

Effect of feed supplementation with docosahexaenoic acid in regio- and enantiopure triacylglycerols on gut metabolome and microbiota in rats

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Table S1. Composition of diet

Ingredients	Contents (g/kg)
Corn starch	397
Casein (> 85% protein)	200
Corn dextrin (90–94% tetrose)	132
Sucrose	100
Oil ¹	70
Fiber	50
Minerals	35.5
Vitamins	10
L-cystinol	3
Choline bitartrate (42% choline)	2.5
Tert-butylhydroquinone	0.014

¹ Low n-3 FA diet (modified AIN-93G diet) for the n3De, Trip, sn1DHA, sn2DHA, and sn3DHA groups contained peanut oil. Standard n-3 AIN-93G diet for the Con group contained soybean oil

Table S2 Fatty acid composition of oils used in the feed. Values are expressed as mean mass percentages of two replicates.

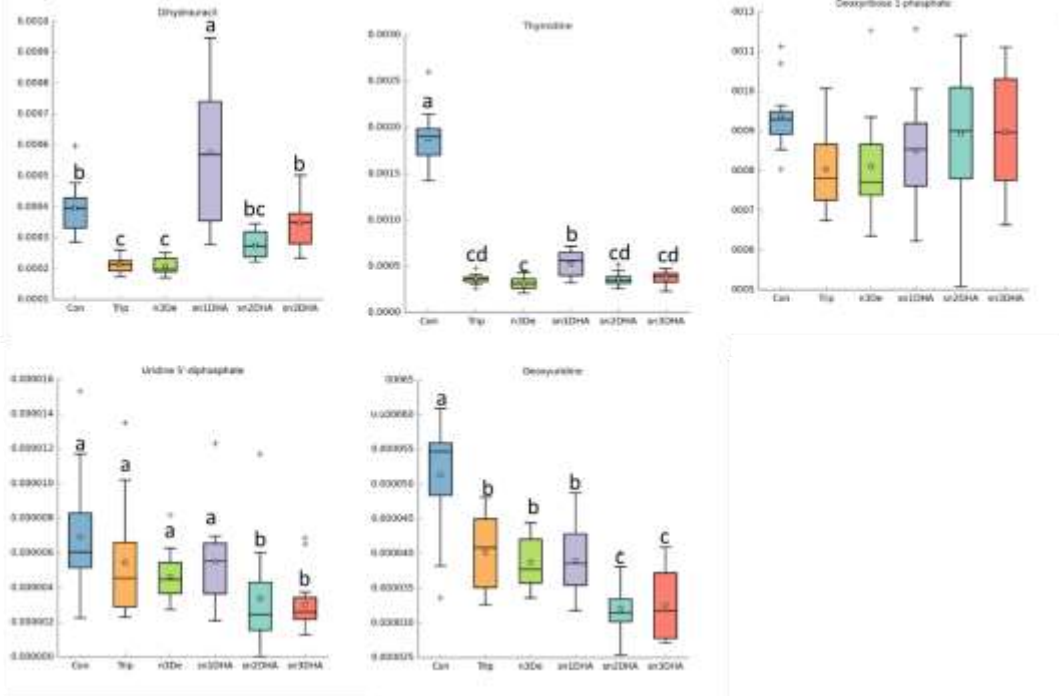
Fatty acid	Soybean oil	Peanut oil
16:0	10.40	9.61
18:0	4.46	3.54
18:1(n-9)	22.95	49.75
18:2(n-6)	53.30	31.29
18:3(n-3)	7.50	0.11
20:0	0.40	1.24
22:0	0.40	2.39
24:0	0.12	1.04
Others ¹	0.47	1.03

¹ This category includes 16:1(n-7), 18:1(n-7), 18:3(n-6), 20:1(n-9) and 23:0.

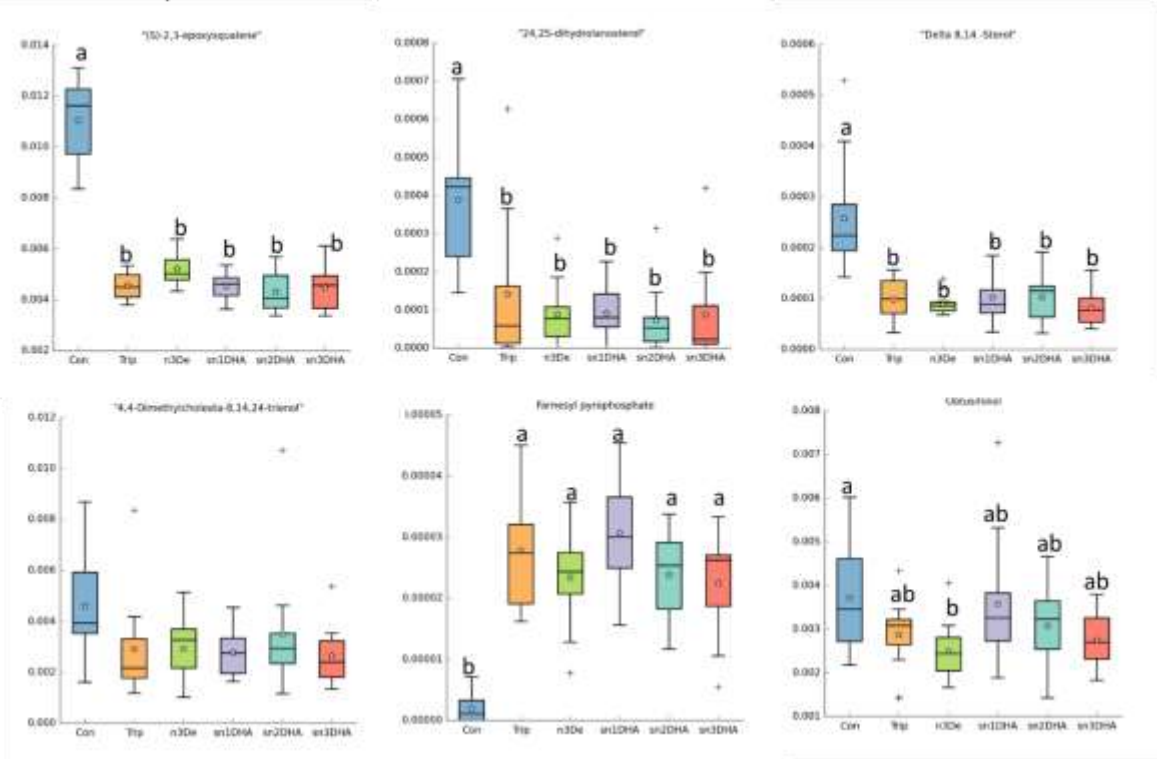
Table S4 Correlation analysis between gut microbiota at species level.

Correlation pairs	Correlation coefficient	P value
('Bacteroides_sp._AM16-15', 'Bacteroides_sp._CAG:98')	0.999591571	1.54356E-52
('Bacteroides_sp._AM22-3LB', 'Bacteroides_sp._CAG:98')	0.99962793	3.31526E-53
('Bacteroides_sp._AM16-15', 'Bacteroides_sp._AM22-3LB')	0.999748845	5.06544E-56
('Bacteroides_sp._AM16-15', 'Bacteroides_sp._AM25-34')	0.999754601	3.45552E-56
('Bacteroides_sp._AM25-34', 'Bacteroides_sp._CAG:98')	0.99977323	9.39371E-57
('Bacteroides_massiliensis', 'Bacteroides_sp._AM16-15')	0.999804403	8.1895E-58
('Bacteroides_massiliensis', 'Bacteroides_sp._CAG:98')	0.999831142	7.24371E-59
('Bacteroides_sp._AM22-3LB', 'Bacteroides_sp._AM25-34')	0.99984375	2.01344E-59
('Bacteroides_massiliensis', 'Bacteroides_sp._AM22-3LB')	0.999845795	1.62004E-59
('Bacteroides_massiliensis', 'Bacteroides_sp._AM25-34')	0.999892545	4.18085E-62

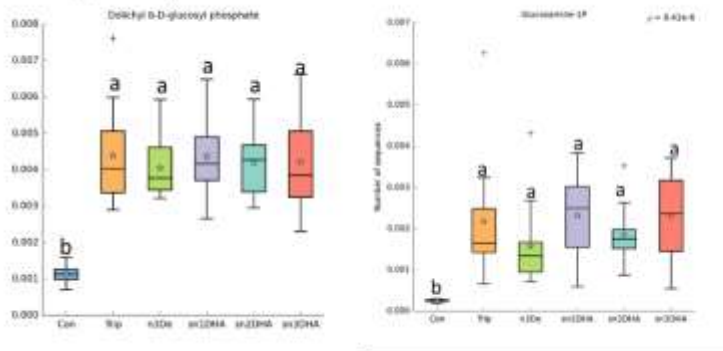
A Pyrimidine metabolism



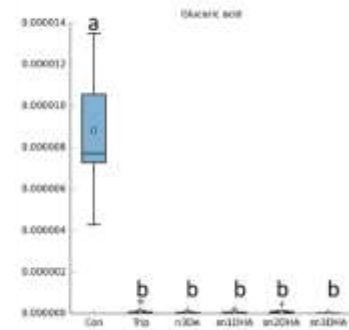
B Steroid biosynthesis



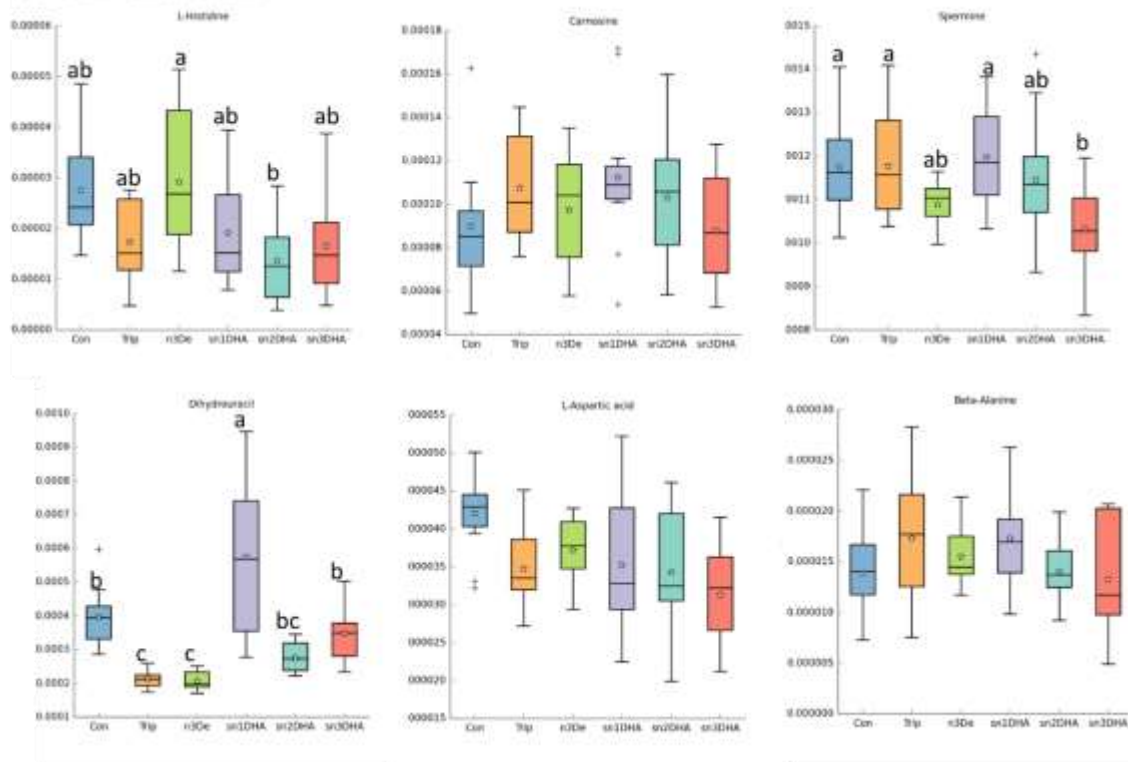
C N-Glycan biosynthesis



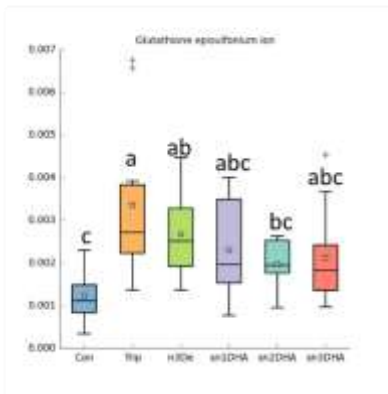
D Ascorbate and aldarate metabolism



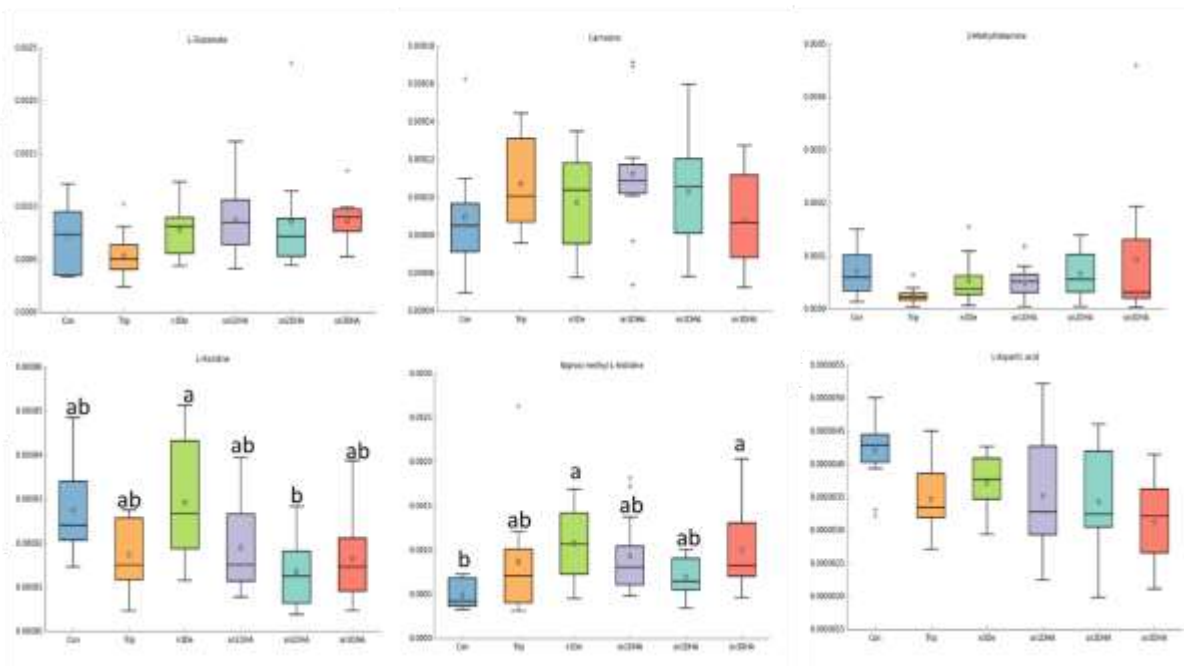
E beta-Alanine metabolism



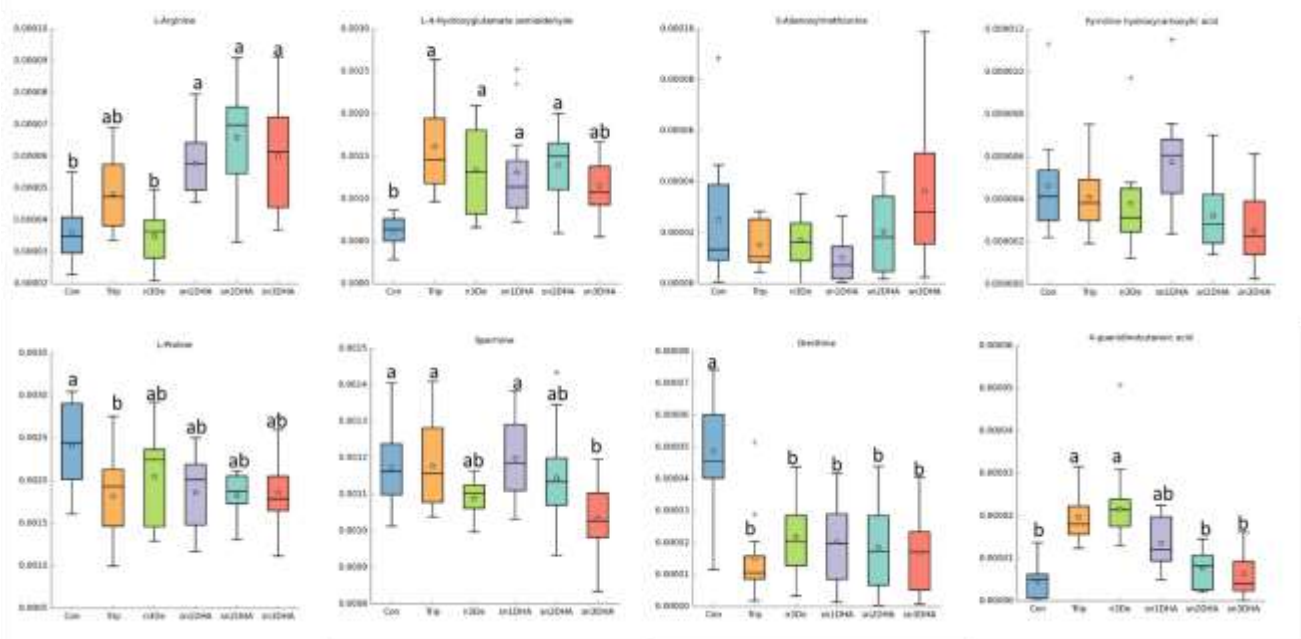
F Metabolism of xenobiotics by cytochrome P450



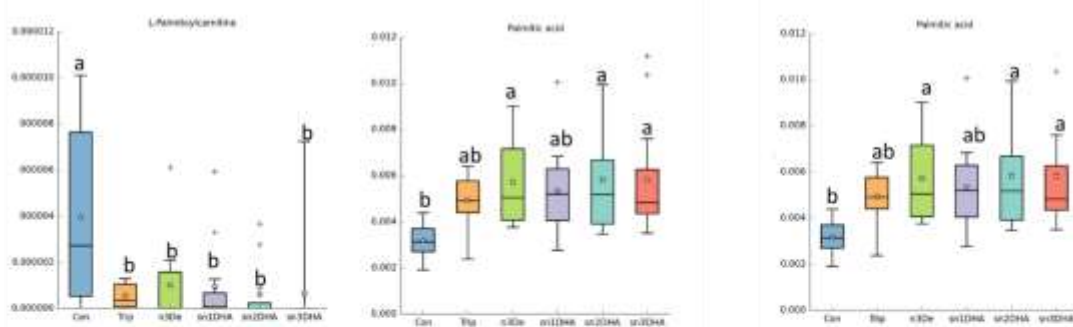
G Histidine metabolism



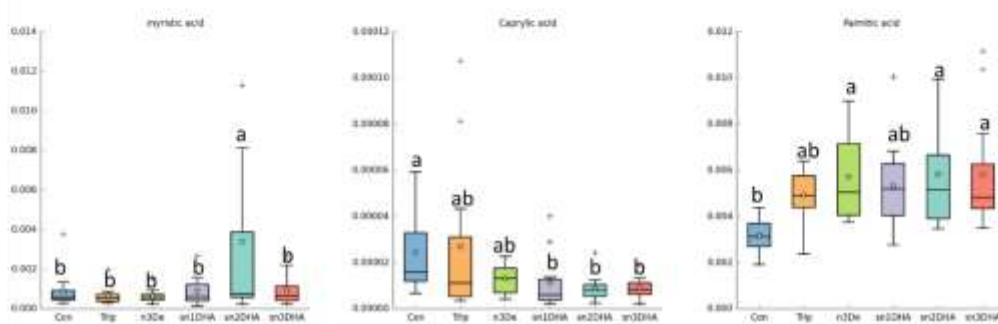
H Arginine and proline metabolism



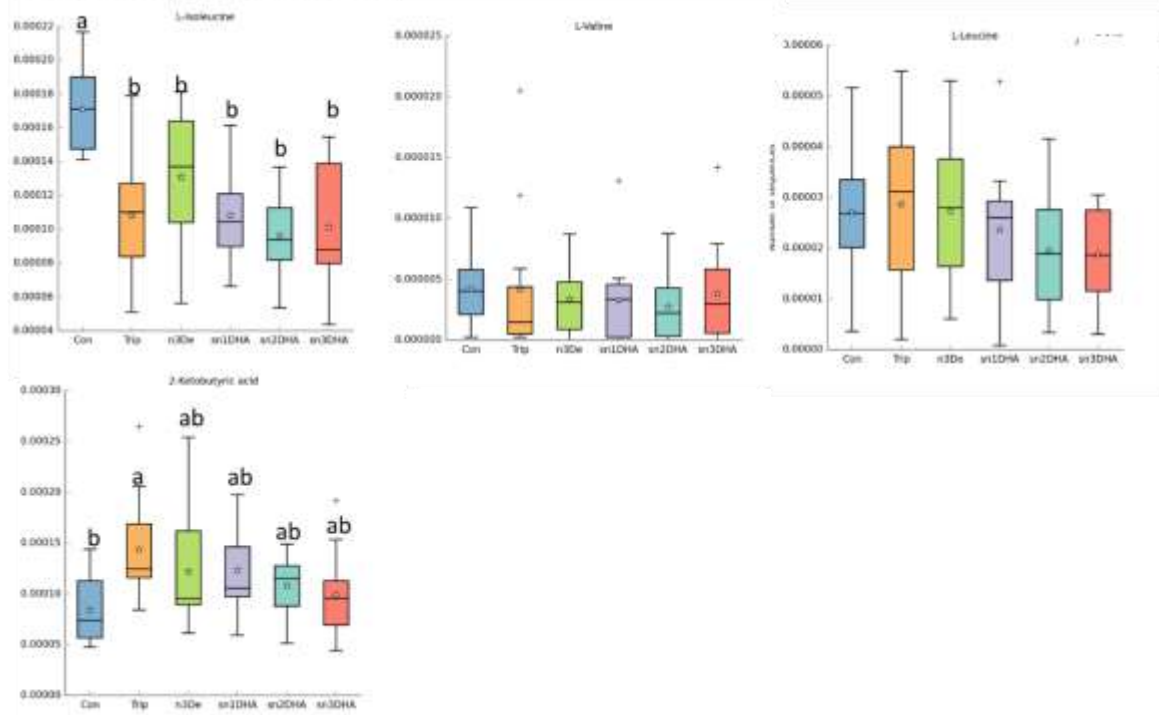
I Fatty acid degradation



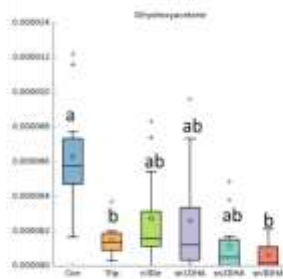
J Fatty acid biosynthesis



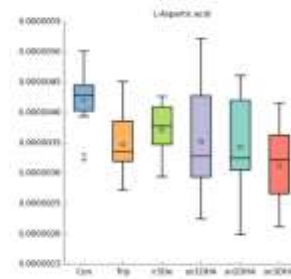
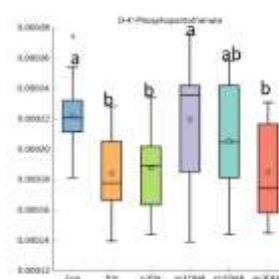
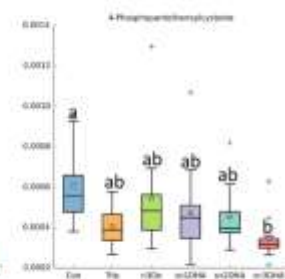
L Valine, leucine, and isoleucine metabolism



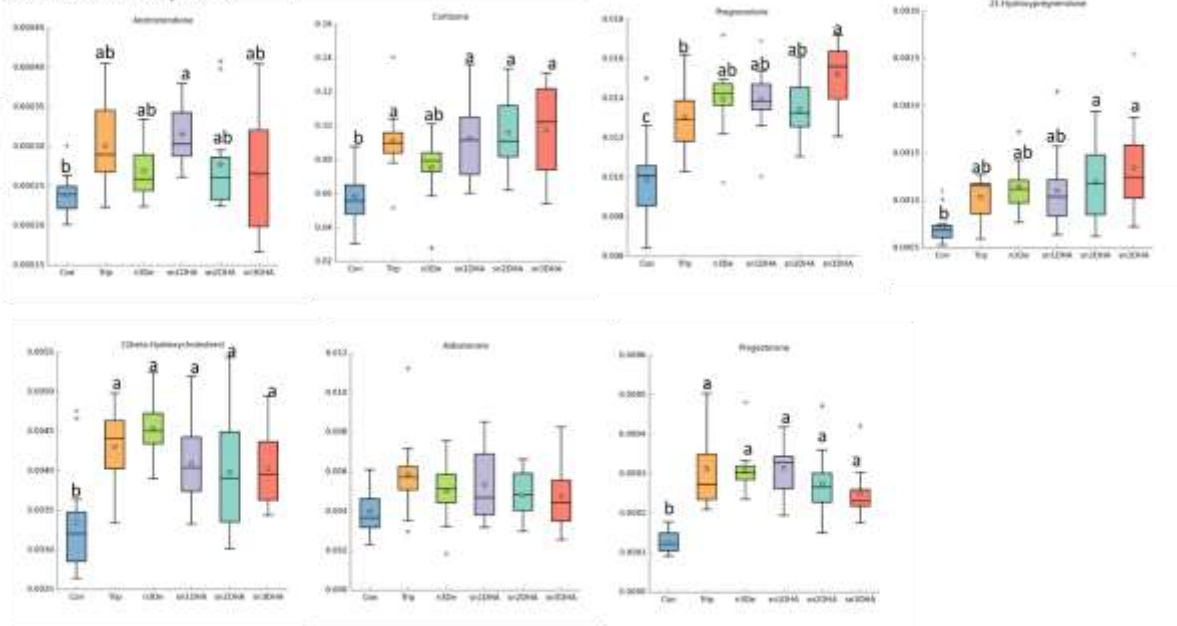
M Glycerolipid metabolism



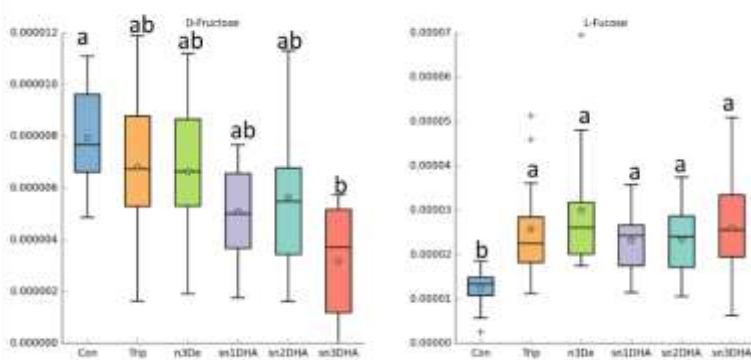
N Pantothenate and CoA biosynthesis



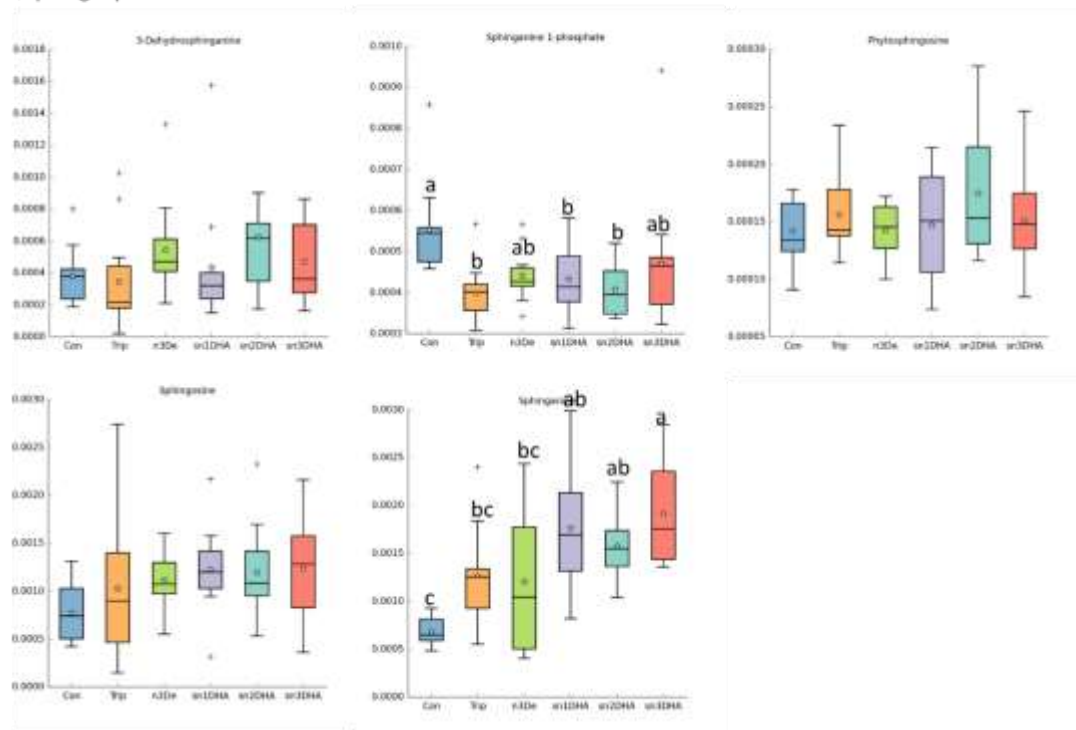
O Steroid hormone biosynthesis



P Fructose and mannose metabolism



Q Spingolipid metabolism



R Glycine, serine and threonine metabolism

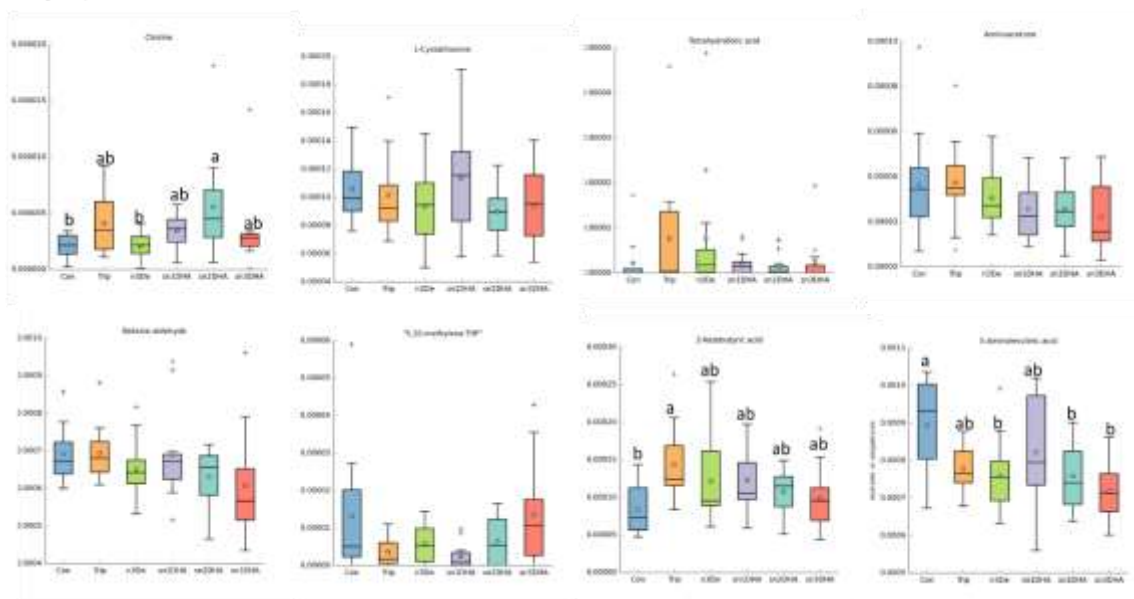


Figure S1. Metabolites mapped to those pathways in Table S3 and their statistical analysis.

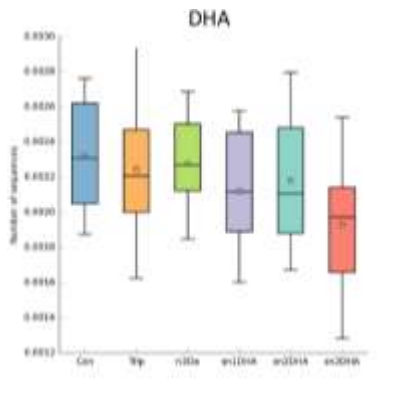
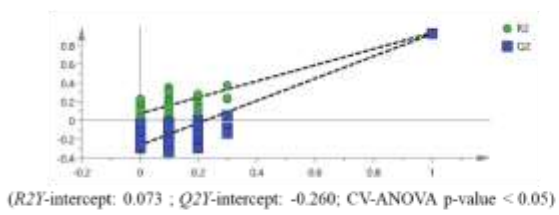
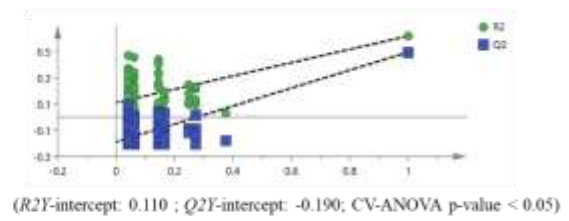


Figure S2. DHA level.

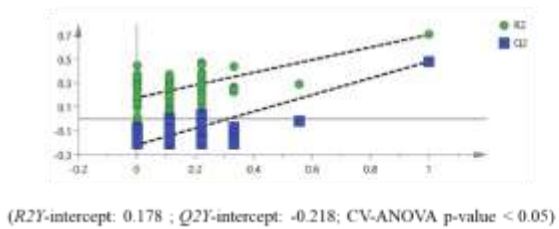
A Model 1



B Model 2



C Model 3



D Model 4

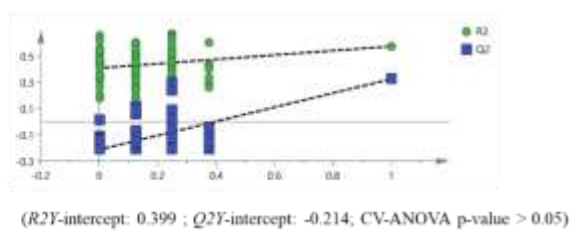


Figure S3. Validation of PLS-DA models by permutation test and CV-ANOVA.

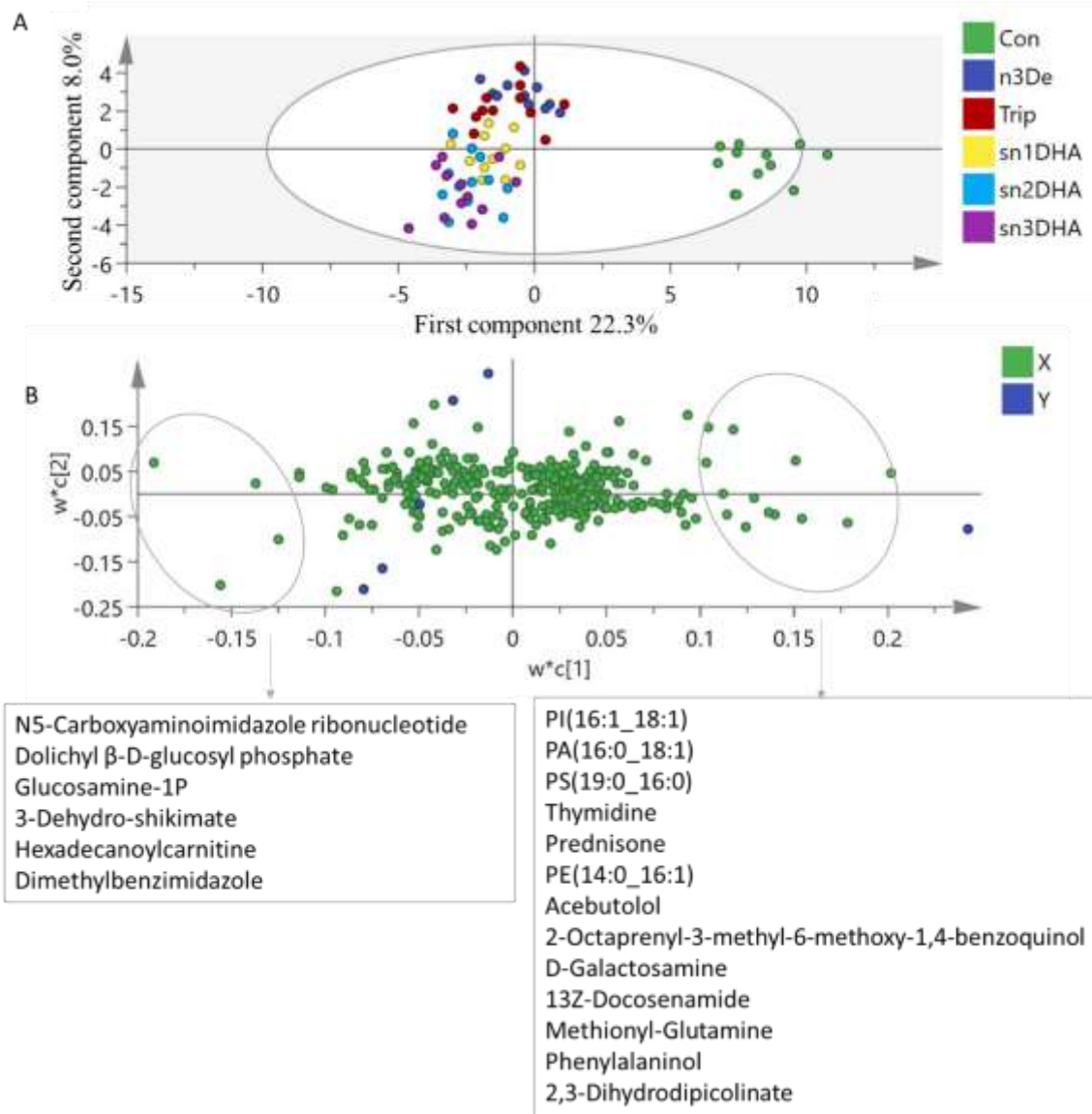


Figure S4. PLS-DA score plot (A) and loading plot (B) based on identified metabolites from all groups. Those metabolites in the right circle with positive w^*c value (a value generated from combination of the X-weights, w^* , and Y-weights, c) such as thymidine, 13Z-docosenamide, and 2,3-dihydrodipicolinate were higher in the Con group compared to the other groups. Those metabolites in the left circle with negative w^*c value such as N5-carboxy aminoimidazole ribonucleotide, glucosamine-1P, and dimethylbenzimidazole were lower in the Con group compared to the other groups fed with n-3 fatty acid deficient peanut-oil-based rodent chow.

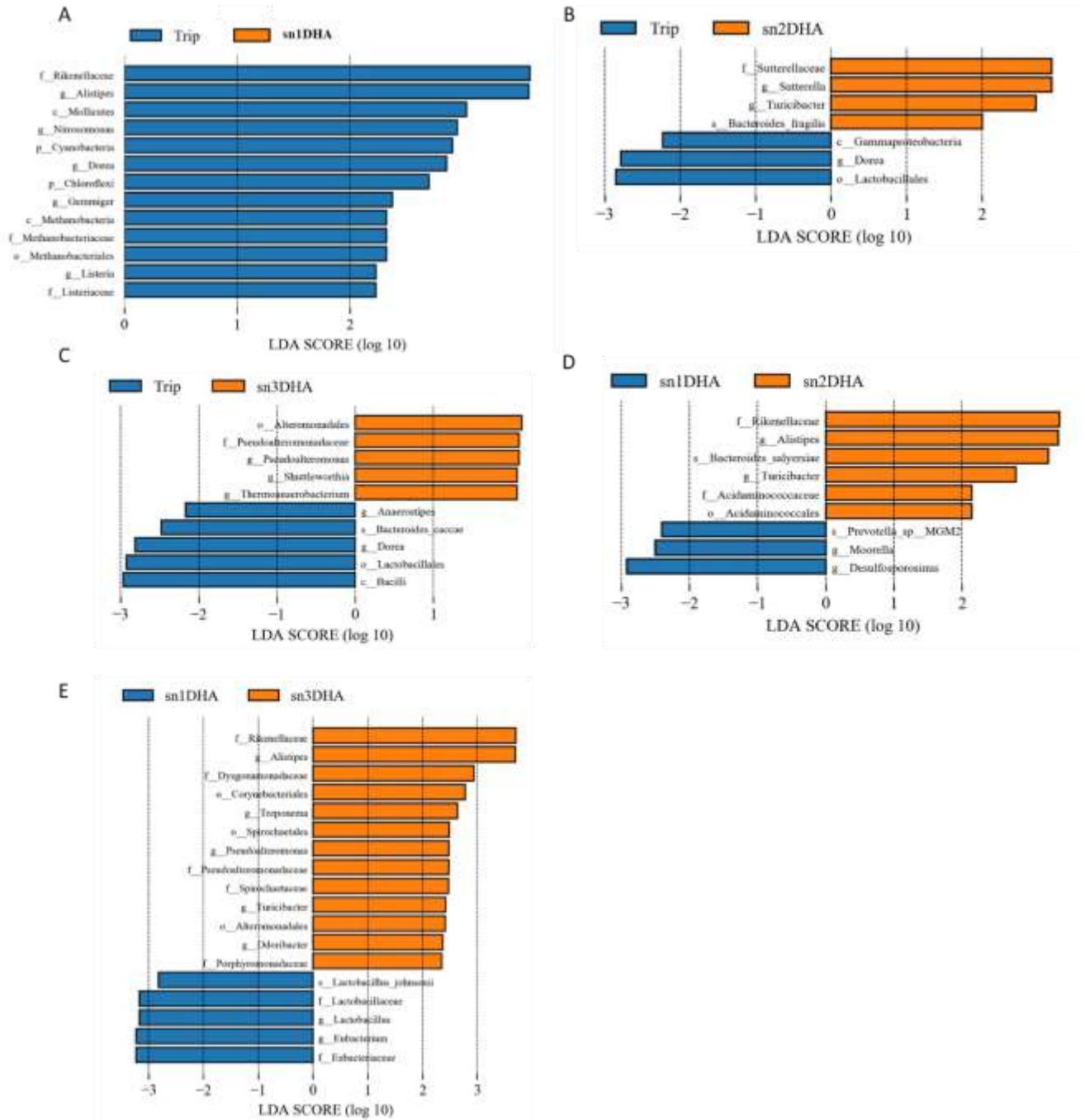


Figure S5. Significantly changed gut microbes revealed by LefSe (LDA Effect Size).

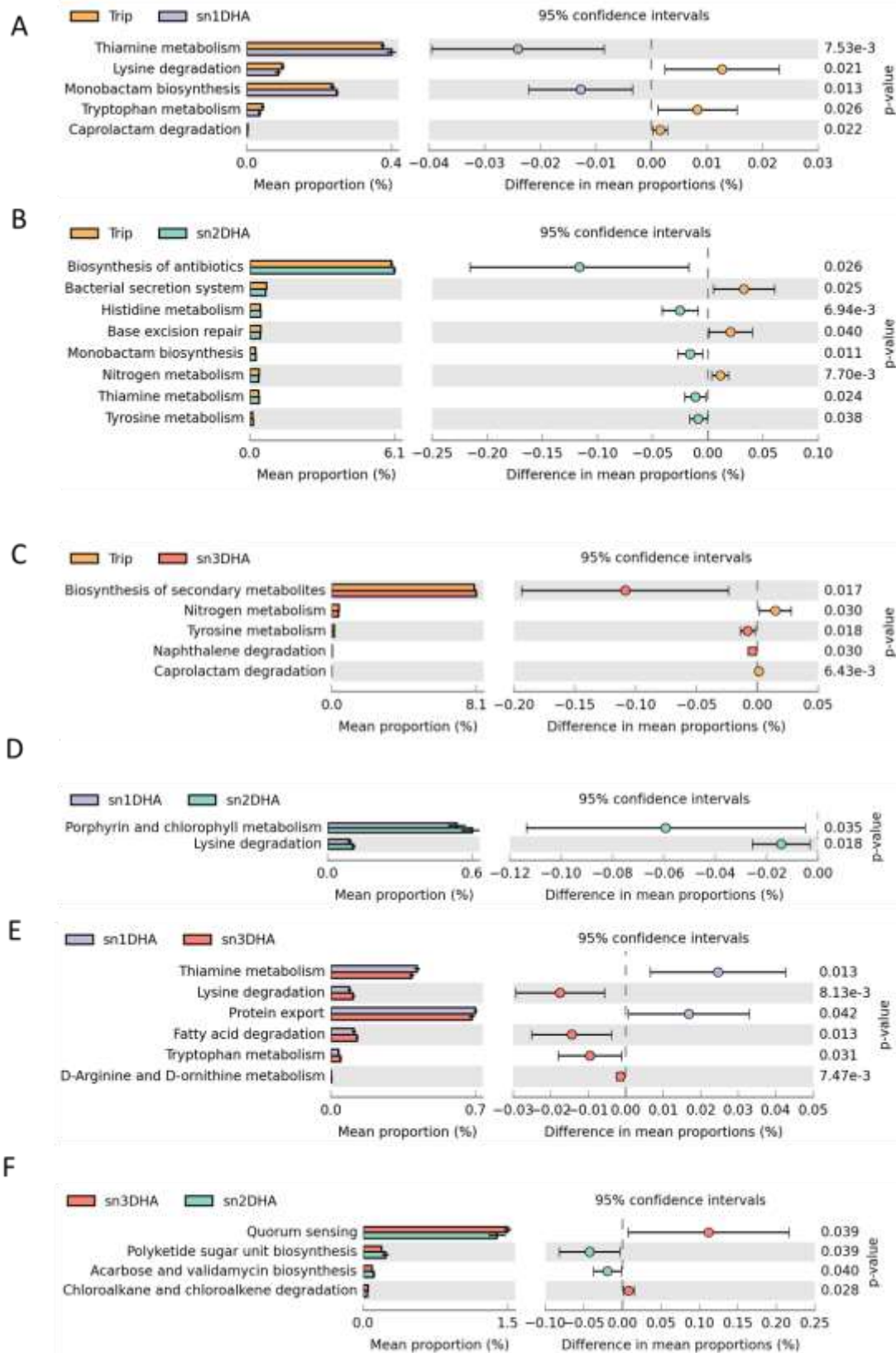


Figure S6. Extended error bar plot identifying significant altered KEGG pathway of gut microbiot

