

Supplementary Table 1. Nutritional composition of grape polyphenol-soy protein isolate (GP-SPI) complex and SPI (per 100 g)

Diet	SPI	GP-SPI
Calories	386	388
Calories from fat	29	34
Ash (%)	3.9	4.0
Moisture (%)	4.6	3.75
Carbohydrates (%)	0	33.6
Total Dietary Fiber	<0.1	24.9
Protein (%)	89.3	54.9
Total Fat (%)	3.24	3.77
Sugars (%)		
Glucose	<0.1	.84
Fructose	<0.1	1.2
Sucrose	<0.1	<0.1
Total Sugars (%)	0	2.5

Supplementary Table 2. Nutritional Composition of Diets

	HFD with 10% SPI (HFD)	HFD with 10% GP-SPI (HFD-GP)	LFD with 10% SPI (LFD)	LFD with 10% GP-SPI (LFD-GP)
Ingredient (g/kg)				
Casein	158.2	157.6	93.5	98.9
Soy protein, Supro 661	0	39.4	0	28.9
DL- methionine	3.9	3.9	2.84	2.84
GP-SPI	0	100	0	100
SPI	100	0	100	0
Corn starch	6.7	0	484	477.5
Maltodextrin 10	162.1	161.5	118.5	118.5
Sucrose	80.5	80.2	58.9	58.9
Dextrose	0.8	0	0.85	0
Fructose	1.2	0	1.23	0
Cellulose	65.0	40.0	47.4	22.6
Lard	318.0	316.5	19.0	19.0
Soybean oil	30.3	28.0	21.7	19.7
Mineral mix S10026	13.0	12.9	9.5	9.5
Dicalcium phosphate	17.0	16.8	12.3	12.3
Calcium carbonate	7.1	7.1	5.2	5.2
Potassium citrate	21.4	21.3	15.6	15.6
Vitamin Mix V10001	13.0	12.9	9.5	9.5
Choline bitartrate	2.6	2.6	1.9	1.9
Red dye #40, FD&C	0.065	0	0	0.04
Blue dye #1, FD&C	0	0	0.04	0.01
Yellow dye #5, FD&C	0	0.065	0.01	0
Total (g)	1000	1000	1000	1000
Gram%				
Protein	23.2	23.1	17.0	17.0
Carbohydrate	26.4	25.6	67.3	66.6
Dextrose	0.1	0.1	0.1	0.1
Sucrose	9.3	9.3	6.9	6.8
Fructose	0.1	0.1	0.1	0.1
Maltodextrin	16.2	16.1	11.8	11.8
Corn starch	0.67	0	48.4	47.8
Fat	35.3	35.2	4.5	4.5
Fiber	6.5	6.5	4.8	4.7
SPI	10.0	0	10	0
GP-SPI	0	10	0	10
kcal (%)				
Protein	18	18	18	18
Carbohydrate	20	20	71	71
Fat	62	62	11	11
Total	100	100	100	100
kJ/g (calculated)	21.64	21.56	15.87	15.81
kCal/g (calculated)	5.17	5.15	3.79	3.78

HFD: high fat diet; LFD: low fat diet

Supplementary Table 3. Serum Biochemistry

Diet	IL-6 (pg/mL)	Insulin (pg/mL)	Leptin (pg/mL)	PYY (pg/mL)
LFD	6.9 ± 0.2	9818 ± 2839	19769 ± 3815	68.6 ± 26
LFD-GP	6.9 ± 0.2	10037 ± 4462	18071 ± 7893	92.5 ± 41

Values (Mean ± S.D) were not significantly different between groups (unpaired, two-tailed, t-tests)

Supplementary Table 4. TaqMan assay primer sets

TaqMan primer	Assay ID
Il-6	Mm00446190_m1
iNOS	Mm00440502_m1
Occludin	Mm00500912_m1
Tjp1 (ZO-1)	Mm00493699_m1
Glut2	Mm00446229_m1
Gcg	Mm01269055_m1
Pcsk1 (Pc1/3)	Mm00479023_m1
Hmbs	Mm01143545_m1
Tnfa	Mm00443258_m1
Angptl4 (Fiaf)	Mm00480431_m1
Muc2	Mm01276696_m1
Muc3	Mm01207064_m1
Nr1h4 (FXR)	<u>Mm00436419_m1</u>
Gpbar1 (TGR5)	<u>Mm04212121_s1</u>
Fgf15	<u>Mm00433278_m1</u>
Nr0b2 (Shp)	Mm00442278_m1
Smpd3	Mm00491359_m1
Cers4	Mm01212479_m1
Sptlc2	Mm00448878_m1
Cyp7a1	Mm00484152_m1
Cyp8b1	Mm00501637_s1
Cyp27a1	Mm00470430_m1
Cyp7b1	Mm00484157_m1
G6pc	Mm00839363_m1
Pck1	Mm00440636_m1
ldh3a	Mm00499674_m1
Ddit3 (Chop)	Mm00492097_m1
Mlxipl (Chrebp)	Mm02342723_m1
Lbp	Mm00493139_m1
Abcb11	Mm00445168_m1
Slc51b	Mm01175040_m1
Obp2a (Lcn13)	Mm00434315_m1
Fabp6 (Ibabp)	Mm00463685_m1

Supplementary Table 5 - Bile acids

No.	Common name	Abbreviation	Source	Catalogue No.	Molecular Formula	[M-H] ⁻	Rt (min)	Internal standard ^a
1	Tauro- ω -muricholic acid	T ω MCA	Steraloids	C1889-000	C26H45NO7S	514.2833	2.862	GCA
2	Tauro- α -muricholic acid	T α MCA	Steraloids	C1893-000	C26H45NO7S	514.2833	3.130	GCA
3	Tauro- β -muricholic acid	T β MCA	Steraloids	C1899-000	C26H45NO7S	514.2833	3.401	GCA
4	Tauroursodeoxycholic acid	TUDCA	Steraloids	C1052-000	C26H45NO6S	498.2884	5.920	GCA
5	Taurohyodeoxycholic acid	THDCA	Steraloids	C0890-000	C26H45NO6S	498.2884	5.655	GCA
6	Taurocholic acid	TCA	Sigma	T4009	C26H45NO7S	514.2833	5.731	GCA
7	Glycocholic acid (internal std)	GCA	Sigma	G2878	C26H43NO6	464.3007	4.440	-
8	ω -muricholic acid	ω MCA	Steraloids	C1888-000	C24H40O5	407.2792	4.560	GCA
9	α -muricholic acid	α MCA	Steraloids	C1890-000	C24H40O5	407.2792	5.061	GCA
10	Taurochenodeoxycholic acid	TCDCa	Sigma	T6260	C26H45NO6S	498.2884	11.481	GCA

11	β -muricholic acid	β MCA	Steraloids	C1895-000	C24H40O5	407.2792	6.028	GCA
12	Taurodeoxycholic acid	TDCA	Sigma	T0875	C26H45NO6S	498.2884	13.091	GCA
13	Cholic acid	CA	Sigma	C1129	C24H40O5	407.2792	8.435	GCDCA
14	Glycochenodeoxycholic acid (internal std)	GCDCA	Sigma	G0759	C26H43NO5	448.3058	10.100	-
15	Hyodeoxycholic acid	HDCA	Sigma	H3878	C24H40O4	391.2843	10.866	GCDCA
16	Tauroolithocholic acid (internal std)	TLCA	Sigma	T7515	C26H45NO5S	482.2935	26.500	-
17	Chenodeoxycholic acid	CDCA	Sigma	C9377	C24H40O4	391.2843	18.808	TLCA
18	Deoxycholic acid	DCA	Sigma	D2510	C24H40O4	391.2843	20.070	TLCA

Bile acids in bold font were used as internal standards and to calculate both matrix effect and percent recovery of Oasis Prime extraction cartridges. All bile acids were injected using negative ion mode. The listed internal standard was used to quantify the given bile acid.

Supplementary Table 6. Bile acid calibration curves for quantification

Bile acid (BA)	Abbreviation	Calibration Curves ^a	R ²	Quality Control ^b C.V. (%)	LoD ^c (μ g/mL)	LoQ ^d (μ g/mL)
Tauro- ω -muricholic acid	<i>TωMCA</i>	$y = 121185(x) - 4123.2$.9999	3.80	0.0125	0.0125
		$y = 112472(x) - 24984$.9924			
		$y = 148899(x) - 5080.8$.9997			
Tauro- α -muricholic acid	<i>TαMCA</i>	$y = 912016(x) - 2502.7$.9999	4.62	0.0098	0.0125
		$y = 870471(x) - 153587$.9957			
		$y = 539340(x) - 10384$.9999			
Tauro- β -muricholic acid	<i>TβMCA</i>	$y = 2E+06(x) - 21894$.9999	5.64	0.0020	0.0125
		$y = 1E+06(x) - 170638$.9969			
		$y = 890379(x) - 15458$.9999			
Tauroursodeoxycholic acid	<i>TUDCA</i>	$y = 4E+06(x) - 12759$.9998	1.04	0.0008	0.0125
		$y = 4E+06(x) - 422385$.9977			
		$y = 2E+06(x) - 72600$.9997			
Taurohyodeoxycholic acid	<i>THDCA</i>	$y = 2E+06(x) - 2294.6$	1	0.16	0.0010	0.0125
		$y = 2E+06(x) - 24982$.9997			
		$y = 1E+06(x) - 1931.6$.9998			

Taurocholic acid	TCA	y = 2E+06 (x) + 28806 y = 1E+06 (x) - 190915 y = 930608(x) - 27195	.9994 .9966 .9999	7.17	0.0125 0.0025 0.0025	0.0125 0.0025 0.0025
ω -muricholic acid	ω MCA	y = 7024.7(x) - 291.36 y = 10378 (x) - 406.74 y = 29100 (x) - 2790.7	.9998 .9997 .9992	7.33	0.1073 0.0834 0.0185	0.1250 0.1250 0.1250
α -muricholic acid	α MCA	y = 57808 (x) - 8794.7 y = 73526 (x) - 18151 y = 33793(x) - 5554.3	.9981 .9921 .9977	3.99	0.0010 0.0331 0.0112	0.1250 0.1250 0.1250
Taurochenodeoxycholic acid	TCDC	y = 2E+06 (x) - 37012 y = 2E+06 (x) - 293535 y = 1E+06 (x) - 42210	.9999 .9962 .9999	1.83	0.0035 0.0007 0.0006	0.0125 0.0125 0.0025
β -muricholic acid	β MCA	y = 25972 (x) - 705.38 y = 25229 (x) - 1367 y = 14633 (x) + 70.839	.9998 .9963 1	5.64	0.0125 0.0317 0.0125	0.0125 0.1250 0.0125
Taurodeoxycholic acid	TDCA	y = 3E+06 (x) -152437 y = 1E+06 (x) - 49312 y = 2E+06 - 326520	.9997 .9955 .9998	0.81	0.0019 0.0006 0.0012	0.0125 0.0025 0.0025
Cholic acid	CA	y = 438813 (x) - 112.07 y = 470718 (x) - 48844 y = 259959 (x) - 1957	.9999 .9977 1	5.05	0.0016 0.0044 0.0030	0.0125 0.0125 0.0125
Hyodeoxycholic acid *	HDCA	y = 1456 (x) - 976.04 y = 1980.7 (x) + 989 y = 871.15 (x) + 220.52	.9835 .9710 .9966	14.81	1.2500 5.0000 2.5000	1.2500 5.0000 2.5000
Chenodeoxycholic acid *	CDCA	y = 4101.7 (x) - 2564.2 y = 6227 (x) - 2429.3 y = 3256.7 (x) -1034	.9818 .9927 .9880	9.74	1.2500 2.5000 5.0000	1.2500 2.5000 5.0000
Deoxycholic acid	DCA	y = 156240 (x) - 8355 y = 224842 (x) +1259.3 y = 93571 (x) - 1366.1	.9997 .9995 .9999	2.90	0.0095 0.0125 0.0116	0.0125 0.0125 0.0125

Bile acids (BAs) with asterisks (*) were not included in the statistical analysis, as these BAs were not detectable or quantifiable within mice serum due to low concentrations and insufficient ionization capability under the conditions we have established for our method.

^aPeak area (μ V*s) of each BA standard concentration was averaged to generate a linear calibration curve (from lowest to highest concentration; 0.0025 μ g/mL, 0.0125 μ g/mL, 0.125 μ g/mL, 1.25 μ g/mL, 2.5 μ g/mL, 5 μ g/mL and 10 ng/ μ L) for each sample set.

A total of three calibration curves were collectively used to quantify BA concentrations (μ g/mL) present within the wash and eluent serum filtrates produced by filtering mice serum through Oasis HLB Prime column cartridges (Waters, Milford MA).

Washes and eluents were injected separately to minimize loss of BAs during sample processing and quantified using the standard curves generated during the respective sample sets.

^bCoefficient of variance (CV) for each bile acid was determined by comparing peak areas (μ V*s) of standard quality control (QC) samples with peak areas (μ V*s) of all BA standards.

^cLimit of detection (LoD) for each standard curve was determined based on the lowest quantifiable concentration discernible via manual integration of BA peak signals with a signal to noise (S/N) ratio (greater than 1.65) for each single ion recording (SIR) of every injection.

^dLimit of quantification (LoQ) for each linear calibration curve is established by the lowest quantifiable concentration of each BA in the mix standards.

