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# Cereal Byproducts Have Prebiotic Potential in Mice Fed a High-Fat Diet

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*S* Supporting Information

**ABSTRACT:** Barley husks, rye bran, and a fiber residue from oat milk production were processed by heat pretreatment, various separation steps, and treatment with an endoxylanase in order to improve the prebiotic potential of these cereal byproducts. Metabolic functions were intended to improve along with improved microbial activity. The products obtained were included in a high-fat mouse diet so that all diets contained 5% dietary fiber. In addition, high-fat and low-fat controls as well as partially hydrolyzed guar gum were included in the study. The soluble fiber product obtained from rye bran caused a significant increase in the bifidobacteria (log copies of 16S rRNA genes; median (25−75 percentile): 6.38 (6.04−6.66) and 7.47 (7.30−7.74), respectively; $p < 0.001$) in parallel with a tendency of increased production of propionic acid and indications of improved metabolic function compared with high-fat fed control mice. The oat-derived product caused an increase in the pool of cecal propionic (from $0.62 \pm 0.12$ to $0.94 \pm 0.08$) and butyric acid (from $0.38 \pm 0.04$ to $0.60 \pm 0.04$) compared with the high-fat control, and it caused a significant increase in lactobacilli (log copies of 16S rRNA genes; median (25−75 percentile): 6.83 (6.65−7.53) and 8.04 (7.86−8.33), respectively; $p < 0.01$) in the cecal mucosa. However, no changes in measured metabolic parameters were observed by either oat or barley products.

**KEYWORDS:** *dietary fiber, xylanase, gut microbiota, short-chain fatty acids, C57BL/6 mice*

## INTRODUCTION

Dietary fiber is a collective term for carbohydrates in plants with three or more monomeric units that are resistant to digestion by human gastrointestinal enzymes. Health benefits of dietary fiber in the human diet, such as reduced risk for developing obesity, diabetes, coronary heart disease, hypertension, and gastrointestinal disorders such as colorectal cancer are well-known.[1,2] In this respect, the viscosity of the dietary fiber seems to play an important role and the higher the viscosity the higher the beneficial effect.[3] However, in recent years, the effect of short-chain fatty acids (SCFA), produced from soluble fiber during colonic fermentation, has been more emphasized. This means that also nonviscous indigestible carbohydrates of lower molecular weight may contribute to reduce the risk of cardiovascular disease and type 2-diabetes.

Cereals such as wheat, rye, barley, and oats are important sources of dietary fiber in the human diet. The hemicelluloses, which have structural functions in the plant cell walls, constitute major components in the fiber fraction. Cereals contain a variety of hemicelluloses with arabinoxylan as an important example. In obese mice, wheat arabinoxylan has been shown to counteract the high-fat-induced change of the gut microbiota in parallel with decreased body weight gain and insulin resistance.[4]

Dietary fiber, which is fermented by the colonic microorganisms, may be an important modulator of the gut microbiota, which in turn is essential for the metabolic function of the host. A beneficial microbiota may inhibit pathogen colonization, modulate the immune system, and metabolize otherwise indigestible carbohydrates.[5] Indigestible carbohydrates can be regarded as prebiotic if they selectively stimulate the growth and activity of intestinal bacteria with health promoting effects for the host. Mostly oligosaccharides such as fructo-oligosaccharides and galacto-oligosaccharides are included in this concept, but in fact all dietary fiber that are fermented in the colon are potential prebiotic components.[6] Also the formation of bacterial metabolites, formed as a consequence of oligosaccharide fermentation, such as SCFA, may be important. Of the SCFA, propionic acid and butyric acid have mostly been associated with beneficial metabolic effects.[7] Interestingly, the monomeric composition and chain length of the oligosaccharides are of utmost importance in this respect. Thus, FOS of higher chain length gave higher formation of propionic acid in rats than FOS of lower chain length, which instead gave higher amounts of butyric acid.[8]

In addition to FOS, several other types of oligosaccharides have promising properties. These include mannooligosaccharides (MOS), xylooligosaccharides (XOS), and arabinoxylooli-

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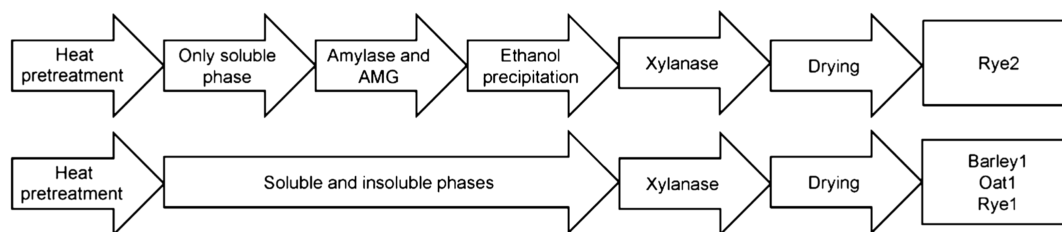


**Figure 1.** Schematic flowchart of the processes used to produce the different products. AMG = amyloglucosidase.

gosaccharides (AXOS). MOS produced from guar gum has been shown to increase the number of bifidobacteria in faeces of humans when incorporated into biscuits.[9] Furthermore, there is now overwhelming evidence that XOS and AXOS are prebiotics.[10] XOS have been shown to be fermented by certain groups of lactobacilli and bifidobacteria present in the human gut microbiota[11,12] XOS have been shown to be fermented by certain strains of the genus *Weissella*, which are putative probiotic bacteria.[13] FOS and XOS were found to stimulate bifidobacteria and lactobacilli, but the stimulation pattern was different.[14]

It should be emphasized that the size and structure of the oligosaccharides are important for their properties in general and for their digestibility by bacteria. Short AXOS have been shown to stimulate digestion by bifidobacteria, while longer ones were shown to decrease protein fermentation (branched SCFA), and it has been suggested that an average degree of polymerization of 5 was optimal for the overall effect on gut health characteristics.[15] It has also been shown that the selectivity for lactobacilli and bifidobacteria, i.e., the prebiotic effect, increased with decreasing size of wheat arabinoxylan.[16]

In the present study, byproducts from industrially processed barley, oats, and rye were treated in different ways, including selective hydrolysis catalyzed by a xylanase for the generation of XOS and AXOS, in order to improve the products' prebiotic potential. The products were evaluated in high-fat fed C57BL/6 mice, a model of obesity and insulin resistance associated with microbial dysbiosis,[17] with special attention on effects on the gut microbiota and generation of SCFA as an improved gut microbial activity was hypothesized to improve metabolic functions of the host. This model was chosen rather than healthy animals in order to get faster effects on physiological parameters and gut microbiota.

## ■ MATERIALS AND METHODS

**Chemicals.** Thermostable amylase (*Bacillus licheniformis*) and amyloglucosidase (*Aspergillus niger*) were purchased from Megazyme (Wicklow, Ireland). Xylanase was from the thermophilic bacterium *Rhodothermus marinus* (*Rm*Xyn10A, catalytic domain[18]) and was produced and purified in-house as described elsewhere.[19] All other chemicals were of analytical grade unless stated otherwise.

**Materials.** Milled barley husks (Lyckeby Stärkelsen, Kristianstad, Sweden), oat fiber residue (Oatly, Landskrona, Sweden), and rye bran (Farina, Kävlinge, Sweden) were included in the study. Partially hydrolyzed guar gum (Guar) was obtained under the trade name Sunfiber R (lot no. 002141) (Taiyo Lucid Pvt. Ltd., Mumbai, India). The Sunfiber has been shown to be polydispersed with an average weight of 20 kDa.[20]

**Processing Steps.** *Heat Pretreatment.* The products Barley1, Oat1, and Rye1 were made from 150 g of dry barley husks (Barley0), oat fiber residue (Oat0), and rye bran (Rye0), respectively (Figure 1). The more refined product Rye2 was made from 1.62 kg of rye bran (Rye0) (Figure 1). The products were mixed with deionized water with a weight ratio of 1:9 and autoclaved in glass bottles at 121 °C for 15 h.

*Treatments of Products Barley1, Oat1, and Rye1.* The autoclaved products were adjusted to pH 6 using NaOH and then heated under stirring to 70 °C. Thereafter, 900 U of the thermostable xylanase was added to the samples. The reaction mixture was maintained at 70 °C for 23 h under constant stirring, and the reaction was terminated by autoclaving at 121 °C for 15 min and the product was freeze-dried (Figure 1).

*Treatments of Soluble Fibers for Product Rye2.* After heat pretreatment of rye bran, the extract was first filtered through a textile cloth and then the filtrate was centrifuged at 10 000 rpm for 30 min to separate the soluble extract from insoluble particles. The soluble extract was concentrated by vacuum evaporation down to 4 L. The extract was treated with 15 kU of amylase at 85 °C. The extract was cooled to 50 °C, 33 kU amyloglucosidase was added, and the reaction mixture was stirred for 60 min. The extract was then precipitated under stirring with ethanol to a final concentration of 80% (v/v), and the precipitate was collected by filtration and air-dried (Figure 1).

The ethanol precipitate was dissolved in 3 L of deionized water and heated under stirring to 70 °C, and the pH was adjusted to 6.5. Then 1.2 kU of xylanase was added. The reaction mixture was maintained at 70 °C for 7 h under stirring, and the reaction was stopped by boiling for 20 min and thereafter the product was freeze-dried.

**Analysis of Final Products.** Protein was analyzed according to Kjeldahl on a FIASTAR 5000 system (FOSS TECATOR). Available starch and total starch contents were analyzed as previously described.[21,22]

Monosaccharide analysis of starting materials and final products was done using a 10 mg sample with 5 mL of 4% sulfuric acid at 100 °C for 3 h according to a modified method by Sluiter et al.[23] After hydrolysis, the hydrolysate was neutralized with 0.1 M $Ba(OH)_2$. Released monosaccharides were analyzed by high-performance anion exchange chromatography with pulsed amperiometric detection (HPAEC-PAD) (DIONEX, California, USA) using a 250 mm × 4 mm i.d., 8.5 μm CarboPac PA10 column and guard column 50 mm × 4 mm of the same material and an isocratic mobile phase of 1 mM NaOH at 1 mL/min.

Oligosaccharides present in the final products were analyzed using a 250 mm × 3 mm i.d., 5.5 μm CarboPac PA200 and a guard column 50 mm × 3 mm of the same material and a mobile phase of 100 mM NaOH and a gradient of sodium acetate of 0−50 mM between 0−20 min at 0.5 mL/min.

Dietary fibers were analyzed according to Asp.[24] The fat content was determined using the Schmid−Bondzynsky−Ratzlaff method (SBR) (NMKL Method no. 88, 1974). The water content of the samples was analyzed by NREL/TP-510-42621 and ash by NREL/TP-510-42622 (http://www.nrel.gov/publications/).

**Mouse Study Procedure.** Male 4 week old C57BL/6JBomTac mice (Taconic, Skensved, Denmark) were housed 10 mice per cage in 10 cages from arrival throughout the study. During acclimatization, they were all fed the low-fat diet (LFD) for 12 days. The animals were maintained in a temperature-controlled room with 12 h light−dark cycle. The study was approved by the local animal ethics committee (M185-11, Lund, Sweden), and the principles of laboratory animal care were followed.[46] After acclimatization, the mice were fed either one of the eight experimental diets with supplement or a control diet without supplement. The mice were fed the different diets ad libitum

**Table 1. Composition of Fiber Rich Products Used in the Study (%)[a]**

| | Oat0 | Oat1 | Barley0 | Barley1 | Rye0 | Rye1 | Rye2 | Guar |
|---|---|---|---|---|---|---|---|---|
| starch and maltodextrin | 5 | 5 | 8 | 6 | 15 | 15 | 0 | |
| monosaccharides and maltose | 5[b] | 3[b] | 1 | 2 | 4 | 10 | 6[c] | |
| dietary fiber | 38 | 38 | 65 | 67 | 41 | 46 | 71 | 80 |
| protein | 30 | 29 | 10 | 10 | 16 | 18 | 4 | 0.6 |
| fat | 13 | 13 | 3 | 3 | 4 | 5 | 1 | |
| ash | 6 | 6 | 7 | 7 | 5 | 5 | 13 | 1 |
| water | 2 | 4 | 6 | 3 | 13 | 3 | 5 | 5 |

[a]Oat: fiber rich residue from oat milk production. Barley: barley husks. Rye: rye bran. Guar: partly hydrolyzed guar gum. 0 = unmodified starting material; 1 = product obtained without separation steps, contains insoluble and soluble material; 2 = soluble product. [b]Mainly maltose. [c]Mainly glucose.

with free access to drinking water for 5 weeks. Body weight and food intake were registered once a week. At the time of sacrifice, mice were fasted for 4 h and thereafter anesthetized with an intraperitoneal injection of midazolam (Midazolam Panpharma 5 mg/mL, Panpharma SA, France) and a mixture of fluanison 10 mg/mL and fentanyl citrate 0.315 mg/mL (Hypnorm, VetPharma, U.K). The mice were weighed, and body fat content and lean body mass were analyzed using dual-energy X-ray absorptiometry (DEXA) technique with a Lunar PIXImus densitometer (GE Medical Systems). Blood was drawn by intraorbital puncture followed by cervical dislocation. The ceca were weighed, content collected and snap frozen for SCFA analysis, and the cecum tissues were rinsed in sterile phosphate buffered saline (PBS; Oxoid, England) and weighed, liquid was thoroughly soaked up by sterile compress, and tissues were snap frozen. All samples were stored at −80 °C until analysis.

*Experimental Diets.* The supplemented cereal byproducts were prepared as described above and analyzed for nutritional content (Table 1). In the eight experimental diets and the high-fat control diet (HFD), 60% of the energy originated from fat while the energy from fat was 10% in the low-fat control diet (LFD). All diets were manufactured by Research Diets (NB, USA). The supplemented products were included in levels so that each product contributed with 5 g/100 g dietary fiber (dry weight basis, dwb, Supporting Information, Table S1), i.e., cellulose was only added in the control HFD and LFD (5 g/100 g, dwb). One of the diets contained guar gum and used as a positive control. All high-fat diets were balanced to contain equal amounts of protein, carbohydrates (starch, maltodextrin, and mono/disaccharides), and fat.

*Plasma Samples.* Blood samples were drawn by intraorbital puncture using an EDTA-coated glass pipet. After immediate centrifugation plasma was collected and stored at −20 °C until analysis. Glucose, total cholesterol, triglycerides, HDL (all from Infinity, Thermo Electron Melbourne, Australia), fructosamine (VetSpec, Catachem Inc., Oxford, CT, USA), and alanine amino-transferase (ALT) (DiscretePak, Catachem Inc.) were measured enzymatically with colorimetric methods. LDL was calculated using Freiwalds formula: LDL = total cholesterol − (triglycerides/2.2 + HDL).[25] Insulin was measured with ELISA (Mercodia, Uppsala, Sweden).

*DNA Extraction from Cecum Tissue.* DNA from cecum tissue was isolated and purified in BioRobot EZ1 (EZ1 DNA Tissue Kit and tissue card; Qiagen, Hilden, Germany). Tissue samples were incubated with 380 μL of Buffer G2 and 30 μL of Proteinase K (Qiagen, Germany) in 56 °C for 6 h. Then 12 glass beads (2 mm in diameter) were added to each sample, which were shaken at 4 °C for 45 min to disintegrate the bacterial cell walls. Then 100 μL of the supernatant and 100 μL sterile PBS were used for extraction in the BioRobot EZ1.

*Microbial Analyses.* The amount of 16S rRNA genes of bacteria belonging to *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae*, and *Clostridium leptum* groups and *Akkermansia muciniphila*-like bacteria were estimated using separate quantitative real-time PCR (qPCR) assays as previously described,[26] with previously published primers.[26−28] Detection limit was $10^2$ genes/reaction for *Lactobacillus*, *Bifidobacterium*, and *A. muciniphila*-like bacteria, while the limit for the *Enterobacteriaceae* assay was $10^4$ genes/reaction. For standard curves, 10-fold dilution series of the target DNA were made in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Number of bacteria was expressed as log 16S rRNA genes/g cecum tissue.

*SCFA in Cecum.* The SCFA (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, caproic, and heptanoic acids) in cecum were analyzed using a GLC method.[29] The intestinal content was homogenized with an Ultra Turrax T25 basic (IKA-Werke, Staufen, Germany) after adding hydrochloric acid (to protonise the SCFA) and 2-ethylbutyric acid (internal standard). The samples were centrifuged (MSE Super Minor, Hugo Tillquist AB, Solna, Sweden) and the supernatant injected on to a fused-silica capillary column with a free fatty acid phase (DB-FFAP 125-3237; J&W Scientific, Agilent Technologies Inc., Folsom, CA, USA). GC ChemStation software (Agilent Technologies Inc., Wilmington, DE, USA) was used for the evaluation. The concentration of each SCFA (μmol/g) was multiplied by the amount of cecal content to obtain the cecal pool (μmol).

**Statistics.** All groups were compared with HFD control. One-way analysis of variance (ANOVA) with Dunnet's multiple comparison post-test was used when the data was Gaussian distributed according to D'Agostino and Pearson omnibus normality test. When not normally distributed, Kruskal−Wallis nonparametric test with Dunn's multiple comparison post-test was used (GraphPad Prism 6.0, GraphPad Software, San Diego, CA, USA or Minitab software, release 16). To find significances between the different oat products and also the three rye products, the general linear model of ANOVA followed by Tukey's procedure was used. These data are not shown in any table but reported in the text under Results and Discussion.

**Principal Component Analysis.** Principal component analysis (PCA) was performed using the software SIMCA (version 13.0.3, Umetrics, Umeå, Sweden) to visualize the relationship between the analyzed bacterial groups, cecal SCFA production, and some physiological parameters in relation to each other and to the various diets.

## RESULTS AND DISCUSSION

In the present study, processed cereal byproducts were used as dietary fiber additives in a high-fat diet (HFD) and their affects were evaluated in a mouse model for human obesity and prediabetes. Three byproducts from industrials processing of cereals were used as raw materials: a fiber-rich residue from the production of oat milk, barley husks, and rye bran. The materials are rich sources of dietary fiber, antioxidants, and possibly also other potentially health-promoting substances. In an attempt to improve their health promoting properties further, the materials were subjected to selective hydrolysis by a thermostable xylanase to generate xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS). A heat pretreatment step was used as pretreatment to solubilize the fibers and make the materials more accessible to the enzymes.

Because no separation steps were included in the processing of three of the four products, the starting materials (Oat0, Barley0, and Rye0) and the xylanase-treated products (Oat1,

Barley1, and Rye1) had similar overall chemical composition (Tables 1 and 2).

**Table 2. Content of Carbohydrates of the Barley, Oat and Rye Products**

| | sugars[a] (mg/g dry weight material) | | | | |
|---|---|---|---|---|---|
| products | Ara | Gal | Glc | Xyl | total |
| Oats0 | 41 | 4 | 190 | 70 | 300 |
| Oats1 | 31 | 4 | 150 | 60 | 240 |
| Barley0 | 35 | 10 | 300 | 180 | 530 |
| Barley1 | 29 | 7 | 280 | 180 | 500 |
| Rye0 | 64 | 9 | 270 | 150 | 490 |
| Rye1 | 54 | 8 | 270 | 140 | 470 |
| Rye2 | 85 | 9 | 240 | 280 | 610 |

[a]Fructose is not included in the analysis because it is degraded to a large extent by sulfuric acid. Values are means, $n$ = 3. SD not more than 3%.

Rye bran was selected as starting material for production of the fourth product (Rye2, Tables 1 and 2), which is a material rich in XOS and AXOS. After heat pretreatment, only the soluble fraction was recovered and further processed. To improve the purity, starch was degraded enzymatically using a combination of $\alpha$-amylase and amyloglucosidase. Precipitation of soluble oligo- and polysaccharides to remove glucose and other small molecular impurities was carried out using ethanol. After redissolving the precipitate, xylanase-catalyzed hydrolysis was carried out. The resulting product (Rye2) had a dietary fiber content of 71% (Table 1) and high amounts of polymeric xylose and arabinose (Table 2). Similar processing has successfully been done with wheat bran to make short chain XOS and AXOS.[30]

The xylanase treatment produced xylobiose from all three tested raw materials, and in most cases longer XOS were formed as well (Figure 2). The xylanase is expected to produce XOS from xylan and from parts of AX without arabinose substituents, and it is thus less efficient in hydrolyzing highly substituted AX.[19] The smallest amount of xylobiose was found in Barley1, although Barley0 was the starting material having the highest content of xylose (Table 2). The main reason was probably the tough, highly cross-linked structure of the barley husk material. In Rye1, the distribution of different XOS was shifted toward the shorter ones, mainly xylobiose and xylotriose, which indicates that the xylanase-catalyzed hydrolysis had proceeded further in this case than in the preparation of Rye2. It has previously been shown that relatively short XOS are efficiently consumed by bifidobacteria and some strains of *Lactobacillus* in vitro.[19] More complex oligosaccharides, tentatively identified as AXOS, were detected in Oat1, Rye1, and in the highest amounts in Rye2.

To assess the effects of the different products in vivo, C57BL/6 mice were fed high-fat diets supplemented with the cereal byproducts. The different diets were evaluated for their effect on metabolic parameters, abundance of selected bacterial groups in the intestinal microbiota, and production of SCFA. The mean body weight of the test diet groups did not differ compared with the HFD control. There were no differences in energy intake, weight gain per energy intake, or body fat mass between the mice fed HFD with or without cereal supplementation. In addition, the measured plasma parameters glucose, insulin, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, as well as HOMA-IR did not differ

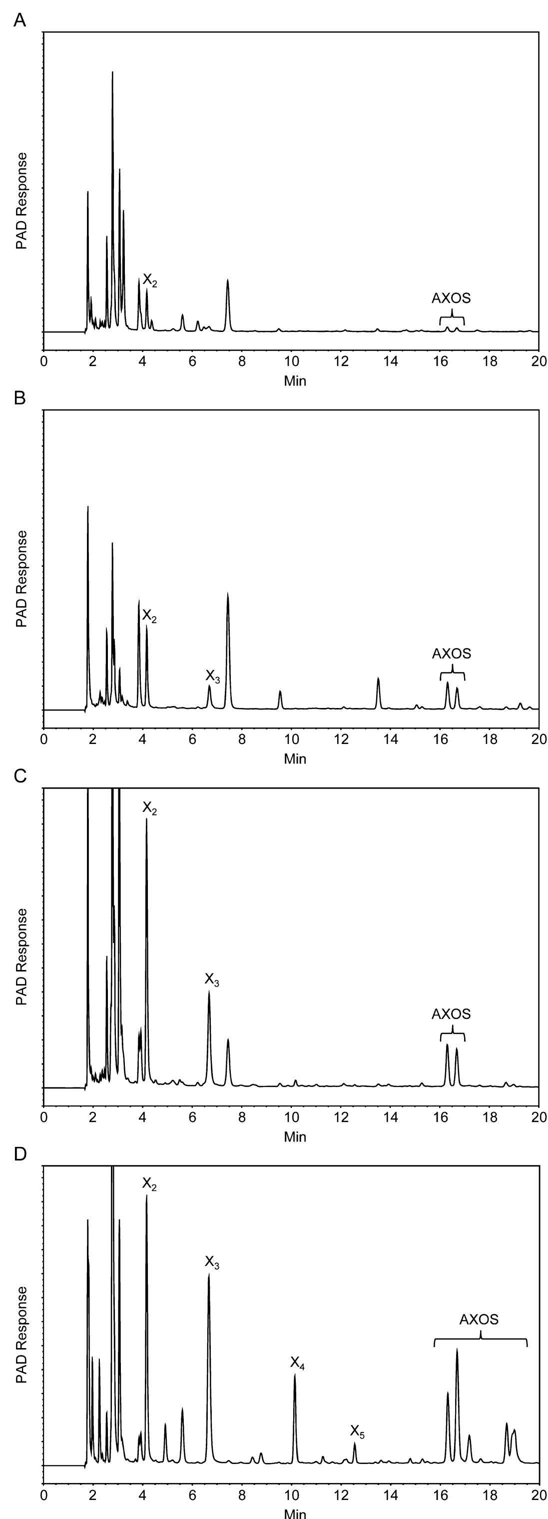


**Figure 2.** HPAEC-PAD analysis of oligosaccharides present in fiber products (A) Barley1, (B) Oat1, (C) Rye1, and (D) Rye2. $M_2$ = maltose; $X_n$ = XOS having $n$ xylose residues.

significantly between the groups fed with fiber-supplemented high-fat diet, compared with the HFD control (Table 4). Mice receiving a diet supplemented with guar gum hydrolysate (Guar, positive control[31]) showed reduced body weight after 5 weeks compared with the HFD control mice group (Table 3). Some minor metabolic changes were observed in mice fed the Rye2 diet. In plasma from Rye2-fed mice, the level of alanine

Table 3. Body Weight and Body Composition after 5 Weeks of Different Diets[a]

| | HFD | Oat0 | Oat1 | Barley0 | Barley1 | Rye0 | Rye1 | Rye2 | Guar | LFD |
|---|---|---|---|---|---|---|---|---|---|---|
| body weight (g) | 30.0 ± 0.8 | 28.9 ± 0.5 | 28.3 ± 0.5 | 31.7 ± 1.3 | 28.2 ± 0.7 | 29.1 ± 0.9 | 28.4 ± 0.5 | 27.6 ± 1.0 | 26.5 ± 0.9[b] | 23.5 ± 0.8[c] |
| body weight gain/feed intake (mg/kcal) | 28.5 ± 2.7 | 30.4 ± 2.5 | 28.6 ± 1.5 | 31.6 ± 4.0 | 26.4 ± 2.1 | 24.6 ± 3.3 | 26.0 ± 1.4 | 24.6 ± 3.5 | 27.2 ± 1.9 | 18.4 ± 4.2 |
| body fat (%) | 28.8 ± 1.7 | 24.1 ± 1.1 | 25.4 ± 2.0 | 27.1 ± 1.6 | 26.4 ± 1.1 | 28.4 ± 1.1 | 27.5 ± 1.0 | 27.7 ± 2.2 | 24.1 ± 1.3 | 18.6 ± 0.7[c] |
| lean body mass (g) | 22.6 ± 10.5 | 23.0 ± 0.4 | 22.6 ± 0.5 | 24.1 ± 0.5 | 22.4 ± 0.4 | 22.2 ± 0.5 | 22.1 ± 0.4 | 21.1 ± 0.5 | 22.2 ± 0.6 | 20.5 ± 0.7 |

[a]Values are means ± SEM, $n = 10$. [b]Indicating significant difference ($p < 0.05$) compared to HFD. [c]$p < 0.001$.

aminotransferase (ALT) was decreased ($p = 0.006$) compared with the HFD control (Table 4), which indicates that Rye2 can counteract HFD-induced liver dysfunction. Also, the Rye2 group had a tendency of lower fructosamine ($p = 0.13$ using ANOVA multiple comparison test and $p = 0.012$ using unpaired $t$ test), which mirrors the blood glucose over the last 2 weeks, indicating an improved glucose control compared to HFD control. As expected, mice fed a low-fat diet (LFD), which was used as an additional control, showed reduced body weight ($p < 0.001$), energy intake ($p < 0.001$), and adiposity ($p < 0.001$) compared with the HFD control.

In a previous study in rats fed a high-fat diet, inclusion of XOS was shown to reduce blood glucose significantly.[32] A few of the differences compared to the present study were that the rat study was somewhat longer (6 weeks), used a pure XOS preparation (obtained from wheat bran), and that the XOS replaced corn starch in the diet, which made the dietary fiber content different in the different groups. In a human study, 4 g/day of XOS for 8 weeks reduced glucose, HbA1c, fructosamine, and blood lipids in type 2 diabetic patients.[33] With these results in mind, there seems to be good reasons to study antidiabetic effects of cereal byproducts enriched in XOS further, although no effects on some of the risk parameters could be seen, and it seems likely that both the dosage and the length of the intake period influence the observed response.

Propionic acid and butyric acid, formed from microbial fermentation of dietary fiber in colon, have been suggested to have positive metabolic effects due to their capacity to modulate pro- and anti-inflammatory markers present in the gut.[3] With this background, it is interesting to see that the cecal amount of SCFA in the mice decreased to almost the half when fed the HFD diet compared with the LFD. When the cellulose in the HFD was replaced by the dietary fiber in the different diets, the cecal amount of total SCFA formed increased in the mice, but it was only significant in mice fed the diet with Oat0 ($p < 0.01$) and guar gum ($p < 0.05$) (Table 5). More significance was seen with the individual SCFA. Guar gum, which was used as a positive control in the experiment, more than doubled the yield of propionic ($p < 0.001$) and butyric acids ($p < 0.01$) compared with the HFD control. Increased amounts of propionic ($p < 0.01$) and butyric ($p < 0.001$) acids could be seen as an effect of Oat0 and Oat1. Interestingly, if comparing the butyric acid formation before and after enzymatic treatment of oats (Oat0 with Oat1) and rye (Rye0 with Rye 1 and Rye2, respectively) there was a lower formation ($p < 0.01$−$p < 0.05$) of butyric acid after enzymatic treatment. However, Rye0 increased the amount of butyric acid ($p < 0.05$) in the cecum of mice compared with the HFD. Enzymatic treatment caused changes in the physicochemical properties of the products. When the insoluble fibers were removed (Rye2), there was an increase of propionic acid in the cecum of mice ($p < 0.05$) compared with Rye0. Similar findings, with an increased amount of propionic acid, have been seen in wheat enzyme-treated in a similar way.[34] None of the barley products had any effects on the amounts of SCFA formed in the cecum of mice compared with the HFD, and the specific SCFA formed were very similar to those formed with the high-fat control diet. The SCFA usually detected in rather low amounts; valeric, iso-valeric, and iso-butyric acid were significantly higher in mice fed the two oat products (Oat0, $p < 0.05$, and Oat1, $p < 0.001$) and with guar gum ($p < 0.001$) compared with the HFD control. Iso-valeric acid was also significantly higher ($p < 0.01$) with Rye2. The physiological impact of this is not known, but higher

Table 4. Plasma Parameters after 5 Weeks of Different Diets[a]

| | HFD | Oat0 | Oat1 | Barley0 | Barley1 | Rye0 | Rye1 | Rye2 | Guar | LFD |
|---|---|---|---|---|---|---|---|---|---|---|
| glucose (mM) | 6.5 ± 0.3 | 6.1 ± 0.2 | 6.1 ± 0.3 | 6.3 ± 0.4 | 6.9 ± 0.3 | 7.2 ± 0.2 | 6.6 ± 0.3 | 6.0 ± 0.2 | 6.0 ± 0.3 | 5.4 ± 0.3 |
| insulin (pM) | 272 ± 39 | 188 ± 30 | 222 ± 46 | 370 ± 58 | 294 ± 54 | 280 ± 36 | 267 ± 38 | 257 ± 42 | 316 ± 31 | 119 ± 12[b] |
| HOMA-IR | 11.2 ± 1.9 | 7.2 ± 1.2 | 8.9 ± 2.3 | 14.0 ± 2.1 | 12.9 ± 2.9 | 12.5 ± 1.7 | 11.1 ± 1.6 | 9.7 ± 1.7 | 11.8 ± 1.4 | 5.8 ± 1.9[b] |
| fructosamine (μM) | 505 ± 17 | 450 ± 9 | 515 ± 25 | 528 ± 21 | 470 ± 11 | 470 ± 16 | 523 ± 25 | 435 ± 18 | 497 ± 40 | 535 ± 14 |
| cholesterol (mM) | 4.4 ± 0.1 | 4.3 ± 0.1 | 4.4 ± 0.2 | 4.2 ± 0.2 | 4.9 ± 0.1 | 4.6 ± 0.2 | 5.1 ± 0.2 | 4.6 ± 0.3 | 4.5 ± 0.1 | 3.4 ± 0.2[b] |
| HDL (mM) | 1.4 ± 0.04 | 1.4 ± 0.03 | 1.5 ± 0.06 | 1.5 ± 0.04 | 1.6 ± 0.04 | 1.5 ± 0.04 | 1.6 ± 0.04 | 1.5 ± 0.06 | 1.4 ± 0.03 | 1.1 ± 0.03[b] |
| LDL (mM) | 2.6 ± 0.1 | 2.5 ± 0.1 | 2.5 ± 0.2 | 2.3 ± 0.2 | 2.9 ± 0.1 | 2.7 ± 0.1 | 3.1 ± 0.2 | 2.7 ± 0.2 | 2.7 ± 0.1 | 1.8 ± 0.1[c] |
| LDL/HDL | 1.9 ± 0.03 | 1.8 ± 0.1 | 1.7 ± 0.1 | 1.5 ± 0.2 | 1.8 ± 0.1 | 1.8 ± 0.1 | 2.0 ± 0.1 | 1.8 ± 0.1 | 1.9 ± 0.1 | 1.5 ± 0.1[b] |
| TAG (mM) | 0.9 ± 0.03 | 1.1 ± 0.05 | 0.9 ± 0.04 | 1.1 ± 0.06 | 1.0 ± 0.05 | 1.0 ± 0.03 | 0.9 ± 0.02 | 0.9 ± 0.04 | 0.9 ± 0.06 | 1.1 ± 0.06 |
| ALT (U/L) | 4.4 ± 0.4 | 4.3 ± 0.4 | 5.8 ± 0.9 | 6.4 ± 1.1 | 4.0 ± 0.3 | 3.1 ± 0.4 | 3.4 ± 0.5 | 1.4 ± 0.2[d] | 2.3 ± 0.4 | 2.9 ± 0.2 |

[a]HFD: high-fat diet. LFD: low-fat diet. HOMA-IR: homeostatic model assessment of insulin resistance. HDL: high-density lipoprotein. LDL: low-density lipoprotein. TAG: triacylglycerol. ALT: alanin aminotransferase. Values are mean ± SEM, $n = 10$. [b]Indicating significant difference ($p < 0.05$) compared to HFD. [c]$p < 0.01$. [d]$p < 0.001$.

Table 5. Pools of SCFA (μmol) in Cecum of Mice[a]

| | HFD | Oat0 | Oat1 | Barley0 | Barley1 | Rye0 | Rye1 | Rye2 | Guar | LFD |
|---|---|---|---|---|---|---|---|---|---|---|
| acetic | 3.06 ± 0.44 | 4.75 ± 0.51 | 4.51 ± 0.51 | 3.38 ± 0.35 | 3.23 ± 0.48 | 2.76 ± 0.17 | 2.53 ± 0.2 | 3.28 ± 0.44 | 5.05 ± 0.69 | 5.85 ± 0.88[c] |
| propionic | 0.62 ± 0.12 | 1.03 ± 0.10[c] | 0.94 ± 0.08[b] | 0.69 ± 0.06 | 0.76 ± 0.10 | 0.55 ± 0.03 | 0.60 ± 0.1 | 0.86 ± 0.13 | 1.62 ± 0.21[d] | 1.17 ± 0.16[c] |
| iso-butyric | 0.05 ± 0.01 | 0.10 ± 0.01[b] | 0.13 ± 0.02[d] | 0.08 ± 0.01 | 0.09 ± 0.01 | 0.05 ± 0.01 | 0.06 ± 0.0 | 0.09 ± 0.02 | 0.16 ± 0.02[d] | 0.12 ± 0.02[c] |
| butyric | 0.38 ± 0.04 | 0.83 ± 0.07[d] | 0.60 ± 0.04[b] | 0.46 ± 0.04 | 0.34 ± 0.04 | 0.68 ± 0.08[b] | 0.36 ± 0.0 | 0.50 ± 0.06 | 0.81 ± 0.13[c] | 0.62 ± 0.08[b] |
| iso-valeric | 0.07 ± 0.01 | 0.11 ± 0.01[b] | 0.16 ± 0.02[d] | 0.09 ± 0.01 | 0.11 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.0 | 0.13 ± 0.03[c] | 0.22 ± 0.03[d] | 0.13 ± 0.02[c] |
| valeric | 0.06 ± 0.01 | 0.10 ± 0.01[b] | 0.12 ± 0.01[d] | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.06 ± 0.00 | 0.07 ± 0.0 | 0.07 ± 0.01 | 0.13 ± 0.02[d] | 0.10 ± 0.02[b] |
| caproic[e] | | | | | | | | | | |
| heptanoic[f] | | | | | | | | | | |
| total | 4.25 ± 0.58 | 6.93 ± 069[b] | 6.45 ± 0.64 | 4.77 ± 0.46 | 4.61 ± 0.64 | 4.18 ± 0.25 | 3.73 ± 0.3 | 4.94 ± 0.67 | 7.98 ± 1.07[c] | 8.01 ± 1.17[c] |

[a]Values are means ± SEM, $n = 10$. Indicating significant difference ($p < 0.05$) from HFD. [b]$p < 0.05$. [c]$p < 0.01$. [d]$p < 0.001$. [e]Values were less than 0.01 μmol. [f]Values were less than 0.004 μmol.

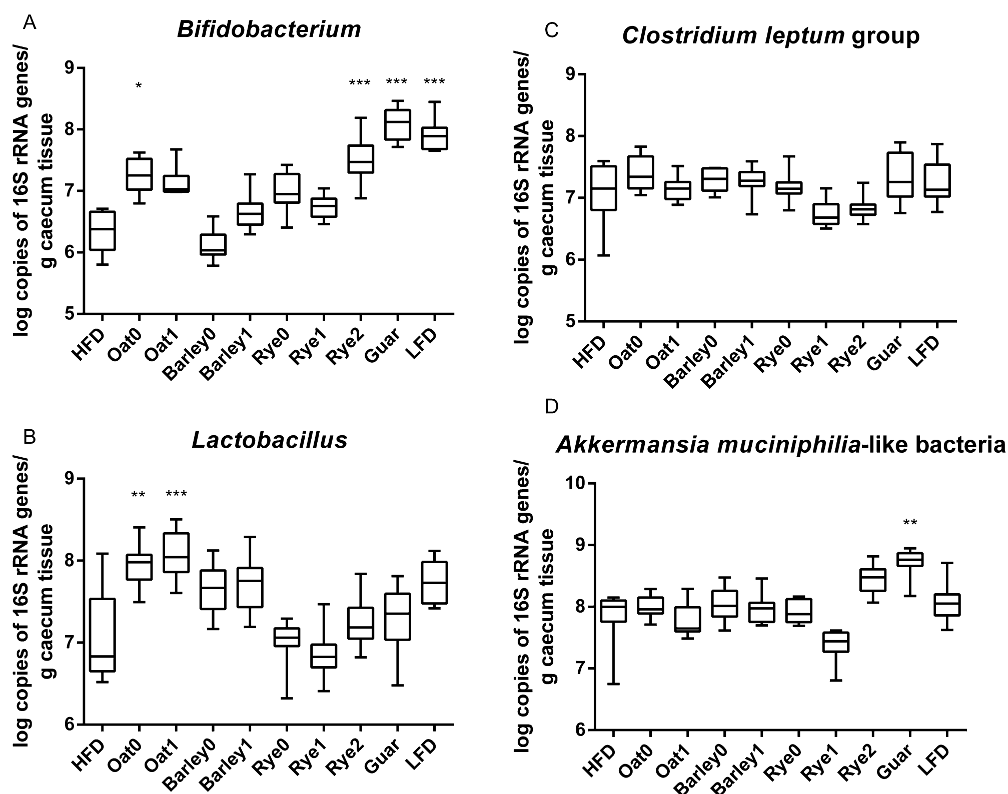


**Figure 3.** Box plots presenting the median, 10th, 25th, 75th, and 90th percentiles of the concentrations of 16S rRNA genes from (A) *Bifidobacterium*, (B) *Lactobacillus*, (C) *Clostridium leptum* group, and (D) *Akkermansia muciniphila*-like bacteria in the cecum tissue of mice on the test diets ($n$ = 7−10 animals/group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

amounts of valeric acid in blood have been seen in microscopic colitis patients, who usually have a low dietary fiber intake compared with healthy controls.[35]

*Lactobacillus* and *Bifidobacterium* are bacterial genera considered beneficial for host health.

The abundance of *Lactobacillus* and *Bifidobacterium* was assumed to be stimulated by the supplemented nondigestible carbohydrates that were used in the diets of the present experimental study. Members of the *C. leptum* group are abundant in the gastrointestinal tract and known to contribute to the intestinal butyrate production and so of interest in the present study.[36] *Akkermansia* is a commensal bacterial genus known to degrade mucin and is commonly found in the gastrointestinal tract and has been negatively correlated to obesity and metabolic disorders.[37,38] *Enterobacteriaceae* is a Gram-negative family with pro-inflammatory lipopolysaccharides in its outer cell membrane. Abundance of *Enterobacteriaceae* can be regarded as an indicator if the microbiota is in balance or if dysbiosis occurs.

In the experimental data, prebiotic potential was shown by diets LFD, Oat0, Rye2, and guar gum because animals on these diets had increased amount of *Bifidobacterium* in cecum mucosa compared to animals on HFD ($p < 0.001$, 0.05, 0.001, and 0.001, respectively) (Figure 3A). Furthermore, the number of 16S rRNA genes of *Lactobacillus* were significantly higher in animals on diets Oat0 and Oat1 compared with HFD ($p < 0.01$ and $<0.001$, respectively) (Figure 3B). The number of 16S rRNA genes from the *C. leptum* group did not differ significantly between the groups (Figure 3C). The number of 16S rRNA genes corresponding to *Akkermansia muciniphila*-like bacteria was increased in animals on a diet supplemented with guar gum compared to animals on HFD ($p < 0.01$) (Figure 3D), possibly indicating a prebiotic potential by enhancing the gut integrity through mucin production.[37] The amount of *Enterobacteriaceae* was below detection limit for all diet groups, so no significant differences between the diet groups could be obtained.

It is obvious that the different diets produced quite different effects on the mucosal bacterial groups quantified in this study. Because the total content of dietary fiber was the same in all cases, it seems that the composition of the fiber had a major influence although other components of the products likely contributed to the observed effects. However, Rye2 and guar gum contained mainly oligosaccharides, so at least in these cases those can be expected to have been of major importance.

The stimulation of bifidobacteria by Rye2 was likely due to its content of XOS and AXOS, which have been shown to have this effect in several other studies. For example, it has been reported that 10% XOS in the diet caused the most efficient stimulation of bifidobacteria in streptozotocin induced diabetic rats.[39] Likewise, XOS caused an increase in bifidobacteria in rats given a Western human diet.[15] In vitro experiments have also shown that XOS are indeed fermented by many intestinal bifidobacteria.[40] The mechanism for XOS utilization has been studied in the strain *Bifidobacterium animalis* subsp. *lactis* BB-12.[41] XOS/AXOS are supposed to be transported into the bacterial cells using an ABC transporter, and they are subsequently degraded intracellularly by xylanolytic enzymes. It is likely that rye fructooligosaccharides also contributed to the stimulation of bifidobacteria, as observed previously.[42] The positive effect of Oat0 on bifidobacteria agrees with previous observations of bifidobacteria stimulation by oat-based


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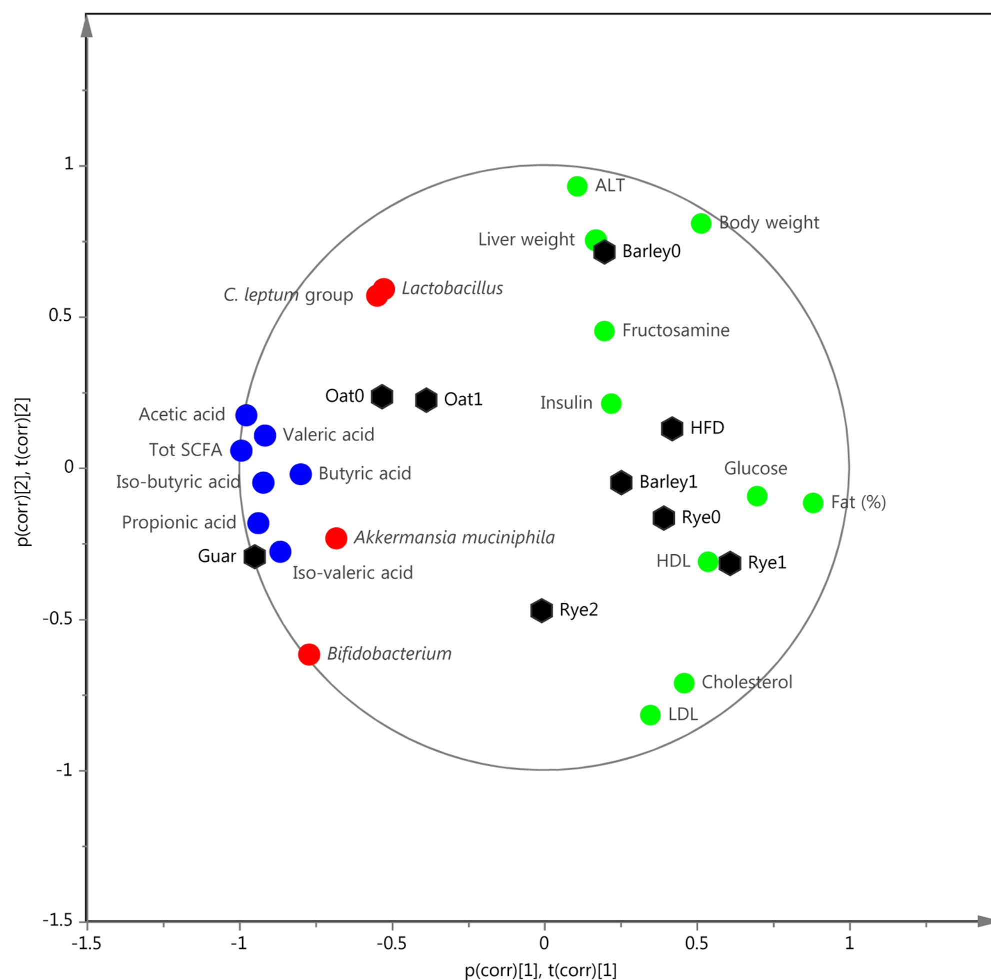


**Figure 4.** To visualize patterns and relationship in the data, a PCA was performed. Data is displayed in a biplot of analyzed bacterial groups, SCFAs, and some physiological parameters in relation to the diet groups. PC1 explained 46.6% and PC2 23.3% of the variation. Blue dots represent mean of cecal pool of analyzed SCFA or total SCFA, red dots represent the mean of the 16S rRNA expression of the analyzed microbial groups, green dots represent mean of the physiological parameters, and black hexagons represent the different diets.

products.[43] It has been reported that $\beta$-glucans from both oats and barley stimulate growth of lactobacilli and bifidobacteria,[44] so that might be at least part of the reason. The third product that caused a significant increase in bifidobacteria compared to HFD was the guar hydrolysate, which is in agreement with results reported by Giannini and co-workers.[45]

The best stimulation of lactobacilli was achieved with the oat-based products. As in the case with bifidobacteria, the stimulation was probably partly due to $\beta$-glucans,[44] but at least some lactobacilli (e.g., *L. brevis* and *L. fermentum*) grow very well on XOS as well.[40] It is interesting that the products composed mainly of oligosaccharides (Rye2 and Guar) favored bifidobacteria, while those based on oats were more efficient for lactobacilli. Notably, the abundance of the *C. leptum* group appeared similar, indicating no major change in this group of butyrate producing bacteria, although some changes of butyrate concentration in cecum content was observed in mice on different diets. Everard et al.[37] suggested *Akkermansia muciniphila* to have extensive prebiotic effects. In the present study, animals on the Guar diet was the only group with significantly higher abundance of *Akkermansia muciniphila*-like bacteria compared to HFD. The Guar group had lower body weight, but no changes of other metabolic parameters could be observed.

Multivariate statistical analysis was performed using PCA to visualize the relationship between SCFA, analyzed microbial groups, and some metabolic parameters (Figure 4). In a biplot, PC1 and PC2 were shown to explain 46.6% and 23.3% of the variation, respectively. The PCA biplot illustrates the fact that the diets supplemented with Guar and Oats (Oat0 and Oat1) cluster with the beneficial bacterial groups and SCFA in cecum but have inverse impact on, for example, body weight, body fat, and blood glucose (Figure 4).

In conclusion, the diet supplemented with Rye2, containing the highest amount of XOS and AXOS, induced bifidobacteria and there was also a tendency to an increased amount of propionic acid in the cecum as compared with the HFD control, indicating a prebiotic effect. Rye2 also significantly reduced the plasma concentration of ALT, indicating that the altered microbiota, with higher amounts of bifidobacteria, had beneficial effects on liver function. In addition, a tendency of reduced fructosamine was observed in the Rye2 group, indicative of improved glucose control compared to HFD control. The lack of other statistically proven beneficial metabolic changes might be due to the relatively short duration of the study. Also, both fractions of oat were shown to increase the amount of lactobacilli and the amounts of propionic and butyric acids in cecum, however, only the nonhydrolyzed Oat0-

supplement increased the bifidobacteria. The present study demonstrated that underutilized byproducts from industrial processing of cereals have a potential to be used as raw materials for production of valuable prebiotic food ingredients.

## ■ ASSOCIATED CONTENT

### Supporting Information

Composition of experimental diets. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

SCFA, short-chain fatty acids; GOS, galactooligosaccharides; FOS, fructooligosaccharides; MOS, mannooligosaccharides; XOS, xylooligosaccharides; AXOS, arabinoxylooligosaccharides; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperiometric detection; HFD, high-fat diet; LFD, low-fat diet; HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerol; ALT, alanine aminotransferase

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## NOTE ADDED AFTER ASAP PUBLICATION

This article published July 29, 2014 with a typo in the fourth paragraph of the Introduction section, errors in Figure 4, and an incorrect initial designation in the Funding statement. The correct version published August 13, 2014.