Cashew apple fiber prevents high fat diet-induced obesity in mice: a NMR metabolomic evaluation

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1. Experimental

The chow used in this study consisted in: moisture (maximum) 12 g.100 g^{-1}; crude protein (minimum) 22 g.100 g^{-1}; ethereal extract (minimum) 5 g.100 g^{-1}; mineral content (maximum) 10 g.100 g^{-1}; fibrous content (maximum) 8 g.100 g^{-1}; and energy content of 3.25 kcal.g^{-1}. In addition, the centesimal composition of HFD and HFD-CABwc are shown in Supplementary Table S1

Table S1. Centesimal composition (%) for cashew fiber without low molecular weight compounds (CABw), high fat diet (HFD) and high fat diet supplemented cashew fiber without low molecular weight compounds (HFD-CABwc): humidity; lipids; protein; ash; total carbohydrates; energy value (kcal/100 g); fiber content.

Samples	*Humidity*	*Lipids*	*Protein*	*Ash*	*Carbohydrates*	*Energy*	*Fiber*
CABw	14,14	0,71	13,25	1,09	70,81	342,63	11,64
HFD	4.57	24.03	17.36	4.17	49.85	485.29	2.95
HFD-CABwc	4.53	23.94	17.15	3.50	50.88	487.58	4.00

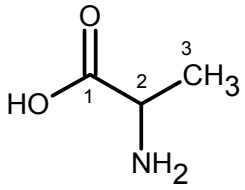
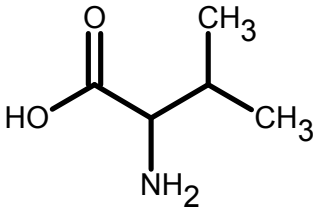
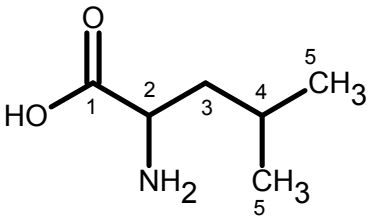
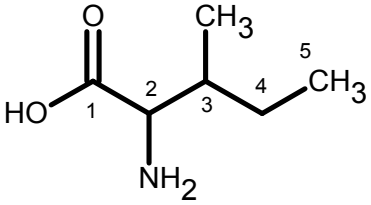
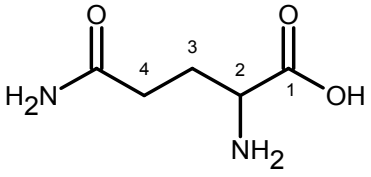
2. NMR data from the identification of the organic compounds

Tables S2 and S3 show the structures, ^{1}H and ^{13}C chemical shifts (δ), multiplicity, correlations, and constant coupling (J in HZ) of the compounds identified in the serum and feces, respectively. The identified compounds exhibit characteristics signals at aliphatic region (between δ 0.66 to 2.60) from aliphatic alicyclic, allylic, β-substituted aliphatic, and alkyne protons; carbinolic region (between δ 2.60 to 5.50) from olefinic, α-monosubstituted and α-disubstituted aliphatic protons; and aromatic region (between δ 6.80 to 9.22) from alkene, heteroaromatic and aldehydic protons [1-8].

Table S2. Organic compounds identified in the mice serum samples.

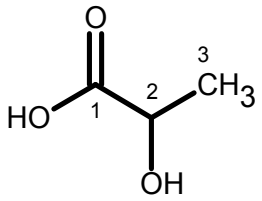
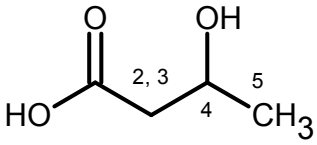
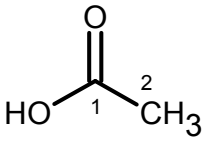
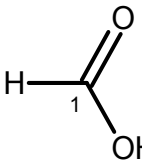
Structures	*δ ^{1}H (multip.* J in Hz)*	*δ ^{13}C (HSQC)*	*δ ^{1}H ref.*	*δ ^{13}C ref.*

AMINO ACIDS

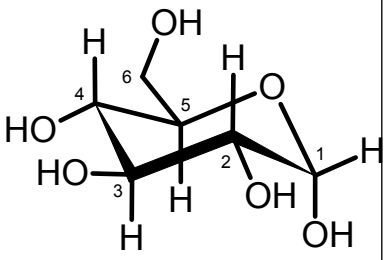
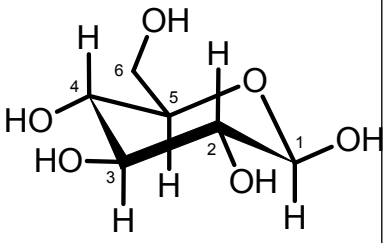
Alanine 	2 -3.80 (o) 3 - 1.49 (*d* 7.2)	no 19.2	3.90 (*q* 7.3) 1.52 (*d* 7.3)	53.4 19.1
Valine 	2 - 3.62 (o) 3 - 2.28 (o) 4 - 0.98 (*d* 7.2) 5 - 1.05 (*d* 7.2)	o o 19,3 20,8	3.82 (*d* 4.4) 2.33 (*m*) 1.02 (*d* 7.1) 1.06 (*d* 7.1)	n 32.0 19.1 20.9
Leucine 	2,3,4 - no 5,6 - 0.97 (*d* 6.0)	no 42.7 23.4 24.7	3.90 (no) 1.73 (*m*) 0.96 (*dt*)	n 42.8 23.9 27.0
Isoleucine 	2 - no 3 – 1.80 (o) 4 – 1.48 (o) 5 - 1.02 (o) 6 - 1.02 (o)	o 37.0 27.7 16.0 17.5	3.65 (*n*) 1.96 (*m*) 1.45 (*m*) 0.92 (*d* 7.1) 0.99 (*d* 7.1)	62.5 38.7 27.0 13.9 17.4
Glutamine 	4 - 2.30 a 2.47 (*m*) 3 - 2.00 a 2.14 (*m*) 2 - 3.73 (o)	34.1 30.0 57.6	3.77 (o) 2.12 (*m*) 2.45 (*m*)	57.2 29.3 33.9

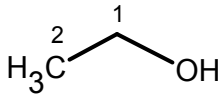
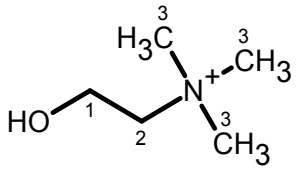
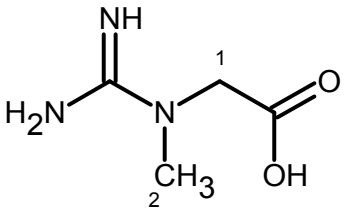
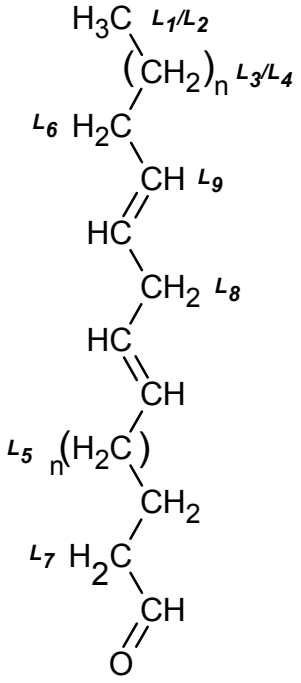
ORGANIC ACIDS

Lactic	2 - 4.11 (*q* 7.20) 3 - 1.33 (*d* 7.20)	71.9 22.9	4.10 (*q* 6.93) 1.32 (*d* 6.93)	71.4 22.9

				
3-hydroxybutyric 	5 - 1.20 (o) 3 - 2.31 (*m*) 2 - 2.40 (*m*) 4 - 4.15 (o)	24.6 49.4 49.4 68.7	1.18 (*d* 6.26) 2.29 (*m*) 2.39 (*m*) 4.14 (*m*)	24.4 49.2 49.2 68.5
Acetic 	2 - 1.92 (*s*)	26.3	1.90 (*s*)	26.1
Formic 	1 - 8.46 (*s*)	no	8.46 (*s*)	173.9

CARBOHYDRATES

α-glucose 	1 - 5.23 (*d* 3.8) 2 - 3.47 (m) 3 - 3.77 (m) 4 - 3.56 (m) 5 - 3.72 (m) 6 - 3.85 (m)	95.0 72.3 75.6 74.0 63.9 75.5	5.25 (*d* 3.80) 3.89-3.36 (o) n n n n	95.4 72.2 76.0 72.8 64.2 74.5
β-glucose 	1 - 4.65 (*d* 7.90) 2 - 3.26 (m) 3 - 3.75 (m) 4 - 3.48 (m) 5 - 3.41 (m) 6 - 3.90 (m)	98.6 77.5 63.6 78.8 72.2 63.7	4.66 (*d* 8.10) 3.25 (*t* 8.40) n n n n	99.2 77.6 56.1 79.0 72.8 63.1

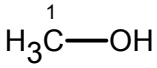
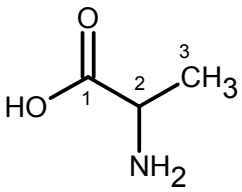
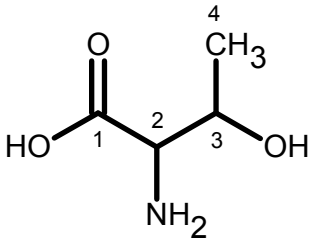
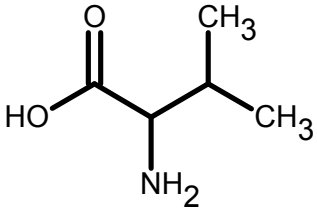
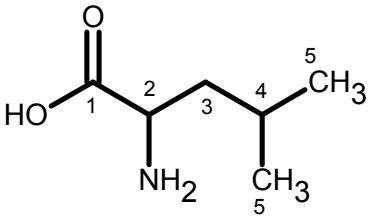
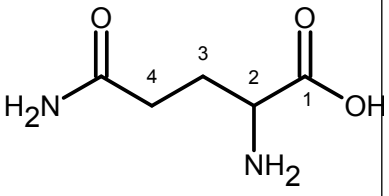
OTHER COMPOUNDS				
Ethanol 	1 - 3.66 (o) 2 - 1.19 (o)	60.3 19.6	3.64 (o) 1.17 (o)	60.3 19.6
Choline 	3 - 3.22 (o) 2 - 3.52 (o) 1 - 4.07 (o)	56.8 70.4 58.8	3.19 (*s*) 3.50 (*dd* 5.8; 4.2) 4.05 (*m*)	56.7 70.1 58.5
Creatine 	2 - 3.04 (*s*) 1 - 3.93 (o)	39.8 56.6	3.03 (*s*) 3.92 (*s*)	39.5 56.4
Fatty acids LDL and VDL[7] 	L1/L2 - 0.86 (o) L3/L4 - 1.27 (o) L5 - 1.70 (o) L6 - 2.01 L7 - 2.25 L8 - 2.76 L9 - 5.29	17.0 25.7; 32.5 29.7 30.5 36.4 20.06 29.0 131.8		

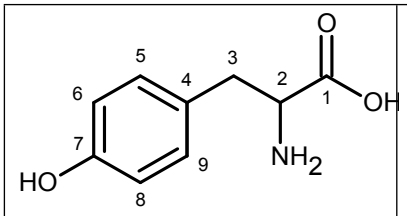
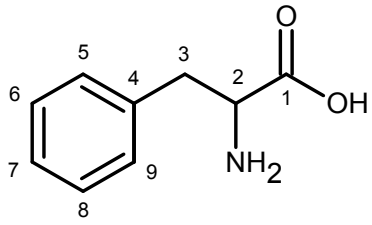
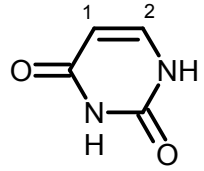
s – simplet; *d* – duplet; *t* – triplet; *q* – quadruplet; *quin* – quintet; *dd* – double of duplets; *dt* – double of triplets; o – overlapping signal; n – no information; no – not observed.

Table S3. Organic compounds identified in the mice feces samples.

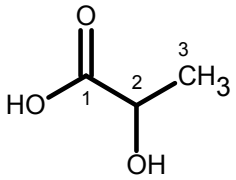
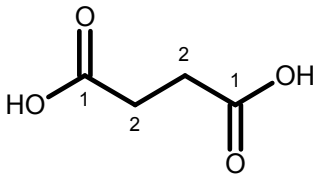
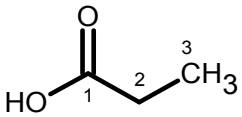
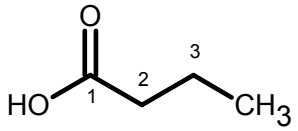
Structures	*δ [1]H (multip.* J in Hz)*	*δ [13]C (HSQC)*	*Ref. [1]H*	*Ref. [13]C*

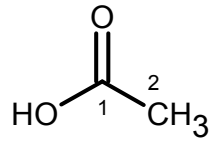
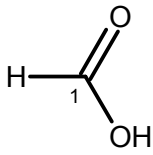
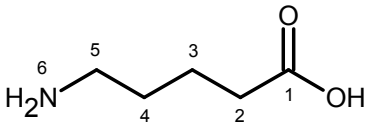
AMINO ACIDS

Methanol 	1 - 3.36 (*s*)	52.6	3.37 (*s*)	51.4
Alanine 	2 - 3.80 (o) 3 - 1.49 (*d* 7.8)	54.1 19.5	3.9 (*q* 7.3) 1.52 (*d* 7.3)	53.4 19.1
Threonine 	2 - 3.9 (o) 3 - 4.3 (o) 4 - 1.33 (*d* 6.6)	63.3 68.9 23.4	3.81 (*d* 4.2) 4.35 (*m*) 1.35 (*d* 6.5)	63.4 69.3 22.3
Valine 	2 - 3.78 (o) 3 - 2.30 (o) 4 -1.00 (o) 5 - 1.05 (o)	63.2 32.3 19.5 20.7	3.82 (*d* 4.4) 2.33 (*m*) 1.02 (*d* 7.1) 1.06 (*d* 7.1)	n 32.0 19.1 20.9
Leucine 	2 - no 3,4 - 1.70 (o) 5,6 - 0.97 (*t* 6.0)	42.8 25.0 23.8	3.90 (no) 1.73 (*m*) 0.96 (*t* 5.9)	42.6 26.8 23.6
Glutamine 	2 - 3.77 (o) 3 - 2.11 (o) 4 - 2.36 (*m*)	57.4 29.8 36.0	3.77 (o) 2.12 (*m*) 2.45 (*m*)	57.2 29.3 33.9
Tyrosine	6,8 - 6.91 (*m*) 5,9 - 7.20 (*m*) 2 - (o)	118.7 133.7 no	6.89 (*m*) 7.19 (*m*) 3.93 (*dd*)	118.9 133.5 59.0

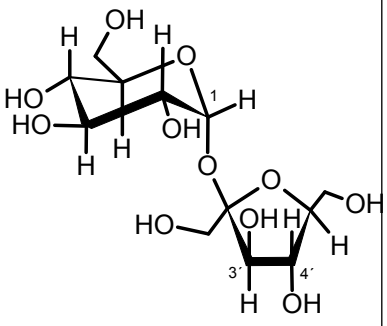
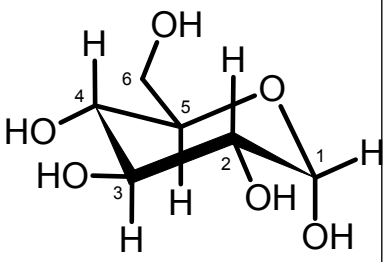
	3 - 3.04 (o)	no	3.06 (*dd*)	38.3
Phenylalanine 	5,9 - 7.24 (*m*) 6,8 - 7.42 (*m*) 7 - 7.32 (*m*)	132.0 131.8 131.7	7.32 (*d* 6.98) 7.42 (*m*) 7.37 (*m*)	132.1 131.8 130.4
Uracyl[1,2] 	1 - 5.80 (*d* 7.80) 2 - 7.54 (*d* 7.80)	104.2 no	5.79 (*d* 7.69) 7.56(*d* 7.69)	103.7 146.2

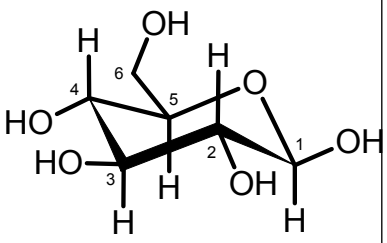
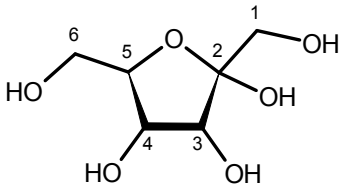
ORGANIC ACIDS

Lactic 	2 - 4.06 (*q* 7.30) 3 - 1.33 (o)	72.1 20.7	4.10 (*q* 6.93) 1.32 (*d* 6.93)	71.4 22.9
Succinic 	2 - 2.42 (*s*)	36.9	2.39 (*s*)	36.8
Propionic 	2 - 2.19 (o) 3 -1.05 (o)	34.1 13.3	2.17 (*q* 7.41) 1.06 (*t* 7.41)	33.4 13.0
Butyric 	2 - 2.19 (o) 3 - 1.56 (*m*) 4 - 0.90 (*t* 7.8)	42.7 22.6 16.6	2.16 (*t* 7.41) 1.56 (*sex* 7.41) 0.90 (*t* 7.41)	42.1 21.9 16.1
Acetic	2 - 1.93 (*s*)	27.2	1.90 (*s*)	26.1

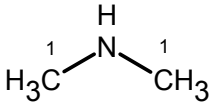
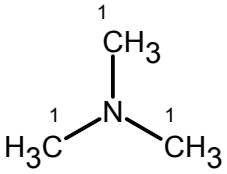
				
Formic 	1 - 8.46 (*s*)	173.5	8.46 (*s*)	173.9
5-Aminovaleric acid 	2 - 40.7 3 - 25.0 4 - 29.8 5 - 42.4	40.7 25.0 29.8 42.4	2.21 (*t* 6.86) 1.62(*m*) 1.65 (*m*) 3.00 (*t*, 7.09)	39.4 25.2 29.3 42.2

CARBOHYDRATES

Sucrose 	1 - 5.42 (*d* 3.70) 2 - 3.56 (o) 3 - 3.76 (o) 4 - 3.48 (o) 5 - 3.85 (o) 6 - 3.82 (o) 1’ - 3.82 (o) 2’ - 3.89 (o) 3’ - 4.05 (*m*) 4’ - 4.22 (*m*) 6’ - 3.68 (*m*)	95.1 74.1 75.5 72.3 75.5 63.1 65.2 84.3 77.0 79.3 64.5	5.44 (*d* 3.80) 3.89-3.57 (*m*) n n n n n n 4.08 (*t* 8.40) 4.24 (*d* 9.0) n	94.7 73.5 75.0 71.8 74.9 62.8 64.0 83.7 76.6 79.0 65.0
α-glucose 	1 - 5.24 (*d* 3.80) 2 - 3.47 (*m*) 3 - 3.77 (*m*) 4 - 3.56 (*m*) 5 - 3.72 (*m*) 6 - 3.85 (*m*)	95.1 72.3 75.6 74.0 63.9 75.5	5.25 (*d* 3.80) 3.89-3.36 (o) n n n n	95.4 72.2 76.0 72.8 64.2 74.5

β-glucose 	1 - 4.66 (*d* 7.90) 2 - 3.26 (*m*) 3 - 3.75 (*m*) 4 - 3.48 (*m*) 5 - 3.41 (*m*) 6 - 3.90 (*m*)	98.8 77.5 63.6 78.8 72.2 63.7	4.66 (*d* 8.10) 3.25 (*t* 8.40) n n n n	99.2 77.6 56.1 79.0 72.8 63.1
Fructose 	3 - 4.12 (o) 4 - 4.12 (o) 5 - 3.81 (o) 1 - 3.48 (o) 6 - 3.82; 3.72 (o)	78.3 77.1 84.2 65.3; 66.5	3 – 4.10 (o) 4 – 4.10 (o) 5 – 3.81 (o) 7 – 3.48 (o) 11 – 3.8; 3.66 (o)	78.2 77.4 83.6 65.4 65.6

OTHER COMPOUNDS

Dimethylamine 	1 - 2.73 (*s*)	37.6	2.76 (*s*)	39.2
Trimethylamine 	1 - 2.91 (*s*)	47.7	2.88 (*s*)	47.2

s – simplet; *d* – duplet; *t* – triplet; *q* – quadruplet; *quin* – quintet; *dd* – double duplet; *dt* – double triplet; o – overlapping signal; n – no information; no – not observed.

Table S4. Statistical parameters of the PLS-DA models from ^{1}H NMR analysis for both serum and feces evaluations (sections 3.2 and 3.3, respectively).

Models	*LV1+LV2+LV3*[a]	*r² cal*[b]	*RMSEC*[c]	*r² val*[d]	*RMSEV*[e]	*SEC/SEV*[f]
Feces alip+carb	93.1 %	0.93	0.21	0.92	0.24	0.88
Feces arom	98.3 %	0.98	0.11	0.95	0.18	0.61
Serum	95.4 %	0.95	0.17	0.94	0.20	0.85

[a] Total variance percent in X matrix refer to the first three Latent Variables (LV); [b] Coefficient of correlation between the real and predicted values during the calibration; [c] Root Mean Square Error of Calibration; [d] Coefficient of correlation between the real and the predicted values during the validation; [e] Root Mean Square Error of Cross Validation; [f] Similarity criterion.

Figure S1 illustrates the loadings of the PC2 and PC3 axes (between δ 0.8 and 5.5 – aliphatic and carbinolic region) plotted in lines from PCA evaluation of serum.

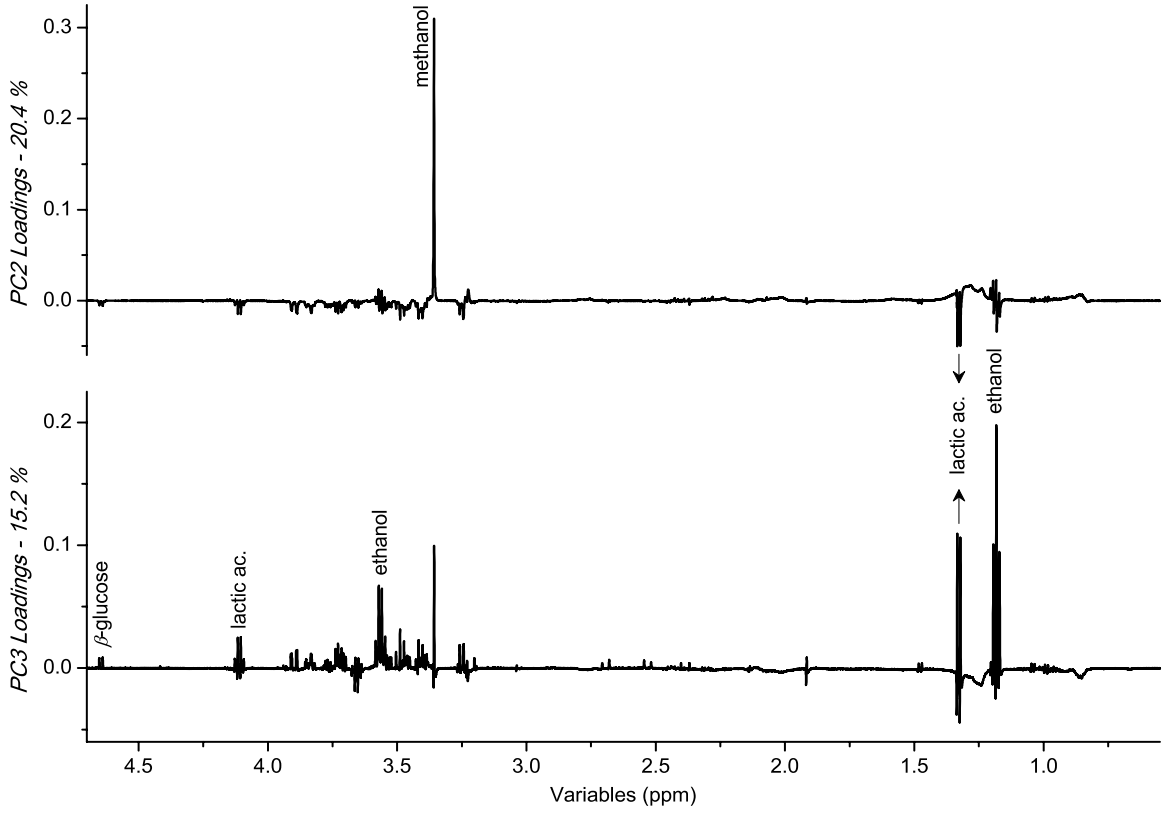


Figure S1. PC2 and PC3 loadings of the aliphatic and carbinolic region plotted in lines from serum evaluation.

Figure S2 illustrates the loadings of the PC1 and PC3 axes (between δ 0.8 and 5.5 – aliphatic and carbinolic region) plotted in lines from PCA evaluation of feces.

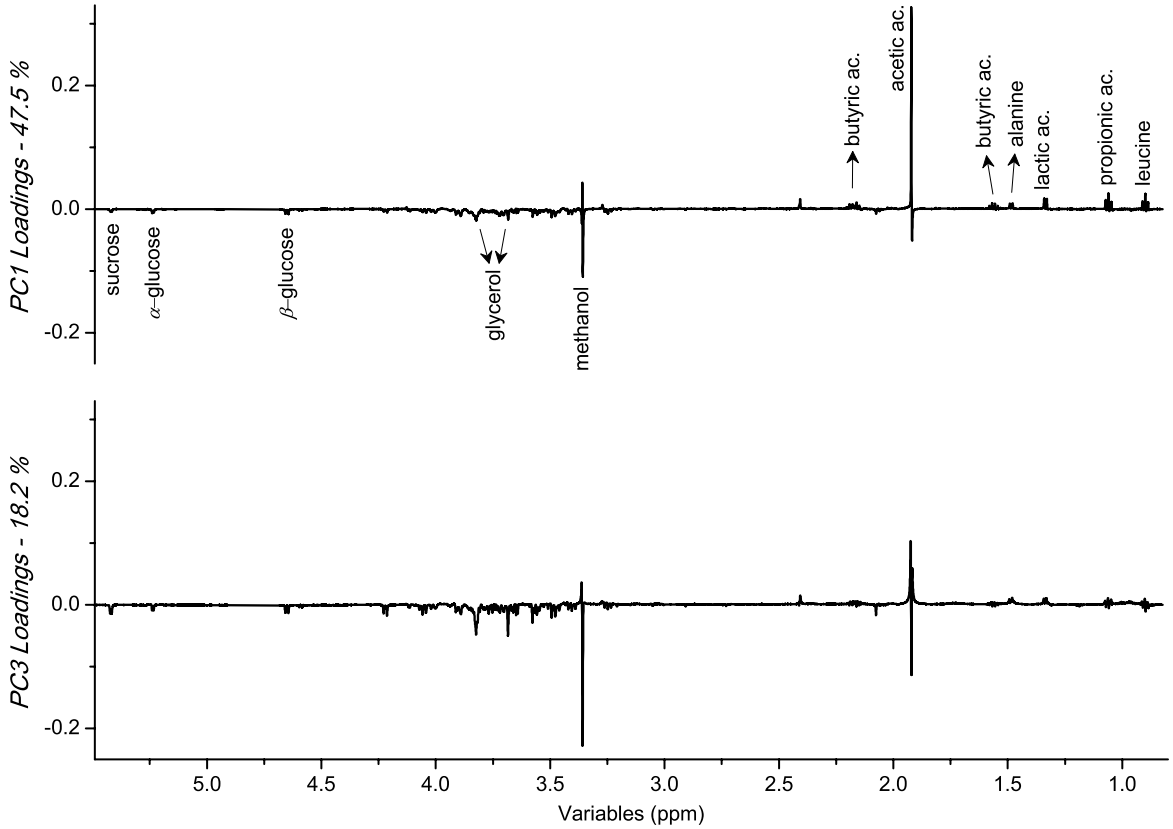


Figure S2. PC1 and PC3 loadings of the aliphatic and carbinolic region plotted in lines from feces evaluation.

Figure S3a illustrates the 3D scores from feces samples using PC1, PC2, and PC3 axes (63.3 % of the total variance) with projections in PC2 × PC3 plane, and Supplementary Figure 4b presents the PC3 loadings plotted in lines that retained the most significant responses. The CD samples were symbolized by blue color, those from HFD in red, and from HFD-CABwc in green. The loading from PC1 and PC2 axes were presented separately at Supplementary Figure S5 due to the low contribution for the experiment.

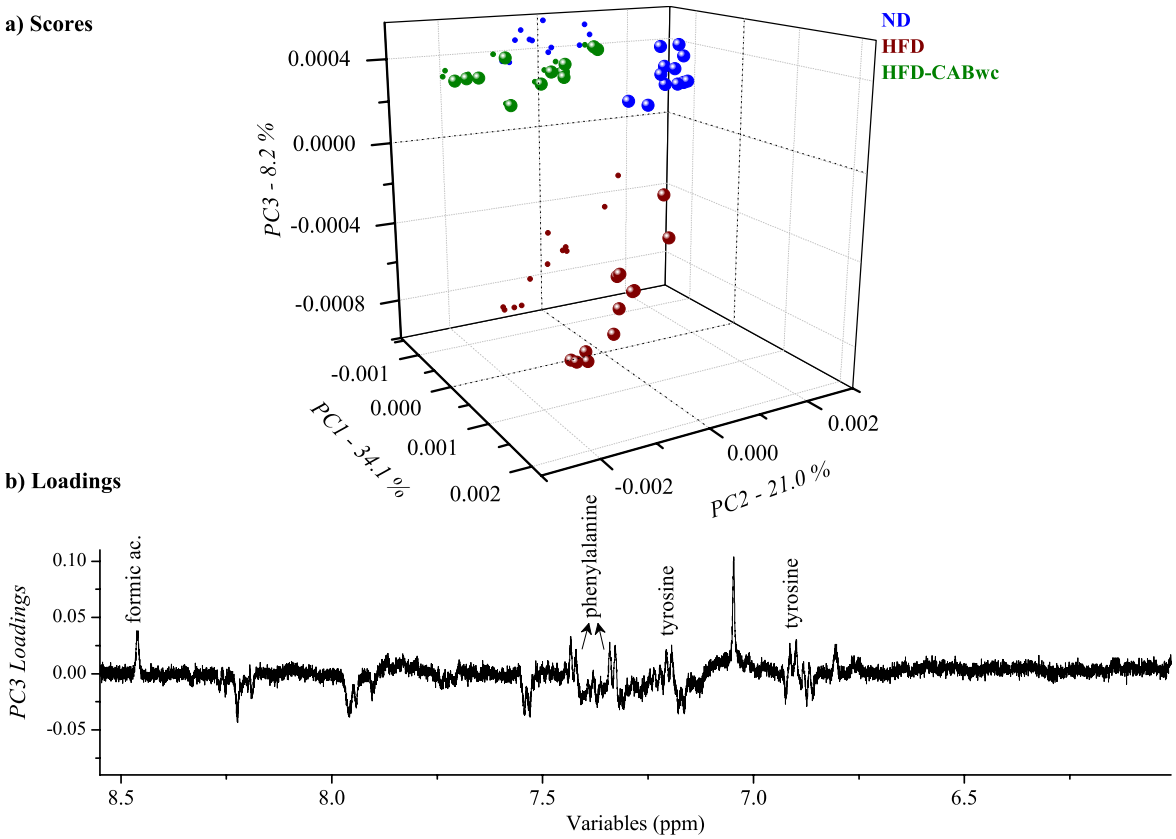


Figure S3 a) PC1 × PC2 × PC3 scores coordinate system from aromatic region, with projections in PC2 × PC3 plane for feces samples: mice fed chow diet (CD) in blue color, mice fed high fat diet (HFD) in red, and mice fed HFD supplemented cashew apple fiber without low molecular weight metabolites (HFD-CABwc) in green; b) PC3 loadings plotted in lines form.

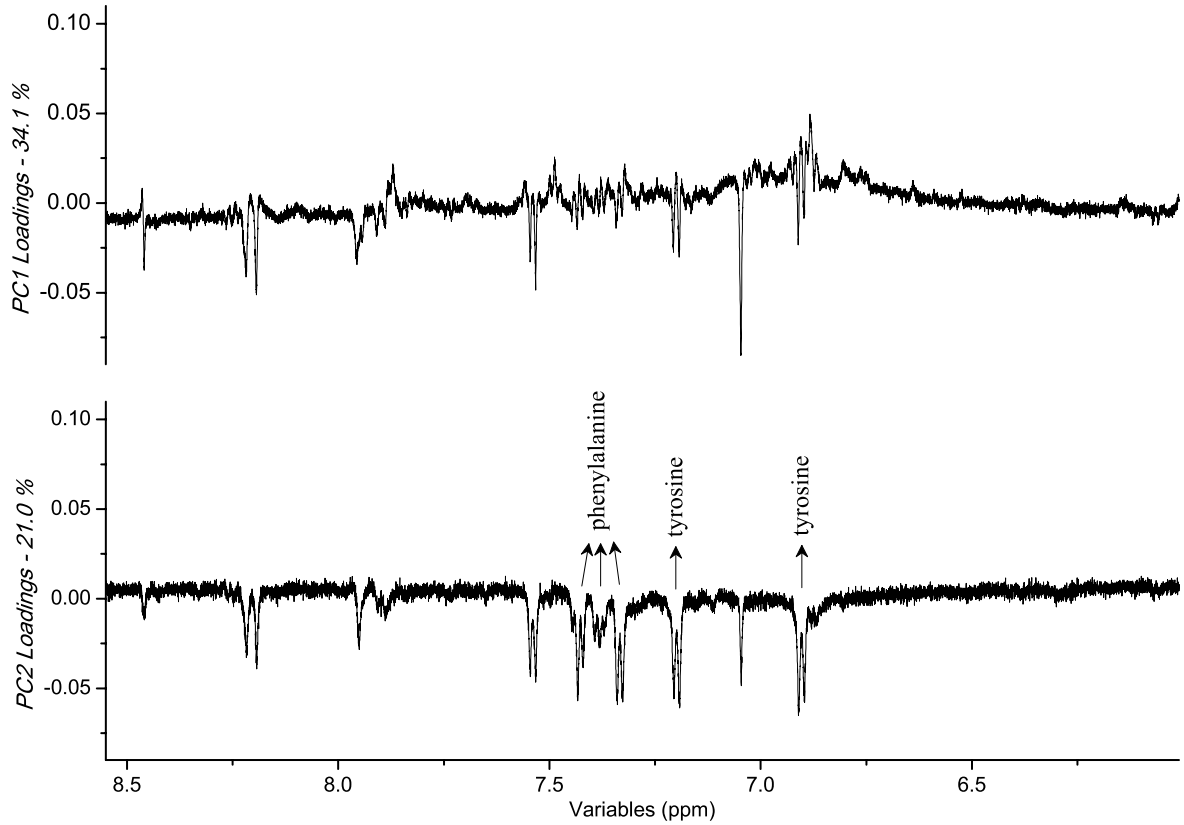


Figure S4. PC1 and PC2 loadings of the aromatic region plotted in lines from feces evaluation.

3. References

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