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Extrusion of Barley and Oat Improves the Bioaccessibility of Dietary Phenolic Acids in Growing Pigs

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ABSTRACT: To evaluate the bioaccessibility of phenolic acids in extruded and nonextruded cereal grains, an in vivo experiment was carried out using growing pigs as a model system. Four diets were prepared containing either whole grain barley (BU), dehulled oat (OU), or their respective extruded samples (BE, OE) according to the requirements for crude protein, mineral, and vitamin contents in pig diets. The total contents of free phenolic acids in the OE and BE diets were 22 and 10%, respectively, higher compared with the OU and BU diets, whereas the level of bound phenolic acids was 9% higher in OE than in OU and 11% lower in BE compared with BU. The total tract bioaccessibilities of bound phenolic acids were 29 and 14% higher for the extruded BE and OE diets, respectively, compared with the nonextruded diets. The results of this study indicate an improved bioaccessibility of phenolic acids in extruded cereal grains.

KEYWORDS: *extrusion, barley, oat, phenolic acids, pig, bioavailability*

INTRODUCTION

Intake of whole grain barley and oat is associated with a decreased risk for coronary heart disease and certain types of cancer, as well as a cholesterol-lowering effect.[1] Thus, barley and oat have been intensively studied as dietary components with health beneficial properties.

Processing of cereal grains is important for sensory properties of cereal-based products. However, the physical, chemical, and nutritional status of the cereal constituents can be modified dramatically in processed food. Significant losses of dietary fiber and associated compounds have been demonstrated during milling and dehulling.[2,3] Levels of bioactive compounds such as phytate, alkylresorcinols, and tocopherols have been shown to diminish significantly during baking, whereas the levels of folate and easily extractable phenolic compounds have been shown to increase during germination and sourdough baking.[4]

Interest in the nutritional aspects of extruded cereals has lately increased due to a broad industrial use. Extrusion is a high-temperature−short-time process used widely in the production of foods and feeds. It has been shown that extrusion of cereal grains can lead to enhanced mineral bioavailability[5] and protein digestibility.[6] Moreover, an increased content of soluble dietary fiber and phenolic acids and a destruction of antinutritional factors such as trypsin inhibitors and phytates in extruded products have been documented.[5,7]

Phenolic acids are known to exhibit good antioxidant activities and to have beneficial health effects.[8] The most abundant dietary phenolic acids in cereal grains include ferulic, caffeic, *p*-coumaric, and sinapic acids. However, the bioavailability of phenolic acids in cereals is low due to their low content in free form. The majority of phenolic acids in cereals is found in bound form esterified to the cell walls. Cereal ester-bound phenolic acids are not hydrolyzed by human digestive enzymes. On the other hand, they can be released by the action of bacterial enzymes in the colon and be further metabolized by bacterial microflora by various reactions and cleavage of functional groups before absorption.[8−10] Subsequently, numerous phenolic metabolites can be detected in the blood after intake of dietary phenolic acids. However, most studies have mainly focused on the analysis of the phenolic acids themselves in biological samples and not their metabolites after consumption of diets enriched with purified phenolic acids or with cereal bran fractions as rich sources of phenolic acids.[11−14] Although the release and metabolism of phenolic acids by colonic microflora have been intensively studied in vitro,[15−17] only a few studies have attempted to follow the fate of ingested phenolic acids and their colonic metabolites in vivo.[9,18] Furthermore, it has not yet been documented whether the increased content of bound phenolic acids in extruded cereal products would lead to their improved bioaccessibility.

Thus, the aim of this study was to investigate the effect of extruded whole grain barley and oat groat based diets, containing increased levels of bound and free phenolic acids, on the bioaccessibility of phenolic acids using growing pigs as a model system. Unextruded whole grain barley and oat groat were used in control diets to evaluate the effect of extrusion of cereals. Data about the content of phenolic acids and their

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Table 1. UPLC and Mass Spectrometric Characteristics of Standards for Phenolic Acids and Their Colonic Microbial Metabolites

no. (Figure1)	trivial name, phenolic acid/phenolic acid metabolite	systematic name, phenolic acid/phenolic acid metabolite	abbrev	$[M-H]^-$ (m/z)	fragment ions (m/z)	RT, min
1	3,4-dihydroxybenzoic	3,4-dihydroxybenzoic (protocatechuic)	PCa	153	109	1.07
2	3,4-dihydroxyphenylacetic	3,4-dihydroxyphenylacetic	DOPAC	167	123	1.24
3	4-hydroxyphenylacetic	2-(4-hydroxyphenyl)acetic	PHPA	151	107	1.41
4	dihydrocaffeic	3,4-dihydroxyphenylpropionic	HCA	181	137	1.49
5	phenylacetic	phenylacetic	PAA	135	91	1.49
6	dihydro-*p*-coumaric	3-(4-hydroxyphenyl)propanoic	HpCA	165	121	2.11
7	vanillic	4-hydroxy-3-methoxybenzoic	Van	167	152	2.17
8	homosyringic	3,5-dimethoxy-4-hydroxyphenylacetic	homoSyr	211	181, 196	2.17
9	benzoic	benzoic	BA	121	77	2.31
10	caffeic	3-(3,4-dihydroxyphenyl)propenoic	CA	179	135	2.64
11	chlorogenic	3-(3,4-dihydroxycinnamoyl)quinic	CGA	353	191, 179	2.76
12	dihydroferulic	3-(4-hydroxy-3-methoxyphenyl)propanoic	HFA	195	136	2.76
13	syringic	4-hydroxy-3,5-dimethoxybenzoic	Syr	197	153, 182	3.06
14	phenylpropionic	3-phenylpropionic	PPA	149		3.64
15	*p*-coumaric	3-(4-hydroxyphenyl)propenoic acid	pCA	163	119	3.75
16	*m*-coumaric	3-(3-hydroxyphenyl)propenoic	mCA	163	119	3.85
IS	*o*-coumaric (IS)	3-(2-hydroxyphenyl)propenoic (IS)	o-CA	163	119	4.17
17	ferulic	3-(4-hydroxy-3-methoxyphenyl)propenoic	FA	193	134	4.82
18	sinapic	3-(3,5-dimethoxy-4-hydroxyphenyl)propenoic	SA	223	149, 208	5.74

metabolites in plasma as well as information about the levels of bound and free phenolic acids excreted with the feces were used for estimation of bioaccessibility of dietary phenolic acids. Growth performance of animals and plasma antioxidant status during experiment were also analyzed.

MATERIALS AND METHODS

The feeding experiment was performed at the Experimental Farm of the Norwegian University of Life Sciences, Ås, Norway. All pigs were cared for according to laws and regulations controlling experiments with live animals in Norway (Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance concerning experiments with animals of January 15, 1996).

Dietary Treatments. Four batches of grain were used in the study: (1) whole grain barley, untreated; (2) whole grain barley, extruded; (3) oat groat, untreated; (4) oat groat, extruded. Each of the batches was produced and pelleted by Lantmännen Cerealia, Moss, Norway. Oat groat samples were produced from the Norwegian oat cultivar Belinda, and whole grain barley samples were produced from the Norwegian barley cultivar Olve. After dehulling, the hull fraction constituted approximately 25% of the grain weight for oat. Whole grain barley and dehulled oat (oat groat) were milled to produce barley flour and oat flour with particle size <300 mm and extruded using a single-screw extruder (Wenger Manufacturing, Inc., Sabetha, KS, USA); approximately 30% water of flour weight was added, and the temperature at the die was 110 °C. After extrusion, samples were dried and cooled to room temperature. Each of the grain batches was mixed with soybean meal, limestone meal, and micromineral and vitamin premix prior to feeding to meet the requirements for protein, minerals, and vitamins.[19] Yttrium oxide (0.1 g kg^{-1} of diet) was used as dietary marker. One kilogram of the diets consisted of 838.7 g of grain, 150.0 g of soybean meal, 13.0 g of limestone meal, 1.6 g of micromineral premix, 0.6 g of vitamin premix, and 0.1 g of yttrium oxide. Before feeding, the pelleted grains were crushed to a fine powder using a hammer mill, model DT900-12 (CPM-Roskamp, Waterloo, IA, USA).

Experimental Animals. A total of 16 female pigs [(Landrace × Yorkshire) × (Landrace × Duroc)] from four litters were used in the experiment. The average initial weight was 46.5 kg, and the average final weight was 57.6 kg. The pigs were blocked by litter and by live weight, and four animals were fed each dietary treatment. Live weight and feed intake were measured for each pig at every week in the experiment.

Experimental Procedure. The total experimental period lasted for 21 days; a 7 day adaptation period followed by a 14 day experimental period. The pigs were fed twice daily (at 8 a.m. and 2 p.m.) according to a restricted Norwegian feeding scale,[20] and they had free access to drinking water. They were kept in pens designed for individual feeding in a room with an average temperature of 18 °C.

Sample Collection. Fecal samples were collected from each of the pigs at the beginning and during the final two days of the experiment. The samples were frozen immediately at −20 °C. The fecal samples were freeze-dried, ground, and pooled for each dietary treatment before being analyzed.

Blood samples were taken at 7 a.m. at the start of the experiment, after 1 week (adaptation period), and at the end of the experiment. The blood samples were centrifuged for 20 min at 3000*g*, and blood plasma was transferred to TT tubes and frozen at −20 °C for analysis of phenolic metabolites.

Analytical Methods. *Feed Analysis.* The four grain samples, the soybean meal and the four diet samples, were analyzed for dry matter (DM; EU Directive 71/393), ash (EU Dir.ective 71/250), crude protein (Kjeldahl-N × 6.25; EU Directive 93/28), crude fat (EU Directive 98/64), crude fiber (EU Directive 92/89), and starch (AOAC 996.11). The four diets were also analyzed for yttrium by inductively coupled plasma mass spectrometry (ICP-AES analysis, Perkin-Elmer Optia 3000DV; Perkin-Elmer, Wellesley, MA, USA) at 371 nm, after mineralization and solubilization in acid of the pooled sample.

In addition, the grain samples and soybean meal were analyzed for β-glucan and nonstarch polysaccharides (total, soluble, and insoluble) as described in ref 21 and for free and bound phenolic acids using the same method as described in our previous study.[22] Each sample was prepared in parallels, which were analyzed in duplicates.

Fecal Analysis. The final fecal samples were analyzed for dry matter and for yttrium as described earlier and for free and bound phenolic acids as described by Hole et al.[22]

Plasma Analysis. Total content of free and conjugated phenolic acids and products of their metabolic transformation were determined in plasma samples after glucuronidase/sulfatase hydrolysis. To 500 μL of plasma were added 20 μL of 100 μg/mL *o*-coumaric acid aqueous solution as internal standard (IS), 20 μL of sulfatase type H-1 solution in 1 M acetate buffer, pH 4.9, containing 2.5×10^5 U/L of sulfatase and 7.5×10^6 U/L of β-glucuronidase, and the mixture was incubated

at 37 °C in a water bath for 2 h. Proteins were further precipitated by 1.25 mL of acetone and removed by centrifugation at 800*g* during 10 min at 20 °C using a centrifuge Multifuge 4 KR (Heraeus, UK). The supernatant was dried under nitrogen at 30 °C, and the dry residue was dissolved in 200 μL of 25% methanol and filtered using a 0.22 μm filter. Analysis was performed using a UPLC system Agilent 1200 series equipped with a quadrupole ion trap mass spectrometer (qTOF) Agilent 6530 with an electrospray ionization source operated in negative ion mode. The nebulizer pressure was 60 psi; drying gas flow rate, 13 L/min; drying temperature, 350 °C; skimmer voltage, 65 V; and fragmentor voltage, 128 V. Analysis was carried out using scan from m/z 50 to 1000. Chromatographic separation was performed on an analytical column Zorbax SB-CN RRHD 3.0 × 100 mm, 1.8 μm (Agilent), kept at 50 °C using 1% acetic acid in Milli-Q water as eluent A and 1% acetic acid in acetonitrile of HPLC grade (Merck, Darmstadt, Germany) as eluent B and the following elution program with a flow rate of 1 mL/min: 5% B (0.8 min), 10% B (2.9 min), 15% B (3.9 min), 15% B (4.9 min), 21% B (5.0 min), 21% B (5.5 min), 27% B (6.0 min), 27% B (7.0 min), 50% B (8.0 min), 100% B (9.0 min), 100% B (10.5 min), and 5% B (13.3 min).

A linear calibration curve for each standard (phenolic acids and their colonic microbial metabolites, Table 1) was established between 0 and 10 μg/mL. Retention time and fragmentation patterns from mass spectrometry of the respective standard were used for identification of the compounds presented in Table 1. Each sample was prepared in parallels and analyzed in duplicates. Data were extracted and processed with Agilent MassHunter software.

Calculation and Statistical Analysis. Total tract bioaccessibility (TTB) of bound and free phenolic acids, using yttrium oxide as a marker, was calculated as described by McDonald et al.[23] according to the equation

$$\mathrm{TTB} = \frac{\frac{a_1}{a_2} - \frac{b_1}{b_2}}{\frac{a_1}{a_2}} \times 100 \quad (1)$$

where a_1 is the concentration of phenolic acid in the diet, a_2 is the analyzed concentration of yttrium in the diet, b_1 is the concentration of phenolic acid in feces, and b_2 is the concentration of yttrium in feces.

The statistical analyses were performed using the GLM procedure of SAS version 9.2 (SAS Institute, Cary, NC, USA) with the Ryan−-Einot−Gabriel−Welsch multiple-range test (REGWQ), when appropriate, and Statistix (Analytical Software, Tallahassee, FL, USA).

To investigate the effect of the design on the detected metabolites in the blood plasma, principal component analysis (PCA) was used (Matlab, v. 7.11, The Mathworks Inc., Natrick, MA, USA). The clustering of groups was studied by score plots and correlation loading plots.[24] In the correlation loading plots the correlations between latent variables and metabolites and design variables are plotted. This allows us to investigate which metabolites are increased or decreased in the respective design groups.

RESULTS AND DISCUSSION

This study was performed to evaluate the effect of extrusion of whole grain barley and oat groat on the bioaccessibility of phenolic acids using growing pigs as a model system. The main objective was to relate the changes in the content of free and bound phenolic acids obtained in cereal samples after extrusion to changes in their bioaccessibility and their metabolite profiles in plasma samples.

Chemical Composition of the Diets and Phenolic Acid Intake. The chemical analysis of the extruded and untreated grains and cereal-based diets is shown in Table 2. In agreement with earlier studies,[25,26] extrusion increased the crude protein (CP) content and caused a shift of insoluble β-glucans to soluble β-glucans for both whole grain barley and oat groat

Table 2. Chemical Composition of the Grains and Diets[a]

		dry matter (g kg^{-1})	crude protein (g kg^{-1})	crude fat (g kg^{-1})	crude fiber (g kg^{-1})	starch (g kg^{-1})	ash (g kg^{-1})	β-glucan (% w/w)			NSP (% w/w)			insoluble AX (% w/w)
								soluble	insoluble	total	soluble	insoluble	total	
grains	barley (U)	865	122	24	18	540	16	3.97	1.31	5.28	6.11	6.03	12.14	4.22
	barley, extruded	915	135	29	12	585	17	4.44	1.07	5.51	6.18	6.15	12.32	4.63
	dehulled oats (U)	882	130	76	11	541	18	2.24	2.47	4.71	3.97	3.82	7.79	2.70
	dehulled oats, extruded	930	150	69	24	579	23	2.89	1.26	4.14	3.63	3.39	7.03	2.50
diets	BU (control B)	870	172	23	26	451	36	NA	NA	NA	NA	NA	NA	NA
	BE	912	183	27	21	488	37	NA	NA	NA	NA	NA	NA	NA
	OU (control O)	884	179	66	20	452	38	NA	NA	NA	NA	NA	NA	NA
	OE	924	196	60	31	483	42	NA	NA	NA	NA	NA	NA	NA

[a]Diet BU, diet based on barley, untreated; diet BE, diet based on barley, extruded; diet OU, diet based on dehulled oats, untreated; diet OE, diet based on dehulled oats, extruded. AX, arabinoxylan, estimated as the sum of arabinose and xylose (data not shown). NA, analysis or calculations not performed.

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Table 3. Content of Phenolic Acids in Barley- and Oat-Based Feeds (mg kg $^{-1}$)[a]

	CA		*p*-CA		FA		SA		5,5-DFA	8-*O*-4-DFA	8,5-DFA	total	
diet	free	bound	free	bound	free	bound	free	bound	bound	bound	bound	free	bound
OU	0.85a	17.22	1.90a	9.67a	3.10a	234.61a	1.58a	42.30a	9.21a	16.13a	5.74a	7.43	334.88
OE	1.87b	17.06	1.95a	14.90b	3.86b	262.69b	1.41a	33.97b	10.31b	18.86b	7.61b	9.08	365.41
BU	0.27c	17.19	1.41b	37.09c	1.96c	462.29c	0.44b	21.67c	22.35c	22.79c	12.53c	4.09	595.90
BU	0.29d	19.84	1.42b	28.25d	2.37d	432.07c	0.43c	3.72d	18.88d	19.75d	10.22c	4.51	532.72

[a]OU and BU, oat- and barley-based feeds, respectively; OE and BE, extruded oat and extruded barley based feeds, respectively. Different letters (a–d) for phenolic acids (within a column) indicate significant difference among diets ($p < 0.05$).

Table 4. Growth Performance of the Pigs[a]

	diet BU	diet BE	diet OU	diet OE	SEM	*p* value
pigs, no.	4	4	4	4		
initial weight, kg	47.0	49.7	45.9	43.5	3.93	0.74
final weight, kg	57.9	59.5	57.6	55.8	4.07	0.93
week 1						
ADFI, kg	1.82a	1.44b	1.80a	1.60ab	0.064	0.004
ADG, kg	0.846ab	0.593b	0.757ab	1.029a	0.095	0.045
F:G, kg/kg	2.17ab	2.45a	2.40a	1.69b	0.171	0.031
week 2						
ADFI, kg	1.92	1.52	1.93	1.77	0.101	0.050
ADG, kg	0.700	0.804	0.914	0.711	0.093	0.37
F:G, kg/kg	2.84	1.96	2.13	2.57	0.233	0.07
weeks 1 + 2						
ADFI, kg	1.87a	1.48b	1.86a	1.69ab	0.077	0.012
ADG, kg	0.773	0.698	0.836	0.870	0.054	0.18
F:G, kg/kg	2.42	2.15	2.32	1.97	0.117	0.10

[a]SEM, standard error of the mean; ADFI, average daily feed intake; ADG, average daily weight gain; F:G, feed conversion ratio, kg feed/kg gain; diet BU, diet based on barley, untreated; diet BE, diet based on barley, extruded; diet OU, diet based on dehulled oats, untreated; diet OE, diet based on dehulled oats, extruded. Different letters (a, b) indicate significant difference among treatments ($p < 0.05$). The means were separated using REGWQ (Ryan–Einot–Gabriel–Welsch multiple-range test).

Table 5. Apparent Total Tract Bioaccessibility (TTB; % DM)[a]

	diet BU	diet BE	diet OU	diet OE
dry matter (DM)[b]	82.8	82.0	87.1	85.1
bound CA	80.13 (±4.39)	85.31 (±2.71)	ND	13.56 (±11.85)
bound FA	61.97 (±5.61)	68.27 (±4.62)	65.23 (±1.44)	68.46 (±5.29)
bound 5,5-DFA	58.24 (±5.06)	76.36 (±4.60)	73.17 (±6.56)	85.49 (±3.64)
bound 8-*O*-4-DFA	34.38 (±9.41)	76.96 (±7.50)	51.00 (±4.45)	86.11 (±7.51)
bound 8,5-DFA	68.99 (±4.59)	75.52 (±5.41)	57.91 (±3.24)	87.74 (±4.93)
total bound PA	50.83c (±5.40)	65.63ab (±1.63)	60.65b (±1.12)	69.23a (±1.47)
total free PA	83.23b (±3.56)	85.68b (±4.76)	93.31a (±0.42)	94.44a (±1.25)

[a]Diet BU, diet based on barley, untreated; diet BE, diet based on barley, extruded; diet OU, diet based on dehulled oats, untreated; diet OE, diet based on dehulled oats, extruded; PA, phenolic acids. Different letters (a–d, within a row) indicate significant difference among treatments ($p < 0.05$). The means were separated by two-way analysis of variance using Minitab 13.30 (Minitab Inc., State College, PA, USA) and SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). [b]estimated for pooled samples for each treatment.

samples. However, no significant effect was obtained for nonstarch polysaccharides (NSP), starch, and crude fiber levels.

The content of phenolic acids in the diets is shown in Table 3. Phenolic acids are mostly found in bound form in cereals, giving a high ratio of bound to free phenolic acids of nearly 50:1 in oat groat diets and 150:1 in whole grain barley diets. Samples from both extruded (BE) and nonextruded whole grain barley based (BU) diets contained higher total amount of phenolic acids than oat groat diets (OE and OU, respectively). This is most likely a result of the hulling process of oat, which is known to decrease the content of phenolic acids, as phenolic acids are concentrated in the outer layers of the cereal kernel. However, the content of free phenolic acids was higher for oat groat diets compared with whole grain barley diets, as oat is known to have a higher content of free phenolic acids than barley.[22] The content of FA and *p*-CA was predominant in all feed samples used (Table 3).

The contents of free phenolic acids in OE and BE diets were 22 and 10%, respectively, higher compared with the OU and BU diets. This is in agreement with our previous study,[7] in which we showed that extrusion can increase the content of free phenolic acids in barley products. In comparison Anson et al.[27] found that combining fermentation with baker's yeast and enzyme treatment increased wheat bran free phenolic acid bioaccessibility by 6-fold compared to native bran.

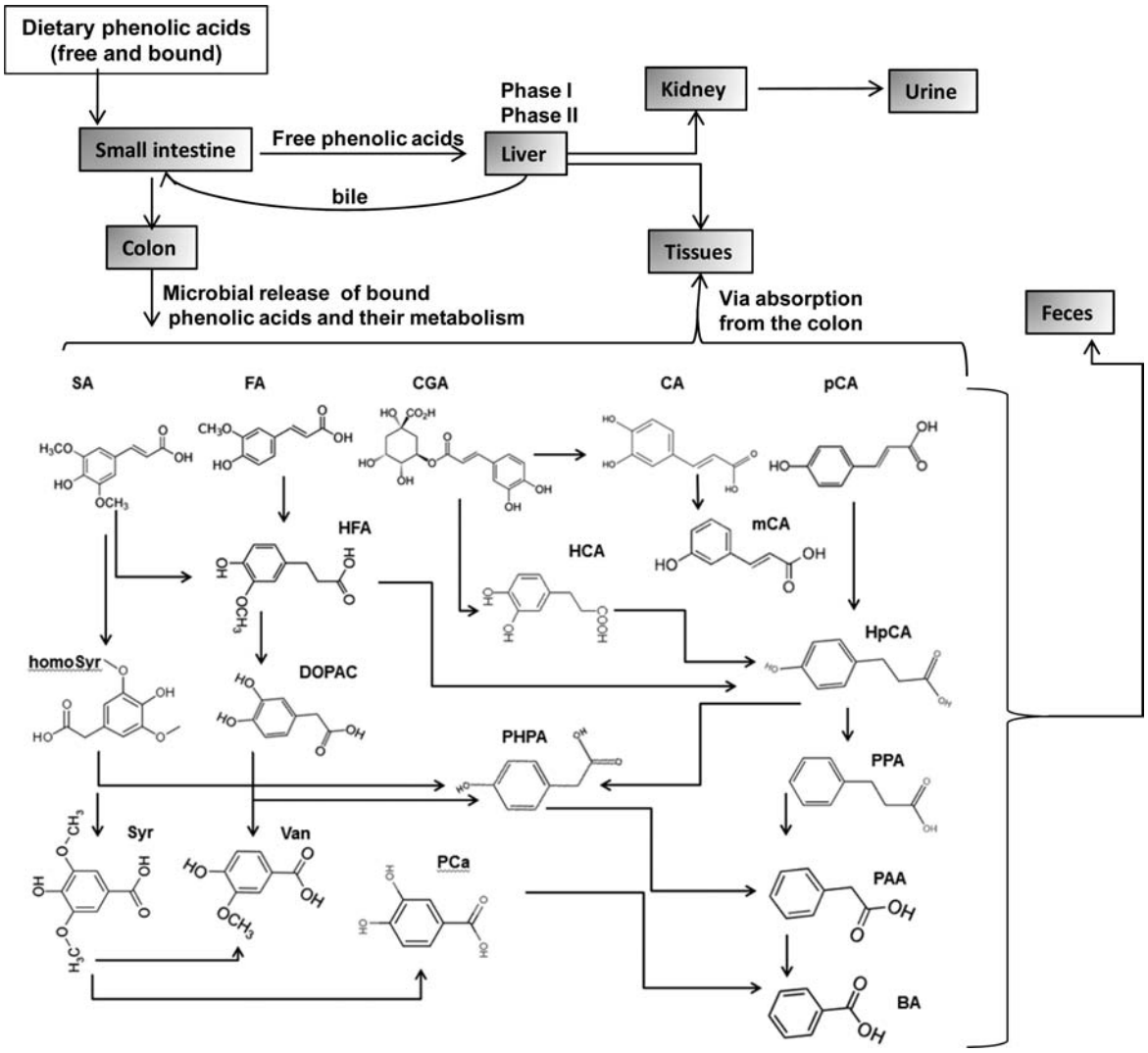

Figure 1. Structures of caffeic, ferulic, *p*-coumaric, and sinapic acids and their major colonic metabolites. For abbreviations, refer to Table 1.

Moreover, extrusion can also improve the accessibility of bound phenolic acids to alkaline hydrolysis,[7] probably due to an increased solubility of dietary fiber.[26] The levels of bound *p*-CA, FA, 8-*O*-4-DFA, 5,5′-DFA, and 8,5-DFA were therefore 54, 12, 15, 12, and 33%, respectively, higher (Table 3) in the OE diet than in the OU diet. This may also be attributed to the decreased level of insoluble arabinoxylans in oat after extrusion (Table 2). However, the total content of bound phenolic acids was decreased in barley diets following extrusion. The reason for this is not understood. One explanation could be the increased content of insoluble AX in extruded whole grain barley, reducing the accessibility of bound phenolic acids to alkaline hydrolysis (Table 2). Thus, the content of bound phenolic acids in the BE diet was 11% lower than in the BU diet, whereas the content of bound phenolic acids in the OE diet was 9% higher compared with that in the OU diet.

Growth Rate, Body Weight, Feed Intake: Growth Performance Parameters. The growth performance parameters (ADFI, ADG, and feed conversion ratio (F:G) presented in Table 4 were within expected ranges for growing pigs, and all pigs were in good health during the experimental period.

Effect of Extrusion of Barley and Oat on Total Tract Bioaccessibility of Phenolic Acids. The total availability of phenolic acids to absorption in any site of the gastrointestinal tract, referred to as the total tract bioaccessibility (TTB), was estimated using the eq 1. The TTB data for total bound phenolic acids and total free phenolic acids consumed with experimental diets are shown in Table 5. The TTB values for bound phenolic acids increased significantly for BE and OE diets compared to respective control BU and OU diets. Moreover, TTB calculations performed for individual bound phenolic acids showed that the increase in total TTB was mainly due to a significant increase in the bioaccessibility of dimers of ferulic acids (DFA). The bioaccessibility of bound 5,5′-DFA, 8-*O*-4-DFA, and 8,5-DFA increased by 24, 96, and 31%, respectively, in extruded diets, whereas total bioaccessibility of bound CA, FA, *p*-CA, and SA increased by only around 7%. TTB values for free phenolic acids also increased, but this effect was less significant.

Effects of Extrusion of Barley and Oat on the Level of Phenolic Acids and Their Metabolites in Plasma. The increased values of TTB for bound phenolic acids (Table 5) indicate their improved availability in BE and OE diets for absorption in the gastrointestinal tract. However, increased TTB values do not necessarily reflect increased levels of phenolic acids in the systemic circulation.

It is known that free phenolic acids absorbed in the upper intestinal tract can be modified by xenobiotic metabolizing

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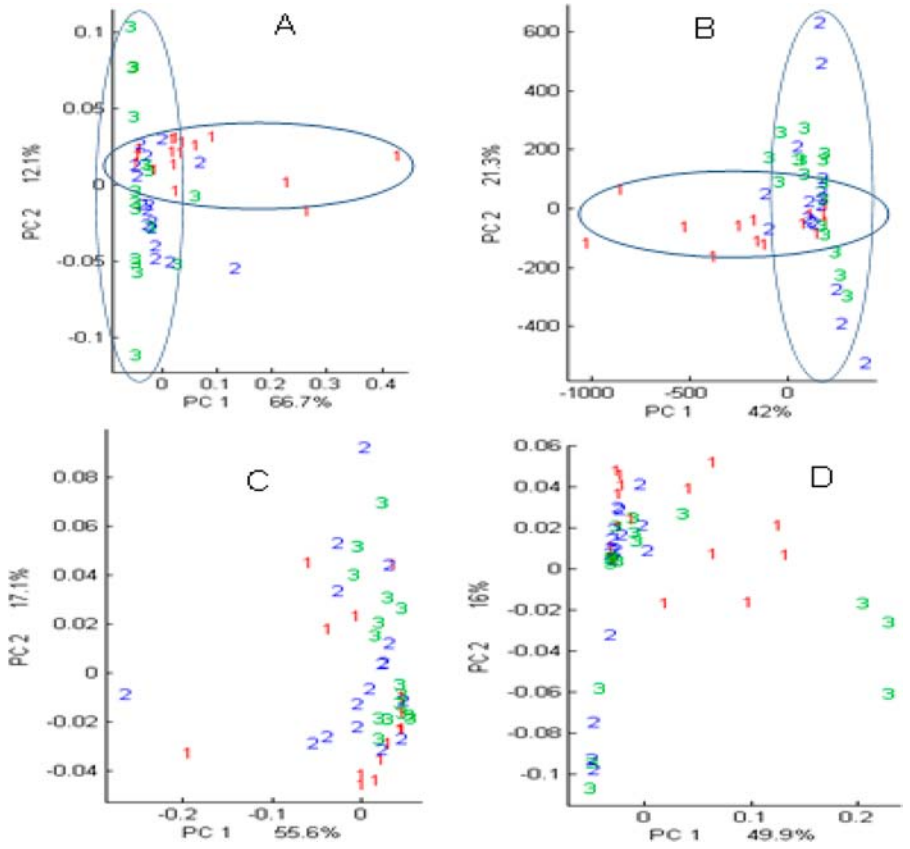

Figure 2. Principal component analysis (PCA) score plots. A total of 48 plasma samples collected from 4 growing pigs before (1), after 1 weeks' (2), and after 3 weeks' (3) consumption of experimental diets BE (A) and OE (B) and control diets BU (C) and OU (D).

enzymes such as CYP450 (phase I) and/or by conjugating enzymes of phase II biotransformation (UDP-glucuonosyltransferases and phenolsulfotransferases) (Figure 1). These metabolites can be excreted into the intestine as components of bile and, together with the phenolic acids released from cereal fiber by the action of bacterial enzymes in the colon, can be further metabolized before reabsorption.[28] Moreover, a number of studies have shown that the majority of phenolic metabolites detected in biological samples are mainly derived from the metabolism of phenolic compounds in the colon and/or from their phase I and II biotransformation. In turn, the level of ingested phenolic compounds themselves is usually much lower in plasma samples.[9] Therefore, in this study phenolic acids and 14 major metabolites (Table 1 and Figure 1), the result of colonic metabolism of both free and bound phenolic acids, were identified and quantified in plasma samples collected at the start of the experiment, after the adaptation period (1 week), and at the end of the experiment (3 weeks) (data not shown). PCA was used for the interpretation of the data due to the large number of samples analyzed and their complexity (Figure 2).

As seen in Figure 2, the PCA score plot based on the concentrations of phenolic metabolites in the plasma samples separated clearly plasma samples collected at the start and at the end of the experimental period for the groups BE and OE. The PCA loading plot (Figure 3) shows that this separation was mainly due to the changes in concentrations of phenylpropionic acid (PPA) and *p*-hydroxyphenylacetic acid (PHPA), products of colonic transformation of cereal phenolic acids as demonstrated in other studies.[15,17,29] Similar results

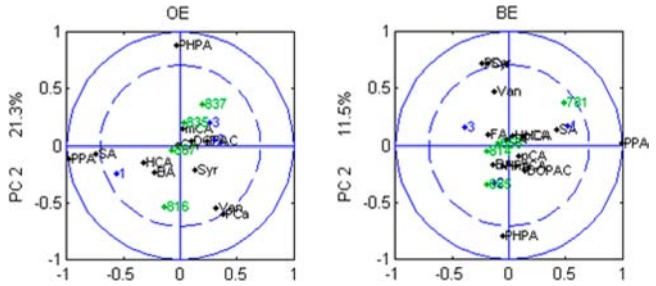

Figure 3. Loading plots showing all 18 phenolic acids (for abbreviations, refer to Table 1) quantified in 48 plasma samples collected from 4 growing pigs before (1), after 1 weeks' (2), and after 3 weeks' (3) consumption of experimental diets BE (A) and OE (B) and control diets BU (C) and OU (D) and their impact on the PCA results (Figure 2).

were obtained by Anson et al.[27] when using a human colonic in vitro model (TIM-2) to study the colonic metabolism of the phenolic compounds in the nonbioaccessible fraction of wheat bran breads. Bran bioprocessing increases colonic end metabolite 3-phenylpropionic acid (PPA). Anson et al.[30] followed the human plasma blood concentrations of phenolic acids and metabolites for 24 h after consumption of 300 g of whole wheat bread containing native bran (control bread) or bioprocessed bran (bioprocessed bread). The bioavailabilities of ferulic acid (FA), vanillic acid (Van), sinapic acid (SA), and 3,4-dimethoxybenzoic acid from the bioprocessed bread were 2−3-fold those from the control bread. In our study we were not able to include 3,4-dimethoxybenzoic acid as a standard (see Table 1). After 3 weeks of consumption of extruded barley

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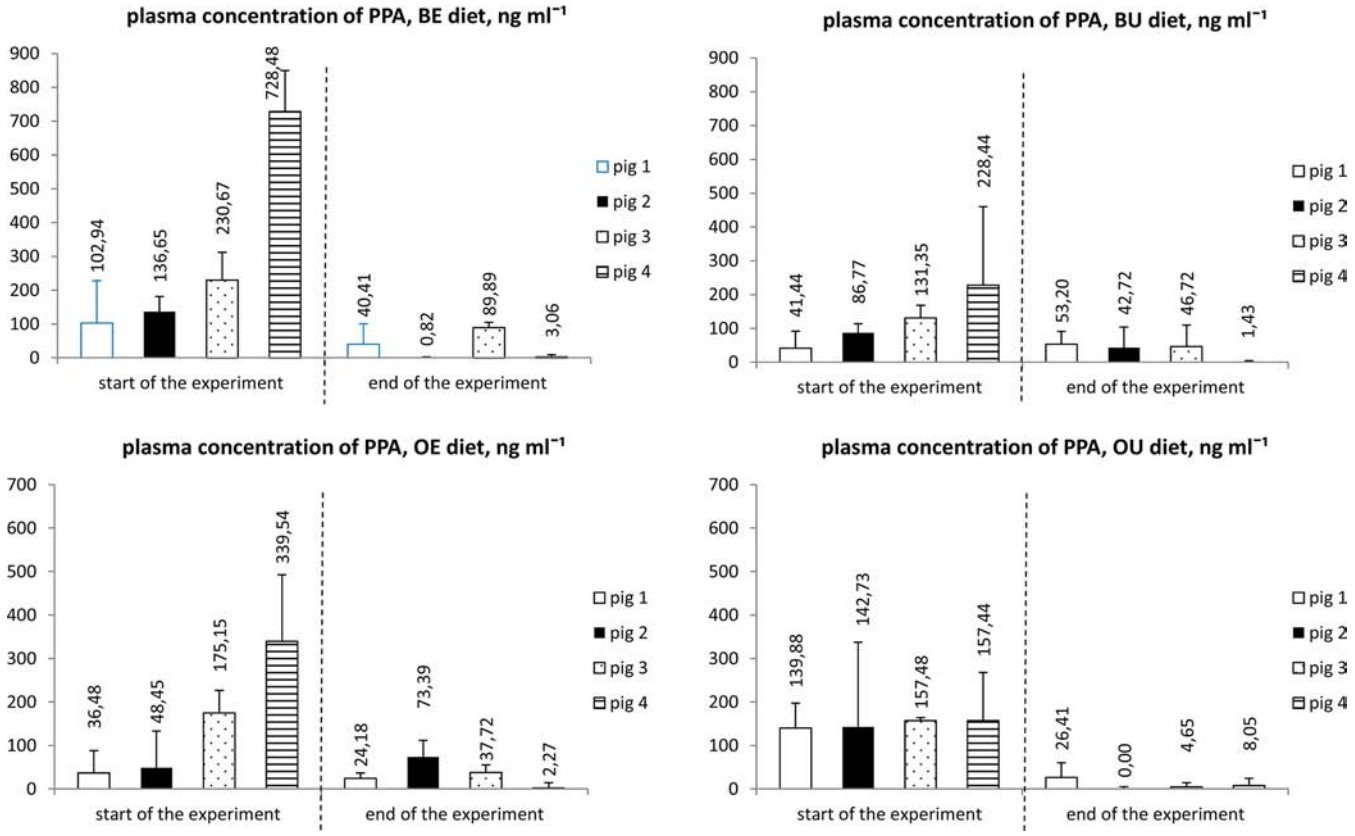

Figure 4. Plasma concentration of PPA for each pig in the group collected at the start and at the end of the experiment.

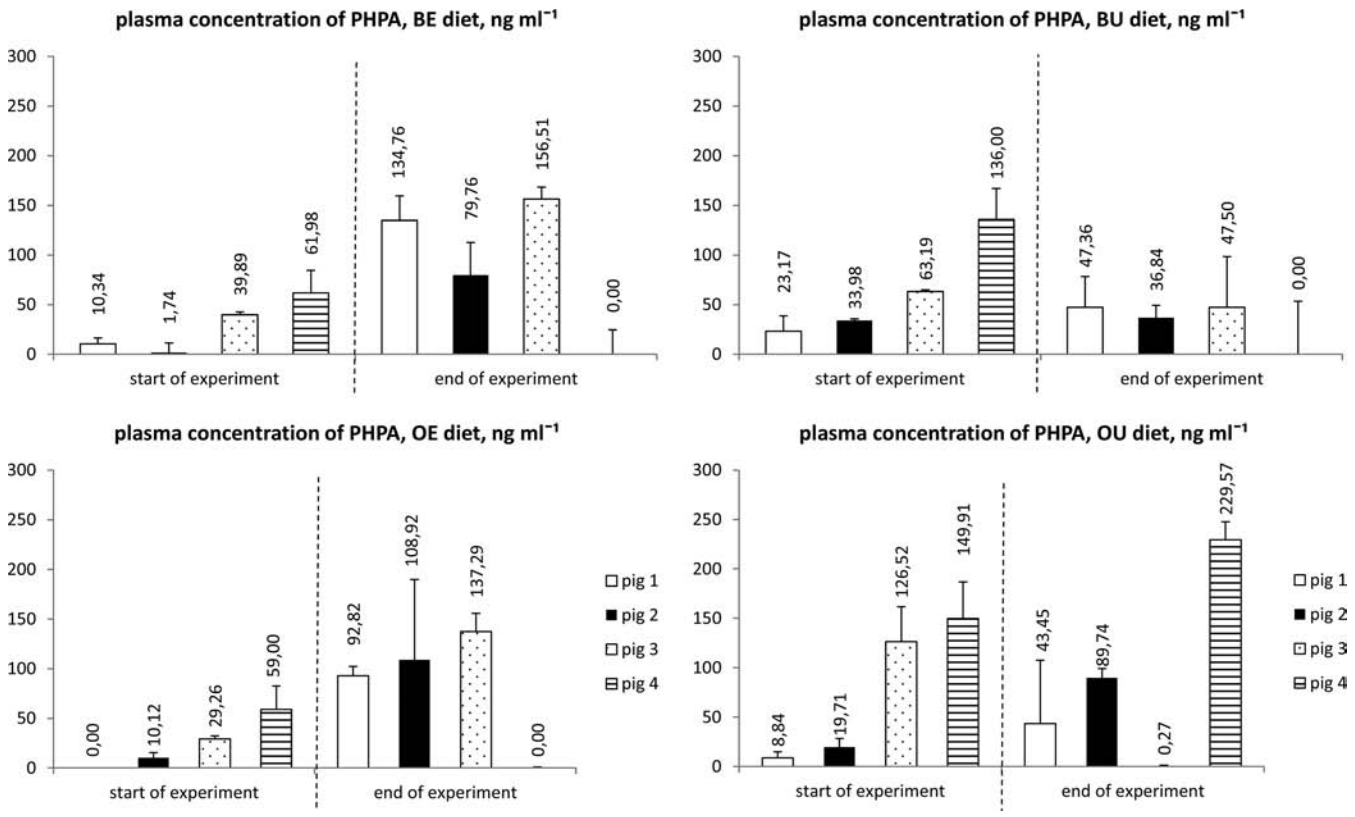

Figure 5. Plasma concentration of PHPA for each pig in the group collected at the start and at the end of the experiment.

and oat, no increase in pig plasma blood concentrations of FA, Van, and SA was observed. In Figure 1 the metabolic route for production of PPA and PHPA is represented. PPA and PHPA were also the abundant phenolic acid components in plasma at

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the end of the experimental period detected in average concentrations of 90 and 69 ng mL^{-1}, respectively. For three pigs in the OE and BE diet groups, a reduction of PPA level between 61 and 99% was detected in plasma samples at the end of the experimental period, whereas the plasma concentration of PHPA was increased more than 300% for at least three pigs in the BE and OE diet groups (Figures 4 and 5). No similar effect was observed for pigs in the control groups given OU and BU diets (Figures 2, 4, and 5).

However, changes in the plasma levels of the other 16 metabolites were not significantly affected by the treatments. In comparison, the studies of Stalmach et al.[31] and Rechner et al.[9] on gut fermentation products of ingested phenolic acids in plasma documented an increased level of dihydroferulic (HFA) and dihydrocaffeic (HCA) acids. It is known that the concentration of specific phenolic metabolites from colonic transformation of hydroxycinnamic acids is time-dependent.[32] The later the fermentation product reaches its maximum concentration in the large intestine, the later it reaches its maximum level in plasma due to a subsequent absorption delay. Plasma levels of HFA and HCA are known to peak 5−6 h after ingestion of phenolic acids,[31] whereas concentrations of PHPA have been shown to increase even 24 h after intake.[32] The plasma samples analyzed in our study were collected 17 h after feed intake, when HFA and HCA presumably can be detected in only low concentrations.

Conclusions. In summary, the bioaccessibility of phenolic acids was greater in growing pigs fed extruded whole grain barley and oat groats than in pigs receiving nonextruded whole grain barley and oat groats. The difference is due to the extrusion process, which affects the content of both free and bound phenolic acids in the diets. Interestingly, both free and bound phenolic acids were more bioaccessible in extruded oat groat diets compared with extruded whole grain barley diets, presumably due to a higher content of insoluble dietary fiber in whole grain barley diets, and as a consequence the diet was less fermentable in the colon. Thus, both extrusion and dehulling/pearling processes of cereal grains should be considered in cereal consumption for the improvement of the bioaccessibility of dietary phenolic acids.

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Notes

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