 ± 0.2^{a}	7.01 ± 0.1^{aB}	7.08 ± 0.1^{a}			
Weight of cecum (g)	0.17 ± 0.0	0.17 ± 0.0^{B}	0.17 ± 0.0^{B}	0.17 ± 0.0			
Ammonia (mg/g digesta)	0.27 ± 0.0^{b}	0.23 ± 0.0^{a}	0.25 ± 0.0^{ab}	0.20 ± 0.0^{cB}			
Weight of digesta (g/100 g BW)	0.27 ± 0.1^{cB}	0.37 ± 0.1^{a}	0.42 ± 0.1^{ab}	0.48 ± 0.1^{bB}			
Dry Matter % of digesta	23.87 ± 0.7^{bB}	25.66 ± 3.7^{ab}	26.52 ± 0.8^{a}	26.18 ± 0.6^{a}			
Weight of organs (g)							
Heart	0.25 ± 0.0^{aB}	0.26 ± 0.0^{a}	0.26 ± 0.0^{a}	0.28 ± 0.0^{bB}			
Kidneys	0.57 ± 0.0^{b}	0.59 ± 0.0^{ab}	0.61 ± 0.0^{aB}	0.62 ± 0.0^{a}			
Liver	3.27 ± 0.1^{ab}	3.15 ± 0.2^{b}	3.31 ± 0.2^{abB}	3.38 ± 0.2^{a}			
Brain	0.56 ± 0.0	0.56 ± 0.0	0.56 ± 0.0	0.56 ± 0.0			
Lungs	0.36 ± 0.0	0.35 ± 0.0	0.36 ± 0.0	0.35 ± 0.0			

The values represent mean and standard deviation for n = 8.
[abc]Mean values in rows with different superscript letters are significantly different ($p \leq 0.05$).
[ABC]Mean values in column with different superscript letters only when are significantly different ($p \leq 0.05$).

most similar body weight. The results were verified for normal distribution and homogeneity of variance. Extremely outlier result (inside the study group) was deleted if it did not achieve the Grubbs' test. The significance of differences between means was analysed by Duncan's test. The interactions between the structure of β-glucans and acidity were determined by two-way ANOVA. The interactions between the structure of β-glucans and acidity of the milk gel were determined by one-way ANOVA. The results were processed in Statistica 13.5 PL software (Statsoft 2017, Krakow, Poland). All data were presented as means ± standard deviation or standard deviation of the mean.

3. Results

3.1. Effect of diet supplementation with acidified and non-acidified milk gels enriched with β–glucans on a daily food intake, body weight gain and gastrointestinal tract parameters

No significant differences were noted in food intake among all rats (Table 2). Body weights of the rats fed the acidified milk gel increased significantly and these of rats fed the non-acidified milk gel decreased compared to the control rats. β-Glucan structure had a significant effect on kidney weight ($p = 0.027$) and liver weight ($p = 0.005$), whereas milk gel acidity on caecal tissue weight ($p = 0.002$). The average weight of caecum tissue in rats fed the acidified milk gels was higher by 6% than in the rats fed the non-acidified milk gels. A significant influence of product acidity ($p < 0.001$) and structure of β-glucans ($p = 0.026$) was also noted on ammonia concentration. Acidity of caecal digesta significantly depended on the structure of β-glucans ($p = 0.045$) and the interaction of β-glucans with milk gels acidity ($p = 0.013$). In rats fed the acidified milk gels, the lowest (0.26 mg/g) ammonia concentration in rat intestine was found with (1-3)-β-glucan used as a dietary additive and the highest one (0.29 mg/g) with (1-3)(1-6)-β-glucan used as a dietary additive. The acidity of the intestinal digesta in rats fed the acidified milk gels with (1-3)(1-4)- and (1-3)(1-6)-β-glucans was comparable and averaged pH = 7.16. In rats receiving (1-3)-β-glucan, digesta acidity was higher ($p < 0.05$) and averaged pH = 6.99 (Table 2).

3.2. Effect of diet supplementation with acidified and non-acidified milk gels enriched with β-glucans on the concentration of short chain fatty acids (SCFAs) and putrefactive SCFAs (PSCFAs) in the caecal digesta

The metabolic activity of the intestinal microbiome resulted in the production of short-chain volatile fatty acids. The concentration of acetic acid ($p = 0.02$), propionic acid ($p < 0.001$), butyric acid ($p = 0.03$) in the caecal digesta significantly depended on the structure of β-glucan. Feeding rats the acidified milk gel enriched with (1-3)(1-6)-β-glucan resulted in reduced concentrations of acetic acid ($p < 0.05$), propionic acid ($p > 0.05$), and butyric acid ($p > 0.05$). In addition, the level of caecal putrefactive SCFAs (PSCFAs), defined as the sum of *iso*-butyric acid, *iso*-valeric acid and valeric acids, significantly depended on the structure of β-glucans and to a lesser extent on the acidity of the milk gel. The structure of β-glucans was found to cause significant changes in concentrations of *iso*-butyric acid ($p = 0.02$) and valeric acid ($p = 0.0246$). Addition of the (1-3)(1-4)-β-glucan to the acidified milk gel resulted in an increase by 25% ($p < 0.05$) while to the non-acidified gel in a decrease by 7.5% of PSCFAs concentration. In rats fed receiving (1-3)(1-4)-β-glucan and in

Table 3
The concentration of short chain fatty acids (SCFAs) and putrefactive SCFAs (PSCFAs) in the caecal digesta of rats fed.

Diets	CONTROL	(1-3)-β-glucan	(1-3)(1-4)-β-glucan	(1-3)(1-6)-β-glucan	acidity effect	β-glucan effect	β-glucan × acidity
SCFAs (μM/g digesta)							
Acidity milk gel							
acetic acid	49.474 ± 3.18^{c}	42.993 ± 6.92^{a}	44.239 ± 5.62^{a}	37.891 ± 2.58^{b}	0.610	0.001	0.100
propionic acid	8.451 ± 0.71^{a}	9.322 ± 0.86^{b}	8.936 ± 0.28^{abA}	7.723 ± 0.19^{cA}	0.001	< 0.001	0.292
butyric acid	5.558 ± 0.57^{A}	4.724 ± 1.28	5.308 ± 0.85	4.981 ± 0.18^{A}	0.636	< 0.001	0.014
izo-butyric acid	0.982 ± 0.12^{a}	1.027 ± 0.13^{a}	1.400 ± 0.30^{bA}	0.986 ± 0.16^{ab}	0.028	0.010	0.001
izo-valeric acid	1.194 ± 0.10	1.160 ± 0.21	1.387 ± 0.16^{A}	1.215 ± 0.29	0.531	0.267	0.205
valeric acid	1.154 ± 0.25^{ab}	1.024 ± 0.20^{aA}	1.278 ± 0.29^{bA}	0.977 ± 0.18^{a}	0.608	0.699	0.003
Total PSCFAs	3.329 ± 0.22^{ab}	3.212 ± 0.39^{b}	4.065 ± 0.42^{cA}	3.178 ± 0.37^{aA}	0.271	0.004	< 0.001
Total SCFAs	63.483 ± 5.75^{A}	57.039 ± 10.11	58.483 ± 2.80	50.595 ± 2.86	0.023	0.003	0.510
Non-acidity milk gel							
acetic acid	44.444 ± 8.48	45.446 ± 2.42	41.487 ± 3.97	40.555 ± 5.13			
propionic acid	8.498 ± 0.87^{ab}	10.069 ± 1.12^{c}	9.642 ± 0.93^{bcB}	8.794 ± 0.62^{abB}			
butyric acid	6.320 ± 0.88^{cB}	5.418 ± 0.68^{a}	5.264 ± 1.22^{a}	3.971 ± 0.49^{bB}			
izo-butyric acid	0.973 ± 0.19	0.955 ± 0.19	0.968 ± 0.15^{B}	1.080 ± 0.18			
izo-valeric acid	1.231 ± 0.22	1.127 ± 0.20	1.174 ± 0.22^{B}	1.295 ± 0.18			
valeric acid	1.076 ± 0.12^{ab}	1.186 ± 0.08^{bB}	0.956 ± 0.16^{aB}	1.115 ± 0.21^{ab}			
Total PSCFAs	3.280 ± 0.30	3.268 ± 0.43	3.098 ± 0.22^{B}	3.489 ± 0.40^{B}			
Total SCFAs	59.261 ± 8.62^{aB}	60.933 ± 5.10^{a}	56.393 ± 5.10^{ab}	53.320 ± 9.17^{b}			

The values represent mean and standard deviation for n = 8.
[abc]Mean values in rows with different superscript letters are significantly different (p ≤ 0.05).
[ABC]Mean values in column with different superscript letters only when are significantly different (p ≤ 0.05).

rats administered (1-3)(1-6)-β-glucan, the concentration of these fatty acids was the highest (4.023 μM/g digesta) and the lowest (2.814 μM/g digesta), respectively. In rats fed the non-acidified milk gel, the effect of β-glucan structure on PSCFAs content varied; use of (1-3)(1-4)-β-glucan as a dietary additive resulted in the lowest (3.098 μM/g), and the use of (1-3)(1-6)-β-glucan in the highest (3.489 μM/g) PSCFAs concentration in the caecal digesta (Table 3).

3.3. *Effect of diet supplementation with acidified and non-acidified milk gels enriched with β-glucans on the activity of bacterial enzymes in caecal digesta*

One of the manifestations of the metabolic activity of intestinal microbiome is the production and secretion of bacterial enzymes outside the cells. It was shown that both the acidity of the product and the structure of β-glucan had a significant ($p < 0.001$) effect on the extracellular activities of α- and β-glucosidase, α- and β-galactosidase, and β-glucuronidase in the last day of the experiment. The effects of glucans on bacterial enzyme activity changes in the caecal digesta were different. The diet of rats supplemented with the acidified milk gel with the addition of (1-3)(1-4)-β-glucan resulted in a significant increase in the activities of α- and β- glucosidase and α- and β-galactosidase. On the other hand, the use of (1-3)(1-6)-β-glucan resulted in a significant reduction of β-glucosidase, β-galactosidase and β-glucuronidase activities. The use of the non-acidified milk gel, enriched with different β-glucans, resulted in suppressed α- and β- galactosidase activities in comparison to the animals fed control gels. Worthy of notice is that α-glucosidase activity in rats fed the control milk gel and activity of β-glucosidase in rats fed the gel with (1-3)-β-glucan did not depend on the acidity of the milk gel (Table 4).

3.4. *Effect of diet supplementation with acidified and non-acidified milk gels enriched with β-glucans on blood and urine biochemistry*

The influence of milk gel acidity and β-glucan structure on liver enzyme activity, i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), in blood plasma was studied. The activity of aspartate aminotransferase (AST) was not dependent on the acidity and the structure of β-glucans. In turn, such a dependency was found in the ALT level. The highest (17.6%; $p < 0.05$) reduction of ALT concentration in plasma was found in rats fed the milk gel with (1-3)(1-4)-β-glucan. The alkaline phosphatase (ALP) activity significantly ($p < 0.013$) depended on the structure of β-glucans. Compared to the control rats, the highest activity of ALP was observed in the rats administered branched (1-3)(1-4)-β-glucan and (1-3)(1-6)-β-glucan, and the lowest one in rats receiving linear (1-3)-β-glucan. In rats fed the acidified milk gel, the ALP activity decreased on average by 10.5% in comparison to the control rats. On the other hand, feeding rats with the non-acidified milk gel resulted in a 17% increase in ALP (Table 4). The concentration of cholesterol in rat blood plasma did not significantly depend on the acidity of the milk gel and significantly ($p = 0.004$) depended on β-glucan structure. The concentration of triglycerides in blood plasma was found to significantly ($p < 0.001$) depend on the acidity of the product and not on the structure of β-glucan. A significant increase in plasma triglyceride levels was found in animals fed the non-acidified milk gel in comparison to the rats fed with the acidified milk gel. The use of (1-3)(1-6)-β-glucan in the diet of rats fed the acidified gel resulted in a significant reduction (by 12%) of triglyceride blood levels; whereas in rats fed the non-acidified milk gel – in a significant increase of their level (by 14%). The above dependency was not observed for the other β-glucans (Table 4). The significant influence of β-glucan structure ($p < 0.001$) and the interaction between β-glucan and acidity ($p < 0.001$) and the creatinine level in urine was noted. A significant decrease in creatinine concentration was found in the urine of rats fed the acidified and non-acidified milk gel with (1-3)-β-glucan (by 54% and 20%, respectively). The decrease in urine level of creatinine was also observed in rats fed the acidified and non-acidified gels with the addition of (1-3)(1-4)- and (1-3)(1-6)-β-glucan; i.e. by 37% and 70%, respectively. Urea concentration in urine depended on the acidity ($p = 0.079$) of the milk gel and structure of β-glucans ($p < 0.001$). Its concentrations decreased in rats receiving (1-3)(1-4)-β-glucan and (1-3)-β-glucan (by 38% and 22%, respectively). The feeding of rats with acidified and non-acidified milk gels enriched with different β-glucans resulted in a reduction of urea concentration by 38% ($p < 0.05$) and 8% ($p > 0.05$), respectively, in comparison to rats fed the control diet.

Table 4
The activity of bacterial enzymes in the caecal digesta and blood and urine parameters in rats.

Diets							
	CONTROL	(1-3)-β-glucan	(1-3)(1-4)-β-glucan	(1-3)(1-6)-β-glucan	acidity effect	β-glucan effect	β-glucan × acidity
Acidity milk gel							
Activity of microbial enzymes (μmol/h/g)							
α-Glucosidase	19.213 ± 1.07^{aA}	30.957 ± 5.90^{aA}	34.314 ± 4.12^{aA}	23.307 ± 1.98^{c}	0.004	< 0.001	< 0.001
ß-Glucosidase	3.210 ± 0.24^{c}	4.928 ± 0.43^{a}	4.707 ± 0.39^{a}	1.951 ± 0.41^{bA}	0.001	< 0.001	0.005
α-Galactosidase	6.680 ± 0.25^{aA}	7.565 ± 0.30^{bA}	7.963 ± 0.75^{b}	6.784 ± 1.24^{a}	0.023	0.001	< 0.001
ß-Galactosidase	59.201 ± 5.79^{aA}	64.205 ± 10.57^{a}	74.001 ± 4.74^{cA}	40.410 ± 1.71^{bA}	0.905	< 0.001	< 0.001
ß-Glucuronidase	31.666 ± 4.97^{cA}	17.703 ± 2.11^{a}	22.248 ± 1.67^{bA}	18.866 ± 0.98^{aA}	0.049	< 0.001	< 0.001
Blood parameters							
AST (U/L)	49.814 ± 3.13	50.283 ± 3.77	51.663 ± 7.66	49.233 ± 2.88	0.850	0.701	0.252
ALT (U/L)	21.250 ± 1.28^{ab}	20.150 ± 1.44^{a}	17.500 ± 1.09^{cA}	22.971 ± 2.71^{bA}	0.375	0.002	0.019
ALP (U/L)	135.279 ± 7.92^{cA}	115.783 ± 12.00^{a}	127.208 ± 7.02^{bc}	120.057 ± 12.90^{abA}	0.131	0.006	< 0.001
Cholesterol (mmol/L)	1.433 ± 0.12^{cA}	1.269 ± 0.13^{a}	1.122 ± 0.03^{bA}	1.249 ± 0.07^{a}	0.928	0.004	< 0.001
Triglycerides (mmol/L)	0.479 ± 0.06^{bA}	0.582 ± 0.05^{a}	0.445 ± 0.04^{abA}	0.422 ± 0.03^{aA}	< 0.001	0.124	< 0.001
Glucose (mmol/L)	13.038 ± 0.84^{a}	12.260 ± 0.93^{ab}	11.763 ± 0.34^{bA}	12.910 ± 1.25^{a}	0.010	0.016	0.009
Urinary parameters							
Creatinine (μmol/L)	6503.833 ± 772.19^{c}	4050.714 ± 831.65^{a}	4091.571 ± 996.81^{aA}	3019.667 ± 688.05^{bA}	0.937	< 0.001	< 0.001
Urea (mmol/L)	337.463 ± 11.09^{bA}	218.659 ± 37.94^{a}	211.672 ± 26.51^{aA}	198.875 ± 50.05^{aA}	0.079	< 0.001	< 0.001
Non-acidity milk gel							
Activity of microbial enzymes (μmol/h/g)							
α-Glucosidase	22.917 ± 3.73^{B}	24.248 ± 5.44^{B}	24.263 ± 2.20^{B}	24.636 ± 3.81			
ß-Glucosidase	3.514 ± 0.79^{a}	5.866 ± 1.77^{b}	4.443 ± 0.97^{a}	3.791 ± 0.55^{aB}			
α-Galactosidase	9.648 ± 1.73^{cB}	6.364 ± 1.25^{aB}	8.231 ± 0.89^{b}	7.246 ± 1.23^{ab}			
ß-Galactosidase	72.924 ± 4.47^{bB}	56.329 ± 9.48^{a}	52.095 ± 8.05^{aB}	55.623 ± 7.51^{aB}			
ß- Glucuronidase	24.910 ± 3.15^{bB}	20.574 ± 4.70^{a}	15.970 ± 2.86^{cB}	22.384 ± 3.79^{abB}			
Blood parameters							
AST (U/L)	48.938 ± 4.99	52.213 ± 6.21	47.704 ± 1.90	51.231 ± 4.92			
ALT (U/L)	21.413 ± 3.89	19.563 ± 2.52	19.317 ± 2.12^{B}	19.494 ± 2.31^{B}			
ALP (U/L)	114.013 ± 4.24^{bB}	124.250 ± 9.44^{c}	133.933 ± 9.18^{a}	140.649 ± 10.16^{aB}			
Cholesterol (mmol/L)	1.271 ± 0.07^{aB}	1.195 ± 0.12^{a}	1.390 ± 0.14^{bB}	1.226 ± 0.06^{a}			
Triglycerides (mmol/L)	0.690 ± 0.06^{abB}	0.592 ± 0.11^{a}	0.615 ± 0.11^{aB}	0.788 ± 0.17^{bB}			
Glucose (mmol/L)	12.688 ± 1.18^{ab}	12.225 ± 1.72^{a}	13.840 ± 0.88^{bcB}	14.175 ± 1.17^{c}			
Urinary parameters							
Creatinine (μmol/L)	6937.979 ± 1901.90^{c}	3130.083 ± 1039.91^{a}	2107.938 ± 361.05^{aB}	5571.719 ± 1031.17^{bB}			
Urea (mmol/L)	214.433 ± 52.07^{aB}	203.773 ± 39.61^{a}	132.027 ± 12.33^{bB}	354.054 ± 23.49^{cB}			

ALT – alanine transaminase, AST – aspartate transaminase and ALP – alkaline phosphatase.
The values represent mean and standard deviation for n = 8.
^{abc}Mean values in rows with different superscript letters are significantly different ($p \le 0.05$).
^{ABC}Mean values in column with different superscript letters only when are significantly different ($p \le 0.05$).

3.5. Effect of diet supplementation with acidified and non-acidified milk gels enriched with β-glucans on intestinal bioavailability of calcium, magnesium, zinc, manganese, iron, phosphorus, and potassium

The bioavailability of calcium significantly ($p < 0.001$) depended on the structure of β-glucan as well as milk gel acidity. The use of the acidified milk gel with the addition of (1-3)(1-4)-β-glucan in rat nutrition increased (by 12.8%) calcium bioavailability compared to the control milk gel (Table 5). Calcium bioavailability was higher for branched β-glucans, but was lower in rats fed a control diet and with the addition of linear (1-3)-β-glucan. A significant correlation was observed in all rats between bioavailability of calcium and contents of valeric acid (r = 0.320) and dry matter (r = 0.277). The bioavailability increased proportionally to the content of protein metabolites (ammonia) only in rats receiving (1-3)(1-4)-β-glucan (r = 0.532) and (1-3)(1-6)-β-glucan (r = 0.704). The average calcium concentration in the blood of rats fed with the acidified milk gels was 125 mg/kg and was by 6% higher than in rats fed the non-acidified milk gels. However, this difference was not significant. The bioavailability of magnesium was not dependent on β-glucans addition of to milk gels (p = 0.09). On the other hand, it was found to significantly (p = 0.012) depend on the acidity of the milk gel. Bioavailability of magnesium was significantly correlated with the content of valeric acid (r = 0.320) and *iso*-valeric acid (r = 0.248). In rats fed the gel with (1-3)(1-4)-β-glucan, its bioavailability was correlated with contents of acetic acid (r = 0.584) and propionic acid (r = 0.607). Magnesium concentration in the blood of rats was significantly ($p < 0.001$) dependent on the structure of β-glucan and acidity of the milk gel. The use of β-glucan significant increased (68%) blood level of magnesium in rats fed the acidified milk gels (Table 6). The bioavailability of manganese significantly ($p < 0.001$) depended on product acidity and β-glucan structure. Its highest (91%) bioavailability was reported in rats fed the acidified milk gel with (1-3)(1-4)-β-glucan, while the lowest one (77%) in rats fed the control non-acidified milk gel. Bioavailability of manganese was strongly correlated with intestinal digesta (r = 0.311) and ammonia (r = 0.255) contents. The use of (1-3)(1-6)-β-glucan and (1-3)-β-glucan as feed additives resulted in a significant increase in manganese level in the blood of rats fed the milk gels (Table 6). The significant ($p < 0.001$) influence of acidity and β-glucan structure on the bioavailability of zinc was noted as well. The use of (1-3)(1-4)-β-glucan and (1-3)(1-6)-β-glucan resulted in ∼5% and ∼2% increase, respectively, while the use of (1-3)-β-glucan in a ∼3% decrease in the bioavailability of zinc in rats receiving the non-acidified milk gels. The addition of non-acidified milk gels, regardless of β-glucan structure, resulted in a ∼9%

Table 5
Intestinal bioavailability of calcium, magnesium, zinc, manganium, iron, phosphorus and potassium in rats fed control and experimental diets.

Mineral		CONTROL	(1-3)-β-glucan	(1-3)(1-4)-β-glucan	(1-3)(1-6)-β-glucan	acidity effect	β-glucan effect	β-glucan × acidity
Bioavailability (%)								
Acidity milk gel	Ca	72.26 ± 4.8^{a}	79.43 ± 3.0^{b}	93.44 ± 2.5^{dA}	84.86 ± 2.1^{cA}	< 0.001	< 0.001	< 0.001
	Mg	73.03 ± 9.5^{aA}	65.42 ± 6.4^{b}	73.97 ± 8.2^{a}	73.41 ± 4.8^{aA}	0.012	0.090	0.417
	Zn	83.35 ± 2.5^{aA}	80.20 ± 2.0^{b}	88.66 ± 2.1^{cA}	85.59 ± 3.0^{aA}	< 0.001	< 0.001	< 0.001
	Mn	85.74 ± 1.8^{aA}	82.68 ± 1.7^{b}	90.63 ± 1.3^{cA}	87.75 ± 3.1^{aA}	< 0.001	< 0.001	< 0.001
	Fe	81.07 ± 3.7^{abA}	78.06 ± 3.0^{a}	86.96 ± 2.3^{cA}	81.96 ± 4.6^{bA}	< 0.001	< 0.001	< 0.001
	P	79.48 ± 5.5^{A}	78.16 ± 4.7	83.70 ± 5.0^{A}	82.59 ± 5.7	< 0.001	0.013	0.173
	K	69.17 ± 15.4	59.91 ± 8.2	65.14 ± 10.3	67.97 ± 6.4^{A}	0.021	0.238	0.699
Non-acidity milk gel	Ca	74.20 ± 1.9^{a}	80.55 ± 2.8^{b}	81.83 ± 2.5^{bB}	76.04 ± 3.9^{aB}			
	Mg	63.30 ± 8.0^{B}	64.94 ± 7.6	68.99 ± 6.6	68.53 ± 9.4^{B}			
	Zn	75.34 ± 1.2^{aB}	81.99 ± 2.6^{b}	81.56 ± 2.6^{bB}	80.86 ± 1.7^{bB}			
	Mn	77.00 ± 1.1^{aB}	84.47 ± 2.2^{b}	84.06 ± 2.7^{bB}	83.40 ± 2.1^{bB}			
	Fe	73.09 ± 2.5^{aB}	79.14 ± 3.4^{b}	77.96 ± 3.9^{bB}	76.76 ± 3.5^{bB}			
	P	72.43 ± 3.6^{aB}	77.27 ± 6.1^{b}	76.51 ± 4.3^{abB}	80.12 ± 3.5^{b}			
	K	57.42 ± 11.6	55.27 ± 13.0	61.87 ± 8.1	61.95 ± 10.7^{B}			

The values represent mean and standard deviation for n = 8.
^{abc}Mean values in rows with different superscript letters are significantly different (p ≤ 0.05).
^{ABC}Mean values in column with different superscript letters only when are significantly different (p ≤ 0.05). Bioavailability was calculated as the difference between the intake of minerals and their quantity excreted with faeces and urine, and expressed in relative (%) values.

increase of zinc bioavailability. Milk gel acidity and β-glucan structure were found to cause no significant effect on zinc concentration in blood of rats (Table 6). The bioavailability of iron significantly depended on both the acidity of the milk gel and β-glucan structure. The use of β-glucan resulted in a significant increase in its bioavailability in rats fed the acidified milk gel with (1-3)(1-4)-β-glucan and in all rats fed the non-acidified milk gels (regardless of β-glucan used). No significant ($p > 0.05$) effect of gel acidity, as well as β-glucan structure was found on blood level of iron (Table 6). The bioavailability of phosphorus significantly depended on β-glucan structure ($p = 0.013$) and gel acidity ($p < 0.001$). The use of (1-3)(1-4)-β-glucan as a feed additive resulted in a significant ~7% increase in phosphorus bioavailability from the acidified milk gels. The concentration of potassium in the blood of rats fed the non-acidified milk gels with (1-3)(1-6)-β-glucan and (1-3)-β-glucan was also found to be significantly lower (approximately 6 mg/kg). There were no significant effects of β-glucans structure and gel acidity on the bioavailability and blood level of potassium.

4. Discussion

Body weight gains reported for rats administered the standard diet supplemented with acidified milk gels enriched with β-glucans, suggest their stimulating effect on the improved bioavailability of nutrients which had been partly hydrolysed upon the action of bacterial enzymes. By affecting a change in the intestinal microbiome and stimulating activities of bacterial enzymes, the *Lactobacillus* sp. and *Streptococcus* sp. bacteria present in the acidified milk gels enhanced the synthesis of short-chain fatty acids and retention of minerals (Onoda et al., 2000; Samolińska and Grela, 2017). This is confirmed by a higher (by ca. 6%) calcium content determined in the femoral bone of rats and a higher force needed to break it in a 3-point bending test (Aljewicz, Tońska, Juśkiewicz, & Cichosz, 2018). Unlike, other authors have demonstrated the addition of inulin to result in rat body weight decrease (Wilczak et al., 2015; Krupa-Kozak et al., 2017). This was, most likely, due to the use of preparations of oligofructose/inulin differing in chain length and form they had been applied in (Shah et al., 2009; Wilczak et al., 2015; Bueno-Vargas et al., 2016).

Although β-glucans are not digested in the gastrointestinal tract,

Table 6
The blood content of calcium, magnesium, zinc, manganium, iron, phosphorus and potassium in rats fed control and experimental diets.

Mineral		CONTROL	(1-3)-β-glucan	(1-3)(1-4)-β-glucan	(1-3)(1-6)-β-glucan	acidity effect	β-glucan effect	β-glucan × acidity
Blood (mg/kg)								
Acidity milk gel	Ca	121.409 ± 9.41	126.950 ± 8.30	127.876 ± 6.40	127.109 ± 13.62^{A}	0.265	0.319	0.007
	Mg	19.065 ± 2.43^{aA}	32.880 ± 4.95^{b}	32.184 ± 2.11^{b}	31.089 ± 5.06^{b}	< 0.001	< 0.001	< 0.001
	Zn	2.120 ± 0.47	1.840 ± 0.34	2.300 ± 0.73	1.991 ± 0.45	0.063	0.536	0.013
	Mn	0.025 ± 0.00^{aA}	0.042 ± 0.02^{b}	0.031 ± 0.00^{aA}	0.035 ± 0.00^{b}	0.113	0.269	0.017
	Fe	1.620 ± 0.26	2.010 ± 0.42	1.958 ± 0.41	1.536 ± 0.28	0.016	0.039	0.990
	P	91.928 ± 4.34^{a}	101.823 ± 7.01^{bA}	87.412 ± 4.17^{aA}	88.500 ± 6.73^{a}	0.601	0.003	< 0.001
	K	158.072 ± 9.42^{aA}	175.269 ± 12.77^{b}	175.469 ± 14.40^{a}	177.027 ± 8.80^{b}	0.001	0.002	0.286
Non-acidity milk gel	Ca	117.610 ± 6.24	118.305 ± 6.55	120.161 ± 9.61	114.109 ± 5.17^{B}			
	Mg	32.908 ± 1.35^{B}	33.871 ± 2.63	30.948 ± 5.28	32.324 ± 2.73			
	Zn	1.750 ± 0.48	1.628 ± 0.07	1.580 ± 0.34	1.598 ± 0.52			
	Mn	0.036 ± 0.01^{B}	0.035 ± 0.00	0.044 ± 0.02^{B}	0.033 ± 0.00			
	Fe	2.003 ± 0.56	2.311 ± 0.38	2.270 ± 0.79	1.867 ± 0.46			
	P	96.234 ± 3.09^{a}	89.610 ± 4.95^{bB}	96.626 ± 3.81^{aB}	89.774 ± 3.68^{b}			
	K	175.380 ± 7.37^{B}	182.014 ± 7.99	185.539 ± 8.51	179.980 ± 13.88			

The values represent mean and standard deviation for n = 8.
^{abc}Mean values in rows with different superscript letters are significantly different (p ≤ 0.05).
^{ABC}Mean values in column with different superscript letters only when are significantly different (p ≤ 0.05).

they are used by the enteric microbiota as a source of energy and as a substrate for the synthesis of short-chain fatty acids (SCFAs) (Leonhard-Marek, Gabel, & Martens, 1998; Wong et al., 2006; Moschen, Bröer, Galic, Lang, & Bröer, 2012). The SCFAs which enter the bloodstream via enterocytes serve as an additional source of energy as well as stimulate the endocrine system, lipolysis and work of skeletal muscles (Ruppin et al., 1980; Wong et al., 2006; Moschen et al., 2012; Krupa-Kozak et al., 2017). Our study demonstrated that dietary administration of β-glucans resulted in decreased concentrations of acetic acid and butyric acid, 60–70% of which are utilised for the energetic needs of the body. The rats with a lower concentration of butyric acid in caecal digesta achieved lower body weight gains, which is consistent with results published by other authors (Heimann et al., 2016). Considering the low (ca. 10%) SCFAs retention in the body, corroborating the roles of butyric acid, acetic acid or propionic acid as energy sources or as lipolysis promoters, requires further investigations (Wong et al., 2006). SCFAs synthesis is determined by: chemical composition and microbiological quality of a product, prebiotic susceptibility to enzymatic hydrolysis, and transport capabilities of the apical membrane of colonic epithelial cells (Ruppin et al., 1980; Moschen et al., 2012), including the activity of transport protein receptors, i.e. GPR41 and GPR43 (Brown et al., 2003). Higher SCFA and PSCFA concentrations were dependent on the acidity of the milk gels and resulted from the use of (1-3)(1-4)-β-glucan and the change in the intestinal microbiome (Yanan et al., 2016). In contrast to the acidified, the non-acidified milk gels were characterized by a higher lactose content, which stimulates the growth of the saccharolytic bacteria in the intestine. A human trial demonstrated that the intake of soluble dietary fibre, including (1-3)(1-6)-β-glucan, resulted in an enhanced growth of saccharolytic bacteria and a significant decrease in the concentration of protein hydrolysis products in the colon (ammonia, *iso*-butyrate or *iso*-valerate) (Macfarlane and Macfarlane 2012, Nyangale, Mottram, & Gibson, 2012). Protein hydrolysis was inhibited by both acidity and the structure of the polysaccharide (Smith and Macfarlane, 1998). Results obtained in our study confirm these findings.

Likewise SCFA content, the content and activity of faecal α- and β-glucosidase as well as α- and β-galactosidase are one of the main determinants of the fermentation activity of intestinal microbiota. A significant (about 50%) increase in β-glucosidase activity in rats fed (1-3)(1-4)- and (1-3)-β-glucan may have been due to the use of milk as a carrier for β-glucan (Mroczynska and Libudzisz, 2010). The activity of β-glucosidase was, most probably, also stimulated by *Bacillus* ssp., which uses β-glucosidase to produce glucose from oligosaccharides formed from (1-3)-and (1-3)-(1-4)-β-glucan (Uhlig, 1998).

The increase in the activities of α- and β-galactosidase determined in the rats fed the acidified milk gels with β-glucans was due to the presence of *Streptococcus thermophilus* (Drouault, Anba & Corthier, 2002; Alvaro et al., 2007) and increased populations of *Lactobacillus* ssp., *Bifidobacterium* ssp., and *Enterococcus* ssp., and to decreased counts of *Escherichia coli*, *Clostridium* spp., *Bacteroides* spp., *Staphylococcus* ssp., and *Eubacterium* ssp. (De Preter et al., 2008; Flores et al., 2012; Hijová et al., 2017).

It should be noted that the use of β-glucans in the rat diet significantly reduced the activity of β-glucuronidase (regardless of lactic gel acidity). Similar results were achieved with phosphor-oligosaccharides from coconut fibre and cellulose (Lindop, Tasman-Jones, Thomsen, & Lee, 1985, Lhoste, Nugon-Baudon, Lory, Meslin, & Andrieux, 2001). This is important because suppression of β-glucuronidase activity results in the reduction of mutagenic, toxic effects on the gastrointestinal tract (Gråsten, Pajarib, Liukkonenc, Karppinenc, & Mykkänena, 2002).

Another, equally important aspect is the effect of prebiotics, including beta-glucans on the biochemical parameters of blood. Our results are consistent with findings reported by El Rabey, Al-Seeni & Amer (2013) and by Andersson et al. (2013), who demonstrated that 10% - 19% addition of barley and oat brans rich in (1-3)(1-4)-β-glucan to diet for rats caused a decrease in cholesterol level in their blood plasma. The reduced cholesterol level determined in the rats fed acidified milk gels with the addition of β-glucans could be due to a higher content of propionic acid which has been implicated in the suppression of cholesterol synthesis and secretion to blood (Lin, Vonk, Slooff, Kuipers, & Smit, 1995). However, considering our study results, cholesterol absorption by β-glucan molecules is more likely (Gao, Lin, Sun, & Zhao, 2017).

The apt course of metabolic transformation and safety of food additives are indicated by levels of hepatic enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT) or alkaline phosphatase (ALP). A small, statistically insignificant, decrease in ALT concentration in rat plasma was due to dietary administration of β-glucans, which is in agreement with a previous work by Hosseini, Talebi, & Taheri (2013). ALP concentration in blood plasma is one of the indicators of osseous tissue metabolism. Results obtained in this study are consistent with findings from our previous research which demonstrated a strict correlation between milk gel acidity and rat femur susceptibility to fracture. The osseous tissue of the rats fed the non-acidified milk gels was characterised by a poorer Ca/P ratio and thinner trabeculae (Aljewicz et al., 2018). Inulin addition to rat diet caused a change not only in the microbiome but also in contents of SCFAs and also increased ALP concentration. This points to the intensified ossification coupled with suppressed secretion of biomarkers indicative of bone resorption (Bueno-Vargas et al., 2016).

The lower dietary fibre content in the control diet for rats resulted in greater acidification of their bodies. To maintain the acid-base balance and to remove non-metabolised protein anions, kidneys intensify glomerular filtration, which increases urinal levels of ammonia and creatinine (Adeva and Souto, 2011). Significant reduction of creatinine in the urine of beta-glucan-fed rats was, most likely, due to its degradation by bacteria colonising intestinal epithelium (Chiavaroli et al., 2015). Our findings are consistent with results reported by Rampton, Cohen & De Crammond, (1984) and El Rabey et al. (2013), as well as with results of *meta*-analysis conducted by Chiavaroli et al. (2015) to compare different types of dietary fibre. The activity of a starter culture during ripening and storage of acidified gel results in a successive degradation of casein and loosening the milk gel structure, which makes it more susceptible to the action of bacterial enzymes. This, in turn, resulted in a lower acidity of intestinal digesta and a higher concentration of ammonia. The higher level of creatinine assayed in the rats fed the acidified milk gels resulted from better retention of nutrients (Napolitano et al., 2009), which was also reflected in their higher body weight gains.

The function of body cells as well as the activity of different enzymes depends on the content of different mineral compounds and trace elements in the body. Calcium is one of the most important minerals for the human body. The demonstrated increase in its bioavailability in rats fed with beta-glucan gels is consistent with findings presented by other authors who demonstrated that diet supplementation with soluble and insoluble dietary fibre caused a significant increase in calcium bioavailability in both rats and humans. This effect appeared stronger along with a shorter polymer chain and shorter experiment duration and with a lower calcium dose in the diet (Spencer, Norris, Derler, & Osis, 1991, Shah et al., 2009, Krupa-Kozak et al., 2017). The reported study demonstrated a high (~80%) bioavailability of calcium. The use of branched (1-3)(1-4)- and (1-3)(1-6)-β-glucan in the acidified milk gels resulted in a significantly higher calcium bioavailability compared to the addition of linear (1-3)-β-glucan. Acidity of the acidified gel (pH = 4.5–4.6) is expected to cause a higher bioavailability of mineral compounds (due to higher solubility and ionization), compared to the non-acidified gel (pH = 6.5). However, the acidified gel contains more lactose which undergoes fermentation already in the intestine, which also effects bioavailability (Coudray et al., 2003). In addition, ammonia, acetate, propionate and butyrate present in the intestines activate ionic channels and increase percentage of protons in cells of the

intestinal epithelium. It results in enhanced penetration of calcium and magnesium ions through intestinal mucosa to blood (Trinidad, Wolever, & Thompson, 1993, Leonhard-Marek et al., 1998). This is confirmed by higher concentrations of calcium and magnesium in blood plasma, and by higher hardness of the femoral bone in the rats receiving acidified milk gels (data, not show).

The passive transport of zinc through cells of erythrocytes is determined by its dietary level. Assessment of zinc bioavailability is more difficult than of other mineral compounds, because it may be secreted to the intestine and because its absorption is affected by diet composition (Roohani et al., 2013). Results obtained in our study point to a significant role of β-glucans in increasing bioavailability of zinc ($p = 0.001$), iron ($p < 0.001$), and manganese ($p = 0.004$). The average bioavailability of zinc from different food products is ca. 50% (WHO/FAO, 2004) and is higher from its average bioavailability (30–40%) from milk (Talsma et al., 2017). According to WHO/FAO Report (2004) and work by Bel-Serrat et al. (2014), if the P:Zn ratio exceeds 15, zinc bioavailability is significantly reduced (to ca. 30%). In our study, the P:Zn ratio in the diet for rats containing acidified and non-acidified milk gels with the addition of β-glucans was significantly higher (ca. 81), and did not diminish zinc bioavailability. Its high (ca. 80%) bioavailability compared to milk resulted from conducting the experiment with rats being at the stage of intensive growth, when zinc is intensively absorbed and metabolised (Roohani et al., 2013). The statistical analysis demonstrated significant correlations between contents of: valeric acid ($r = 0.536$) in the rats fed (1-3)(1-4)-β-glucan, *iso*-valeric acid ($r = -0.558$) in the rats fed (1-3)-β-glucan and ammonia ($r = 0.817$) in the rats fed (1-3)(1-6)-β-glucan, which confirm the stimulating effect of β-glucans on caecal microbiome activity. Microbiome stimulation causes intensified peptydolytic transformations, which result in the synthesis of free amino acids or other low-molecular peptides which maintain the ionised form of zinc in the product, thereby increasing its bioavailability (Lönnerdal, 2000, Rosado et al., 2005; Roohani et al., 2013).

The high bioavailability of iron (70–80%) was due to erythropoiesis processes ongoing during intensive growth of rats (Reece, 2015). The significantly higher (by 10% on average) iron bioavailability from acidified gels resulted from transcytosis (García-Nebot, Barberá & Alegríam, 2013) and from the presence of iron phospocaseinate (b-CN (1-25)4P) produced primarily from β-casein by *Lactobacillus dulbrecki* peptidolytic enzymes (Kibangou et al., 2005). In addition, the primary transport of iron, mediated by enterocytes, may be intensified via long-term prebiotic supplementation, e.g. β-glucan. The mechanism of bioavailability stimulation by β-glucan is, most likely, the same as in inulin and is associated with enhanced expression of genes (mucin genes, ferroportin, DMT1, Dcytb, transferrin receptor) responsible for iron accumulation and transport in cells of erythrocytes (Tako et al., 2008). This is confirmed by our study results indicating increased iron absorption ($p < 0.05$) and increased iron concentration in blood plasma ($p > 0.05$) of rats administered β-glucan, and by findings of other authors (Samolińska and Grela, 2017).

High (approx. 83%) bioavailability of phosphorus was a consequence of standard diet supplementation with casein-rich milk gels. During digestion, casein is hydrolysed to phosphopeptides and nucleopeptides, which stimulate phosphorus release and bioavailability (Gaucheron, 2011). No significant effect of β-glucans on phosphorus bioavailability was due to their chemical structure. Higher (statistically insignificant) bioavailability of potassium from the acidified milk gels could be due to its ionisation and increased solubility. Potassium is well soluble and absorbed in the upper section of the gastrointestinal tract (Stone, Martyn, & Weaver, 2016), whereas β-glucans are hydrolysed in its lower sections. This explains no significant effect of β-glucans on potassium bioavailability.

5. Conclusion

Physiological effects were determined by β-glucan structure and, to a lesser extent, by acidity of the milk gels. Study results demonstrated that β-glucans addition to rat diet enhanced the activity of bacterial enzymes and affected blood and urine biochemistry. SCFAs synthesis was stimulated by milk gel starter cultures and lactic acid. Owing to increased solubility of calcium, manganese, zinc or iron, the acidity was a significant factor which determined their bioavailability. The intake of acidified dairy products with the addition of β-glucans may result in beneficial changes of intestinal microbiome, better retention of nutrients, and increased bioavailability of mineral compounds. The diet supplemented with a milk gel enriched (1-3)(1-4)-β-glucans contributed to the best digestibility of nutrients, improved blood biochemical markers, and increased bioavailability of mineral compounds. Their bioavailability was affected to the least extent by the addition of (1-3)-β-glucan.

Acknowledgments

We gratefully acknowledge financial support from the NCN (National Science Center, Warsaw, Poland; Grant SONATA No. 2014/15/D/NZ9/02886).

Conflict of interest

The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results. The authors declare no conflict of interest.

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