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The Effect of Dietary Fiber from Wheat Processing Streams on the Formation of Carboxylic Acids and Microbiota in the Hindgut of Rats

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ABSTRACT: Colonic fermentation of dietary fiber produces carboxylic acids and may stimulate the growth of beneficial bacteria. This study investigated how byproducts of wheat processing (distillers' grains and two fractions from the wet fractionation to starch and gluten, one of which was treated with xylanase) affect the composition of the cecal microbiota and the formation of carboxylic acids in rats. Differences were mostly found between diets based on supernatants and pellets, rather than between fiber sources. Cecal pools and levels of most carboxylic acids in portal blood were higher for rats fed the supernatant diets, while cecal pH and ratios of acetic to propionic acid in portal blood were lower. The diet based on supernatant from distillers' grains gave the highest level of bifidobacteria. Molecular weight and solubility are easier to modify with technological processes, which provides an opportunity to optimize these properties in the development of health products.

KEYWORDS: fructan, arabinoxylan, wheat, distillers' grains, short-chain fatty acids, carboxylic acids, lactobacilli, bifidobacteria

INTRODUCTION

Colonic fermentation of dietary fiber and other indigestible carbohydrates produces short-chain fatty acids (SCFA) and other carboxylic acids (CA) as the main end products, some of which are thought to have health-promoting effects. While acetic acid, the major acid formed, is the primary substrate for hepatic cholesterol synthesis, propionic acid may inhibit the production of cholesterol from acetic acid.[1] Butyric acid is mainly used as an energy source by the epithelial cells, but it may also offer protection against colon cancer, have an anti-inflammatory effect, and strengthen the colonic defense barrier.[2] Less is known about the role of the other CA formed. However, they contribute to a decrease in pH, which may have beneficial effects such as reducing the growth of pathogenic bacteria and increasing mineral absorption.[1] In addition, dietary fiber may have a prebiotic effect through selectively stimulating bacteria that are considered beneficial, such as bifidobacteria and lactobacilli.[3]

One source of dietary fiber may be byproducts of industrial wheat processing. Distillers' grains (DG) and a process stream from the wet fractionation of wheat have been shown to contain significant amounts of dietary fiber, including fructan and arabinoxylan (AX).[4] Dietary fiber in DG also includes (1→3) and (1→6) β-glucans and mannan from yeast cells.[5]

Inulin-type fructans, consisting of linear chains with (1→2)-linked β-fructose units, from chicory root have been found to give different patterns of colonic SCFA depending on molecular weight.[6] A low degree of polymerization (DP = 2−8) gave large amounts of butyric acid, while a high DP (10−60) gave particularly high levels of propionic acid. Furthermore, bifidobacteria specifically use short-chain inulin as a substrate, but cannot metabolize highly polymerized inulin.[7] Little is known about the physiological effects of wheat fructans, which are graminan-type fructans with both (1→2) and (6→2) linkages with a degree of polymerization (DP) of up to 19.[8] However, *in vitro* studies have shown that bifidobacteria are able to metabolize fructan from wheat.[9] Recently, several studies have focused on the potentially beneficial health effects of AX and arabinoxylan oligosaccharides (AXOS). Certain species of bifidobacteria and lactobacilli have been found to grow on AX and AXOS *in vitro*, showing a higher selectivity for AX with a lower molecular mass.[10] *In vitro* studies suggest that the fermentability is lower for AXOS with a higher degree of substitution.[11,12]

The aim of this study was to investigate the possibility of changing the formation of carboxylic acids and the cecal composition of the microbiota in the hindgut by feeding healthy rats wheat processing fractions containing dietary fiber with different compositions and physicochemical properties. For this purpose, distillers' grains and waste products from the wet fractionation of wheat were used. All products were centrifuged in order to separate the fiber into fractions with different molecular weights.

MATERIALS AND METHODS

Dietary Fiber Fractions. Wheat flour (*Triticum aestivum*, cultivar Harnesk, extraction rate 79%) was fractionated with and without endoxylanase (Shearzyme, Novozymes A/S, Bagsvaerd, Denmark) in a starch and gluten wet fractionation plant run by Lantmännen Reppe (Lidköping, Sweden). Sample material was taken from a byproduct fraction separated in a tricanter (fractions C and CX; X denoting a xylanase-treated C fraction). DG produced from wheat flour (organically grown Harnesk, extraction rate 79%) were also obtained from Lantmännen Reppe. The material was frozen after sampling and

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Table 1. Composition of the Test Diets

component	amount (g/kg dry wt)
dietary fiber fraction[a]	139−436
casein	120
DL-methionine	1.2
maize oil	50
mineral mixture[b]	48
vitamin mixture[c]	8
choline chloride	2
sucrose	100
wheat starch[a]	234−532

[a] The dry matter content was adjusted with wheat starch to obtain a concentration of 80 g of dietary fiber/kg of diet (dwb). [b] Containing (g/kg) 0.37 $CuSO_4 \cdot 5H_2O$, 1.4 $ZnSO_4 \cdot 7H_2O$, 332.1 KH_2PO_4, 171.8 $NaH_2PO_4 \cdot 2H_2O$, 324.4 $CaCO_3$, 0.068 KI, 57.2 $MgSO_4$, 7.7 $FeSO_4 \cdot 7H_2O$, 3.4 $MnSO_4 \cdot H_2O$, 0.020 $CoCl \cdot 6H_2O$, 101.7 NaCl. [c] Containing (g/kg) 0.62 menadion, 2.5 thiamine hydrochloride, 2.5 riboflavin, 1.25 pyridoxine hydrochloride, 6.25 calcium pantothenate, 6.25 nicotinic acid, 0.25 folic acid, 12.5 inositol, 1.25 *p*-aminobenzoic acid, 0.05 biotin, 0.00375 cyanocobalamin, 0.187 retinol palmitate, 0.00613 calciferol, 25 D-α-tocopheryl acetate, 941.25 maize starch.

thawed before centrifugation (1000*g*, 20 min). Pellets and supernatants were freeze-dried separately, and the six resulting fiber fractions were ground to a powder in an ultracentrifugal mill with a 0.5 mm ring sieve (Retsch GmbH, Haan, Germany). The freeze-dried supernatant fraction of DG (DG-S) did not become completely dry because of the high level of low-molecular-weight carbohydrates and was therefore extracted twice with 90% ethanol. The ethanol was added to the freeze-dried material in the morning. The mixture was stirred several times during the day and was left to stand until the next morning. The ethanol was discarded and the residue washed twice with more ethanol and then centrifuged. The pellet was air-dried, redissolved in water, and freeze-dried a second time. Before milling, DG-S was lightly mortared and mixed with starch (136 g/kg dry weight basis (dwb) of the final weight) to avoid caking.

Preparation of Diets. One test diet was prepared for each dietary fiber fraction, in total six diets. Each diet contained casein (Sigma Chemical Company, St Louis, MO), sucrose (Danisco Sugar, Malmö, Sweden), maize oil, DL-methionine (Sigma Chemical Company), choline chloride (Aldrich Chemie, Steinheim, Germany), a vitamin mixture, and a mineral mixture (both from Apoteket, Malmö, Sweden). The fiber fractions were added at a level of 80 g of dietary fiber/kg of diet (dwb). Wheat starch (Cerestar Deutschland GmbH, Krefeld, Germany) was used to adjust the dry matter content (see Table 1). This type of starch has been shown to be completely digested and therefore does not contribute to any SCFA in the hindgut of rats.

Experimental Design. Forty-two male Wistar rats (Scanbur, Sollentuna, Sweden), about 4 weeks old, with an average weight of 86 g (standard deviation 4.4 g), were divided into six groups of seven animals. Each group was assigned one of the diets, and the rats were fed 12 g of dry matter/day and had unlimited access to water. The rats were allowed to adapt to the diet for 7 days before the experimental period of 5 days. Weight gain and food consumption during the experimental period were measured. After the experimental period the animals were anesthetized by subcutaneous injection of a mixture of Hypnorm (Division of Janssen-Cilag Ltd., Janssen Pharmaceutica, Beerse, Belgium), Dormicum (F. Hoffman-La Roche AG, Basel, Switzerland) and sterile water (1:1:2) at a dose of 1.5 mL/kg body weight. Blood was taken from the hepatic portal vein for SCFA analysis, placed in EDTA tubes, centrifuged, and transferred to vials for storage at −40 °C. The cecum was removed, and a small portion of the content was transferred to sterile test tubes containing freezing medium, placed in liquid nitrogen, and stored at −80 °C for later analysis of bacterial counts. Cecal pH, tissue weight, and content weight were determined. The remaining cecal content, proximal colon, and distal colon were stored at −40 °C for later analysis of CA. The Malmö/Lund Ethical Committee on Animal Experiments approved the animal experiments, reference numbers M40-06 and M96-07.

Analyses of Fiber Fractions. Total carbohydrates were determined by gas−liquid chromatography for neutral sugars, spectrophotometry for uronic acids, and gravimetrical determination of Klason lignin[13] with some modifications to the method. Initial starch degradation with Termamyl and amyloglucosidase was omitted. Samples were extracted with 20 mL of water (40 °C, 15 min) and centrifuged at 1000*g* and the supernatants removed to prevent low-molecular-weight material from being degraded by the 12 M sulfuric acid used in the following incubation stage. The pellets were freeze-dried prior to incubation with sulfuric acid, which was carried out according to the original method. The pellets were then combined with their respective supernatants before hydrolysis, from which point the original method was followed. The fructan content was determined with the enzymatic/spectrophotometric AOAC method 999.03,[14] as described previously.[8] Starch, including maltooligosaccharides and glucose, was analyzed using the method described by Holm et al.[15] Glucose, fructose, sucrose, and arabinose contents were analyzed with HPLC by AnalyCen (Lidköping, Sweden). Digestible carbohydrates were defined as the sum of starch (including maltooligosaccharides and glucose), fructose, and sucrose. Indigestible carbohydrates were defined as the sum of fructan, neutral sugar residues, uronic acid residues, and Klason lignin, and corrected for starch (including glucose) and the glucose part of fructans, approximating the average DP to 6.[8] Arabinoxylan and arabinogalactan (AG) contents were calculated from arabinose, xylose, and galactose residues, as suggested by Delcour et al.,[16] assuming arabinose to be included in AG with an Ara/Gal ratio of 0.7. The contents of protein (Kjeldahl, N × 6.25 for DG and N × 5.70 for the C fractions), crude fat (Schmid−Bondzynski−Ratzlaff[17]), and ash were analyzed by AnalyCen (Lidköping, Sweden).

Analysis of Carboxylic Acids. The amounts of CA in the cecum and colon were determined by gas−liquid chromatography (HP 6890, Hewlett-Packard, Wilmington, DE) using an HP-5 column (Hewlett-Packard).[18] The SCFA in portal blood were analyzed with the same equipment using a DB-FFAP 125−3237 column (J&W Scientific, Agilent Technologies Inc., Folsom, CA).[19] An internal standard (2-ethylbutyric acid) was added to the sample at the start of the analysis. Prior to analysis, the serum was separated from the plasma by adding one drop of a solution containing calcium chloride (1%, w/w) and bovine thrombin (10 000 units per 6 mL water) to 1 mL of plasma. The fibrinogen coagel was removed and the tube centrifuged for 10 min at 1000*g* with an Eppendorf Minispin centrifuge (Eppendorf AG, Hamburg, Germany).

Microbial Analysis. The cecal microbiota was studied by determining the viable counts of lactobacilli and bifidobacteria. Lactobacilli and bifidobacteria were incubated anaerobically at 37 °C for 72 h on Rogosa agar and modified Wilkins−Chalgren agar,[20] respectively (both obtained from Oxoid Ltd., Hampshire, U.K.). Selected colonies of bifidobacteria were studied by microscopy for verification.

Calculations and Statistical Analysis. Cecal pools of CA were calculated as the concentration of each acid multiplied by the weight of the cecal content. The results were extrapolated to complete intake of the food given. Viable counts of lactobacilli and bifidobacteria were expressed as log CFU/g cecal content. Isobutyric, isovaleric, valeric, heptanoic, and succinic acids were present at levels close to the detection limit of about 0.05 μmol/g. The number of rats in the group in which the acid was not detected is given in the tables. When the acid was not detected, a concentration of 0 μmol/g was used to calculate mean values.

Two-way ANOVA with Tukey's test was used to evaluate the effect of fiber source (C, CX, and DG) and solubility (supernatant and pellet) on

Table 2. Composition of Each of the Fiber Fractions Studied, Including the Dietary Fiber Content (g/kg dwb) and the Proportion of Each Carbohydrate (%)[a]

	C		CX		DG	
	P	S	P	S	P	S
dietary fiber	200	289	183	332	458	576
fructan %	5.0	20.7	5.1	20.4	0.7	1.7
arabinose %	15.7	17.3	13.7	18.5	20.6	34.1[b]
xylose %	23.6	23.2	18.5	25.6	14.3	16.4
mannose %	2.8	4.3	3.5	5.0	8.6	10.3
galactose %	3.9	7.5	4.8	7.1	2.2	3.9
glucose %	38.2	21.8	32.7	19.3	46.9	30.0
Klason lignin %	7.0	1.2	17.6	0.3	4.4	0.1
uronic acids %	3.8	4.0	4.2	3.8	2.2	3.5
AX %[c]	36.6	34.0	28.7	38.2		
AG %[c]	6.6	12.8	8.2	12.0	3.8	6.6
Ara/Xyl[c]	0.55	0.47	0.56	0.49		
digestible carbohydrates	668	469	683	441	63	124
starch %[d]	97.0	80.9	98.1	83.2	72.1	65.7
glucose %	2.1	12.5	1.4	10.6	11.9	15.1
fructose %	1.0	6.5	0.6	5.9	13.2	15.1
sucrose %	nd	0.2	nd	0.3	2.7	4.1
crude fat	33	15	52	12	88	29
protein	104	173	95	148	383	234
ash	12	45	12	47	26	61
total	1016	990	1026	980	1019	1025

[a] C, untreated C fraction; CX, C fraction partly degraded by xylanase; DG, distillers' grains; P, pellet; S, supernatant; nd, not detected. [b] Of which 2 percentage units were monomeric arabinose. [c] AX = % Ara − 0.7 × % Gal + % Xyl, AG = (1.7 × % Gal), Ara/Xyl = (% Ara − 0.7 × % Gal)/% Xyl,[16] excluding monomeric arabinose. Not calculated for DG because of an unknown source of arabinose. [d] Including maltooligosaccharides and maltose.

feed intake, weight gain, cecal content, cecal tissue weight, cecal pH, CA, lactobacilli, and bifidobacteria. Significances ($P < 0.05$) were only considered possible to draw conclusions from with $P_{source \times sol} > 0.1$ (significance level for interaction). When $P_{source \times sol} < 0.1$, the same parameters were evaluated with one-way ANOVA with Tukey's test ($P < 0.05$). Before statistical evaluation, levels of acetic, propionic, and butyric acid (μmol/g) and the sum of these acids were transformed with the Box−Cox transformation when not normally distributed according to the Anderson−Darling normality test. Other data not normally distributed were not transformed because it made little difference to the *P*-values. Using the value 0 for CA present at amounts below the detection limit made transformation with the Box−Cox method impossible. Differences between levels of SCFA in the cecum and distal colon were analyzed with the paired *t* test ($P < 0.05$). All statistical tests were performed with Minitab statistical software (Release 16).

RESULTS

Composition of Wheat Fractions. The content of dietary fiber in the six fractions ranged from 183 to 576 g/kg dwb (Table 2). The supernatants from the C and CX fractions contained high amounts of fructan (CX-S, 68 g/kg, and C-S, 60 g/kg dwb), which corresponded to about 20% of the dietary fiber in these two diets. However, the presence of fructose in these fractions (26 g/kg and 30 g/kg dwb, in CX-S and C-S, respectively) indicates that some degradation of fructans had taken place during processing.

The xylanase treatment of the C fraction decreased the molecular weight of the AX, as evidenced by the higher content in the supernatant and lower content in the pellet from the xylanase-treated batch. Furthermore, the viscous layer formed when centrifuging the untreated C fraction was not seen in the CX fraction. No difference was seen in the Ara/Xyl ratio as a result of the enzymatic treatment.

The sum of all the analyzed components was close to 1000 g/kg in all fractions, but with values slightly above 1000 g/kg for the pellet fractions and DG-S. A large amount of arabinose (196 g/kg dwb, 11 g/kg of which could be attributed to free arabinose) was found in the DG-S fraction, which has previously been shown to contain an unknown source of highly soluble arabinose.[4] DG-S and DG-P both contained more arabinose than xylose, in contrast to the C fractions. The proportions of mannose and glucose were higher in the DG fractions than in the C fractions.

Feed Intake, Weight Gain, Cecal Content, Cecal Tissue Weight and Cecal pH. Feed intake ranged from 10.1 to 11.8 g/day, with a lower intake by rats fed the C-S and CX-S fractions than the groups fed the pellet diets (Table 3). Rats fed C-S showed a lower weight gain, calculated per gram of feed, than those fed C-P, DG-P or DG-S. The cecal content of the rats fed the supernatant diets was higher than those fed the pellet diets, although the difference between C-P and C-S was not significant. The DG-S diet led to a higher cecal content than all other diets. Lower cecal pH and higher cecal tissue weight were found in the supernatant groups. Total cecal pools were negatively correlated to cecal pH ($P = 0.007$, $R^2 = 20\%$; and $P < 0.001$, $R^2 = 35\%$ when excluding one rat from the C-P group with high pH and high cecal pool).

Carboxylic Acids in the Cecum and Colon. Cecal pools of CA (Table 4) were generally higher in rats fed the supernatant diets than in those fed the pellet diets, except for valeric, caproic, and heptanoic acids, where the cecal pools were lower in rats fed the supernatant diets. No differences were found in the pools between fiber sources.

The general trend for both main and minor SCFA was toward lower levels in groups fed the supernatant diets than in those fed the pellet diets, throughout the gut (Table 5, data not shown for minor SCFA). The only significant differences in levels of individual CA between fiber sources were found between DG and CX, and between DG and C, i.e. no significant differences were detected between the C and CX diets. Regarding levels of the main SCFA, acetic, propionic, and butyric acid, differences between the DG-P and DG-S diets were larger than between the other pellet and supernatant fractions, often due to very low concentrations with the DG-S diet. This was reflected in low *P*-values for factor interaction, making it difficult to draw conclusions about the main effects of fiber source and solubility in many cases. The same trend was seen for the minor SCFA, but with more pronounced statistical evidence for the differences (less interaction between factors). Succinic acid was an exception, showing a higher level in the cecum of rats fed C and CX supernatant diets than in those fed the corresponding pellet diets ($P_{sol} = 0.008$, $P_{source \times sol} = 0.156$ excluding DG diets).

The proportions of acetic (69−71%), propionic (20−21%), and butyric (9−10%) acids in the cecum were very similar among the C fractions (data not shown). The DG-S diet led to a significantly higher proportion of propionic acid in the cecum than DG-P (24 vs 18%), the proportions of acetic acid being 67 vs

Table 3. Feed Intake, Weight Gain, Cecal Content, Cecal Tissue Weight and Cecal pH in Rats Fed Dietary Fiber from Various Wheat Processing Fractions[a]

	C		CX		DG		P-value		
	P	S	P	S	P	S	source	solubility	source × sol
feed intake (g/day)	11.5 ± 0.4 a	10.1 ± 0.2 c	11.8 ± 0.1 a	10.3 ± 0.3 bc	11.6 ± 0.2 a	11.4 ± 0.2 ab	0.034	<0.001	0.028*
wt gain (g/g of feed)	0.23 ± 0.01 a	0.10 ± 0.04 b	0.19 ± 0.01 ab	0.18 ± 0.03 ab	0.24 ± 0.02 a	0.24 ± 0.03 a	0.017	0.027	0.019*
cecal content (g)	2.0 ± 0.2 cd	3.4 ± 0.4 bc	1.8 ± 0.2 d	4.0 ± 0.7 b	1.7 ± 0.1 d	7.2 ± 0.4 a	<0.001	<0.001	<0.001*
cecal tissue (g)	0.58 ± 0.04 b	0.89 ± 0.04 a	0.51 ± 0.03 a	0.91 ± 0.07 b	0.55 ± 0.02 a	0.90 ± 0.05 b	0.875	<0.001*	0.614
cecal pH	7.2 ± 0.2 a	6.8 ± 0.1 ab	7.2 ± 0.1 a	6.8 ± 0.1 ab	7.3 ± 0.3 a	6.5 ± 0.1 b	0.793	<0.001*	0.454

[a] All feed weights are given on a dry weight basis. Results are given as means ± SEM, $n = 4-7$. C, untreated C fraction; CX, C fraction partly degraded by xylanase; DG, distillers' grains; P, pellet; S, supernatant. Asterisks indicate significant effects of fiber source (C, CX or DG) or solubility (S or P) alone, or interactions between them (source × sol). Means that do not share a letter are significantly different (one-way ANOVA, $P < 0.05$, only performed when the interaction was significant). Significance levels for source and solubility $P < 0.05$, and for interaction $P < 0.1$.

Table 4. Cecal Pools (μmol) of Carboxylic Acids in Rats Fed Dietary Fiber from Various Wheat Processing Fractions[a]

	C		CX		DG		P-value		
	P	S	P	S	P	S	source	solubility	source × sol
formic	4.8 ± 0.6 b	5.5 ± 0.7 b	3.8 ± 0.8 b	7.7 ± 1.9 ab	3.7 ± 0.3 b	11 ± 0.9 a	0.082	<0.001	0.007*
acetic	91 ± 26	108 ± 6.5	76 ± 16	139 ± 22	64 ± 8.9	113 ± 12	0.526	0.003*	0.369
propionic	29 ± 8.4	33 ± 2.5	21 ± 4.1	45 ± 8.5	16 ± 2.2	42 ± 6.9	0.818	0.001*	0.137
isobutyric	2.4 ± 0.5	3.0 ± 0.4	1.8 ± 0.3	3.4 ± 0.7	1.9 ± 0.2	2.9 ± 0.3	0.834	0.004*	0.503
butyric	14 ± 5.4	15 ± 0.8	10 ± 1.9	21 ± 4.9	10 ± 2.5	16 ± 1.8	0.754	0.040*	0.259
isovaleric	1.3 ± 0.2	2.1 ± 0.3	1.1 ± 0.2	2.5 ± 0.5	1.1 ± 0.1	2.5 ± 0.3	0.901	<0.001*	0.453
valeric[b]	2.0 ± 0.7	1.5 ± 0.6	1.8 ± 0.4	0.6 ± 0.1	1.9 ± 0.2	0.1 ± 0.1 (5)	0.200	0.002*	0.393
caproic	0.4 ± 0.1	0.3 ± 0.0	0.9 ± 0.3	0.3 ± 0.0	1.1 ± 0.3	0.3 ± 0.0	0.126	0.002*	0.224
heptanoic[b]	0.1 ± 0.1 (5)	0.0 ± 0.0 (7)	0.2 ± 0.1 (2)	0.0 ± 0.0 (6)	0.2 ± 0.1 (2)	0.0 ± 0.0 (7)	0.343	<0.001*	0.552
succinic	5.7 ± 1.7	13 ± 2.9	3.5 ± 0.4	18 ± 3.8	2.8 ± 0.9	11 ± 1.3	0.149	<0.001*	0.165
total	151 ± 43	180 ± 12	119 ± 23	237 ± 40	102 ± 14	199 ± 22	0.777	<0.001*	0.484

[a] Results are given as means ± SEM, $n = 7$. C, untreated C fraction; CX, C fraction partly degraded by xylanase; DG, distillers' grains; P, pellet; S, supernatant. Asterisks indicate significant effects of fiber source (C, CX or DG) or solubility (S or P) alone, or interactions between them (source × sol). Means that do not share a letter are significantly different (one-way ANOVA, $P < 0.05$, only performed when the interaction was significant). Significance levels for source and solubility $P < 0.05$, and for interaction $P < 0.1$. [b] The numbers in parentheses indicate the number of rats in the group in which the acid could not be detected at the detection limit of about 0.05 μmol/g.

72% and of butyric acid 10 vs 9%. Supernatant fractions led to a lower proportion of butyric acid in the proximal and distal colon ($P_{sol} = 0.003$, $P_{source\times sol} = 0.953$ and $P_{sol} < 0.001$, $P_{source\times sol} = 0.853$, respectively), and a higher proportion of acetic acid in the distal colon ($P_{sol} < 0.001$, $P_{source\times sol} = 0.525$).

Levels of acetic, propionic, and butyric acid were significantly lower in the distal colon than in the cecum for all groups except those given the C-S diet (acetic and propionic acid) and the DG-P diet (butyric acid), where the differences were not significant.

Short-Chain Fatty Acids in Portal Blood. Levels of SCFA in portal blood (μmol/g) were higher in rats fed supernatant diets than in those fed pellet diets for propionic, isobutyric, and isovaleric acids, but significantly lower for valeric acid (Table 6). No differences were seen between dietary fiber sources. The high mean values of acetic and butyric acid in the DG-P group compared with the DG-S group could mainly be explained by very high values from one rat in the DG-P group for both acids, and a very low value for acetic acid in one rat in the DG-S group. Removing the results from these rats gave lower mean values for acetic and butyric acids for the DG-S diet than for the DG-P diet (644 ± 18 vs 686 ± 22 μmol/g for acetic acid and 16 ± 2 vs 19 ± 4 μmol/g for butyric acid), but the differences between supernatant and pellet diets for these acids remained nonsignificant. The ratio of acetic to propionic acid was significantly lower in rats fed the supernatant diets.

Correlations. Levels of propionic, isobutyric, butyric, isovaleric, and valeric acids in portal blood showed a linear relationship with cecal pools ($P < 0.05$, $R^2 = 9-41\%$) (Figure 1). The correlation for acetic acid was also close to being significant ($P = 0.077$), and became significant ($P = 0.032$, $R^2 = 13\%$) upon removing the two rats from the DG-P and DG-S groups with particularly high and low values, respectively, of this acid in portal blood.

Bacterial Counts. The DG-S diet was found to result in the highest level of bifidobacteria as well as a high level of lactobacilli (Table 7). More lactobacilli were found in rats in the DG supernatant group than in the pellet group, while the opposite was true for the C groups. No difference was seen between the CX groups.

DISCUSSION

The aim of the present study was to examine the prebiotic and carboxylic acid-forming potential of carbohydrate-rich waste

Table 5. Levels (μmol/g wet content) of Acetic, Propionic and Butyric Acids in Different Parts of the Hindgut of Rats Fed Dietary Fiber from Various Wheat Processing Fractions[a]

	C		CX		DG		P-value		
	P	S	P	S	P	S	source	solubility	source × sol
cecum									
acetic	39 ± 5.4 a	28 ± 2.8 a	40 ± 5.3 a	32 ± 4.4 a	37 ± 4.4 a	15 ± 1.0 b	<0.001	<0.001	0.001*
propionic	12 ± 2.0	8.5 ± 0.8	11 ± 1.4	9.5 ± 1.1	9.1 ± 1.0	5.4 ± 0.7	0.026*[b]	0.005*	0.650
butyric	5.8 ± 1.4 a	3.9 ± 0.5 a	5.1 ± 0.6 a	4.7 ± 1.0 a	5.6 ± 1.3 a	2.1 ± 0.2 b	0.033	0.001	0.039*
total	57 ± 8 a	41 ± 4 a	57 ± 7 a	46 ± 6 a	52 ± 6 a	22 ± 2 b	0.001	<0.001	0.004*
proximal colon									
acetic	20 ± 4.0 a	17 ± 0.9 a	23 ± 3.4 a	20 ± 2.8 a	15 ± 1.6 a	6.0 ± 0.6 b	<0.001	<0.001	0.000*
propionic	5.7 ± 1.4	4.0 ± 0.6	5.1 ± 0.9	4.5 ± 0.9	3.2 ± 0.5	1.5 ± 0.2	<0.001*[b]	0.011*	0.187
butyric	3.0 ± 1.0	1.6 ± 0.4	2.0 ± 0.3	1.1 ± 0.3	1.6 ± 0.6	0.4 ± 0.1	0.006*[c]	0.001*	0.480
total	28 ± 6 a	23 ± 2 a	30 ± 4 a	26 ± 4 a	19 ± 3 a	8 ± 1 b	0.000	<0.001	0.000*
distal colon									
acetic	20 ± 3.8 ab	23 ± 2.9 a	25 ± 2.4 a	24 ± 6.2 a	20 ± 2.4 ab	7.2 ± 0.6 b	0.006	0.173	0.074*
propionic	5.4 ± 1.0 a	4.8 ± 0.9 a	6.1 ± 0.7 a	4.7 ± 1.6 a	4.5 ± 0.4 a	1.6 ± 0.2 b	0.002	0.001	0.041*
butyric	2.8 ± 0.8	1.6 ± 0.5	2.9 ± 0.4	0.9 ± 0.4	2.5 ± 0.4	0.5 ± 0.1	0.198	<0.001*	0.283
total	29 ± 5 a	29 ± 4 a	34 ± 3 a	30 ± 8 a	27 ± 3 a	9 ± 1 b	0.000	<0.001	0.000*

[a] Results are given as means ± SEM, n = 5−7. C, untreated C fraction; CX, C fraction partly degraded by xylanase; DG, distillers' grains; P, pellet; S, supernatant. Asterisks indicate significant effects of fiber source (C, CX or DG) or solubility (S or P) alone, or interactions between them (source × sol). Means that do not share a letter are significantly different (one-way ANOVA, $P < 0.05$, only performed when the interaction was significant). Significance levels for source and solubility $P < 0.05$, and for interaction $P < 0.1$. [b] DG differed from C and CX. [c] DG differed from C.

Table 6. Levels (μmol/L) of SCFA in Portal Blood from Rats Fed Dietary Fiber from Various Wheat Processing Fractions[a]

	C		CX		DG		P-value		
	P	S	P	S	P	S	source	solubility	source × sol
acetic	679 ± 60	710 ± 32	629 ± 55	710 ± 83	723 ± 81	642 ± 47	0.926	0.843	0.412
propionic	62 ± 15	85 ± 9	59 ± 24	99 ± 19	58 ± 16	82 ± 18	0.868	0.044*	0.867
isobutyric	7.5 ± 0.8	10 ± 0.7	6.9 ± 0.8	12 ± 3.0	8.1 ± 0.8	8.9 ± 1.0	0.851	0.015*	0.311
butyric	22 ± 6.6	26 ± 2.9	19 ± 6.8	30 ± 6.1	30 ± 14	17 ± 3.5	0.997	0.943	0.330
isovaleric	11 ± 0.9	16 ± 1.3	10 ± 1.0	18 ± 3.3	12 ± 1.0	15 ± 1.7	0.856	0.001*	0.242
valeric	3.7 ± 1.3	1.5 ± 0.9	4.9 ± 2.1	0.7 ± 0.4	5.4 ± 2.3	0.0 ± 0.2	0.982	0.003*	0.514
total	785 ± 82	848 ± 43	730 ± 87	869 ± 111	838 ± 114	765 ± 65	0.926	0.843	0.467
Ace:Pro	14 ± 3.0	8.6 ± 0.6	15 ± 2.5	7.6 ± 0.8	15 ± 1.7	9.8 ± 1.7	0.848	0.001*	0.847

[a] Results are given as means ± SEM, n = 5−7. C, untreated C fraction; CX, C fraction partly degraded by xylanase; DG, distillers' grains; P, pellet; S, supernatant. Asterisks indicate significant effects of fiber source (C, CX or DG) or solubility (S or P) alone, or interactions between them (source × sol). Significance levels for source and solubility $P < 0.05$, and for interaction $P < 0.1$.

fractions from wheat processing. Our previous study has shown that the C fraction is particularly rich in fructan and arabinoxylan, while an unknown low-molecular-weight arabinose-containing compound has been found in DG.[4] In this study, the original fractions were further separated by centrifugation into a supernatant and a pellet fraction to study the physiological properties of the low- and high-molecular-weight components of the fractions separately.

The dietary fiber composition of the DG-P and DG-S fractions differed from that of the C fractions. Both DG fractions contained more arabinose than xylose, while the C fractions contained more xylose than arabinose. The DG-S fraction is known from previous studies to contain a low-molecular-weight carbohydrate consisting of arabinose, which is soluble in 80% ethanol.[4] The solubility of this compound in 90% ethanol is not known, and it is thus possible that it was partly removed during the 90% ethanol extraction before the second freeze-drying stage. The higher proportions of mannose and glucose in the DG fractions could be explained by the presence of mannoproteins and glucans from the yeast.[4]

The xylanase treatment decreased the molecular weight of AX, and the distribution of AX was thus shifted to the soluble fraction. Apart from this, the composition was similar for the C-P and CX-P diets and for the C-S and CX-S diets. This suggests that it would be possible to detect physiological effects due to the xylanase treatment. Studies have shown that the molecular weight of AX and AXOS affects the formation of SCFA[21] and growth of bacteria[10,21] in the colon. Pretreatment of water-unextractable AX with endoxylanase has been shown to give oligomers that were better utilized by gut bacteria.[22] However, no differences could be detected between the xylanase-treated and untreated C fractions in this study. This suggests that the main

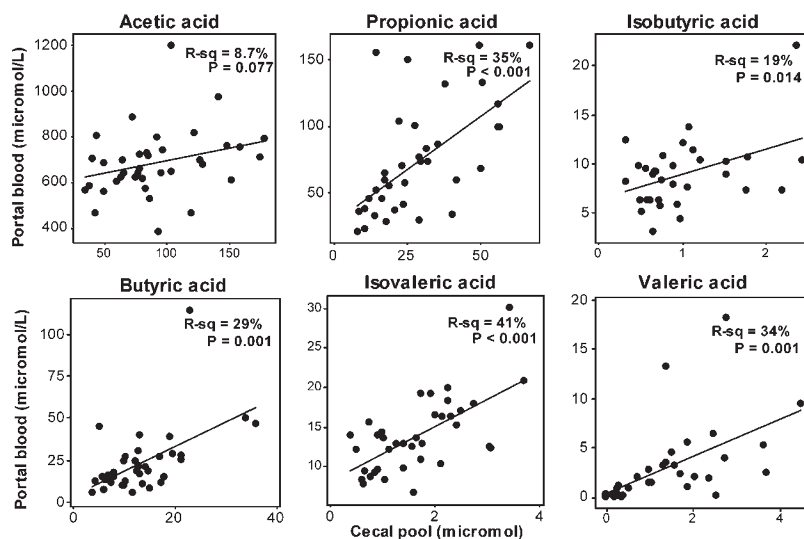

Figure 1. Correlations between SCFA in cecal pools and levels in portal blood.

Table 7. Bacterial Counts (log CFU/g cecal content) of Bifidobacteria and Lactobacilli[a]

	C		CX		DG		*P*-value		
	P	S	P	S	P	S	source	solubility	source × sol
bifidobacteria	7.2 ± 0.2 b	7.6 ± 0.3 b	7.4 ± 0.4 b	8.2 ± 0.3 b	7.3 ± 0.3 b	9.5 ± 0.1 a	0.003	<0.001	0.008*
lactobacilli	8.2 ± 0.1 a	6.9 ± 0.5 bc	7.7 ± 0.2 ab	7.7 ± 0.2 ab	6.4 ± 0.1 c	8.5 ± 0.3 a	0.601	0.261	0.000*

[a] Results are given as means ± SEM, $n = 5-7$. C, untreated C fraction; CX, C fraction partly degraded by xylanase; DG, distillers' grains; P, pellet; S, supernatant. Asterisks indicate significant effects of fiber source (C, CX or DG) or solubility (S or P) alone, or interactions between them (source × sol). Means that do not share a letter are significantly different (one-way ANOVA, $P < 0.05$, only performed when the interaction was significant). Significance levels for source and solubility $P < 0.05$, and for interaction $P < 0.1$.

effect of the xylanase was to increase the solubility of the AX, but that the degradation was not extensive enough to have a significant effect on the physiological properties of the soluble AX. It is also possible that differences resulting from the enzymatic treatment could not be detected when AX made up only 30−40% of the dietary fiber. On the other hand, with a larger proportion of highly soluble dietary fiber in the CX fraction than in the C fraction, a difference may have been found if these byproducts had been directly compared without separation into supernatant and pellet fractions.

In this study, differences between the diets were mostly seen between supernatants and pellets, i.e. the fractions reflecting low- and high-molecular-weight dietary fiber, rather than between fiber sources. Thus, the different composition of the DG fractions compared with the corresponding C and CX fractions was not well reflected in the physiological parameters measured in the rats, especially regarding DG-P. Even clearer differences between the fractions may have been found if the separation between the supernatant and pellet had been more complete, for example by including a washing step. Fructans, for example, were present at proportions of 5% of the dietary fiber in C pellet fractions (compared with 20% in supernatant fractions), in spite of being highly soluble in water.

Cecal pH was lower and cecal pools and levels of most CA in portal blood were higher in rats fed the supernatant diets. This may be due to the higher fermentability of carbohydrates of lower molecular weight. Levels of SCFA in portal blood were correlated to cecal pools, showing that the concentration in the portal blood reflects the total amount formed. On the other hand, CA levels in the cecum and the colon were generally lower, and the cecal content higher in rats fed the supernatant diets, which may be explained by higher water content due to osmosis. Levels of acetic, propionic and butyric acid were lower in the distal colon than in the cecum for all groups, with a few exceptions where the results were not significant. This indicates that all substrates were easily fermented early in the colon.

Supernatant diets led to a lower ratio of acetic to propionic acid in portal blood. This is interesting because a decrease in this ratio has been proposed as the cause of the hypolipidemic effects of inulin and oligofructose.[23] One candidate for the promotion of propionic acid is AX, as AX[24,25] and AXOS[21,26] have been shown to promote the formation of propionic acid in several studies. In that case, the similar AX content in the C-S and C-P diets indicates that soluble/low-molecular-weight AX forms propionic acid to a higher extent than insoluble/high-molecular-weight AX. However, the data in the literature are not conclusive on this point, and other studies suggest an increase in the formation of butyric acid with AX.[10,27] It is also possible that wheat fructans promote the formation of propionic acid, an effect that has been seen with inulin.[6] However, fructans were only found at trace amounts in DG-S and could not be responsible for the increase in propionic acid with this diet.

While DG-S behaved similarly to C-S and CX-S regarding many parameters, this diet led to a higher cecal content and a higher amount of bifidobacteria. In contrast, differences between C supernatant and pellet fractions were not significant regarding

levels of bifidobacteria. Previous studies have indicated that bifidobacteria may grow on AX, with a higher selectivity for AX with decreasing molecular weight, but not to the same extent as on AXOS and xylooligosaccharides.[10,28,29] In other studies, AXOS with a DP ≤ 5 but not DP ≥ 15 led to increased levels of bifidobacteria.[21,26] The better bifidogenic effect of oligosaccharides may be explained by the fact that bifidobacteria do not have endo-1,4-xylanase activity, but contain several enzymes that detach xylose and arabinose units from the ends of the chains.[26] It may thus be speculated that the low-molecular-weight arabinose in the DG-S diet was responsible for the bifidogenic effect of this fraction, although the structure of this compound is not known. Furthermore, AX from the C supernatant fractions may have been slightly more bifidogenic than AX from the pellet fractions, although the AX fragments were not short enough to produce a significant effect. Our earlier results indicate that only insignificant amounts of AXOS with a DP ≤ 5 were present in the C fraction.[4] Bifidobacteria have been shown to grow on wheat fructans *in vitro*,[9] but the difference in concentration between pellet and supernatant (5 vs 20% of the indigestible carbohydrates) may have been too small to reveal significant differences.

Lactobacilli levels varied depending on both fiber source and solubility, in contrast to most other parameters. The DG-P diet led to a lower level of lactobacilli than the C-P and CX-P diets. Furthermore, the C-P diet led to a higher level of lactobacilli than the C-S diet, while more lactobacilli were seen following the DG-S diet than the DG-P diet. No difference in lactobacilli was seen between the CX-P and CX-S diets. A possible explanation of this could be that the lactobacilli are not related to AX or fructan, as has been suggested for bifidobacteria. This is supported by previous findings that inulin-type fructans stimulate the growth of lactobacilli to a lesser extent than bifidobacteria,[30] and the same seems to be the case for AX and AXOS. Although lactobacilli were shown to ferment a 66-kDa AX fraction *in vitro*,[10] other studies have not reported the promotion of lactobacilli with AX or AXOS.[9,21,31,32]

In conclusion, this study shows that waste fractions from wheat processing may have positive physiological effects. The prebiotic potential of the DG-S fraction appears to be particularly interesting because of its bifidogenic effect. As the dietary composition of the diets was complex, further studies with purified fractions are required to confirm the effects of specific components. In this study it was found that molecular weight and solubility were of greater importance than the chemical composition of the dietary fiber, and these are also easier to modify with technological processes, providing the opportunity to optimize these properties in the development of health products. A greater degree of xylanase degradation in the wet fractionation process could possibly have positive physiological effects.

■ ASSOCIATED CONTENT

Supporting Information. Table of levels of minor acids in different parts of the hindgut. This material is available free of charge via the Internet at http://pubs.acs.org.

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