† Electronic Supplementary Information (ESI)

Structurally different mixed linkage β-Glucan supplements differentially increase secondary bile acid excretion in hypercholesterolaemic rat faeces

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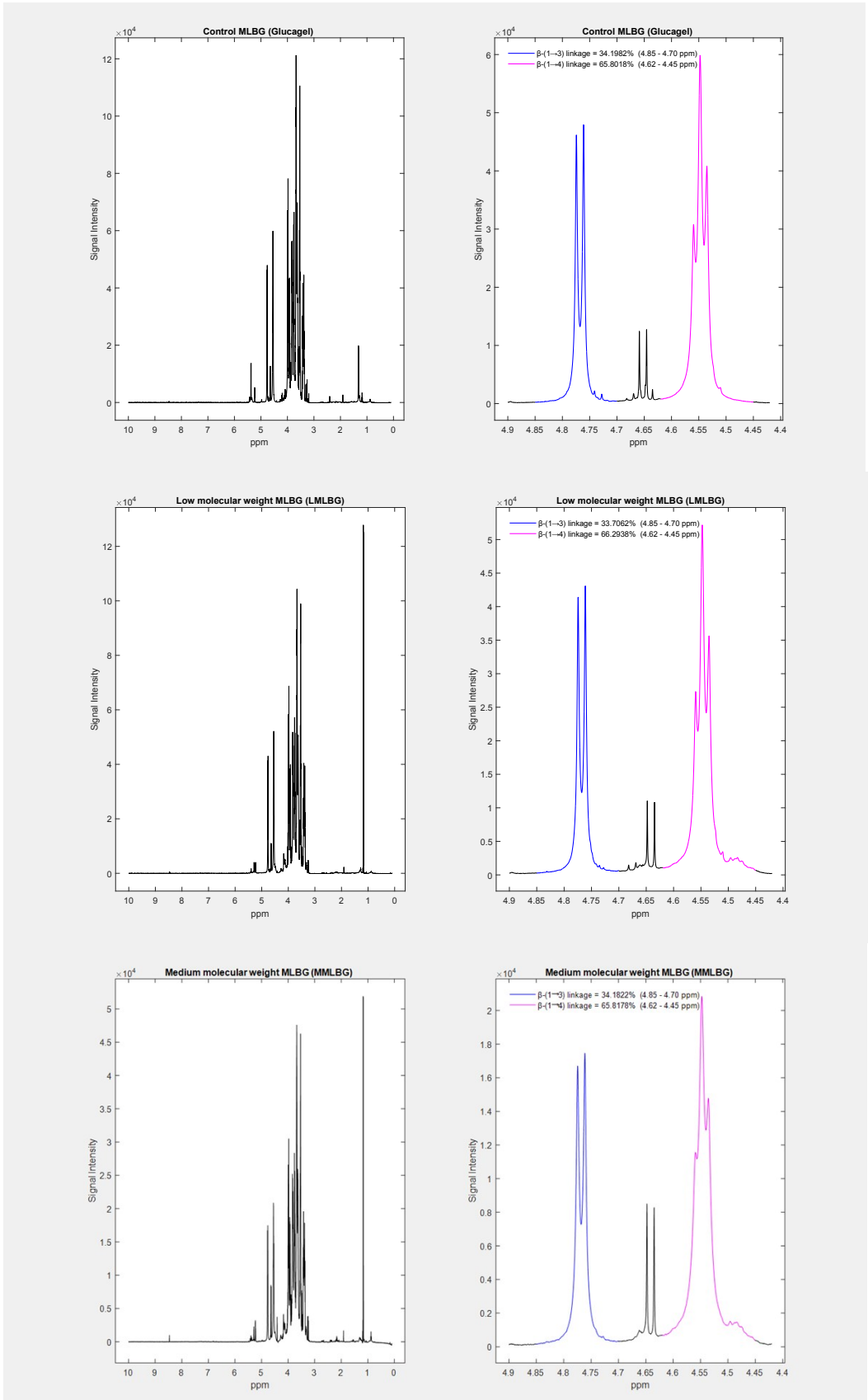

Figure S1. Percentage ratio of β-(1→3)/ β-(1→4) glycosidic bonds in BG.

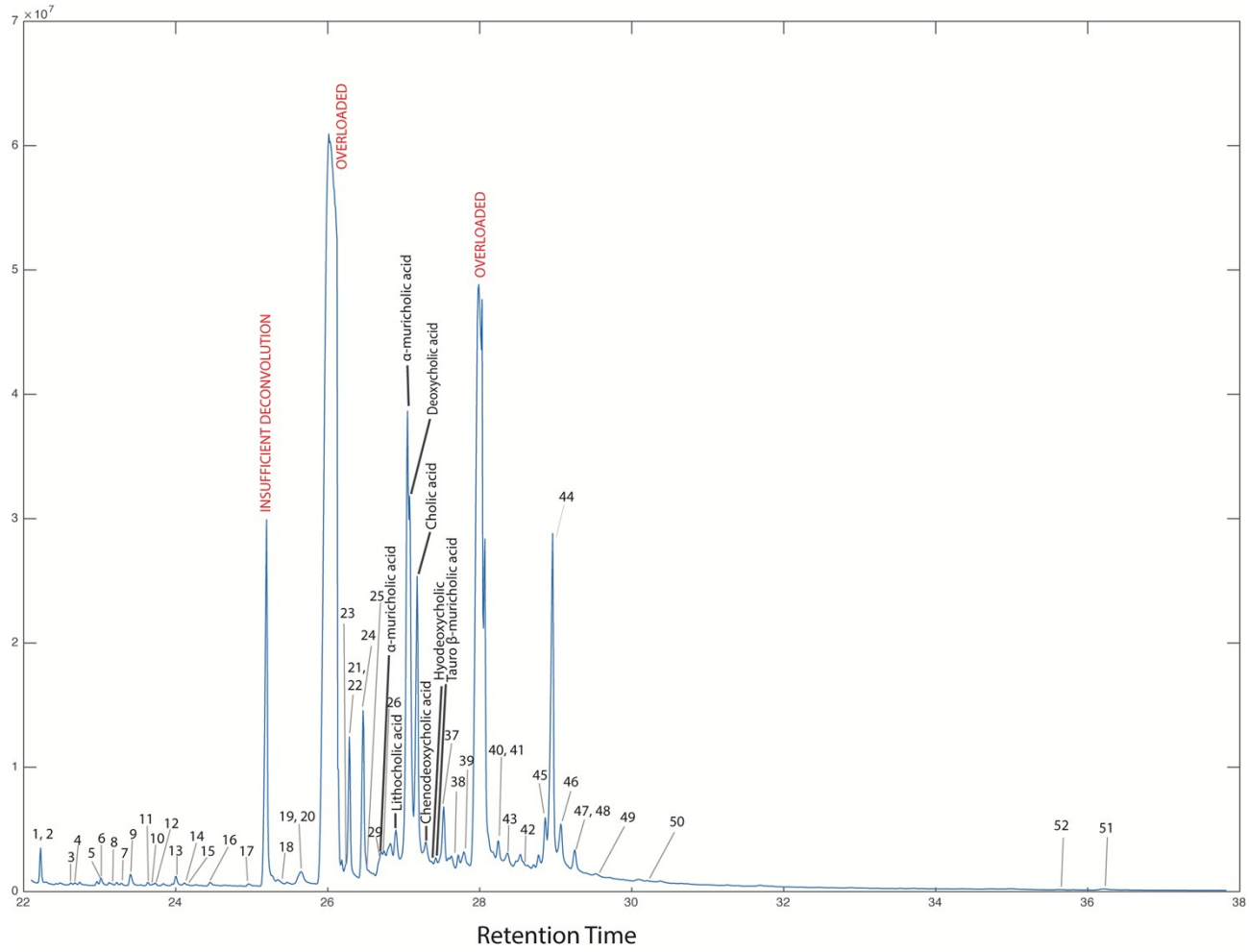

Figure S2. Representative chromatogram of a pooled faecal sample.

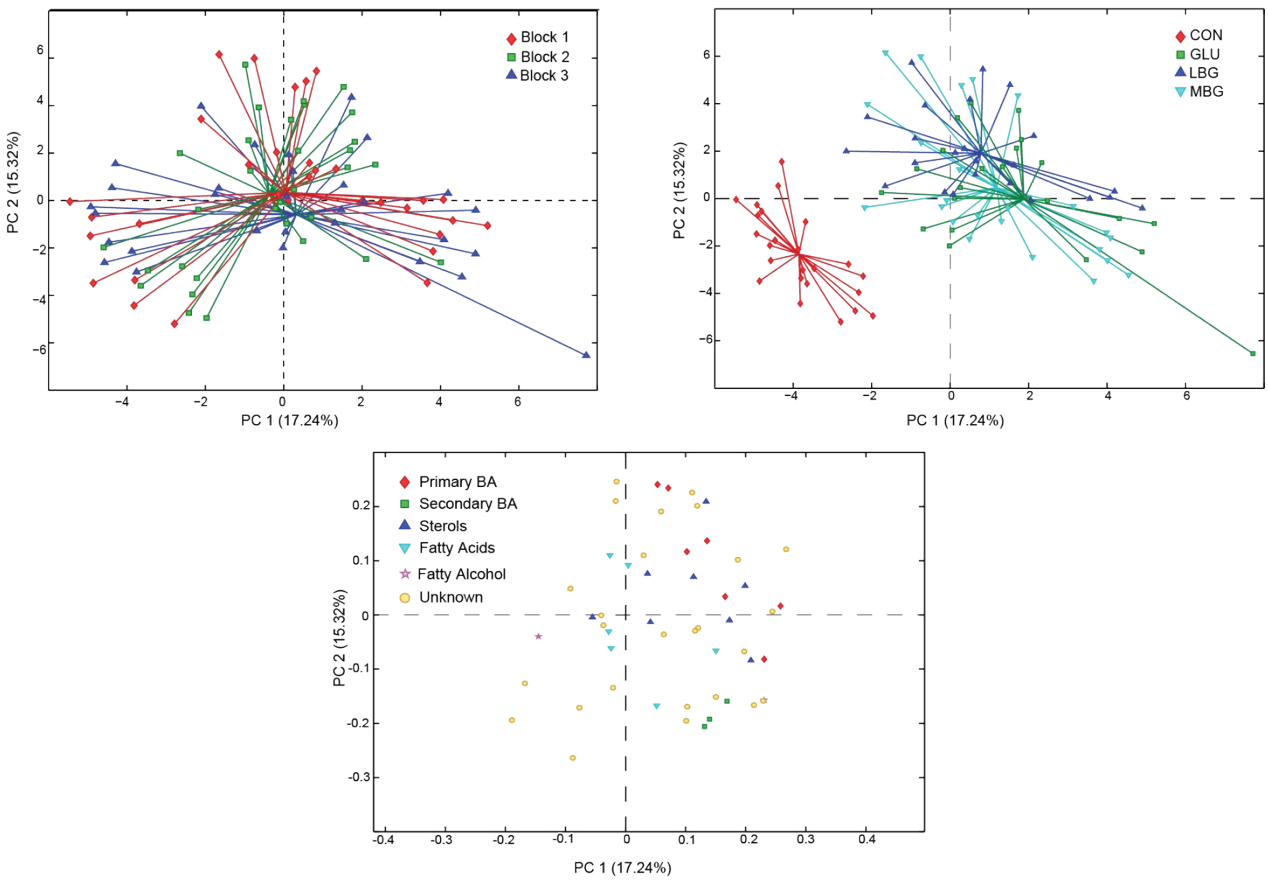

Figure S3. Scores (top) and loadings (bottom) plots derived from the PCA model, including all the studied groups, obtained by the deconvolution of the GC-MS data. The scores plot have been coloured according to the batch (upper left corner) and the diets (upper right corner).

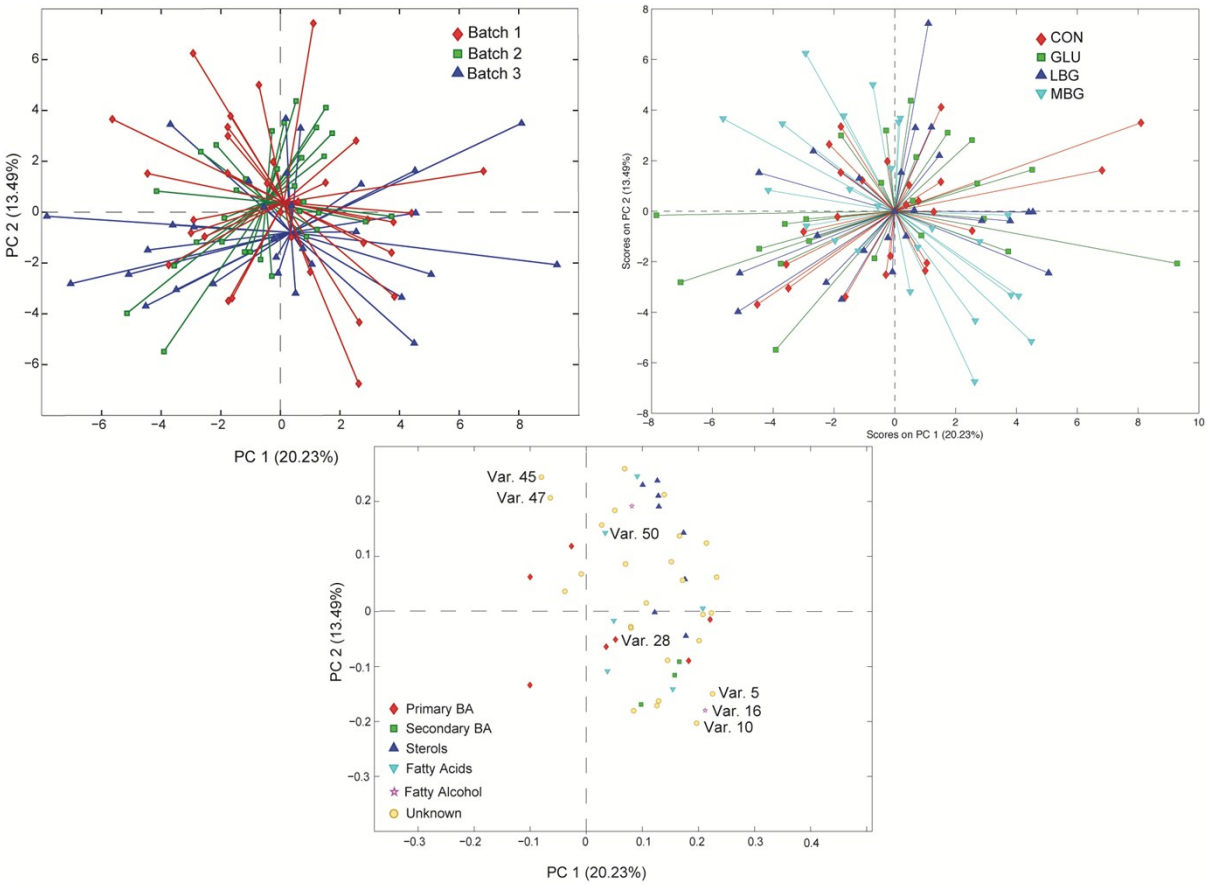

Figure S4. Scores (top) and loadings (bottom) plots derived from the PCA model, including all the studied groups, obtained by batch effect separated matrix (X_{batch}) using ASCA. The scores plot have been coloured according to the batch (upper left corner) and the diets (upper right corner).

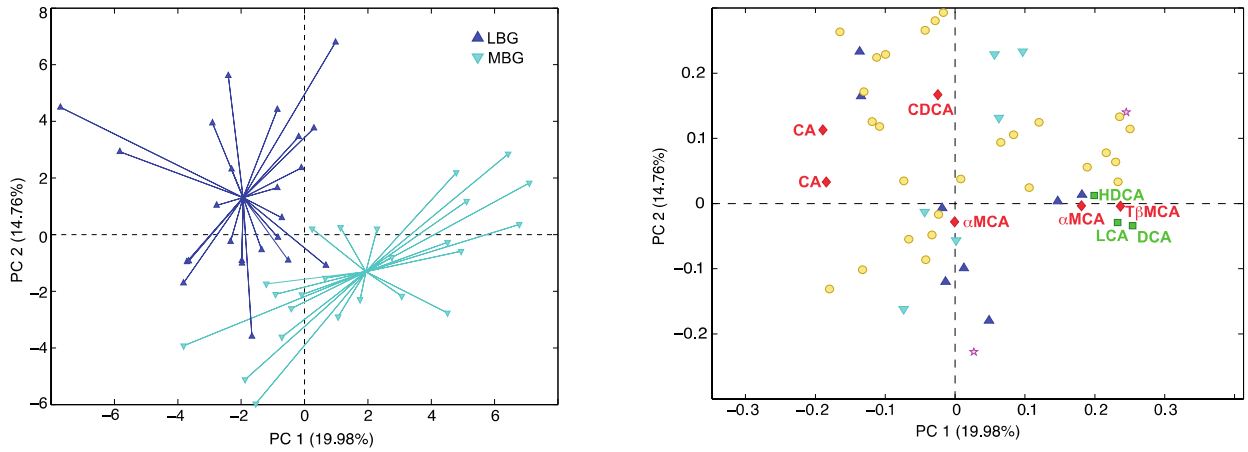

Figure S5. Scores (left) and loadings (right) plots derived from the PCA model, including only LBG and MBG groups, obtained by diet effect separated matrix (X_{diet}) using ASCA.

Table S1. Composition of the four experimental diets.

	CON	GLU	LBG	MBG
Ingredients (%, w/w)				
White wheat flour	35.4	33	34.2	32.5
Casein	19	18	19	17.8
Sucrose	12	12	12	12
Soybean oil	6	6	6	6
Lard	14	14	14	14
Cholesterol	2	2	2	2
Cellulose	6.5			
GLU		7.4		
LBG			7.7	
MBG				10.6
AIN 93G Mineral mix[1]	3.5	3.5	3.5	3.5
AIN 93G Vitamin mix[1]	1.0	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3	0.3
Choline bitartrate	0.25	0.25	0.25	0.25

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Table S2. List of 52 peaks deconvoluted using PARAFAC2 based processing of the raw GC-MS data.

Metabolite №	PARAFAC2 IDs (Int_PC)[a]	Metabolite full name from NIST[b]	Metabolite short name[c]	Metabolite Class[d]	Retention Time (min)[e]	Retention Index (calculated)[f]	Retention Index (reported)[g]	ΔRI[h]	Identification Level[i]	NIST Match[l]	ChEBI[m]	One-way ANOVA on Treatments[n]		One-way ANOVA on Blocks[n]	
1	int1_1	un1	un1	6	22.22	2666	0			0	0	P < 0,0000000037977	12 13 14	P > 0.05	N.S.
2	int1_2	un2	un2	6	22.21	2665	0			0	0	0.006448	14	P > 0.05	N.S.
3	int3_4	Tetracosan-1-ol trimethylsilyl ether	Tetracosan-1-ol	5	22.62	2721	2749	-28	3	67	77413	P < 0,02735	12 13 14 24	P > 0.05	N.S.
4	int3_5	Unknown fatty acid	Unknown fatty acid	6	22.66	2728	0			0	0	P < 0,004281	12 13 14	P > 0.05	N.S.
5	int4_3	Unknown fatty alcohol	Unknown fatty alcohol	6	22.96	2769	0			0	0	P > 0.05	N.S.	P < 0,01136	12 23
6	int4_4	Unknown steroid	Unknown steroid	6	23.01	2777	0			0	0	P < 0,01507	12 13 14	P > 0.05	N.S.
7	int5_4	cis-15-Tetracosenoic acid, trimethylsilyl ester	Tetracosenoic acid	4	23.28	2814	2809	5	3	79	44247	P > 0.05	N.S.	P > 0.05	N.S.
8	int6_1	Tetracosanoic acid, trimethylsilyl ester	Tetracosanoic acid	4	23.15	2828	2829	-1	2	87	28866	P > 0.05	N.S.	P > 0.05	N.S.
9	int6_3	Tetracosanoic acid, trimethylsilyl ester	Tetracosanoic acid	4	23.40	2829	2829	0	3	71	28866	P > 0.05	N.S.	P > 0.05	N.S.
10	int7_3	Unknown fatty alcohol	Unknown fatty alcohol	6	23.68	2866	0			0	0	0.006857	12	P < 0,03560	12 23
11	int7_4	5-β-cholestan-3α-ol, butyrate	5-β-cholestan-3α-ol, butyrate	3	23.63	2859	2809	50	3	81	0	P < 0,02466	12 13 14 34	P > 0.05	N.S.
12	int7_5	Unknown sterol	Unknown sterol	6	23.73	2872	0			0	0	0.01923	13 14 24	P > 0.05	N.S.
13	int8_4	Cholesta-3,5-diene	Cholesta-3,5-diene	3	24.00	2907	2895	12	2	91	0	P < 0,00631	12 13 14	P > 0.05	N.S.
14	int8_5	un3	un3	6	24.11	2923	0		2	91	0	0.01915	13	P > 0.05	N.S.
15	int8_6	un4	un4	6	24.15	2929	1258	1671	3	84	28837	P > 0.05	N.S.	P > 0.05	N.S.
16	int10_3	1-Tetracosanol, tert-butyldimethylsilyl ether	1-Tetracosanol	5	24.45	2967	2988	-21	3	79	77413	0.009544	12	P < 0,0315	12 23
17	int11_3	un5	un5	6	24.95	3030	0			0	0	P < 0,000001438	12 13 14	P > 0.05	N.S.
18	int12_4	Unknown disaccharide	Unknown disaccharide	6	25.37	3082	0			0	0	0.0005934	12 13 24 34	P > 0.05	N.S.
19	int13_1	Unknown sterol	Unknown sterol	6	25.64	3114	0			0	0	P < 0,04379	14 34	P > 0.05	N.S.
20	int13_4	un6	un6	6	25.65	3116	0			0	0	P < 0,008389	12 13	P > 0.05	N.S.
21	int15_1	Desmosterol, TMS	Desmosterol	4	26.28	3193	3169	24	2	91	17737	P > 0.05	N.S.	P > 0.05	N.S.
22	int15_3	Desmosterol, TMS	Desmosterol	4	26.29	3194	3169	25	2	80	17737	P > 0.05	N.S.	P > 0.05	N.S.
23	int15_4	7β-Hydroxycholesterol, bis(trimethylsilyl) ether	7β-Hydroxycholesterol	3	26.24	3188	3254	-66	3	81	42989	P < 0,01741	12 13 24 34	P > 0.05	N.S.
24	int16_1	Lanosterol, trimethylsilyl ether	Lanosterol	3	26.46	3215	3178	37	3	89	16521	P > 0.05	N.S.	P > 0.05	N.S.
25	int16_6	7β-Hydroxycholesterol, bis(trimethylsilyl) ether	7β-Hydroxycholesterol	3	26.50	3219	3254	-35	3	81	42989	P < 0,01723	12 14	P > 0.05	N.S.
26	int17_1	Campesterol, TMS	Campesterol	3	26.74	3248	3220	28	3	77	28623	P < 0,04793	13 14	P > 0.05	N.S.
27	int17_2	Lithocholic acid	Lithocholic acid	2	26.90	3267	3262	5	1	84	16325	P < 0,01723	14 34	P > 0.05	N.S.
28	int17_3	α-Muricholic acid	α-Muricholic acid	1	26.70	3243	3275	-32	1	86	81243	P < 0,009636	12 13 24 34	0.0205	23
29	int17_6	7β-Hydroxycholesterol, bis(trimethylsilyl)	7β-Hydroxycholesterol	3	26.67	3240	3253	-13	2	89	42989	P < 0,04398	14 24	P > 0.05	N.S.

		ether													
30	int19_1	Deoxycholic acid	Deoxycholic acid	2	27.08	3288	3280	8	1	89	28834	P < 0,01938	12 23 34	P > 0.05	N.S.
31	int19_2	α-Muricholic acid	α-Muricholic acid	1	27.04	3284	3275	9	1	88	81243	P < 0,0000001082	12 13 14	P > 0.05	N.S.
32	int20_1	Cholic acid	Cholic acid	1	27.18	3300	3291	9	1	84	16359	P < 0,005713	12 13 34	P > 0.05	N.S.
33	int20_2	Cholic acid	Cholic acid	1	27.18	3300	3291	9	1	89	16359	P < 0,01584	12 13 34	P > 0.05	N.S.
34	int21_2	Chenodeoxycholic acid	Chenodeoxycholic acid	1	27.29	3314	3308	6	1	90	16755	P < 0,008246	12 13	P > 0.05	N.S.
35	int21_3	Tauro β-Muricholic acid	Tauro β-Muricholic acid	1	27.31	3316	3317	-1	1	75	133057	P < 0,01444	12 14 34	P > 0.05	N.S.
36	int22_2	Hyodeoxycholic acid	Hyodeoxycholic acid	2	27.42	3329	3326	3	1	88	52023	0.0114	23	P > 0.05	N.S.
37	int23_2	β-Sitosterol trimethylsilyl ether	β-Sitosterol	3	27.52	3341	3284	57	2	90	27693	P < 0,04022	12 23 24	P > 0.05	N.S.
38	int24_1	un7	un7	6	27.72	3365	0			75	0	P < 0,0249	12 13 14 34	P > 0.05	N.S.
39	int24_4	un8	un8	6	27.77	3371	0			0	0	P < 0,002288	12 13 34	P > 0.05	N.S.
40	int26_2	un9	un9	6	28.24	3422	0			0	0	P < 0,03236	12 13 14	P > 0.05	N.S.
41	int26_3	un10	un10	6	28.24	3422	0			0	0	0.03669	13	P > 0.05	N.S.
42	int27_2	un11	un11	6	28.54	3452	0			0	0	P < 0,04986	12 13 14 23	P > 0.05	N.S.
43	int27_4	Unknown steroid	Unknown steroid	6	28.36	3434	0			0	0	P < 0,04400	13 23 34	P > 0.05	N.S.
44	int28_1	Unknown bile acid	Unknown bile acid	6	28.95	3492	0			0	0	0.01687	23	P > 0.05	N.S.
45	int28_3	Unknown steroid	Unknown steroid	6	28.86	3484	0			0	0	P > 0.05	N.S.	P < 0,03659	12 13
46	int29_1	Unknown bile acid	Unknown bile acid	6	29.07	3504	0			0	0	P < 0,001076	14 34	P > 0.05	N.S.
47	int30_2	un12	un12	6	29.25	3522	0			0	0	P > 0.05	N.S.	P < 0,02408	12 13
48	int30_3	un13	un13	6	29.25	3522	0			0	0	P < 0,000000003768	12 13 14	P > 0.05	N.S.
49	int31_2	un14	un14	6	29.70	3566	0			0	0	P > 0.05	N.S.	P > 0.05	N.S.
50	int32_2	un15	un15	6	30.16	3608	0			0	0	P > 0.05	N.S.	0.02299	13
51	int33_2	un16	un16	6	36.18	3974	0			0	0	P < 0,000000003871	12 13 14	P > 0.05	N.S.
52	int33_3	un17	un17	6	35.77	3953	0			0	0	P > 0.05	N.S.	P > 0.05	N.S.

[a]PARAFAC2 IDs are unique to each peak, indicating which models they derived from.

[b]Full names of metabolites are directly extracted from the NIST11 metabolite database.

[c]Metabolite short names are IUPAC or generic names of the identified metabolites.

[d]Identified metabolites were grouped into 6 different classes based on chemical class they belong to. Class 1: primary bile acids, class 2: secondary bile acids, class 3: sterols, class 4: fatty acids, class 5: fatty alcohols, class 6: unknown compounds.

[e]Retention times of deconvoluted peaks were calculated as a mean of RTs of each peak across all samples.

[f]Based on these RTs, retention indices (RI) of metabolites were estimated using all even alkane mixture sample (C10-C40).

[g]Reported RIs of identified metabolites were extracted from NIS11.

[h]Difference between the reported RI and the calculated RI.

[i]Identification level of the metabolites. Level 1 if the peaks are confirmed using authentic standards; Level 2 when the peaks are identified based on their EI-MS ≥ 80 (%) and RI match (±30); Level 3 when the peaks are identified based on their EI-MS ≥ 65 (%).

[l]EI-MS spectral match of identified metabolites with NIST11.

[m]Chemical Entities of Biological Interest (ChEBI) IDs of identified metabolites.

[n]One-way ANOVA test were performed to see if there are some variables significantly different among the diets: control, glucagel, low MW beta-glucan, medium MW beta-glucan and among the blocks (block 1, 2 and 3). Significance of effects within levels of the factors (diets, blocks) is evaluated by P-values. For example the diet factor has 4 levels, control=1, glucagel=2, low MW beta-glucan=3, and medium MW beta-glucan=4. Thus "12" means that the variable was significantly different between control and glucagel, "23" means that a variable was significant between glucagel and low MW beta-glucan. In the same manner for the block, which has 3 levels.