Electronic Supplementary Material (ESI) for Food & Function.
This journal is © The Royal Society of Chemistry 2022

SUPPLEMENTARY DATA

Saffron extract (Safr'Inside^{TM}) improves anxiety related behaviour in a mouse model of low-grade inflammation through the modulation of the microbiota and gut derived metabolites.

Matthew Pontifex[1], Emily Connell[1], Gwenaelle Le Gall[1], Line Pourtau[2], David Gaudout[2], Cristina Angeloni[3], Lorenzo Zallocco[4], Maurizio Ronci[5], Laura Giusti[6], Michael Müller[1] and David Vauzour[1]*

[1] *Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom.*

[2] *Activ'Inside, Beychac et Caillau, France*

[3] *Department for Life Quality Studies, Alma Mater Studiorum, University of Bologna, Corso d'Augusto 237, 47921 Rimini (RN), Italy*

[4] *Department of Pharmacy, University of Pisa, 56126 Pisa (PI), Italy*

[5] *Department of Pharmacy, University G. d'Annunzio of Chieti-Pescara, Chieti, Italy.*

[6] *School of Pharmacy, University of Camerino, Via Gentile III da Varano, 62032 Camerino (MC), Italy*

* To whom correspondence should be addressed: Dr David Vauzour; Email: D.Vauzour@uea.ac.uk

Table S1: Full dietary composition

Diet	D10012M AIN-93M		D21011503 (44mg Safr’Inside™)	
	gm %	Kcal %	gm %	Kcal %
Protein	14	15	14	15
Carbohydrate	73	76	73	76
Fat	4	9	4	9
Total		100		100
Kcal/gm	3.8		3.8	
Dietary component	gm	Kcal	gm	Kcal
Casein	140	560	140	560
L-Cystine	1.8	7.2	1.8	7.2
Corn starch	495.692	1983	495.692	1983
Maltodextrin 10	125	500	125	500
Sucrose	100	400	100	400
Cellulose BW200	50	0	50	0
Soybean Oil	40	360	40	360
t-Butylhydroquinone	0.008	0	0.008	0
Mineral mix S10022M	35	0	35	0
Vitamin mix V10037	10	40	10	40
Choline Bitartrate	2.5	0	2.5	0
Safr’Inside™	0	0	0.044	0
FD&C Blue Dye #1	0	0	0.05	0
Total	1000	3850	1000.094	3850

Table S2: Bacterial abundance across treatment groups.

	Sham	**LPS**	**LPS SAFF**	**P value**	**FDR**
Phylum					
Bacteroidota	2096 ± 311	829 ± 99.5	2170 ± 345	0.003379	0.024421
Firmicutes	48667 ± 1164	54004 ± 492	50608 ± 607	0.005427	0.024421
Verrucomicrobiota	1063 ± 450	117 ± 31.4	2259 ± 676	0.011951	0.035854
Actinobacteriota	3648 ± 1150	1001 ± 343	875 ± 165	0.019305	0.043437
Family					
Rhizobiaceae	17 ± 3.48	31 ± 3.24	2 ± 1.12	0.001446	0.031211
Rikenellaceae	430 ± 38.5	150 ± 18.0	365 ± 133	0.004171	0.031211
Clostridia_UCG_014	60 ± 5.06	68 ± 10.8	217 ± 39.6	0.004479	0.031211
Anaerovoracaceae	23 ± 4.65	28 ± 5.74	69 ± 5.92	0.005061	0.031211
Prevotellaceae	55 ± 13.7	12 ± 3.07	125 ± 17.2	0.005061	0.031211
Muribaculaceae	1403 ± 276	552 ± 93.3	1456 ± 192	0.005959	0.031499
Christensenellaceae	2 ± 0.80	1 ± 0.55	13 ± 2.57	0.010253	0.04422
Akkermansiaceae	1063 ± 450	117 ± 31.4	2259 ± 676	0.011951	0.04422
Genus					
Christensenellaceae_R_7_group	0 ± 0.00	0 ± 0.00	7 ± 1.54	0.001097	0.034042
Anaerostipes	0 ± 0.00	0 ± 0.00	120 ± 47.6	0.001796	0.034042
Ruminococcus	0 ± 0.00	0 ± 0.00	43 ± 15.7	0.001796	0.034042
Eubacterium_nodatum_group	2 ± 0.91	5 ± 1.65	20 ± 3.16	0.003241	0.034042
Family_XIII_UCG_001	3 ± 0.76	6 ± 1.29	13 ± 2.56	0.003651	0.034042

Rikenella	27 ± 7.51	16 ± 7.54	0 ± 0.00	0.003919	0.034042
Clostridia_UCG_014	60 ± 5.06	68 ± 10.8	217 ± 39.6	0.004479	0.034042
Coriobacteriaceae_UCG_002	161 ± 43.7	103 ± 19.4	2 ± 1.02	0.004705	0.034042
Muribaculum	40 ± 9.04	6 ± 1.52	23 ± 5.92	0.004838	0.034042
Alloprevotella	55 ± 13.7	12 ± 3.1	125 ± 17.2	0.005061	0.034042
Rikenellaceae_RC9_gut_group	27 ± 7.51	16 ± 7.54	0 ± 0.00	0.007149	0.041107
Eubacterium_brachy_group	191 ± 35.7	47 ± 8.93	127 ± 58.5	0.01109	0.046275
Negativibacillus	0 ± 0.00	1 ± 0.3	3 ± 1.28	0.011323	0.046275
Anaeroplasma	21 ± 8.99	8 ± 2.77	0 ± 0.00	0.011426	0.046275
A2	79 ± 41.3	326 ± 52.7	197 ± 42.0	0.011897	0.046275
Akkermansia	1063 ± 450	117 ± 31.4	2259 ± 676	0.011951	0.046275
Staphylococcus	3 ± 0.93	9 ± 1.30	4 ± 1.32	0.012921	0.046275
Lactococcus	39 ± 10.4	123 ± 24.6	53 ± 6.55	0.013124	0.046275
Desulfovibrio	597 ± 533	42 ± 6.68	9 ± 2.43	0.013413	0.046275

Table S3: Full statistical analysis of metabolomic data

Metabolite	f.value	p.value	FDR	Fisher's LSD
Urocanate	16.164	0.0003	0.016	LPS - LPS SAFF; Sham - LPS; Sham - LPS SAFF
Cytidine	15.657	0.0003	0.016	LPS SAFF - LPS; LPS - Sham; LPS SAFF - Sham
Putrescine	11	0.0016	0.044	LPS - Sham; LPS SAFF - Sham
Valine	10.487	0.0019	0.044	LPS SAFF - LPS; Sham - LPS; Sham - LPS SAFF
Histidine	9.1322	0.0033	0.050	Sham - LPS; Sham - LPS SAFF
Ornithine	9.0624	0.0034	0.050	LPS SAFF - LPS; Sham - LPS
Guanosine	8.7775	0.0039	0.050	LPS SAFF - LPS; LPS SAFF - Sham
Alanine	7.802	0.0059	0.063	Sham - LPS; Sham - LPS SAFF
Guanosine phosphate derivate	7.6816	0.0063	0.063	LPS - Sham; LPS SAFF - Sham
Dimethylamine	6.5439	0.0108	0.097	LPS - LPS SAFF; Sham - LPS SAFF
Leucine	6.244	0.0126	0.098	LPS SAFF - LPS; Sham - LPS
Threonine	6.1647	0.0131	0.098	LPS SAFF - LPS; Sham - LPS

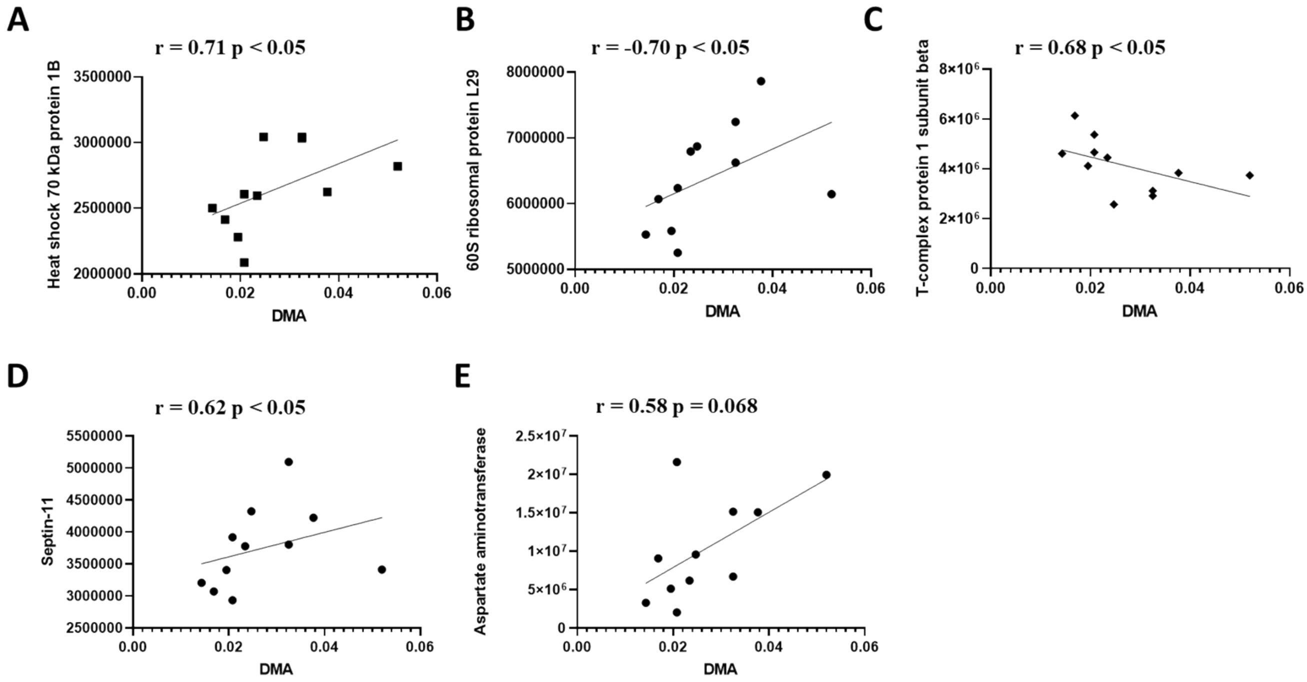

Figure S1: DMA correlates with several significantly modulated proteins