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SUPPLEMENTARY MATERIAL SUBMITTED TO METABOLITES (ISSN: 2218-1989) Article

Digesta and Plasma Metabolomics of Rainbow Trout Strains with Varied Tolerance of Plant-Based Diets Highlights Potential for Non-Lethal Assessments of Enteritis Development

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Supplementary Table S1. Amino acid profile (%; as fed) of the two diets administered. Values listed as mean from four technical replicates.

Amino Acid (%)	PM Diet	FM Diet
Pro	2.47	2.60
Tau	0.43	0.37
Asp	1.18	1.33
Thr	2.01	2.10
Ser	4.21	4.12
Glu	3.73	3.22
Gly	1.03	0.86
Ala	2.38	2.23
Cys	1.95	2.04
Val	0.69	0.48
Met	1.07	1.06
Ile	2.05	2.27
Leu	2.36	2.71
Tyr	4.43	4.51
Phe	9.20	8.11
His	1.78	2.17
Trp	2.43	2.11
Lys	2.31	2.21
Arg	0.53	0.42

Supplementary Table S2. Growth performance data, including feed conversion ratio (FCR).

Strain	Diet	Timepoint	Start #	Morts	End #	Tank Density (g/L)	Tank FCR
ARS_*Sel*	PM	3 months	30	3	27	55,1	0,79
ARS_*Sel*	FM	3 months	30	5	25	50,4	0,98
CS_1	PM	3 months	30	1	29	49,9	1,01
CS_1	FM	3 months	30	2	28	48,9	0,80
CS_2	PM	3 months	30	2	28	51,4	0,99
CS_2	FM	3 months	30	1	29	67,2	0,78
ARS_*Sel*	PM	9 months	27	5	22	100,0	1,29
ARS_*Sel*	FM	9 months	25	0	25	108,5	1,10
CS_1	PM	9 months	29	1	28	102,1	1,42
CS_1	FM	9 months	28	5	23	95,1	1,82
CS_2	PM	9 months	28	1	27	96,5	1,73
CS_2	FM	9 months	29	3	26	131,0	1,63

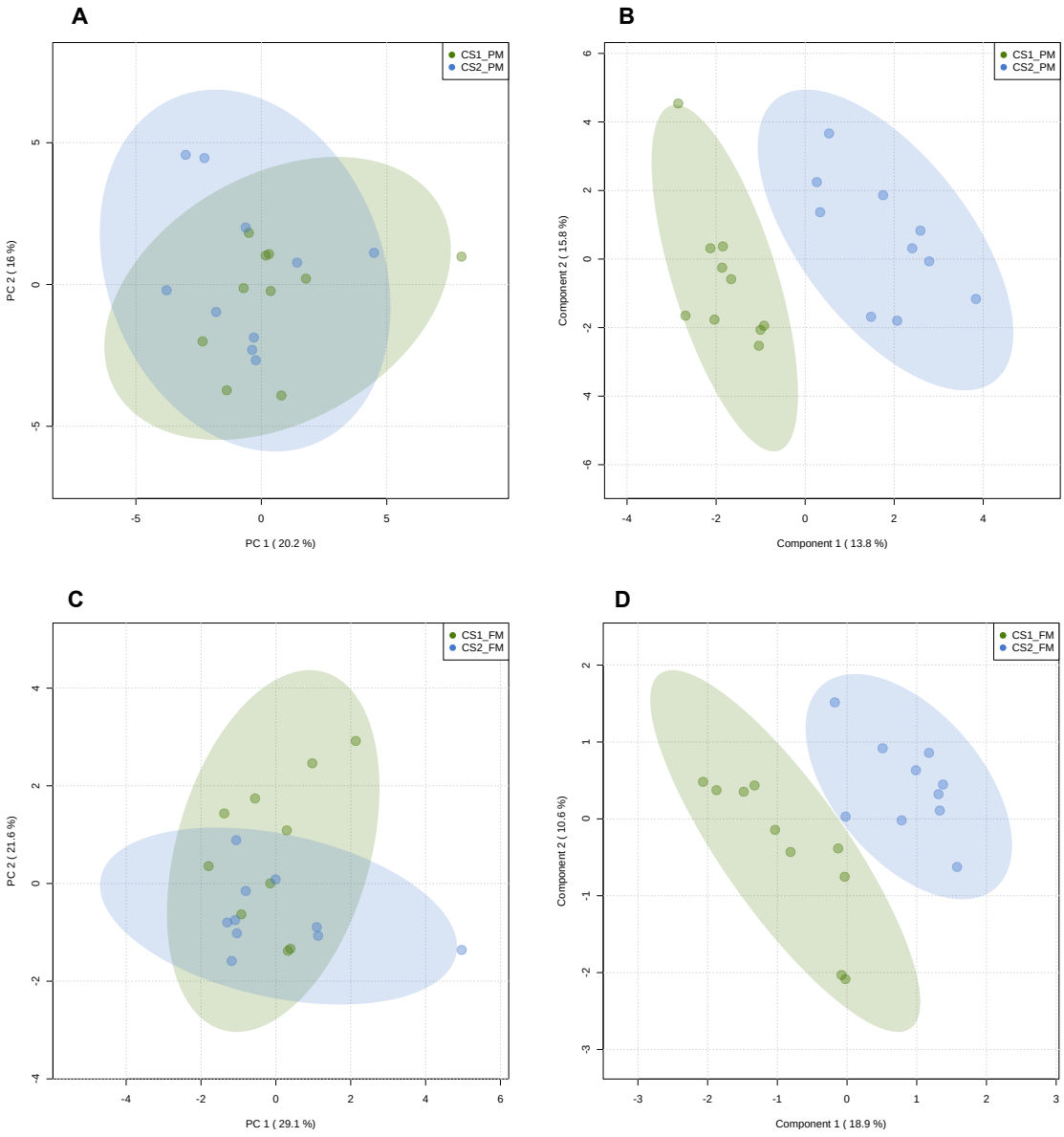
Supplementary Table S3. Metabolites identified in blood plasma, average concentration (mM) and standard deviation (SD) by experimental group.

	ARS-Sel_PM		ARS-Sel_FM		CS-1_PM		CS-1_FM		CS-2_PM		CS-2_FM	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
3-Hydroxyisobutyrate	27.4	19.7	20.1	18.0	20.0	10.8	26.0	16.2	19.4	17.1	29.7	21.7
Butyrate	8.4	4.6	12.1	8.3	11.4	6.4	11.0	6.3	10.3	5.8	12.0	8.0
Acetic acid	6.9	0.7	7.0	1.1	7.7	2.4	6.1	1.6	7.7	1.6	6.3	1.0
Betaine	39.6	9.0	264.2	123.6	38.9	10.2	166.9	56.1	92.5	35.2	340.3	122.1
Acetoacetate	21.8	11.2	9.6	4.7	15.2	2.9	11.4	3.2	14.5	3.8	8.2	4.6
Carnitine	0.0	0.0	4.3	4.2	1.0	1.6	4.0	5.9	0.0	0.0	15.6	9.3
Creatine	65.7	23.0	109.4	60.8	90.7	50.5	100.6	42.4	35.6	9.5	89.7	44.5
Dimethylamine	4.1	1.9	12.8	3.3	5.5	4.1	12.4	2.7	3.8	2.2	12.3	5.1
Citric acid	40.3	16.3	32.4	11.7	33.8	10.6	35.7	8.9	26.6	9.4	30.3	9.3
Choline	11.8	1.5	11.9	5.1	12.0	2.7	17.4	4.0	17.6	2.9	17.8	7.7
D-Glucose	3367.2	850.7	3983.8	935.2	3738.5	1039.7	4853.9	1090.5	4293.5	1137.1	7566.3	4586.0
Glycine	611.4	145.1	747.2	293.5	467.0	65.8	513.6	125.4	401.8	98.1	496.6	97.0
Formate	108.3	15.7	87.7	18.8	113.3	20.3	97.2	9.3	114.4	14.1	105.5	14.6
L-Glutamic acid	129.9	33.8	102.5	34.2	118.4	50.2	75.8	21.4	117.0	30.4	99.9	19.6
Tyrosine	45.3	14.1	36.6	14.8	48.0	9.6	38.0	4.7	49.4	14.4	31.1	11.4
L-Phenylalanine	235.5	51.0	222.3	59.0	231.2	22.1	192.0	35.2	226.8	44.9	188.3	35.1
L-Alanine	193.4	44.5	122.1	18.2	136.6	23.5	122.3	36.4	133.4	32.2	126.2	20.5
L-Proline	114.5	52.7	37.9	22.0	69.7	37.3	44.7	19.0	98.2	41.5	44.6	19.4
L-Threonine	489.8	172.3	268.9	62.1	484.0	178.2	237.0	55.6	385.2	72.2	351.9	158.6
L-Asparagine	129.4	42.7	115.0	31.2	127.2	28.1	107.3	28.6	103.6	34.9	98.0	36.8
Isoleucine	278.1	99.2	175.9	36.7	218.6	31.1	187.3	34.5	280.5	64.5	186.1	40.0
L-Histidine	391.4	107.3	273.4	115.6	451.7	167.2	378.3	113.1	361.1	154.3	435.9	166.2
L-Lysine	234.9	55.1	127.8	26.5	159.9	41.2	99.1	34.0	191.6	59.3	102.8	28.7
L-Lactic acid	1683.7	310.0	2037.1	172.6	2244.6	281.3	2693.6	564.4	2019.5	448.0	2340.4	697.1
Aspartate	28.7	10.5	21.6	11.3	23.6	10.1	23.7	9.7	30.3	15.9	18.6	11.3
Myo-inositol	158.4	30.5	86.2	46.0	158.2	37.8	147.9	58.5	205.7	40.1	172.2	72.0
Pyruvic acid	12.1	4.6	10.0	3.8	14.1	3.0	12.7	7.9	11.6	4.5	13.7	9.1
Taurine	1063.4	239.1	396.1	131.3	1004.1	311.4	324.3	72.8	1379.2	334.8	530.6	261.5
Succinate	2.3	2.1	2.0	1.5	2.4	1.6	2.1	1.5	2.1	1.3	2.2	1.0
Sarcosine	20.4	5.4	34.8	11.4	14.2	2.6	32.5	6.8	16.2	3.5	40.9	9.1
L-Arginine	134.8	27.4	95.8	35.8	78.9	26.5	63.9	39.7	92.0	40.8	50.4	27.9
Creatinine	6.3	5.2	6.1	4.0	7.0	4.7	6.5	5.2	9.3	2.9	7.5	5.5
L-Glutamine	554.7	93.2	466.1	179.5	465.2	98.0	412.3	116.5	490.5	131.1	450.2	114.3
L-Leucine	554.2	201.1	319.8	56.2	415.2	74.9	337.7	61.6	550.3	138.7	357.1	70.7
Methionine	628.3	136.2	222.2	52.7	491.0	128.1	193.9	40.1	521.5	97.2	218.6	57.0
Valine	530.2	183.2	422.9	73.5	439.2	55.9	423.7	62.8	538.9	119.3	469.8	81.8

Supplementary Table S4. Metabolites identified in digesta, average concentration (mM) and standard deviation (SD) by experimental group.

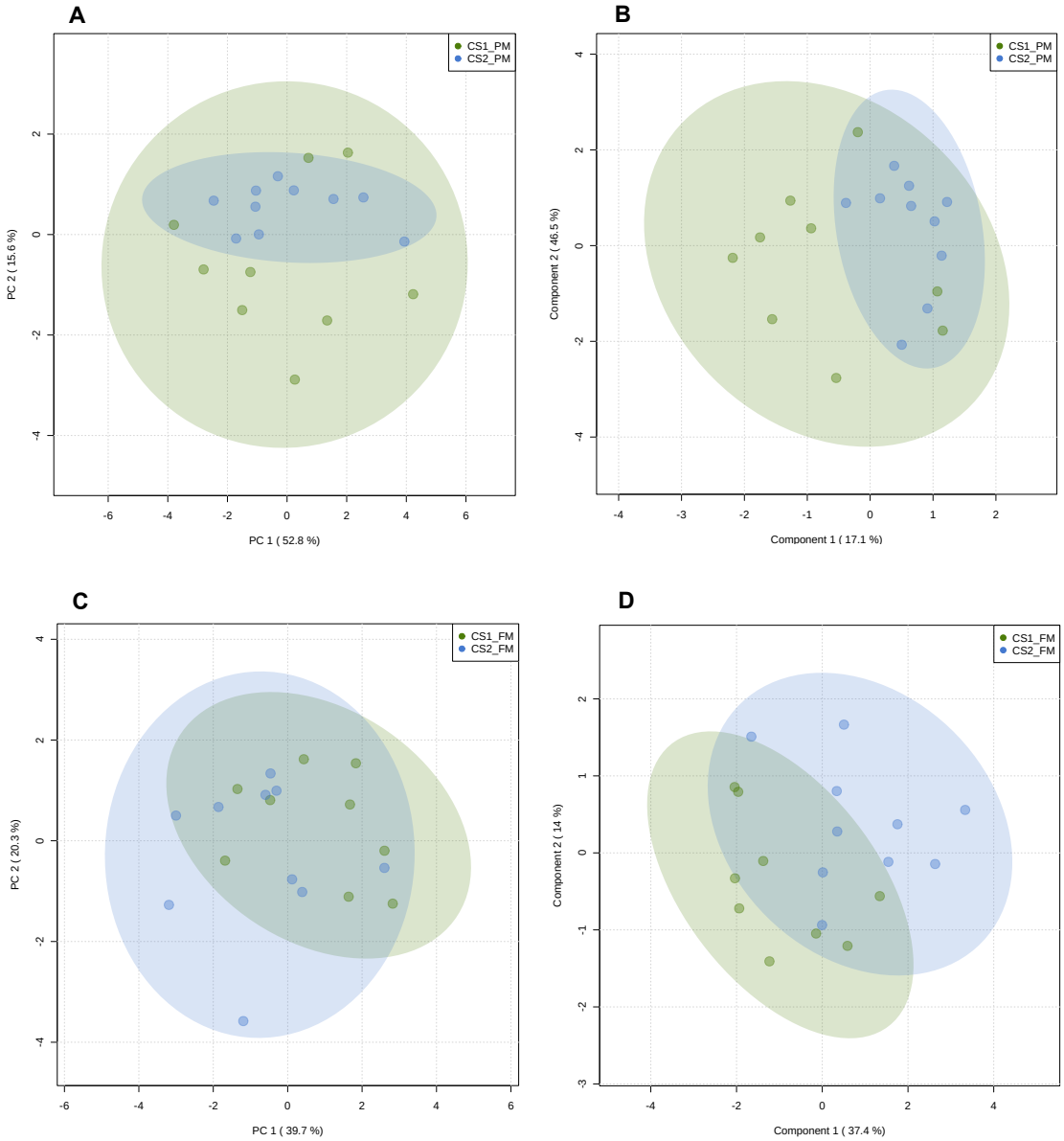
	ARS-Sel_PM		ARS-Sel_FM		CS-1_PM		CS-1_FM		CS-2_PM		CS-2_FM	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Valine	15.2	6.3	5.0	2.9	10.0	6.9	4.7	1.6	6.0	2.8	7.9	4.9
Leucine	12.8	4.9	4.3	2.0	10.0	7.8	4.4	1.5	9.4	4.9	8.4	5.0
Isoleucine	20.5	10.7	6.6	2.4	12.4	7.0	5.6	2.9	5.9	1.1	14.4	11.6
Threonine Lactate	5.6	3.2	2.3	1.2	4.0	2.8	3.0	1.3	3.0	1.6	4.4	2.3
Alanine	12.5	4.5	4.3	2.5	8.7	6.4	4.6	1.9	7.8	4.2	8.8	4.8
Acetate	10.6	9.6	2.7	2.2	5.8	3.2	1.7	0.6	2.7	0.9	2.3	1.1
Methionine	11.9	5.1	3.3	1.4	9.4	5.7	3.9	1.9	6.0	2.9	8.0	4.7
Glutamate	13.8	11.3	6.2	4.5	18.2	12.8	8.3	3.4	13.2	7.2	18.5	11.2
Succinate	0.2	0.3	0.0	0.0	0.5	0.4	0.1	0.1	0.3	0.3	0.2	0.2
Aspartate	4.3	6.3	1.6	2.0	6.2	6.9	2.7	2.6	3.8	3.1	6.4	4.7
Creatine/Creatine-P/Creatinine	1.0	0.9	0.7	0.6	1.1	1.0	1.3	0.7	0.4	0.3	1.7	0.9
Dimethyl sulfone	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.4
Choline/Carnitine	1.2	1.4	5.2	5.4	1.0	1.0	7.5	4.3	0.4	0.3	8.8	6.6
Glycerol	86.3	66.8	26.3	21.5	42.8	43.5	22.7	27.4	28.4	13.3	46.6	44.2
Glycine	48.4	37.5	20.0	16.2	26.8	13.2	9.7	5.1	16.6	9.6	19.1	10.0
Glucose 6P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	1.8	0.6	0.9
Glucose	36.6	23.5	18.1	14.2	10.8	9.6	15.8	13.9	9.4	5.6	28.1	24.5
Sucrose	41.5	60.5	18.5	17.7	3.5	4.3	3.9	3.6	2.7	2.2	10.0	9.8
Tyrosine	1.3	2.0	0.3	0.5	1.4	1.6	0.8	0.7	1.4	1.1	0.6	0.6
Phenylalanine	2.5	4.5	2.0	2.5	4.4	2.5	2.0	1.7	4.1	2.7	2.8	1.3
Formate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0

Figure S1



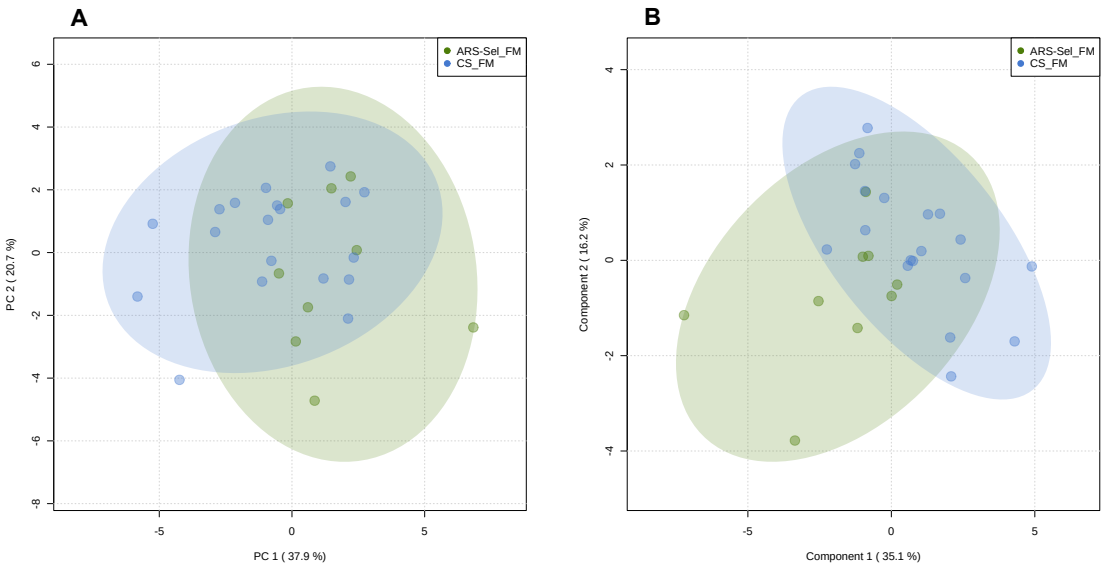
Supplementary Figure S1. Pairwise multivariate analysis of the groups CS-1 and CS-2 by diet, in plasma. **A)** PCA scores plot of the CS-1_PM and CS-2_PM groups; **B)** PLS scores plot of the CS-1_PM and CS-2_PM groups, model not validated (NC = 2; R^2 = 0.883; Q^2 = 0.499; 1000 permutations: p = 0.388); **C)** PCA scores plot of the CS-1_FM and CS-2_FM; **D)** PLS scores plot of the CS-1_FM and CS-2_FM, model not validated (NC = 4; R^2 = 0.922; Q^2 = 0.582; 1000 permutations: p = 0.728).

Figure S2



Supplementary Figure S2. Pairwise multivariate analysis of the groups CS-1 and CS-2 by diet, in digesta: **A)** PCA scores plot of the CS-1_PM and CS-2_PM; **B)** PLS scores plot of the CS-1_PM and CS-2_PM, model not validated (NC = 4; R^2 = 0.788; Q^2 = 0.044; 1000 permutations: p = 0.317); **C)** PCA scores plot of the CS-1_FM and CS-2_FM; **D)** PLS scores plot of the CS-1_FM and CS-2_FM, model not validated (NC = 1; R^2 = 0.339; Q^2 = 0.026; 1000 permutations: p = 0.127).

Figure S3



Supplementary Figure S3. Multivariate analysis of groups ARS_FM and CS_FM in digesta: **A)** PCA scores plot; **B)** PLS scores plot, model not validated (NC = 1; R^2 = 0.299; Q^2 = 0.082; 1000 permutations: p = 0.604).