Electronic Supplementary Material (ESI) for Food & Function.

Supplemental Table 1. Purified standard diet (SD) composition and nutrient information.

FORMULA	**g/Kg**
Casein	200
L-cystine	3.00
Corn starch	397
Maltodextrin	132
Sucrose	100
Soybean oil	70.0
Cellulose	50.0
Mineral mix, AIN-93G-MX (94046)	35.0
Vitamin Mix, AIN-93-VX (94047)	10.0
Choline bitartrate	2.50
TBHQ, antioxidant	0.01

Purified STD diet	**% by weight**	**% Kcal from**
Protein	17.7	18.8
Carbohydrate	60.1	63.9
Fat	7.20	17.2
Kcal/g	3.80	

Whole grain barley	**% by weight**	**% Kcal from**
Protein	13.6	14.7
Total carbohydrate*	75.0	81.3
Total fat	1.60	3.90
Kcal/g	3.69	
Ash	1.60	
Moisture	8.20	
Major dietary fiber (β-glucan + arabinoxylans)	12.2	

*Carbohydrate by calculation = (100-Ash%-total fat%-protein%-moisture%).

The moisture content was evaluated using the Moisture Tester MT-CA (Brabender GmbH&Co KG, Duisburg, Germany). The ash content was determined by incineration (ISO 2171:2023). The Dumas combustion method was used to quantify the nitrogen contents using a Nitrogen/Protein Analyzer (CN628, LECO Corporation, St. Joseph, MI, U.S.A.) following AACC Method 46–30.01. The nitrogen contents were converted to crude protein by multiplying with a conversion factor of 5.88 (ISO 16634-1: 2008). Soxhlet method was applied for the determination of total fat, adapted by the Randall extraction method (ISO 11085:2015). Briefly, 3 g sample were extracted with hexane in the Det-Gras device (JP SELECTA, Spain), the solvent was evaporated, the extracted fat was dried, and the % fat was obtained by gravimetry. The β-glucan and arabinoxylan contents were determined by means of the mixed-linkage β-glucan assay (K-BGLU) and D-xylose assay (K-XYLOSE) kits from Megazyme (Wicklow, Ireland).

Supplemental Table 2. SRM conditions used for the quantification of (poly)phenolic compounds in barley samples and its generated metabolites in urine samples.

(Poly)phenolic compounds and its generated metabolites	SRM quantification	CV (V) / CE (eV)
Benzene-1,2-diols		
Benzene-1,2-diol-*O*-sulphate	189 > 109	20 / 15
Methyl benzene-1,2-diol-*O*-sulphate	203 > 123	20 / 15
Benzoic acids		
4-Hydroxybenzoic acid	137 > 93	30 / 15
Hydroxybenzoic acid	137 > 93	30 / 15
4-Hydroxybenzoic acid-*O*-sulphate	217 > 137	35 / 15
Hippuric acid	178 > 134	40 / 10
3,4-Dihydroxybenzoic acid	153 > 109	45 / 15
3,4-Dihydroxybenzoic acid-*O*-sulphate	233 >153	35 / 15
4-Hydroxy-3-methoxybenzoic acid	167 > 123	30 / 10
4-Hydroxy-3-methoxybenzoic acid-*O*-sulphate	247 > 167	30 / 25
3,5-Dimethoxy-4-hydroxybenzoic acid	197 > 182	30 / 10
Hydroxycinnamic acid derivatives		
Cinnamic acid	147 > 103	45 / 15
Hydroxycinnamic acid (2'-,4')	163 > 119	35 / 10
Hydroxycinnamic acid-*O*-sulphate	243 > 163	35 / 15
3',4'-Dihydroxycinnamic acid	179 > 179	35 / 15
3',4'-Dihydroxycinnamic acid-*O*-sulphate	259 > 179	35 / 15
4'-Hydroxy-3'-methoxycinnamic acid	193 > 134	30 / 15
3'-Hydroxy-4'-methoxycinnamic acid	193 > 134	30 / 15
3,5-dimethoxy-4-hydroxycinnamic acid	223 > 164	45 / 15
4'-Hydroxy-3'-methoxycinnamic acid glycine	250 > 149	40 / 15
8/5-5'-Diferulic acid	385 > 341	40 / 15
Diferulic acid (decarboxylated form)	341 > 282	40 / 15
Triferulic acid	577 > 355	50 / 20
4'-Hydroxy-3'-methoxycinnamic acid-*O*-sulphate	273 > 193	40 / 10
3'-Hydroxy-4'-methoxycinnamic acid-*O*-sulphate	273 > 193	40 / 10
4'-Hydroxy-3'-methoxycinnamic acid-*O*-glucuronide	369 > 193	40 / 10
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	195 > 135	35 /15
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid-*O*-sulphate	275 > 195	35 / 15
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid-*O*-glucuronide	371 > 195	35 / 15
Flavan-3-ols		
Catechin	289 > 245	45 / 15
Catechin-*O*-glucoside	451 > 289	45 / 15
Procyanidin diglucoside	613 > 451	45 / 15
Procyanidin B2	577 >289	45 / 20
Prodelphinidin B3 (GC-C)	593 > 289	45 / 20
Procyanidin B3 (C-C)	577 > 289	45 / 20
Prodelphinidin B4 (GC-C)	593 > 289	45 / 20
Procyanidin C2 (C-C-C)	865 > 289	60 / 30
Prodelphinidin C2 (GC-GC-C)	897 > 593	60 / 25
C-C-GC/GC-C-C	881 > 577	60 / 25
C-GC-C	881 > 593	60 / 25
Catechin-*O*-sulphate	369 > 289	40 / 20
Epicatechin-*O*-sulphate	369 > 289	40 / 20
Methyl catechin-*O*-sulphate	383 > 303	40 / 15
Methyl epicatechin-*O*-sulphate	383 > 303	40 / 15
Methyl catechin-*O*-glucuronide	479 > 303	40 / 20
Methyl epicatechin-*O*-glucuronide	479 > 303	40 / 20
Phenyl-γ-valerolactone derivatives		
5-(4'-Hydroxyphenyl)-γ-valerolactone-*O*-sulphate	271 > 191	40 / 20
5-(3',4'-Dihydroxyphenyl)-γ-valerolactone-*O*-sulphate	287 > 207	40 / 15
5-(3',4'-Dihydroxyphenyl)-γ-valerolactone-*O*-glucuronide	383 > 207	40 / 20
5-(3',4'-Dihydroxyphenyl)-γ-valerolactone-*O*-sulphate-*O*-glucuronide	463 > 207	40 / 20
Phenylacetic acids		
Hydroxyphenylacetic acid-*O*-glucuronide	327 > 151	20 / 15
Dihydroxyphenylacetic acid-*O*-sulphate	247 > 167	30 / 15
Phenylpropanoic acids		
Hydroxyphenylpropanoic acid-*O*-sulphate	245 > 165	35 / 15
Hydroxyphenylproppanoic acid-*O*-glucuronide	341 > 165	40 / 25
Dihydroxyphenylpropanoic acid-*O*-sulphate	261 > 181	40 / 15

Flavone glucosides		
Isoorientin (luteolin-6-C-glucoside)	447 > 357	50 / 20
Isovitexin-*O*-glucoside	593 > 431	60 / 20
Isovitexin-*O*-rutinoside	739 > 431	60 / 20
Apigenin-*O*-glucoside	431 > 269	45 / 15
Apigenin-6-*C*-arabinoside-8-*C*-glucoside	563 > 353	60 / 20
Isoscoparin-7-*O*-glucoside	623 > 341	60 / 20
Isoscoparin-7-*O*-rutinoside	769 > 461	60 / 20
Luteolin-*O*-glucoside	447 > 285	45 / 25
Anthocyanins *		
Cyanidin-3-*O*-arabinoside	419 > 287	40 / 20
Cyanidin-3-*O*-glucoside	449 > 287	40 / 20
Cyanidin-*O*-malonylglucoside	593 > 287	40 / 15
Cyanidin-*O*-dimalonylglucoside	621 > 287	40 / 20
Peonidin-3-*O*-glucoside	463 > 301	40 / 20
Peonidin-*O*-malonylglucoside	549 > 301	40 / 20
Peonidin-*O*-dimalonylglucoside	635 > 301	40 / 20
Peonidin-3-*O*-glucuronide	477 > 301	40 / 20
Delphinidin-3-*O*-glucoside	465 > 303	40 / 20
Pelargonidin-3-*O*-glucoside	463 > 271	40 / 20
Pelargonidin-*O*-malonylglucoside	519 > 271	40 / 25
Malvidin-3-*O*-glucoside	493 > 331	40 / 20

CV: Cone voltage; CE: Collision energy; Anthocyanins*: positive ionization

Supplemental Table 3. Instrumental response of the main generated (poly)phenolic metabolites in mice urine samples after the sustained intake of standard diet (SD) and the barley supplemented diet (WGB).

(Poly)phenolic metabolites	SD	WGB	% Signal increase
Anthocyanins			
Cyanidin-3-*O*-glucoside	n.d.	887	100
Cyanidin-3-*O*-6”-*O*-malonylglucoside	n.d.	2059	100
Cyanidin-3-*O*-(3”,6”)-dimalonylglucoside	n.d.	1577	100
Peonidin-3-*O*-glucoside	n.d.	3241	100
Peonidin-3-*O*-6”-*O*-malonylglucoside	n.d.	4642	100
Peonidin-3-*O*-(3”,6”)-dimalonylglucoside	n.d.	2108	100
Peonidin-3-*O*-glucuronide	n.d.	4939	100
Pelargonidin-3-*O*-6”-*O*-malonylglucoside	n.d.	181	100
Benzene-1,2-diols			
Benzene-1,2-diol-*O*-sulphate (*aka* catechol)	2478	37994	93
Methyl benzene-1,2-diol-*O*-sulphate (*aka* Me catechol S)	29711	41710	29
Phenolic acids			
Hydroxybenzoic acid-*O*-sulphate	7096	24923	72
3,4-Dihydroxybenzoic acid-*O*-sulphate (*aka* PCA S)	5632	54770	90
3,5-Dimethoxy-4-hydroxybenzoic acid (*aka* Syr)	138	449	69
4-Hydroxy-3-methoxybenzoic acid-*O*-sulphate (*aka* VA S)	1450	11944	88
Hippuric acid	49173	299964	84
4’-Hydroxycinnamic acid-O-sulphate (aka coumaric acid S)	9945	20200	51
3’,4’-Dihydroxycinnamic acid (*aka* CA)	27087	40975	34
3’,4’-Dihydroxycinnamic acid-O-sulphate (*aka* CA S)	n.d.	3804	100
4’-Hydroxy-3’-methoxycinnamic acid (*aka* FA)	169	454	63
4’-Hydroxy-3’-methoxycinnamic acid-O-sulphate (*aka* FA S)	24814	133015	81
3’-Hydroxy-4’-methoxycinnamic acid-O-sulphate (*aka* isoFA S)	3345	10235	67
4’-Hydroxy-3’-methoxycinnamic acid-O-glucuronide (*aka* FA G)	91	667	86
3-(4’-Hydroxy-3’-methoxyphenyl)propanoic acid (*aka* dihydroFA)	n.d.	154	100
3-(4’-Hdroxy-3’-methoxyphenyl)propanoic acid-O-sulphate (*aka* dihydroFA S)	3366	85240	96
3-(4’-Hydroxy-3’-methoxyphenyl)propanoic acid-O-glucuronide (*aka* dihydroFA G)	n.d.	458	100
4’-Hydroxy-3’-methoxycinnamic acid glycine (*aka* FA Gly)	n.d.	1000	100
3,5-Dimethoxy-4-hydroxycinnamic acid (sinapic acid)	n.d.	862	100
Hydroxyphenylacetic acid-O-glucuronide	n.d.	273	100
Dihydroxyphenylacetic acid-O-sulphate	392	8258	95
Hydroxyphenylpropanoic acid-O-sulphate	27786	779707	96
Hydroxyphenylpropanoic acid-*O*-glucuronide	n.d.	234	100
Dihydroxyphenylpropanoic acid-*O*-sulphate	2750	24923	91
Flavan-3-ols			
Catechin-*O*-sulphate	n.d.	1651	100
Epicatechin-*O*-sulphate	n.d.	776	100
Methyl catechin-*O*-sulphate	n.d.	1174	100
Methyl epicatechin-*O*-sulphate	n.d.	1297	100
Methyl catechin-*O*-glucuronide	n.d.	1429	100
Methyl epicatechin-*O*-glucuronide	n.d.	5612	100
5-(4’-Hydroxyphenyl)-ɣ-valerolactone-*O*-sulphate	n.d.	35573	100
5-(3’,4’-Dihydroxyphenyl)-ɣ-valerolactone-*O*-sulphate	n.d.	380911	100
5-(3’,4’-Dihydroxyphenyl)-ɣ-valerolactone-*O*-glucuronide	n.d.	2618	100
5-(3’,4’-Dihydroxyphenyl)-ɣ-valerolactone-*O*-sulphate-*O*-glucuronide	n.d.	2089	100

The percentage increase in instrumental response is calculated by taking the difference between the barley instrumental response and the standard diet instrumental response, dividing this difference by the barley instrumental response, and then multiplying by 100.