

Table S1 Compositions analysis of HB, HB-1, HB-2 and HB-3

Name	Method	Standard
β -glucan (g/100g)	Ultraviolet spectrophotometry method	/
Total polysaccharides (g/100g)	Phenol-sulfate method	DB12/T 847-2018
Dietary fiber (g/100g)	Enzyme hydrolysis	GB 5009.88-2014
Total anthocyanin (mg/g)	pH-differential method	/
Free phenol (g/100g)	Colorimetric method	/
Combined phenol (g/100g)	Colorimetric method	/
Moisture (g/100g)	Direct drying method	GB 5009.3-2016
Ash (g/100g)	Direct ignition method	GB 5009.4-2016
Protein (g/100g)	Kjeldah method	GB 5009.5-2016
Fat (g/100g)	Soxhlet extraction method	GB 5009.6-2016
Total starch (g/100g)	Acid hydrolyzation	GB 5009.9-2016

HB: Highland barley; HB-1: Microwave fluidized highland barley; HB-2: Extruded and puffed highland barley; HB-3: Ultrafine pulverized highland barley.

Table S2 Mouse basic diet composition and nutrition level

Product	NCG		FG		HBG		HB-1		HB-2		HB-3	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	19	20	22.62	19.97	22.62	19.97	22.62	19.97	22.62	19.97	33.04%	27.68%
Carbohydrate	67	70	45.51	40.18	45.51	40.18	45.51	40.18	45.51	40.18	20.41%	17.10%
Fat	4	10	20.06	39.85	20.06	39.85	20.06	39.85	20.06	39.85	29.30%	55.22%
Total	/	100	/	100	/	100	/	100	/	100		100
kcal/gm	3.85	/	4.53	/	4.53	/	4.53	/	4.53	/	4.78	
Ingredient	gm	kcal	gm	kcal	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800	169	676	148	592	120	480	87	348
L-Cystine	3	12	3	12	3	12	3	12	3	12	3	12
Corn Starch	386.15	1544.6	212	848	0	0	0	0	0	0	0	0
Maltodextrin	125	500	71	284	0	0	0	0	0	0	0	0
Sucrose	200	800	124.41	497.64	124.41	497.64	124.41	497.64	124.41	497.64	124.41	497.64

Sample Fat	0	0	0	0	6	54	11	99	12	108	28	252
Sample Protein	0	0	0	0	31	124	52	208	80	320	113	452
Sample starch	0	0	0	0	283	1132	283	1132	283	1132	0	0
Cellulose	50	0	50	0	50	0	50	0	50	0	50	0
Soybean Oil	25	225	25	225	19	171	14	126	13	117	0	0
Lard	20	180	155	1395	155	1395	155	1395	155	1395	152	1368
Mineral Mix S10020	5	0	5	0	5	0	5	0	5	0	5	0
Calcium Phosphate	13	0	13	0	13	0	13	0	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0	5.5	0	5.5	0	5.5	0	5.5	0
Potassium Citrate,1 H ₂ O	16.5	0	16.5	0	16.5	0	16.5	0	16.5	0	16.5	0
Sodium Chloride	2.59	0	2.59	0	2.59	0	2.59	0	2.59	0	2.59	0
Vitamin Mix V10001C	1	4	1	4	1	4	1	4	1	4	1	4
Choline Bitartrate	2	0	2	0	2	0	2	0	2	0	2	0

Cholesterol	0	0	11.25	0	11.25	0	11.25	0	11.25	0	11.25	0
FD&C Yellow Dye # 5	0	0	0	0	0	0	0	0	0	0	0	0
FD&C Red Dye # 40	0	0	0.05	0	0.05	0	0.05	0	0.05	0	0.05	0
FD&C Blue Dye # 1	0.1	0	0.05	0	0.05	0	0.05	0	0.05	0	0.05	0
Total	1054.84	4065.6	897.35	4065.64	897.35	4065.64	897.35	4065.64	897.35	4065.64	614.35	2933.64

All mice AIN-93M diet, XT108C HFCD and XT108C mixed HFCD were provided by Jiangsu Synergy Medicine Bioengineering Co., Ltd (Jiangsu, China). HB, HB-1, HB-2 and HB-3 XT108C mixed HFCD were produced according to components in 4 HB including fat, protein and starch. The grouping arrangement was as follows:

Normal control group (NCG, AIN-93M diet).

XT108C HFCD group (FG).

Highland barley group (HBG, XT108C HFCD + Highland barley).

Fluidized highland barley group (HB-1, XT108C HFCD + Microwave fluidized highland barley).

Extruded and puffed highland barley group (HB-2, XT108C HFCD + Extruded and puffed highland barley).

Ultrafine pulverized highland barley group (HB-3, XT108C HFCD + Ultrafine pulverized highland barley).

Table S3 Compositions levels of HB, HB-1, HB-2 and HB-3

Samples	β -glucan (g/100g)	Total polysaccharides (g/100g)	Dietary fibers (g/100g)	Total anthocyanins (mg/g)	Free phenols (g/100g)	Bound phenols (g/100g)	Moisture (g/100g)	Ash (g/100g)	Protein (g/100g)	Fat (g/100g)	Total starch (g/100g)
HB	4.01 ± 0.012^d	51.39 ± 2.34^{cd}	17.42 ± 0.29^b	0.0095 ± 0.003^b	318.07 ± 1.42^c	952.76 ± 2.00^b	9.89 ± 0.418^{ab}	1.15 ± 0.072^d	11.10 ± 0.721^b	2.68 ± 0.111^b	63.19 ± 1.243^a
		56.99 ± 1.45^a	19.88 ± 0.30^a	0.0328 ± 0.001^b	465.23 ± 6.70^a	1249.35 ± 1.31^a	5.12 ± 0.238^c	1.31 ± 0.043^{bc}	11.06 ± 0.057^b	2.59 ± 0.009^b	56.13 ± 0.976^c
HB-1	5.06 ± 0.09^a	54.20 ± 1.89^b	14.63 ± 0.44^c	0.0084 ± 0.001^b	346.50 ± 6.29^b	1235.99 ± 4.33^a	10.46 ± 0.203^a	1.56 ± 0.023^{bc}	4.48 ± 0.329^c	2.50 ± 0.057^c	61.01 ± 0.696^b
		50.25 ± 2.56^d	16.96 ± 0.21^b	0.0564 ± 0.001^a	345.51 ± 5.25^b	845.39 ± 3.69^c	8.42 ± 0.139^b	2.14 ± 0.002^a	14.21 ± 0.360^a	6.81 ± 0.117^a	55.40 ± 1.155^c
HB-2	4.21 ± 0.045^c										
HB-3	4.99 ± 0.023^b										

HB: Highland barley; HB-1: Microwave fluidized highland barley; HB-2: Extruded and puffed highland barley; HB-3: Ultrafine pulverized highland barley.

Table S4 Statistics data of important differential metabolites between HB-1 and FG in metabolomics

Name	VIP	Fold Change	log2(FC)	<i>P</i> -value	FDR
Deoxycholic acid	1.6597419	534380000	28.993	0.0253699	0.7251173
Myclobutanil	1.8242696	7026900	22.744	0.007495	0.525878
Dibutyl phthalate	1.0234929	24.65	4.6235	0.0367139	0.7251173
Pseudouridine	1.4690636	21.665	4.4373	0.0121858	0.6741645
2-Methoxyestrone	1.7488782	17.659	4.1424	0.0367139	0.644535
3-Hydroxyflavone	1.7655092	16.924	4.081	0.0121858	0.6741645
Primaquine	2.2552686	10.288	3.3629	0.0121858	0.525878
Retinoyl b-glucuronide	1.8110645	9.8694	3.303	0.0367139	0.644535
Phenylacetic acid	1.5401845	9.8256	3.2965	0.0215717	0.6797544
1,2-Bis-O-sinapoyl-beta-D-glucose	1.6607275	8.7885	3.1356	0.0215717	0.6797544
(3S,5S)-Carbapenam-3-carboxylic acid	2.0035514	8.5773	3.1005	0.0121858	0.525878
Melphalan	1.5080654	8.5744	3.1	0.0121858	0.525878
3-Methylindole	1.2927387	8.19	3.0339	0.0215717	0.6113888
Alpha-Linolenic acid	2.3540227	7.9162	2.9848	0.0121858	0.6741645
Prednisone	2.425408	6.7697	2.7591	0.0121858	0.525878

Yohimbine	2.4254812	6.0324	2.5927	0.0121858	0.6741645
9,10-Epoxyoctadecenoic acid	2.3254404	5.9362	2.5695	0.0215717	0.6797544
Pyroglutamic acid	1.5998864	5.5753	2.479	0.0367139	0.644535
Uridine	2.0427261	5.3634	2.4232	0.0367139	0.7251173
13S-hydroxyoctadecadienoic acid	1.9249181	5.1117	2.3538	0.0367139	0.644535
Juvenile hormone III acid	1.8216045	5.0885	2.3472	0.0367139	0.644535
trans-Ferulic acid	1.7673251	4.9338	2.3027	0.0367139	0.7251173
Eicosadienoic acid	2.2603013	4.8641	2.2822	0.0215717	0.6113888
Abscisic acid glucose ester	2.0873504	4.7405	2.245	0.0215717	0.6797544
Traumatic Acid	2.6050778	4.5757	2.194	0.0121858	0.525878
L-Gulose	2.1917674	4.3023	2.1051	0.0121858	0.6741645
D-Mannose 1-phosphate	2.2697046	4.2638	2.0921	0.0215717	0.6797544
11Z-Eicosenoic acid	2.3234835	4.2408	2.0843	0.0215717	0.6113888
Fluvoxamine	2.3140472	4.0738	2.0264	0.0215717	0.6113888
Qing Hau Sau	1.5552796	4.0177	2.0064	0.0367139	0.644535
Neocembrene	2.221389	3.5167	1.8142	0.0367139	0.644535
Jasmone	2.5709506	3.3697	1.7526	0.0121858	0.525878

D-Glucuronic Acid	1.5203682	3.2298	1.6914	0.0367139	0.7251173
Dihydrocapsaicin	1.9672658	3.2182	1.6862	0.0121858	0.525878
Prostaglandin E2	2.2017988	3.1558	1.658	0.0215717	0.6113888
N,N-Diethyl-m-toluamide	2.1961334	3.1393	1.6504	0.0367139	0.644535
(R)-Coclaurine	1.9874395	3.1165	1.6399	0.0215717	0.6113888
D-Ribose	1.9202865	3.0659	1.6163	0.0367139	0.7251173
2-Ketobutyric acid	1.9146926	2.9436	1.5576	0.0367139	0.644535
Benzatropine	2.0784541	2.9265	1.5492	0.0367139	0.644535
Enoxacin	2.0164192	2.885	1.5286	0.0367139	0.644535
Enalaprilat	2.2963836	2.7716	1.4707	0.0215717	0.6797544
Gulonic acid	1.7887896	2.7491	1.459	0.0367139	0.7251173
N-Carbamoylputrescine	1.8562015	2.7427	1.4556	0.0367139	0.644535
Adipate semialdehyde	2.5596184	2.6552	1.4088	0.0121858	0.6741645
Phosphorylcholine	1.8740901	2.6364	1.3986	0.0367139	0.644535
3beta,5beta-Ketotriol	1.2770584	2.5977	1.3773	0.0367139	0.644535
Thymol	1.759263	2.5808	1.3678	0.0367139	0.644535
Luteolin	2.1711299	2.4017	1.2641	0.0367139	0.7251173

Gingerol	1.9141183	2.3821	1.2522	0.0121858	0.525878
Propafenone	1.9775482	2.3627	1.2405	0.0367139	0.644535
L-Homophenylalanine	1.4932073	2.3427	1.2282	0.0367139	0.644535
Azelaic acid	2.2869302	2.3236	1.2164	0.0215717	0.6797544
4-Oxoglutaramate	1.2546392	2.2406	1.1639	0.0121858	0.525878
Punicic acid	2.1262048	2.1887	1.1301	0.0215717	0.6113888
(S)-beta-Tyrosine	1.9570738	2.1812	1.1251	0.0367139	0.644535
Gibberellin A3	2.0051878	2.1475	1.1026	0.0367139	0.7251173
Fexofenadine	2.6370433	2.0938	1.0661	0.0121858	0.525878
L-Fuconate	2.2095451	2.0435	1.031	0.0215717	0.6797544
Arachidic acid	1.8551746	2.0109	1.0078	0.0367139	0.7251173
3,5-Diiodo-L-tyrosine	2.2905764	1.9924	0.9945	0.0215717	0.6797544
2-Biphenylol	2.0481549	1.9732	0.98057	0.0215717	0.6113888
Resveratrol	2.0786855	1.8781	0.9093	0.0215717	0.6113888
Plenolin	1.9916162	1.872	0.90456	0.0367139	0.644535
Isochavicol	2.2997776	1.8639	0.89833	0.0215717	0.6113888
o-Xylene	1.7973459	1.7883	0.8386	0.0367139	0.644535

beta-Asarone	1.926198	1.7552	0.81161	0.0367139	0.644535
trans-Isoasarone	2.132304	1.7394	0.79857	0.0121858	0.525878
1D-chiro-Inositol	2.274245	1.7134	0.77686	0.0367139	0.644535
N,N-Dimethylsphingosine	1.9704947	1.7031	0.76814	0.0367139	0.644535
Deoxyinosine	2.2344438	1.6981	0.76391	0.0367139	0.7251173
Arachidonic acid	1.8330845	1.6447	0.71781	0.0367139	0.644535
N6-Acetyl-N6-hydroxy-L-lysine	2.3347895	1.6444	0.71753	0.0121858	0.525878

FG (XT108C HFCD group), HB-1 (XT108C HFCD + Microwave fluidized highland barley).

P-value: Statistically significant difference, $P\text{-value} \leq 0.05$.

VIP: OPLS-DA first principal component variable importance value projection, $VIP \geq 1$.

Fold change: Ploidy change between two groups, Fold change ≥ 1.5 .

Table S5 Metabolic pathway analysis between HB-1 and FG based on significantly differential metabolites

Name	Total	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact	compounds	pathway
Arachidonic acid metabolism	75	15	0.007428	4.9026	1	1	0.13613	C00427;C00584;C05950;C05961;C05964;C06315;C14717;C14732;C14748;C14749;C14773;C14774;C14775;C14794;C14810	mmu00590
ABC transporters	138	7	0.1775	1.7288	1	1	0.050725	C00089;C00121;C00123;C00299;C00719;C05349;C05512	mmu02010
Biosynthesis of unsaturated fatty acids	74	5	0.10013	2.3013	1	1	0.083333	C00219;C06425;C06427;C16525;C16526	mmu01040
Linoleic acid metabolism	28	3	0.065245	2.7296	1	1	0.10526	C00219;C14762;C14825	mmu00591
Ascorbate and aldarate metabolism	50	3	0.23375	1.4535	1	1	0.31818	C00191;C00800;C15923	mmu00053
Bile secretion	97	3	0.63813	0.44922	1	1	0.027523	C00584;C04483;C06999	mmu04976
Steroid hormone biosynthesis	99	3	0.65197	0.42775	1	1	0.034632	C05299;C05497;C18040	mmu00140

Total, the total number of metabolites in the target metabolic pathway; Hits, the number of differential metabolites in the target metabolic

pathway, Raw p, the p value of the hypergeometric distribution test; $-\log(p)$: Negative value for the natural logarithm of the p value; Holm adjust, Holm false positive corrected P value; FDR, false positive corrected value; Impact, metabolic pathway impact value; compounds, metabolite KEGG ID; pathway, metabolite metabolic pathway ID.