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RESEARCH ARTICLE

Prebiotic effects and intestinal fermentation of cereal arabinoxylans and arabinoxylan oligosaccharides in rats depend strongly on their structural properties and joint presence

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Scope: Cereal arabinoxylan (AX) is one of the main dietary fibers in a balanced human diet. To gain insight into the importance of structural features of AX for their prebiotic potential and intestinal fermentation properties, a rat trial was performed.

Methods and results: A water unextractable AX-rich preparation (WU-AX, 40% purity), water extractable AX (WE-AX, 81% purity), AX oligosaccharides (AXOS, 79% purity) and combinations thereof were included in a standardized diet at a 5% AX level. WU-AX was only partially fermented in the ceco-colon and increased the level of butyrate and of butyrate producing Roseburia/E. rectale spp. Extensive fermentation of WE-AX and/or AXOS reduced the pH, suppressed relevant markers of the proteolytic breakdown and induced a selective bifidogenic response. Compared with WE-AX, AXOS showed a slightly less pronounced effect in the colon as its fermentation was virtually complete in the cecum. Combining WU-AX and AXOS caused a striking synergistic increase in cecal butyrate levels. WU-AX, WE-AX and AXOS together combined a selective bifidogenic effect in the colon with elevated butyrate levels, a reduced pH and suppressed proteolytic metabolites.

Conclusion: The prebiotic potential and fermentation characteristics of cereal AX depend strongly on their structural properties and joint presence.

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1 Introduction

Unlike herbivores, omnivores – including man – are incapable of withdrawing substantial amounts of energy from the fermentation processes that occur in their large bowel [1]. Nevertheless, fermentation in the gut and the bacterial population responsible for it are increasingly considered to be important to humans [2]. In this respect, dietary fiber, which is fermented instead of digested, has (re)gained interest. Its consumption is believed to counteract the consequences of a typical unhealthy Western diet [3].

In grains of wheat and related cereals, dietary fiber is predominantly composed of arabinoxylans (AXs) [4]. These cell wall components typically consist of a linear backbone of

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Abbreviations: avDAS, average degree of arabinose substitution; **avDF,** average degree of fermentation; **avDP,** average degree of polymerization; **AX,** arabinoxylan; **AXOS,** arabinoxylan oligosaccharides; **EU,** enzyme units; **WE-AX,** water extractable arabinoxylan; **WU-AX,** water unextractable arabinoxylan

β-(1–4)-D-xylopyranosyl units, which may be substituted by α-L-arabinofuranosyl units [4]. The structure and the physicochemical properties of AX from different layers in cereal kernels are very diverse. Whereas AX in wheat pericarp, for example, has an average degree of arabinose substitution (avDAS) of approximately 1, AX in wheat aleurone layer is characterized by a much lower avDAS (0.2–0.4) [5, 6]. In most cereals, the predominant fraction of AX is water unextractable (WU-AX) due to covalent cross-links and non-covalent interactions with each other and other cell wall components [4]. A large part of WU-AX typically remains unaffected during its passage throughout the intestinal tract, causing a bulking effect, often enhanced by its high water holding capacity. In this respect, it can be reasoned that WU-AX, like other types of insoluble fiber, can reduce intestinal transit time and soften stool consistency [7]. Despite limited breakdown, in vitro tests suggest that the consumption of WU-AX may stimulate the production of short-chain fatty acids (SCFA) and promote bacterial growth [8].

The limited number of studies on the gastrointestinal effects of AX, however, has so far primarily focused on water extractable AX (WE-AX). In this view, Garcia et al. suggested that the intake of WE-AX is associated with improved glucose metabolism [9].

The use of AX-hydrolyzing enzymes in wheat-based food production processes reduces the molecular weight of AX and can shift the balance from unextractable to extractable AX. Extensive hydrolysis leads to the formation of AX oligosaccharides (AXOS), components that are increasingly studied for their prebiotic properties. Prebiotics, such as fructo- and galacto-oligosaccharides, are defined as non-digestible compounds that induce selective shifts in the composition and/or activity of the gastrointestinal microbiota, thereby contributing to the consumer's health and well-being [10, 11]. In several animal studies, AXOS consumption was associated with multiple effects often related to health promotion such as elevated levels of SCFA and a higher concentration of bifidobacteria [12, 13]. Consistent with observations made in animal and in in vitro studies, Cloetens et al. demonstrated that in humans urinary *p*-cresol excretion significantly decreased after 2 wk of AXOS intake, and that bifidobacteria levels in the feces were significantly increased [14].

The possible prebiotic nature of WE-AX and WU-AX has not been considered to any considerable extent. Vardakou et al. did suggest that partial enzymatic hydrolysis of WU-AX to AXOS prior to administration results in a higher prebiotic index [8, 15]. This coincides with the suggestion that the average degree of polymerization (avDP) strongly affects the prebiotic potential of AXOS, and by extension possibly AX, creating an opportunity to tune its fermentation dynamics and hence, health-promoting effects [12]. Spatial regulation of the fermentation of prebiotics in the intestinal tract is indeed of particular interest since most severe chronic colonic diseases (e.g. colon cancer) predominantly originate in the distal colon [16]. However, current prebiotics are predominantly fermented in the proximal colon. A combination of different types of prebiotics is often suggested to prolong their beneficial impact in the hindgut [17].

Against this background, this study investigates the intestinal fermentation behavior of cereal derived WU-AX, WE-AX and AXOS and their impact on the intestinal microbial composition, including bifidobacteria, lactobacilli and *Roseburia* species in rats. Furthermore, it evaluates whether combination of AXOS with WE-AX and/or WU-AX will affect their impact on the ceco-colonic ecosystem.

2 Materials and methods

2.1 Materials

Wheat bran was from Dossche Mills & Bakery (Deinze, Belgium). Wheat flour was from Ceres (Brussels, Belgium). Enzyme preparations used were heat-stable α-amylase (termamyl 120 L, Novozymes, Bagsvaerd, Denmark) and a bacterial protease (neutrase 0.8 L, Novozymes). Enzyme units (EU) were as defined by the suppliers. All chemicals, solvents and reagents were of at least analytical grade and supplied by Sigma-Aldrich (St. Louis, USA).

2.1.1 Preparation of WU-AX-rich material

Wheat bran was suspended in water (1:10, w/v) and treated with termamyl 120 L (216 EU/kg bran) at 90°C for 90 min to remove starch. After boiling (20 min) and centrifugation (10 min; 10 000 g), the destarched residue was washed and resuspended in deionized water (1:12, w/v). The suspension was incubated (55°C; 4 h) with neutrase 0.8 L (50 EU/g destarched residue) at a constant pH of 5.0 to remove proteins. The enzymes were inactivated by boiling (40 min). After centrifugation (10 min; 10 000 g), the residue was freeze-dried and is further referred to as WU-AX.

2.1.2 Preparation of WE-AX

Wheat flour was suspended in water (1:5, w/v) and treated with termamyl 120 L (8500 EU/kg flour) at 90°C for 60 min. After boiling (20 min) and centrifugation (32 200 × *g*) using a continuous, self-cleaning disk separator (Westfalia Separator, type SA 1-02-575, Oelde, Germany), the residue was washed and resuspended in deionized water (1:5; w/v). The suspension was incubated (50°C; 20 h) with neutrase 0.8 L (16 EU/g destarched residue) at a constant pH of 5.0. The enzymes were inactivated by boiling (30 min), and the supernatant was discarded after centrifugation (32 200 × *g*) (Westfalia Separator). The destarched and deproteinized residue was washed with water and resuspended in deionized water. The pH of the suspension was adjusted to 12.0

(8.0 M NaOH) to start alkaline hydrolysis (20°C; 18 h). After neutralization with HCl (10.0 M) and centrifugation (32 200 × g) (Westfalia Separator), the AX in the supernatant was precipitated and dried with ethanol (3:1, v/v), acetone (2:1, v/v) and diethyl ether (2:1, v/v). The residual ether portion was evaporated by spreading the material as a thin layer (≤0.5 cm) on a filter paper below a heated air stream (30°C; 12 h). A batch of 351 g of AX was obtained. This batch was combined with a batch of rye flour AX (99 g) with nearly identical composition (76.5% pure AX, avDAS 0.49), to obtain the required amount of AX. After homogenization in water, the AX was dried as described above. Finally, the dry WE-AX, further referred to as WE-AX, was milled (Bosch MKM1000, Stuttgart, Germany) to a powder with a maximum particle size of 200 μm.

2.1.3 Preparation of AXOS

The AXOS preparation (avDP 5; avDAS 0.24) used in this study, Brana Vita™ 200, was obtained by xylanolytic hydrolysis of wheat bran, essentially as described by Swennen et al. [18] and was provided by Fugeia NV (Leuven, Belgium).

2.2 Methods

2.2.1 Characterization of the AX(OS) compounds

Moisture and ash contents were determined according to AACCI methods 44-19 and 08-01, respectively [19]. Protein contents were determined according to the Dumas combustion method, an adaptation of the AOAC official method for protein determination [20] and using 5.7 and 6.25 as the nitrogen protein conversion factor for flour and bran-derived AX(OS), respectively.

Total and reducing end sugar contents were determined by GC analysis as described earlier [18]. In short, the hydrolysis of AX preparations (10.0–20.0 mg) was performed in 2.0 M trifluoroacetic acid (TFA; 5.0 mL) at 110°C for 60 min. Sugars were subsequently reduced with $NaBH_4$ and acetylated to alditol acetates, which were separated on a Supelco SP-2380 column (30 m × 0.32 mm id, 0.2-μm film thickness; Supelco, Bellefonte, PA, USA) with helium as the carrier gas in a Agilent 6890 series chromatograph (Agilent, Wilmington, DE, USA) equipped with an autosampler, splitter injection port (split ratio 1:20) and a flame ionization detector. Separation was at 225°C with injection and detection set at 270°C. β-D-Allose was used as the internal standard, and calibration samples containing D-arabinose, D-xylose, D-mannose, D-galactose and D-glucose were included with each set of samples. For the analysis of reducing end sugar content, reduction was performed prior to hydrolysis and derivatization. All chemicals and reagents were of at least analytical grade.

The average degree of AX polymerization (avDP) and the average degree of arabinose substitution (avDAS) of AX(OS) were calculated using formulae 1 and 2, respectively. The total AX content of the preparations was calculated using formula 3.

$$\text{avDP} = (\%\ \text{arabinose} - 0.7 \times \%\ \text{galactose} + \%\ \text{xylose})/\%\ \text{reducing end xylose} \quad \text{(Formula 1)}$$

$$\text{DAS} = (\%\ \text{arabinose} - 0.7 \times \%\ \text{galactose})/\%\ \text{xylose} \quad \text{(Formula 2)}$$

$$\text{AX (dry basis)}\ (\%) = (\%\ \text{arabinose} - 0.7 \times \%\ \text{galactose}) \times 132/150 + [132 \times (\text{avDP} - 1) + 150]/[150 \times \text{avDP}] \times \%\ \text{xylose} \quad \text{(Formula 3)}$$

The formulae contain a correction for the presence of galactose in water extracts since wheat and rye contain extractable arabinogalactan peptides with an arabinose-to-galactose ratio of 0.7 [21]. The factors 132 and 150 in formula 3 reflect the molecular mass of anhydropentose sugars and pentose sugars, respectively. As the anhydroxylose and anhydroarabinose units in AX(OS) are hydrated upon hydrolysis, a correction for this molecular mass shift must be incorporated in the calculations.

Total, soluble and insoluble dietary fiber content of the WU-AX enriched preparation was analyzed with the total dietary fiber assay procedure (Megazyme, Bray, Ireland), which is based on AOAC method 991.43 [20].

2.2.2 Rat trial design

A completely randomized controlled rat trial was set up to evaluate the shifts in fermentation pattern (including average degree of AX fermentation (avDF) and fermentation metabolites) and microbial composition in the cecum and colon of rats following administration of AXOS, WU-AX, WE-AX and their combinations, at a total fixed AX level of 5%. Seventy 6-wk-old male rats (Wistar, Elevage Janvier, Saint-Berthein, France) were housed in plastic cages (two rats per cage) of which the bottoms were covered with sawdust. The cages were kept at 22°C with a 14/10 h light/dark cycle. During 6 days, rats consumed a well-balanced basal diet (Table 1) that was prepared and analyzed by Ssniff Spezialdiäten (Soest, Germany). The diet was designed and produced following the general feed standards for laboratory animals and met all nutritional requirements for rats. After the six initial days of adaptation, the rats were randomly assigned to one of seven different treatment groups (ten rats per group). All groups were given free access to the pellets of the basal formulation or of the basal formulation to which AXOS, WE-AX and WU-AX, as such or in combination, were added at a 5% AX level (Table 2). For the latter, the pregelatinized starch in the basal diet was replaced with the

Table 1. Composition of the control and experimental diets used in the rat experiment

Diet ingredients	Control	WU-AX	WE-AX	AXOS	WU-AX+AXOS	WE-AX+AXOS	WU-AX+WE-AX+AXOS
					g/kg diet		
Corn starch, pregel[a)]	745.4	628.8	683.7	684.2	656.5	683.6	665.5
Soybean protein isolate	140.0	140.0	140.0	140.0	140.0	140.0	140.0
Vitamin – mineral mix[b),c)]	52.0	52.0	52.0	52.0	52.0	52.0	52.0
Soybean oil	35.0	32.0	35.0	35.0	32.0	35.0	32.0
Wheat gluten	9.2	–	9.2	9.2	4.6	9.2	6.1
Dicalcium phosphate	7.0	7.0	7.0	7.0	7.0	7.0	7.0
L-lysine HCL	3.6	3.6	3.6	3.6	3.6	3.6	3.6
L-Threonine	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Choline chloride	2.0	2.0	2.0	2.0	2.0	2.0	2.0
DL-Methionine	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-Cysteine	1.2	1.2	1.2	1.2	1.2	1.2	1.2
L-Tryptophan	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Butylhydroxytoluol	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Chromium(III) oxide	0.2	0.2	0.2	0.2	0.2	0.2	0.2
AXOS	–	–	–	61.2	30.6	30.9	20.7
WU-AX	–	125.9	–	–	62.9	–	41.8
WE-AX	–	–	61.7	–	–	30.9	20.6

a) Pregelatinized corn starch.
b) Vitamin – mineral/trace element mix.
c) Supplied the following (to provide mg/kg diet, except as noted): all-*trans* retinol acetate, 4.5; cholecalciferol, 25 μg; all-*rac*-α-tocopheryl acetate, 100; menadion sodium bisulfate, 5; thiamin hydrochloride, 12; riboflavin, 20; pyridoxine hydrochloride, 15; cyanocobalamin, 100 μg, calcium DL-pantothenate, 30; nicotinic acid, 60; folic acid, 6; D-biotin, 300 μg, *myo*-inositol, 100; Fe, 100; Cu, 5; Zn, 50; Mn, 30; Co, 2; I, 2; Se, 0.10.

Table 2. Compositional and structural properties (A) and target dose (B) of different AX preparations used in the different diets

(A)	Compositional and structural properties of AX preparations							
Ingredient	Arabinose	Xylose	Glucose	Ash	Protein	AX(OS)	avDP AX(OS)	avDAS AX(OS)
	% dm							
WU-AX	17.9	28.39	0.9	9.8	6.8	40.2	284	0.59
WE-AX	29.5	63.3	14.8	<0.1	0.7	81.3	233	0.51
AXOS	14.8	74.9	15.8	0.1	0.3	79.1	5	0.24

(B)	Target dose of AX ingredient (%)		
Diet	WU-AX (pure)	WE-AX (pure)	AXOS (pure)
WU-AX	5.00	–	–
WE-AX	–	5.00	–
AXOS	–	–	5.00
WU-AX+AXOS	2.50	–	2.50
WE-AX+AXOS	–	2.50	2.50
WU-AX+WE-AX+AXOS	1.67	1.67	1.67

avDP, average degree of polymerization; avDAS, average degree of arabinose substitution

appropriate amount of AX(OS) preparations. Chromium (III) oxide was included (0.02%) in all diets as a non-digestible marker. Rats were weighed and feed intake was measured twice a week. During the test period, feces samples, found at the bottom of the cages, were collected. Unfortunately, at day 11 of the test period, one cage of the group consuming AXOS exhibited a much lower feed intake, caused by a wet ground cover in turn due to a leaking water bottle. Results obtained from this cage were excluded. After 14 days of treatment, rats were weighed and killed with CO_2 gas. The rats were dissected to collect the ileum, cecum and colon contents. Immediately after sampling, aliquots were stored at −30°C until further analysis.

The experimental protocol was approved by the Ethical Committee on Animal Experiments of the Katholieke Universiteit Leuven.

2.2.3 Metabolic activity analysis

Samples of cecum content, colon content and feces were dried in an oven (65°C; 72 h) prior to the determination of the degree of AX(OS) fermentation. The concentration of AX in the dried samples (approximately 10.0 mg) was determined by GC analysis as described above. Cr_2O_3 content in the samples (approximately 10.0 mg) was analyzed by the conversion of Cr_2O_3 to $Cr_2O_7^{2-}$ according to the method of Fisher et al. [22] and subsequently analyzing elemental Cr(VI) by inductively coupled plasma-atom emission spectroscopy (ICP-AES). Standard solutions, which contained, respectively, 0, 8, 15, 25 and 50 μg Cr(VI)/mL aqueous sulfuric acid (1 M) were prepared. The initial concentration of Cr_2O_3 in the sample was calculated from the measured concentration of elemental Cr(VI) in the solution. The average degree of fermentation of AX(OS) (avDF) was calculated with Formula 4

$$\text{Average degree of AX(OS) fermentation (avDF)} = 1 - ([Cr_2O_3]_{diet} \times [AX(OS)]_{sample})/([Cr_2O_3]_{sample} \times [AX(OS)]_{diet}) \quad \text{(Formula 4)}$$

with $[Cr_2O_3]_{diet}$ and $[Cr_2O_3]_{sample}$ the concentration (g/kg) of Cr_2O_3 in the diet and in the sample (cecum, colon, feces), respectively, and $[AX(OS)]_{diet}$ and $[AX(OS)]_{sample}$ the concentration (g/kg) of AX(OS) in the diet and in the sample (cecum, colon or feces), respectively.

The pH of fresh cecal content was measured with a pH meter (Hanna Instruments HI 9025, Woonsocket, USA).

Non-branched and branched SCFA in cecal and colonic samples were extracted with diethyl ether and analyzed with GC as described earlier [12]. SCFA levels were expressed as the total pool of the particular SCFA within the site (colon or cecum).

2.2.4 Microbiological analysis

Quantification of the levels of bacteria from the *Bifidobacterium*, *Lactobacillus* and *Roseburia/E. rectale* group and the total number of bacteria in cecum and colon was performed by quantitative polymerase chain reaction as described by Van Craeyveld et al. [12]. The primers 243f/243r [23], LactoF/LactoR [23] and RrecF/Rrec630mR [24] were added at 0.6 μM for the detection of bacteria from the *Bifidobacterium*, *Lactobacillus* and *Roseburia/E. rectale* group, respectively. The primer sets 338f [25] and 518r [26] (0.3 μM) were used for the detection of the total number of bacteria. Calibration was performed with standard series (triplicates) consisting of tenfold dilutions (1–10^6 CFU/well) of a positive control. Hereby, stock solutions were applied with a known amount of DNA of *Bifidobacterium animalis* (subspecies lactis) (LMG 18314), *Lactobacillus plantarum* (LMG 9211) or *Roseburia intestinalis* (DSM 14610) for the detection of bacteria from the *Bifidobacterium*, *Lactobacillus* and *Roseburia/E. rectale* group, respectively. Bacteria numbers were expressed as the total number of bacteria of a species within the site (colon or cecum).

2.3 Statistical analysis

Statistical evaluation of the results was performed with SAS software 8.1 (SAS Institute, Cary, NC, USA). The Kolmogorov–Smirnov test for normality pointed out that the response variables – except for feed intakes, body weights and intestinal contents – were not normally distributed. Therefore, a non-parametric ANOVA test was applied to analyze the results as described earlier [12].

Values in the text represent medians, except feed intakes, body weights and (moisture) contents in ileum, cecum and colon, which are expressed as means ± SD.

3 Results

3.1 Diets and structural characterization of bioactive compounds

Tables 1 and 2 list the different diets with their target dose of different AX along with the analyzed AX(OS) content and the results of avDP and avDAS determination for the different AX preparations. WE-AX and AXOS preparations contained approximately 80% AX and only a minor fraction of polymeric glucose. Since the preparation of WE-AX and AXOS included removal of starch and free glucose, the residual glucose measured in these ingredients was mainly derived from β-glucan. The WU-AX-enriched preparation comprised 40.2% AX. Total dietary fiber analysis showed that, apart from AX, this preparation additionally contained approximately 33.1% insoluble fiber and a minor fraction of soluble fiber (2.1%). The major part of the insoluble dietary fiber fraction consists of cellulose material.

3.2 Diets, feed intakes and body weights

During the adaptation wk, daily feed intake per cage (47.2 ± 4.1 g) did not differ among groups. In the first wk of the test period, a lower feed intake was observed in the WE-AX group (45.8 ± 3.8 g) compared with the control group (52.6 ± 1.4 g; $p = 0.02$). In the second week, the groups receiving the WE-AX and WE-AX+AXOS diet consumed less (46.8 ± 3.9 and 47.5 ± 1.9 g, respectively) than the control group (53.2 ± 0.8 g; $p = 0.01$).

Body weights of the rats at the start of the adaptation and the test period were 243.9 ± 7.6 and 298.2 ± 17.8 g, respectively. At the end of the first and second week of the test period, average body weights were 352.8 ± 20.4 and 397.8 ± 23.1 g, respectively. Body weights did not differ among the groups during the experiment.

3.3 Ileum, cecum and colon content

The different dietary treatments had no significant effect on the ileum contents (3.50 ± 0.19 g). Consumption of WE-AX and WE-AX+AXOS led to higher amounts of bulk material in the cecum (3.87 ± 1.53 and 5.02 ± 1.20 g, respectively) than consumption of the control diet (2.16 ± 0.61 g; $p = 0.04$ and 0.01, respectively).

A larger colon content was observed after the intake of the WU-AX and the WU-AX+WE-AX+AXOS diet (2.07 ± 0.53 and 2.02 ± 0.36 g, respectively) compared with the control diet (1.20 ± 0.34 g; $p = 0.03$).

The moisture contents of the cecal contents of the groups consuming WU-AX (79.0 ± 1.8%) and WU-AX+AXOS (79.2 ± 1.6%) was significantly lower compared with those of the animals receiving the control diet (83.1 ± 1.9%; $p < 0.05$). Colonic contents of the AXOS group had higher moisture contents (75.2 ± 5.7%) compared with the control group (67.5 ± 6.1%). Apart from these differences, moisture contents of cecal and colonic contents were not significantly different among the treatment groups ($p = 0.03$).

3.4 AX(OS) fermentation pattern and cecal pH

In the cecum, the highest avDF was observed for the AXOS and WE-AX+AXOS diets (95.8 and 96.3%, respectively), whereas it was somewhat lower for the WE-AX diet (84.0%) (Fig. 1A). The WU-AX fraction was the most resistant to breakdown, as only 27.2% of its AX was fermented in the cecum. Combining WU-AX with AXOS resulted in an increase in its avDF of AX(OS) to 69.9%, while 75.1% of the AX(OS) in the WU-AX+WE-AX+AXOS diet was fermented in the cecum (Fig. 1A).

In the colon, the AX(OS) in the WE-AX diet was further fermented resulting in similar avDF levels to these observed for the AXOS and WE-AX+AXOS diet (91.9, 96.4 and 96.8%, respectively) (Fig. 1B). Conversely, the majority of the WU-AX fraction withstood degradation in the colon (avDF of 42.7%). The degree of fermentation of WU-AX+AXOS and WU-AX+WE-AX+AXOS in the colon was 75.0 and 78.8%, respectively (Fig. 1B).

In the feces, the degrees of AX(OS) fermentation for the different AX preparations were similar to these in the colon (results not shown).

In general, clearly higher avDAS values were measured in the cecum and colon of the animals compared with t he avDAS measured in the diets (Table 3). The avDAS of

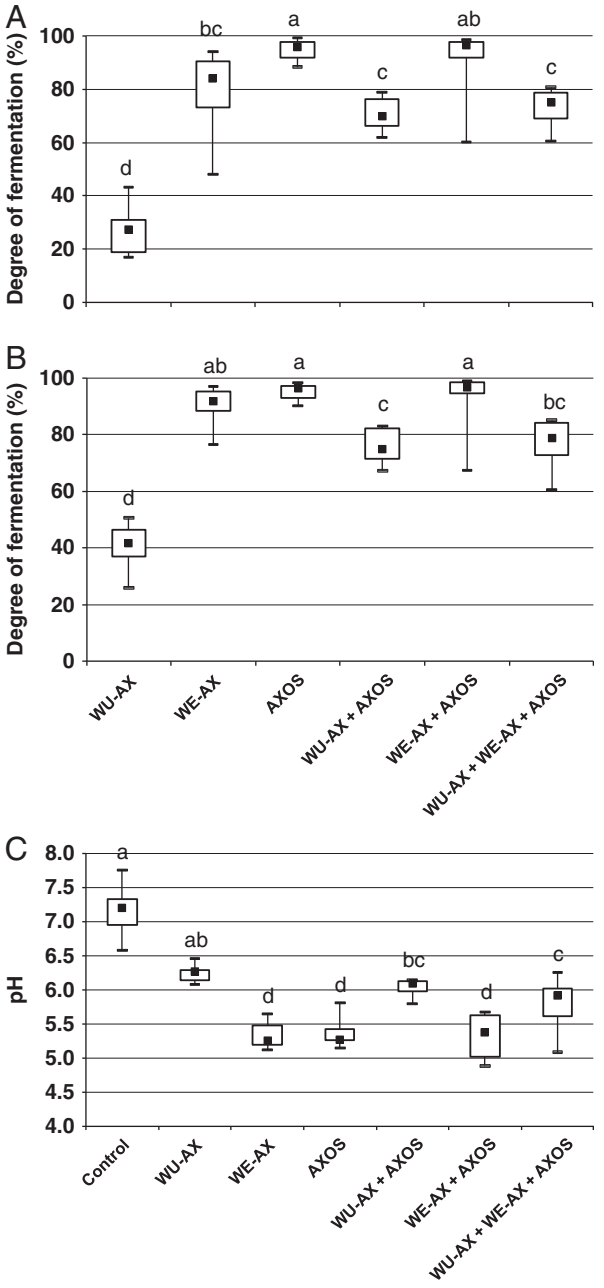

Figure 1. Degree of fermentation of AX in the cecum (A) and colon (B) and pH in the cecum (C) of rats fed the control diet or diets containing different types of AX. The box represents the 25th and 75th quartiles; the median is the square in the box; the whiskers are at the minimum and maximum values. Medians ($n = 10$) shown on the same graph, without a common letter differ significantly at $p < 0.05$. *Values for the AXOS group are based on a group of eight animals instead of ten ($n = 8$).

WU-AX containing feeds increased from approximately 0.50–0.70 in the diet to 0.80–0.90 in the intestinal tract, whereas the avDAS of AXOS and WE-AX increased from, respectively, 0.24 and 0.51 in the diets to approximately 0.54 and 0.64 in the hindgut (Table 3).

Table 3. Average degree of arabinose substitution (avDAS) of the AX analyzed in the diets containing different AX types and avDAS of AX retrieved in the cecum and colon of the rats fed these experimental diets

Ingredient	WU-AX	WE-AX	AXOS	WU-AX+AXOS	WE-AX+AXOS	WU-AX+WE-AX+AXOS
				avDAS		
Diet	0.65	0.51	0.27	0.43	0.40	0.50
Cecum	0.78	0.54	0.63[a)]	0.91	0.64	0.80
Colon	0.87	0.61	–	0.92	0.60	0.74

Data for cecum and colon represent medians ($n = 10$).
a) Values are based on a group of eight animals instead of ten ($n = 8$).

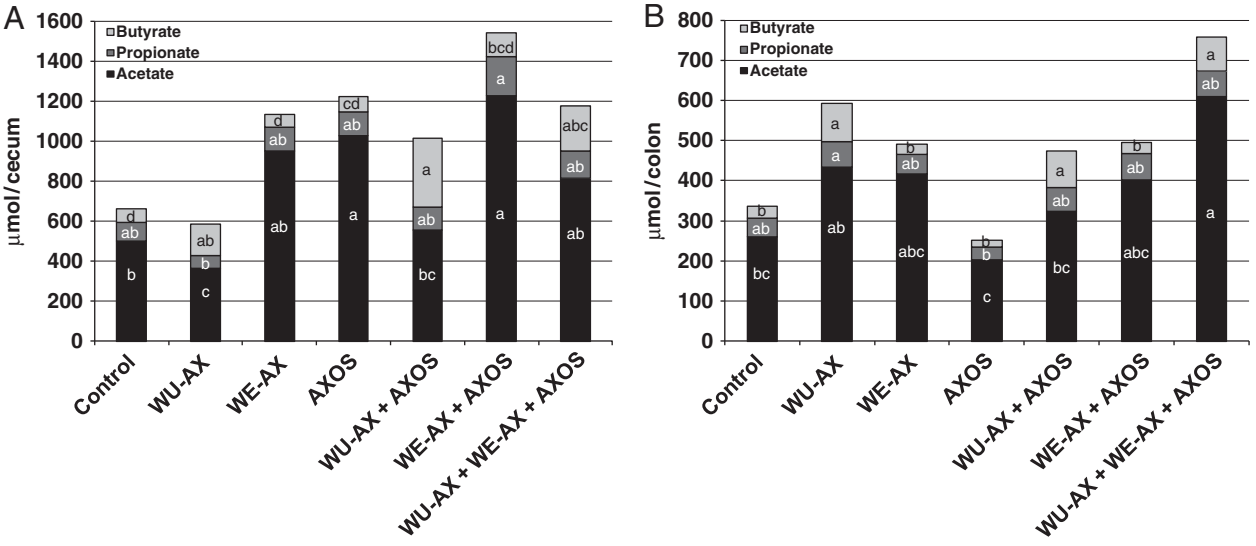

Figure 2. Total SCFA levels and absolute levels of acetate, propionate and butyrate in the cecum (μmol/cecum) (A) and colon (μmol/colon) (B) of rats fed the control diet or diets containing different types of AX. Bars and individual fractions represent the medians ($n = 10$) of the total amount and the relative proportions of the different SCFA, respectively. Fractions, denoting the same SCFA proportion, without a same letter differ significantly at $p < 0.05$. *Values for the AXOS group are based on a group of eight animals instead of ten ($n = 8$).

The average pH of the cecal content in the control group was 7.2 (Fig. 1C). In all AX(OS) consuming groups, except for the animals consuming WU-AX, significant lower pH values were measured in the cecum ($p < 0.01$). Consumption of the WE-AX, AXOS and WE-AX+AXOS diets resulted in the largest drop in pH while the preparations containing WU-AX had a lower impact on the cecal pH (Fig. 1C).

3.5 Fermentation products

3.5.1 Saccharolytic fermentation

Cecal contents of acetate were significantly higher in rats consuming AXOS or WE-AX+AXOS than in the control, WU-AX and WU-AX+AXOS diet groups (Fig. 2A; $p < 0.012$). In contrast, fermentation of the WU-AX diet resulted in significantly higher butyrate levels relative to the control group ($p = 0.003$), while fermentation of diets containing AXOS and WE-AX did not stimulate butyrate production (Fig. 2A). The highest cecal butyrate levels, however, were measured in rats fed with the diet containing WU-AX and AXOS. The consumption of the WU-AX containing diet resulted in lower levels of cecal propionate than the intake of WE-AX+AXOS ($p = 0.014$). Apart from this, there were no differences in cecal propionate levels among the treated groups and the control group (Fig. 2A).

Colonic levels of acetate in any of the treatment groups did not differ significantly from the control group, except for the group consuming WU-AX+WE-AX+AXOS ($p < 0.01$; Fig. 2B). The animals that consumed the WU-AX diet had higher butyrate levels in the colon than the control, WE-AX and AXOS groups (Fig. 2B; $p < 0.025$). Unlike in the cecum, the WU-AX, WU-AX+AXOS and WU-AX+WE-AX+AXOS groups all had about equally high colonic butyrate levels (Fig. 2B). The rats consuming the WU-AX diet had a higher colonic propionate content than the group consuming the AXOS diet ($p = 0.011$), whereas all other differences in colonic propionate levels were non-significant (Fig. 2B).

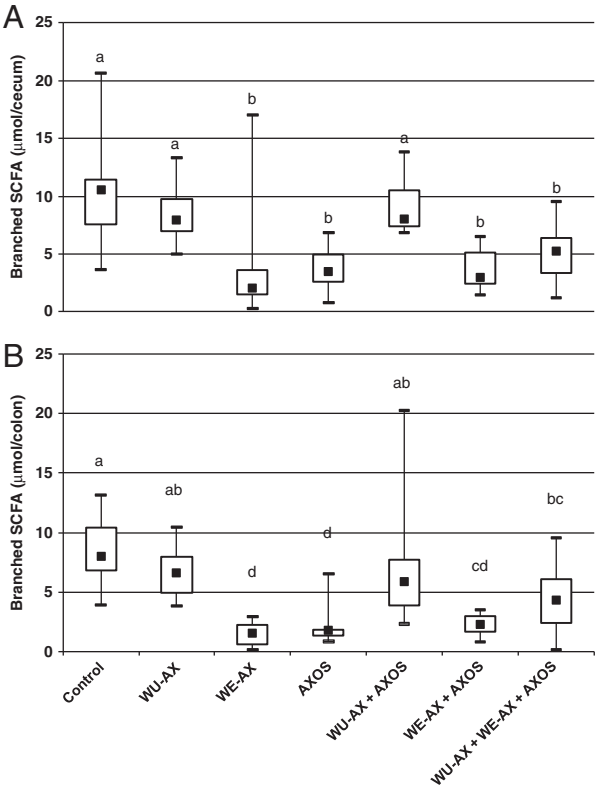

Figure 3. Branched SCFA levels (isovalerate+isobutyrate) in the cecum (A) and colon (B) of rats fed the control diet or diets containing different types of AX. Data are expressed per fresh cecum or colon. The box represents the 25th and 75th quartiles; the median is the square in the box; the whiskers are at the minimum and maximum values. Medians ($n = 10$) without a common letter differ significantly at $p < 0.05$. *Values for the AXOS group are based on a group of eight animals instead of ten ($n = 8$).

3.5.2 Proteolytic fermentation

Cecal and colonic branched SCFA levels (isobutyrate+isovalerate) in the WE-AX, AXOS, WE-AX+AXOS and WU-AX+WE-AX+AXOS groups were significantly lower than those in the control rats ($p < 0.03$; Fig. 3). The reduction was the strongest in rats fed with the WE-AX diet. The consumption of WU-AX and WU-AX+AXOS had no impact on the levels of branched SCFA in the cecum and colon.

A strong correlation ($r = 0.76$; $p < 0.05$) between the levels of isobutyrate and the cecal pH was observed (results not shown).

3.6 Microbiological analyses

Apart from the WU-AX diet, all AX(OS) containing diets stimulated the growth of bifidobacteria in the cecum compared with the control ($p < 0.004$; Fig. 4A). The increase was highest for the WE-AX, AXOS and WE-AX+AXOS diet groups. In the colon, the differences in the bifidobacteria content among the groups were smaller, but still higher bifidobacteria levels were measured in the WE-AX and WE-AX+AXOS diet groups than in the control, WU-AX and WU-AX+WE-AX+AXOS diet groups ($p < 0.015$; Fig. 4B).

Rats consuming the WU-AX+AXOS containing diet had higher lactobacilli contents in their cecum than the control rats ($p < 0.03$). The consumption of the AXOS diet on the contrary resulted in lower numbers of lactobacilli compared with the control group ($p = 0.034$; Fig. 4C). In the colon, lactobacilli levels did not differ significantly among the groups (results not shown).

Numbers of bacteria from the *Roseburia/E. rectale* group in the cecum of WU-AX, WU-AX+AXOS or WU-AX+WE-AX+AXOS treated rats were significantly higher than in the control rats ($p < 0.03$; Fig. 4D), whereas in the colon no differences were observed between the control and the treated groups (results not shown).

The consumption of WU-AX and WU-AX+AXOS increased the total number of bacteria in the cecum ($p < 0.025$; Fig. 4E). In the colon, the fermentation of the WU-AX+AXOS diet led to higher numbers of bacteria compared with the AXOS, WE-AX+AXOS and WU-AX+WE-AX+AXOS diets ($p < 0.03$; Fig. 4F).

Table 4 presents the proportions of bifidobacteria to total bacteria in the cecum and the colon of the different rat groups. The consumption of WE-AX, AXOS and WE-AX+AXOS selectively stimulated the growth of bifidobacteria in the cecum, in contrast to the intake of WU-AX or WU-AX+AXOS. The highest relative increase in bifidobacteria occurred in the AXOS-treated group. A similar situation was observed in the colon. In addition, in the colon, the intake of WU-AX+WE-AX+AXOS resulted in higher bifidobacteria levels without affecting the total number of bacteria. There were no differences in the proportions of lactobacilli or *Roseburia/E. rectale* species among the different groups (results not shown).

4 Discussion

Several studies suggested that the consumption of AX induces health-promoting effects [9, 27, 28]. This study shows that the physiological impact of AX consumption strongly depends on their structures and properties as different types of AX have a different impact on the microbial population and fermentation products in the intestinal tract of rats.

4.1 WU-AX

Despite the more limited breakdown of WU-AX, a marked shift was observed in the type of fermentation products

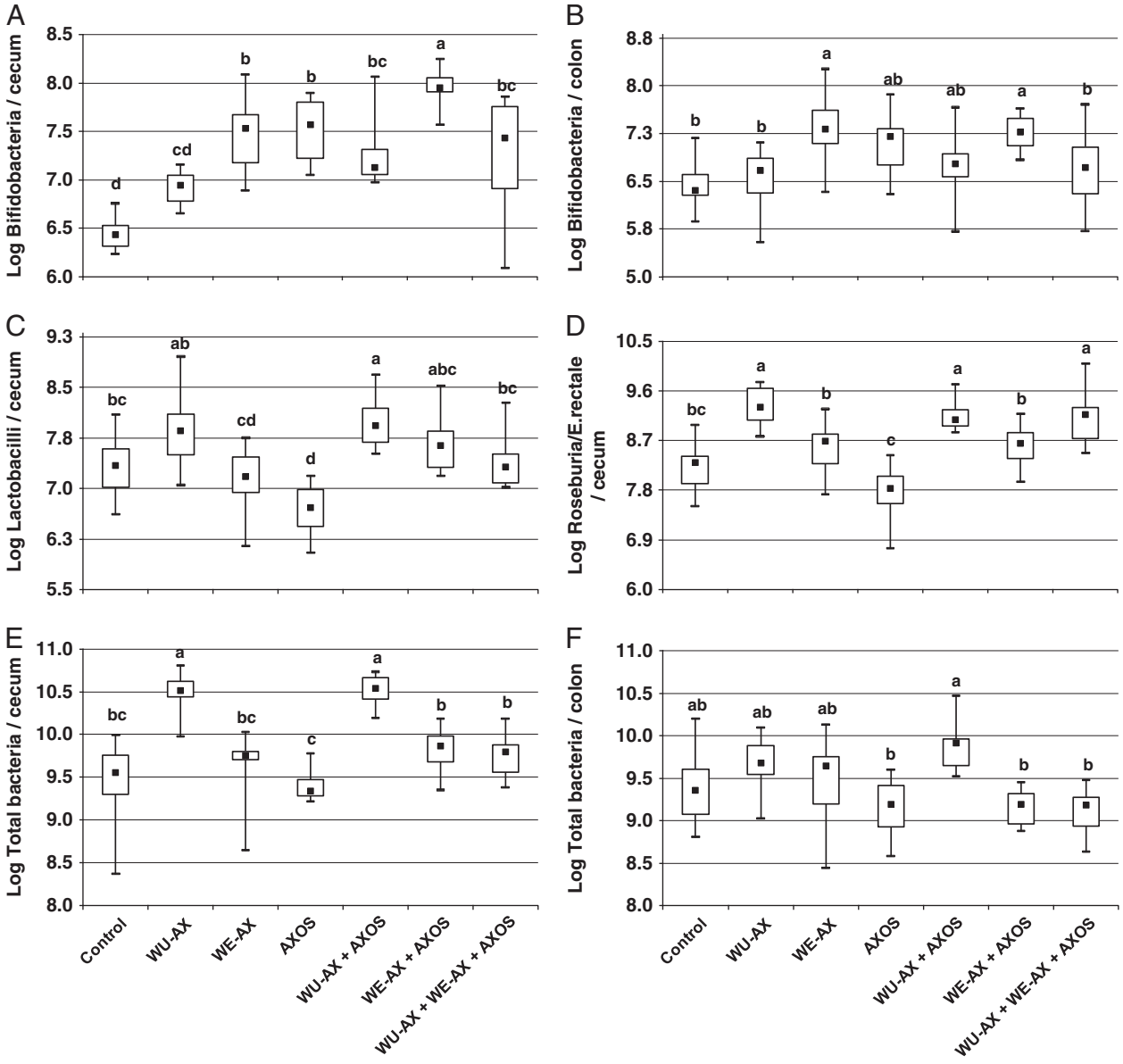

Figure 4. Levels of bifidobacteria in the cecum (A), bifidobacteria in the colon (B), lactobacilli in the cecum (C), *Roseburia/E. rectale* species in the cecum (D) and total bacteria levels in cecum (E) and colon (F) of rats fed the control diet or diets containing different AX types. Data are expressed per total cecum or colon. The box represents the 25th and 75th quartiles; the median is the square in the box; the whiskers are at the minimum and maximum values. Medians ($n = 10$) without a common letter differ significantly at $p < 0.05$. *Values for the AXOS group are based on a group of eight animals instead of ten ($n = 8$).

present in cecum and colon, with higher absolute and relative levels of butyrate after the consumption of the WU-AX enriched diet. At this point, we cannot exclude that the shift in fermentation pattern was partially due to the presence of lignin and cellulose in this preparation. These fibers are mainly derived from the pericarp layers in wheat bran and formed the main other types of indigestible carbohydrates in the WU-AX fraction. However, several studies indicated that lignin and cellulose do not considerably affect the metabolic or microbiological balance since they are virtually non-degradable in the intestinal tract [29–32]. Consistent with our observation for WU-AX, Carneiro et al. found that wheat bran stimulated the production of butyrate in the cecum of pigs [33]. Butyrate is considered an important SCFA with regard to health aspects as it helps to maintain the integrity of the intestinal barrier [34].

Intake of WU-AX induced an unselective microbial growth promoting response. Glitso et al. already indicated that the breakdown of pericarp AX requires a battery of

Table 4. Ratios of bifidobacteria/total bacteria (%) in the cecum and colon of rats fed the control diet or diets containing different AX types

Group	Bifidobacteria (%)	
	Cecum	Colon
Control	0.08 (cd)	0.11 (b)
WU-AX	0.03 (e)	0.12 (b)
WE-AX	0.66 (ab)	0.88 (a)
AXOS[a)]	1.86 (ab)	1.52 (a)
WU-AX+AXOS	0.05 (de)	0.07 (b)
WE-AX+AXOS	0.96 (a)	1.14 (a)
WU-AX+WE-AX+AXOS	0.45 (bc)	0.85 (a)

Medians ($n = 10$) are shown. Results within the same column without a common letter differ significantly at $p < 0.05$.

a) Values are based on a group of eight animals instead of ten ($n = 8$).

enzymes to hydrolyze the linkages within the AX structure as well as bonds between AX and other cell wall compounds [29]. Hence, it can be reasoned that with regard to WU-AX, a diverse population is encouraged to cooperate resulting in a cross-feeding mechanism thereby slowly breaking down the WU-AX substrate. Bifidobacteria, however, did not account for the elevated level of total bacteria as their number remained unaffected by the intake of WU-AX. Similarly, Vardakou et al. reported that unlike partially degraded WU-AX, the administration of intact WU-AX did not induce any bifidogenic effect [8]. As a possible explanation, Broekaert et al. suggested that bifidobacteria lack endoxylanases, enzymes required to degrade AX effectively, but are capable of uptake and breakdown of low molecular weight AXOS [34]. The higher levels of *Roseburia/E. rectale* species observed in the cecum of WU-AX consuming rats can at least be partially explained by the increase in total bacteria in the cecum of these rats. Duncan et al. reported that *Roseburia/E. rectale* spp. are one of the most important butyrate-producing bacteria in humans [35]. In accordance with this observation, the present study points to a clear coincidence between butyrate production and the occurrence of *Roseburia/E. rectale* species in rats. Higher levels of these butyrogenic bacteria occurred exclusively in the WU-AX consuming rats, which also showed increased levels of butyrate.

4.2 WE-AX

The consumption of WE-AX increased the mass of the cecal contents, but so less than that of the colon contents. It is known that the consumption of soluble fermentable fiber by rodents causes an increase in the mass of the cecum and cecum content [36]. Since WE-AX consumption did not entail an increase in the total bacteria counts of the cecum, the increase in the cecal content may be a physiological effect triggered by a trophic effect of SCFA on the cecum mucosa. In our experiments, the values for cecal content mass correlated well with cecal SCFA levels ($r = 0.84$, results not shown).

The breakdown of WE-AX went hand in hand with a significant drop of the pH (<5.5). This can be explained by the formation of SCFA, in particular acetate, which was by far the most dominant fermentation product of WE-AX. Lowering the pH is an effective manner to prevent the growth of several pathogens [37]. Cherrington et al. reported that a low pH due to high concentrations of propionate or formate inactivates *E. coli* and *Salmonella* in vitro [37]. In addition, fermentation of WE-AX appears to diminish protein breakdown as lower levels of isovalerate and isobutyrate were observed in the cecum and colon. These branched SCFA, originating from the catabolism of valine and leucine, are generally accepted as markers for the proteolytic fermentation [12]. An effective suppression of protein fermentation is considered to be relevant from a gut health perspective since some of the protein fermentation end products (e.g. phenolic compounds, ammonia) are potentially harmful [38]. Interestingly, the occurrence of branched SCFA was strongly related to the cecal pH as the lowest levels of isobutyrate and isovalerate were observed in samples characterized by a low pH. Acidification reduces the activity of proteolytic enzymes in the large bowel as microbial proteases present in the hindgut typically have an optimal activity near neutral pH [39].

Finally, the potential of WE-AX in improving gut health was emphasized by the selective increase in bifidobacteria levels.

4.3 AXOS

Degradation of AXOS yielded a fermentation pattern comparable to the one observed after intake of WE-AX. Higher production of SCFA, mainly acetate, was observed and correlated with a lower cecal pH (<5.5). However, AXOS was less resistant to breakdown than WE-AX, as the fermentation of the former was virtually completed in the cecum. Similarly, Kelly et al. reported that the fermentation of FOS primarily occurred in the proximal colon while inulin, having a higher molecular weight, was further degraded in the hindgut [17].

The lower avDAS of AXOS in comparison with WE-AX may also contribute to a higher fermentation rate. Indeed, higher branched AX fractions were typically recovered during passage through the intestinal tract, suggesting that AX with a lower avDAS is more susceptible to degradation. In agreement with these findings, Grootaert et al. demonstrated that the intestinal microbial population preferentially degrades AXOS with low avDAS in vitro [40]. Similar to WE-AX, AXOS suppressed the levels of branched SCFA and induced a selective bifidogenic effect. In agreement with this observation, several studies observed increased

concentrations of bifidobacteria upon administration of wheat bran-derived AXOS preparations [12–14]. Consequently, WE-AX and AXOS exhibited quite similar prebiotic properties in rats. This seems in direct contrast with the results from Van Craeyveld et al. showing that low molecular weight AXOS induced a bifidogenic effect while AXOS with a higher avDP did not [12]. It is important to remark, though, that unlike in the experiment of Van Craeyveld et al., in the present study no sources of undigestible carbohydrate fractions were included in the diets other than the AX test products. Hence, it can be hypothesized that microbiota in rats consuming such diets are able to degrade a larger part of the WE-AX population compared with diets with a higher and more diverse dietary fiber content.

In accordance with the present observations, it was suggested that also the health-promoting potential of inulin-type fructans, the key representatives of prebiotics, depends on their structural properties. In this context, Van de Wiele et al. suggested that inulin-type fructans with longer DP (3–60) exert more pronounced prebiotic effects in vitro than oligofructose with a DP ranging from 2 to 20. Consequently, it was suggested that inulin is a more interesting functional food ingredient than oligofructose based on its lower fermentation rate and higher prebiotic potency [41]. However, Ito et al. recently suggested that mainly inulin-type fructans with low avDP between 4 and 8 stimulated the number of lactobacilli as well as mucosal immune functions and IgA secretion to the highest extent [42].

4.4 WU-AX+AXOS

Interestingly, this study revealed that partial replacement of WU-AX with AXOS had a synergistic effect on cecal butyrate production as it increased the butyrate levels observed after the consumption of the WU-AX diet only with a factor of 2. The observed effect is a true synergistic one, as the doubled butyrate production is achieved with AXOS and WU-AX doses in the combined AXOS+WU-AX diet that are half those in the diets with only AXOS or only WU-AX, respectively. It can be reasoned that the reduction in the intestinal pH that arises after the intake of AXOS induces a strong butyrogenic response as butyrate-producing bacteria may outcompete pH-sensitive organisms in more acidic environments [43]. Moreover, Duncan et al. stated that in the human colon butyrate-producing bacteria require acetate for optimal growth [35]. They further demonstrated that when xylan was fermented in continuous cultures, the contribution of acetate to butyrate formation was 90% [35]. Hence, the fermentation of AXOS, which yields large amounts of acetate, might further stimulate the activity and/or growth of butyrate-producing bacteria in rats. In our case, microbiological analyses indicated that this synergistic effect on butyrate production was not associated with higher numbers of *Roseburia/E. rectale* species. Consequently, the partial replacement of WU-AX by AXOS may boost the metabolic activity of *Roseburia/E. rectale* bacteria or promote the growth or activity of other organisms involved in the butyrate production.

4.5 WE-AX+AXOS

Similar to the combination WU-AX+AXOS, simultaneous administration of WE-AX and AXOS may boost the positive effects of the individual AX types. Although WE-AX was only slightly more resistant in the hindgut of rats than AXOS, it can be hypothesized from this and other experiments that its presence may prolong the health-promoting effects of AXOS to a more distal portion of the colon [12, 17, 29]. In agreement with the observations for the AXOS and the WE-AX consuming rats, WE-AX+AXOS reduced the formation of branched SCFA significantly. In the cecum, the WE-AX and AXOS combination tended to increase the cecum content mass and SCFA the most. In addition, the strongest stimulation in bifidobacteria growth in the cecum was observed after the intake of WE-AX+AXOS. Moreover, the increase in bifidobacteria counts was significantly synergistic versus the increase observed for the groups treated with AXOS and WE-AX alone.

4.6 WU-AX+WE-AX+AXOS

Finally, simultaneous addition of WU-AX, WE-AX and AXOS led to an unique combination of effects neither observed in the individual AX preparations nor in the other combinations analyzed. It is the only treatment that caused a concomitant increase in the levels of bifidobacteria and *Roseburia/E. rectale* species in the cecum without increasing total bacteria. In the ceco-colon, it uniquely resulted in significant higher SCFA and butyrate levels, while suppressing branched SCFA levels. In the colon, the WU-AX, WE-AX and AXOS combination achieved the highest SCFA production, suggesting that it has the farthest reaching effect in the gastrointestinal tract of all AX preparations tested.

As a final remark, it is interesting to reflect on the fact that cereal bran, a rich source of WU-AX, is used in different food products for human consumption, like bread, pasta, cookies and ready-to-eat cereals, in view of improving fecal bulk and gastrointestinal transit. The drawback of adding bran in those products is that, it unfavorably disrupts the textural properties and palatability of the food product. The present study indicates that the WU-AX, WE-AX and AXOS combination results in nearly equal colon content mass (2.02 g per colon), a proxy for fecal mass, as WU-AX alone (2.07 g per colon). It is noteworthy that the content of WU-AX in the triple combination diet was only one third of that in the diet containing WU-AX alone. Likewise, similar if not higher increases in cecal and colonic butyrate production were observed for the triple combination as well as for

the WU-AX+AXOS double combination relative to the single WU-AX treatment, despite a reduction by 66 or 50% of the WU-AX doses in the triple and double combination diets, respectively. When translated to a human diet context, this would imply that the addition of AXOS to a bran-containing food product, naturally containing WU-AX, would allow to lower bran levels in the product while maintaining or even raising its health-related physiological benefits, thereby improving the sensorial properties of the food product.

In this view, it is important to note that a partial shift from WU-AX to higher levels of AXOS can also be obtained by enzymatic modification of the native AX population during processing of cereal-based foods [44].

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The authors have declared no conflict of interest.

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