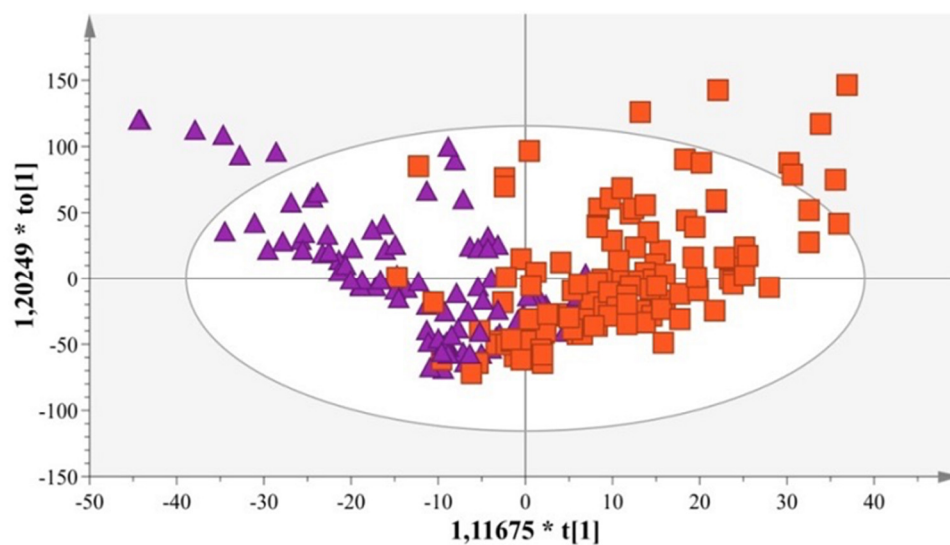
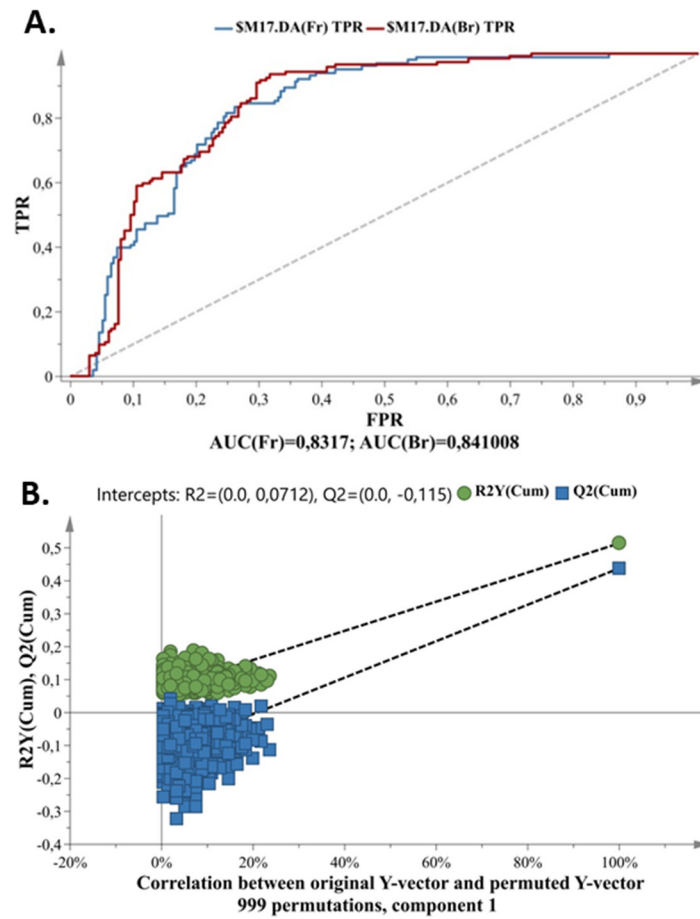


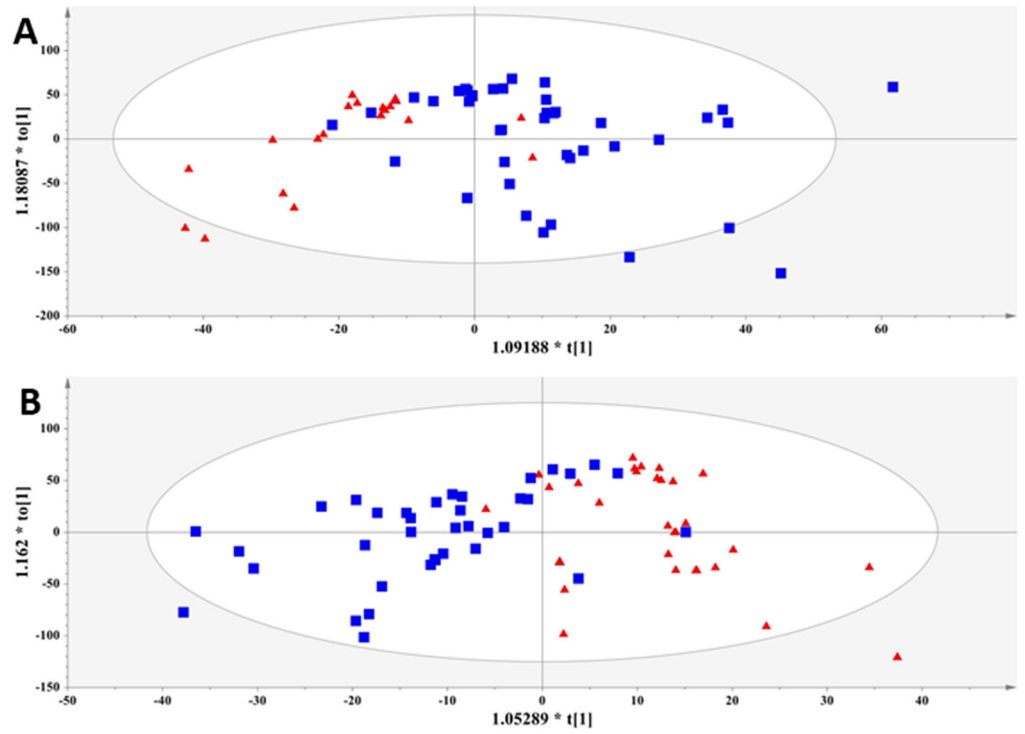
**Figure S1.**  $^1\text{H}$  NMR spectra of a urine sample with annotation on the identified metabolites. 1:lactate; 2:alanine; 3:3-amino-isobutyrate; 4:valine; 5:isoleucine; 6:leucine; 7:isobutyrate; 8: succinic acid; 9:L-fucose; 10:lysine; 11:bile acids (2-hydroxybutyrate); 12:3-methyl-2-oxovalerate; 13:3-hydroxyisovalerate; 14:2-hydroxyisobutyrate. 15: $\beta$ -alanine; 16:2-hydroxyglutarate; 17:lysine; 18:citrate; 19:acetate; 20:N-acetylaspartate; 21:L-glutamine; 22:acetone; 23:aminoadipate; 24:acetoacetate; 25:pyroglutamate; 26:pyruvate; 27:isobutyrate; 28:dimethylamine; 29:methylguanidine; 30:creatine; 31:creatinine; 32:trimethylamine; 33:dimethylglycine; 34: isocitrate; 35:urea; 36:D-glucose; 37:sucrose; 38: L-cystine; 39:gluconate; 40:glycolate; 41:serine; 42:hippurate; 43:trigonelline; 44:threonine; 45:choline; 46:trimethylamine-N-oxide; 47:betaine; 48:taurine; 49:guanidoacetate; 50:glycine; 51:formate; 52: $\tau$ -methylhistidine; 53:N-phenylacetylglutamine; 54:phenol; 55:tyrosine; 56:histidine; 57:  $\pi$ -methylhistidine, 58: trigonelline



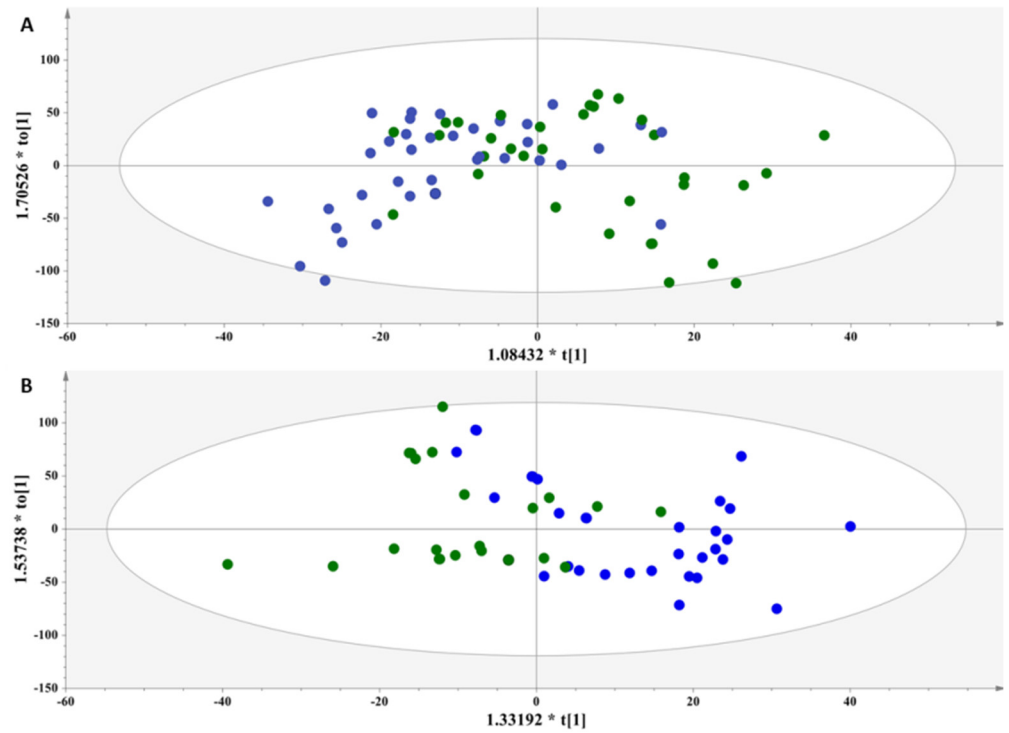
**Figure S2.** OPLS-DA scores plot (breast vs formula-fed), with  $A=1+1+0$ ,  $N=225$ ,  $R^2X(\text{cum})=0.52$ ,  $R^2Y(\text{cum})=0.51$ ,  $Q^2(\text{cum})=0.44$  for pareto scaling and 95% confidence level,  $p\text{-value}= 6,27\text{E-}33$ . (breast-fed samples: squares, formula-fed samples: triangles).



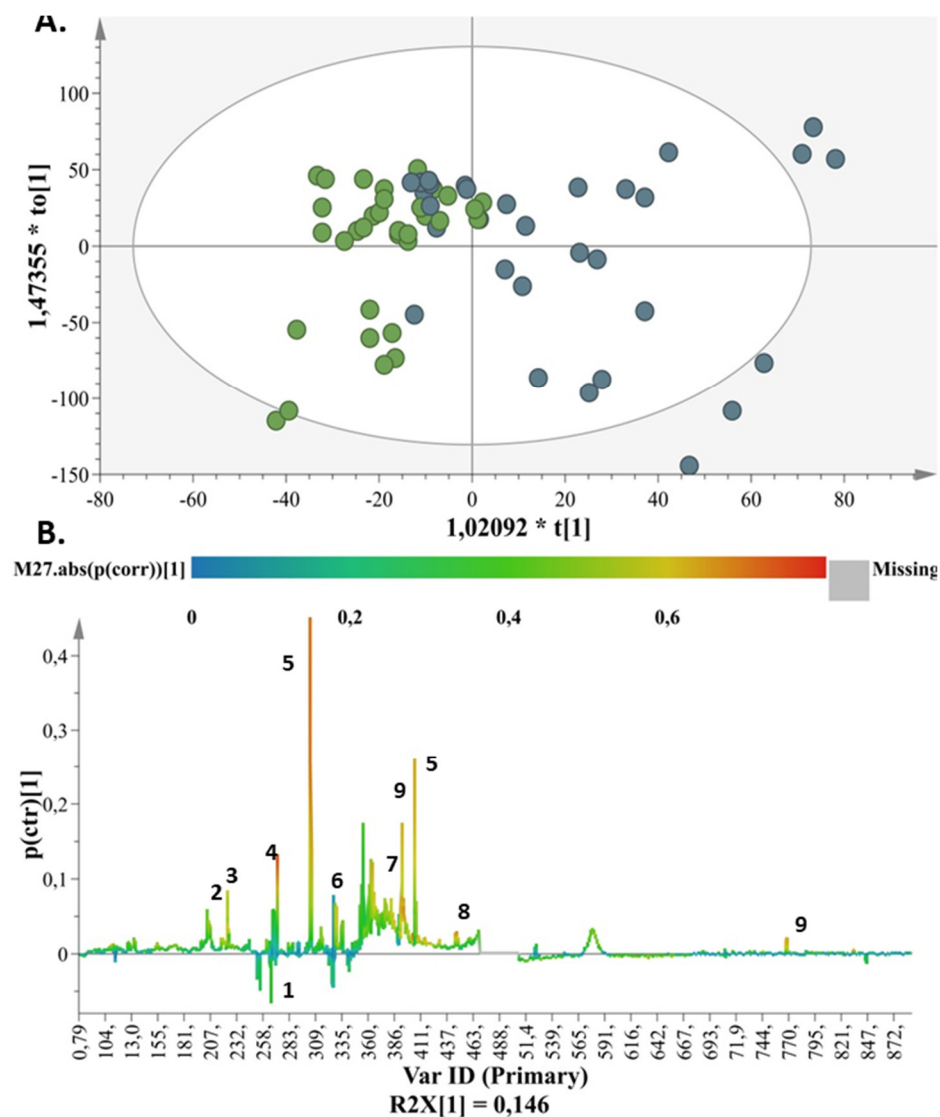
**Figure S3. (a)** ROC curve AUC (Synbiotic formula) = 0.8317 and AUC(Breastfeeding)=0.841 **(b)** Permutation testing for the OPLS-DA model in Figure S2, Synbiotic formula vs Breastfeeding.



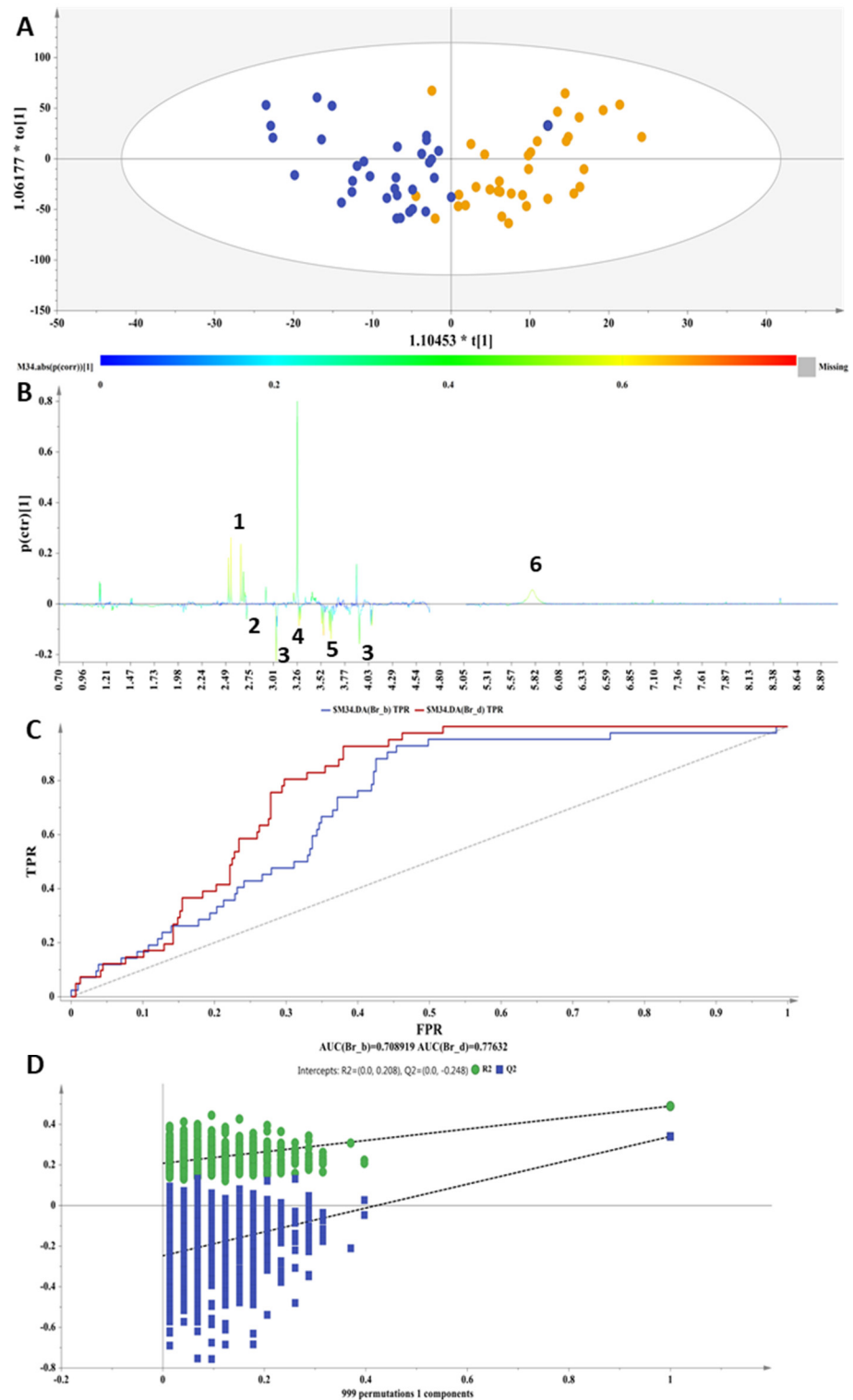
**Figure S4. (a)** OPLS-DA scores plot for day 3 regarding breast- and formula-fed samples, with  $A=1+1+0$ ,  $N=64$ ,  $R^2X(\text{cum})=0.61$ ,  $R^2Y(\text{cum})=0.46$ ,  $Q^2(\text{cum})=-0.23$  for pareto scaling and 95% confidence level,  $p\text{-value}>0.05$ . (Breast-fed samples: blue squares, formula-fed samples: red triangles); **(b)** OPLS-DA scores plot for day 15 regarding breast- and formula-fed samples, with  $A=1+1$ ,  $N=67$ ,  $R^2X(\text{cum})=0.61$ ,  $R^2Y(\text{cum})=0.55$ ,  $Q^2(\text{cum})=0.17$  for pareto scaling and 95% confidence level,  $p\text{-value}>0.05$ . (Breast-fed samples: blue squares, formula-fed samples: red triangles)



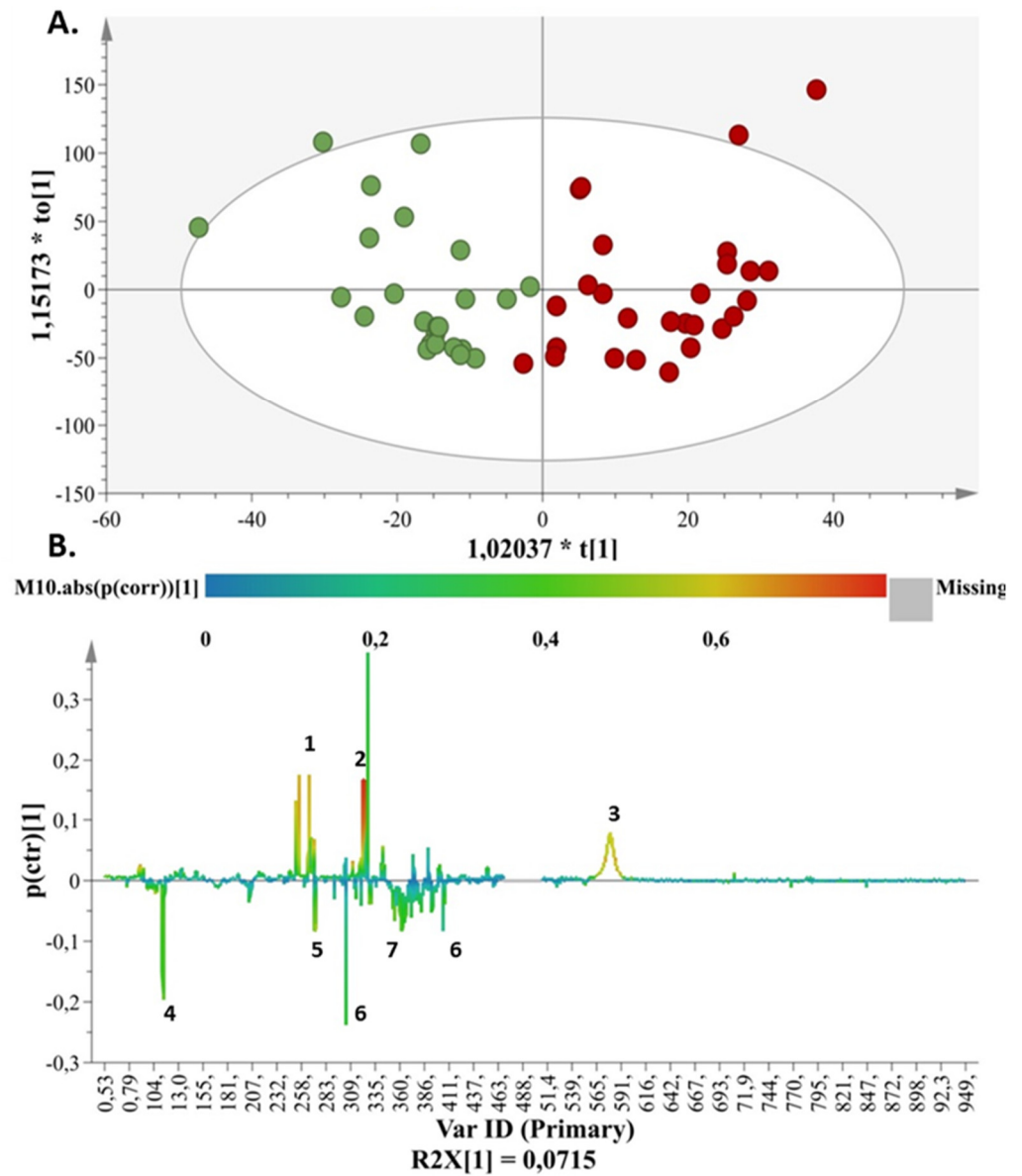
**Figure S5. (a)** OPLS-DA scores plot for breast-fed samples regarding day 3 and day 15, with  $A=1+1+0$ ,  $N=64$ ,  $R^2X(\text{cum})=0.61$ ,  $R^2Y(\text{cum})=0.46$ ,  $Q^2(\text{cum})=-0.23$  for pareto scaling and 95% confidence level,  $p\text{-value}>0.05$ . . (Breast-fed samples: blue squares, formula-fed samples: red triangles); **(b)** OPLS-DA scores plot for formula-fed samples regarding day 3 and day 15, with  $A=1+1$ ,  $N=67$ ,  $R^2X(\text{cum})=0.61$ ,  $R^2Y(\text{cum})=0.55$ ,  $Q^2(\text{cum})=0.17$  for pareto scaling and 95% confidence level,  $p\text{-value}>0.05$ . (Breast-fed samples: blue squares, formula-fed samples: red triangles)



**Figure S6. (a)** OPLS-DA scores plot for breast-fed samples (day 3 vs month 2), with  $A=1+1+0$ ,  $N=64$ ,  $R^2X(\text{cum})=0,61$ ,  $R^2Y(\text{cum})=0,48$ ,  $Q^2(\text{cum})=0,33$ , for pareto scaling and 95% confidence level,  $p\text{-value}=3,32E-05$ . (day 3: blue circles, month 2: green circles); **(b)** S-line plot demonstrates the metabolites responsible for discrimination (1. citric acid, 2. glutamine, 3. acetoacetate, 4. dimethylamine, 5. creatinine, 6. betaine, 7. taurine, 8. threonine, 9. hippurate).

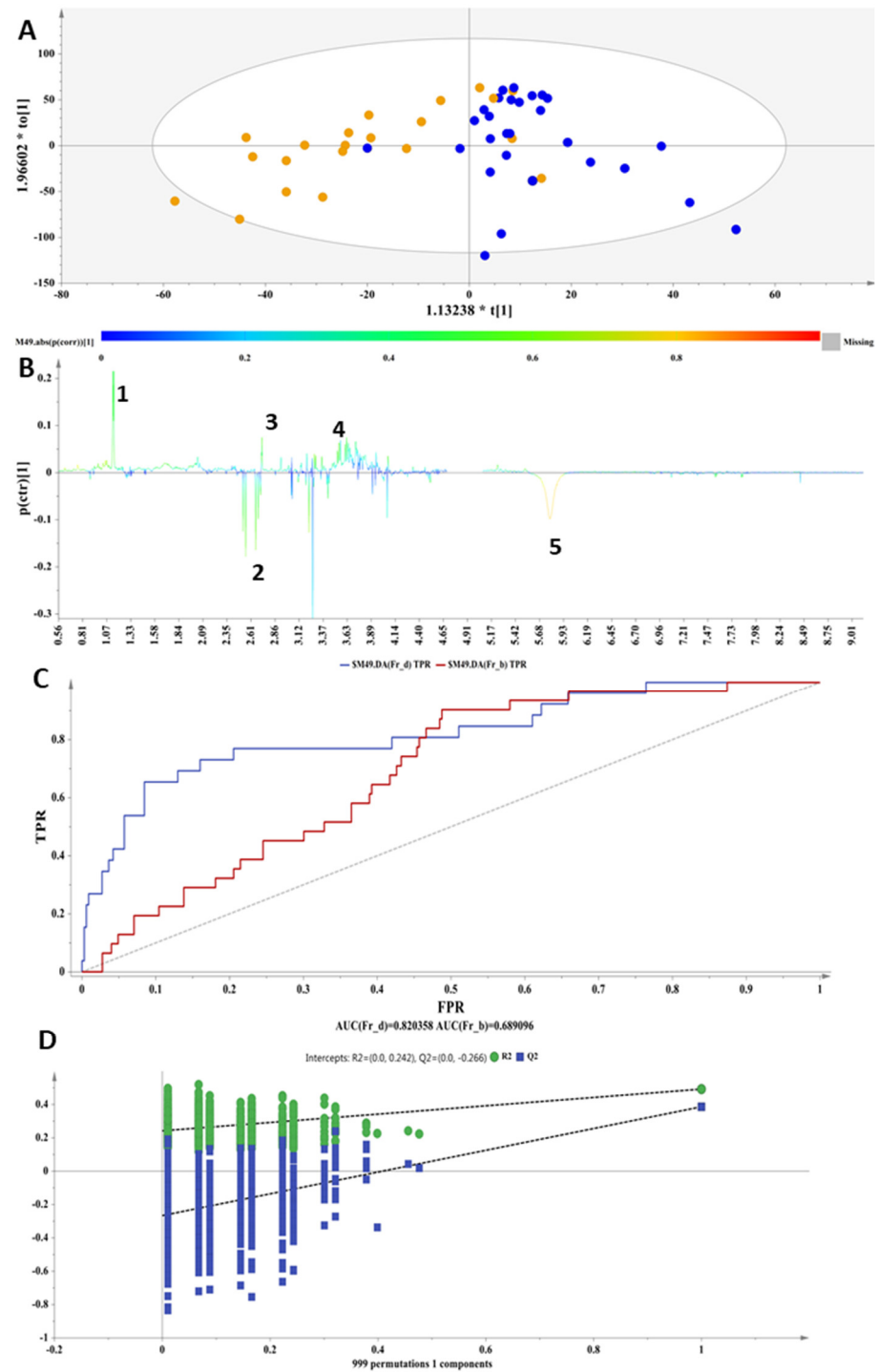


**Figure S7.** (a) OPLS-DA scores plot for breast-fed samples regarding day 15<sup>th</sup> and month 3, with  $A=1+1+0$ ,  $N=67$ ,  $R^2X(\text{cum})=0.55$ ,  $R^2Y(\text{cum})=0.49$ ,  $Q^2(\text{cum})=0.34$  for pareto scaling and 95% confidence level,  $p\text{-value}=9.21236e-006$ . (yellow squares: month 3, blue squares: day 15); (b) S-line plot demonstrates the metabolites responsible for discrimination. 1. citric acid, 2. dimethylamine, 3. Betaine, 4. Creatinine, 5. taurine, 6. urea. (c) ROC curve AUC (15<sup>th</sup> day) = 0.709 and AUC (3<sup>rd</sup> month) = 0.776; (d) permutation testing for the OPLS-DA model for breast-fed 15<sup>th</sup> day vs 3<sup>rd</sup> month.

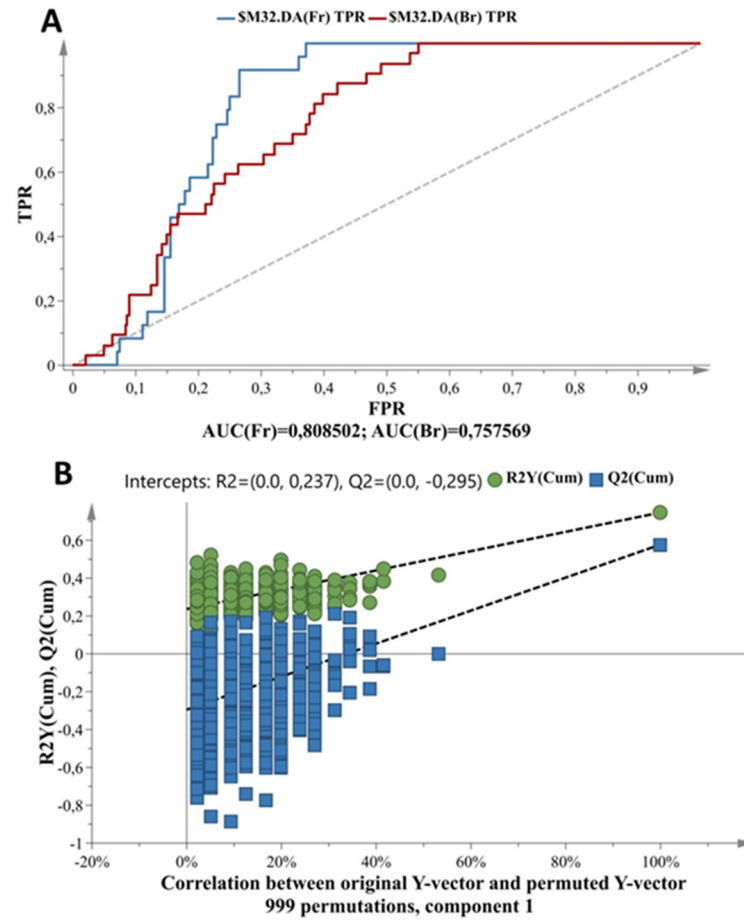


**Figure S8.** (a) OPLS-DA scores plot for formula-fed samples (day 3 vs month 2), with  $A=1+1+0$ ,  $N=53$ ,  $R^2X(\text{cum})=0.53$ ,  $R^2Y(\text{cum})=0.75$ ,  $Q^2(\text{cum})=0.55$  for pareto scaling and 95% confidence level,  $p\text{-value}=7.33\text{E-}12$ , (day 3: green circles, month 2: red circles); (b) S-line plot demonstrates the metabolites responsible for discrimination. (1. citric acid, 2. betaine, 3. urea, 4. methyl succinate, 5. dime-thylamine, 6. creatinine, 7. Taurine).

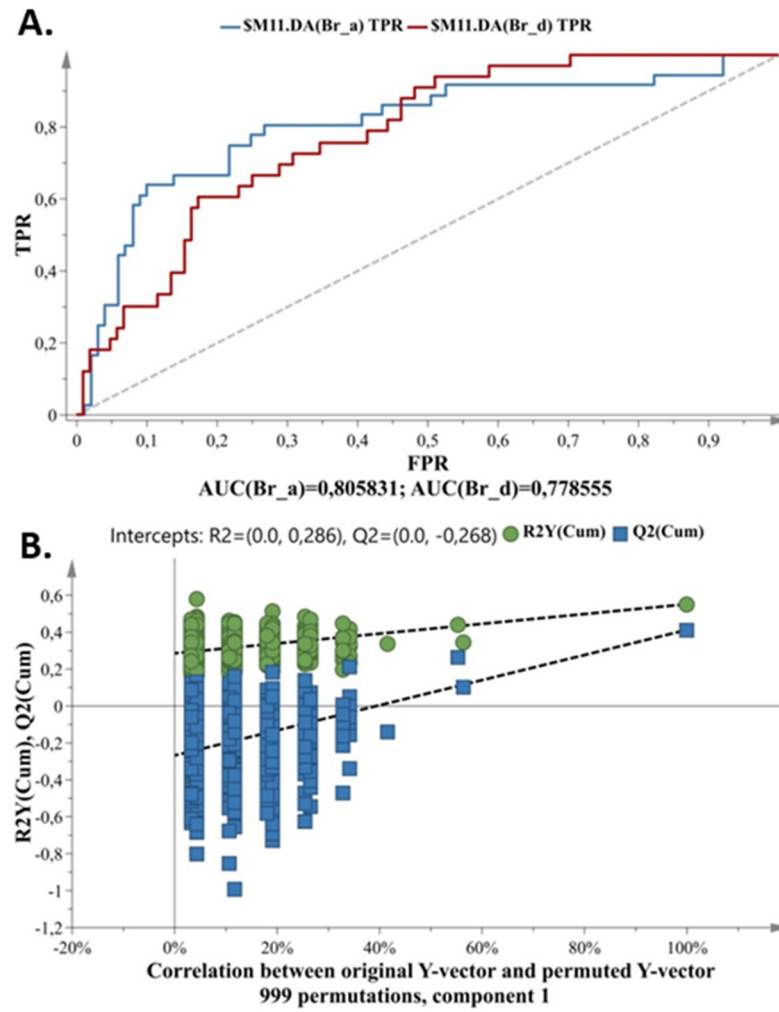




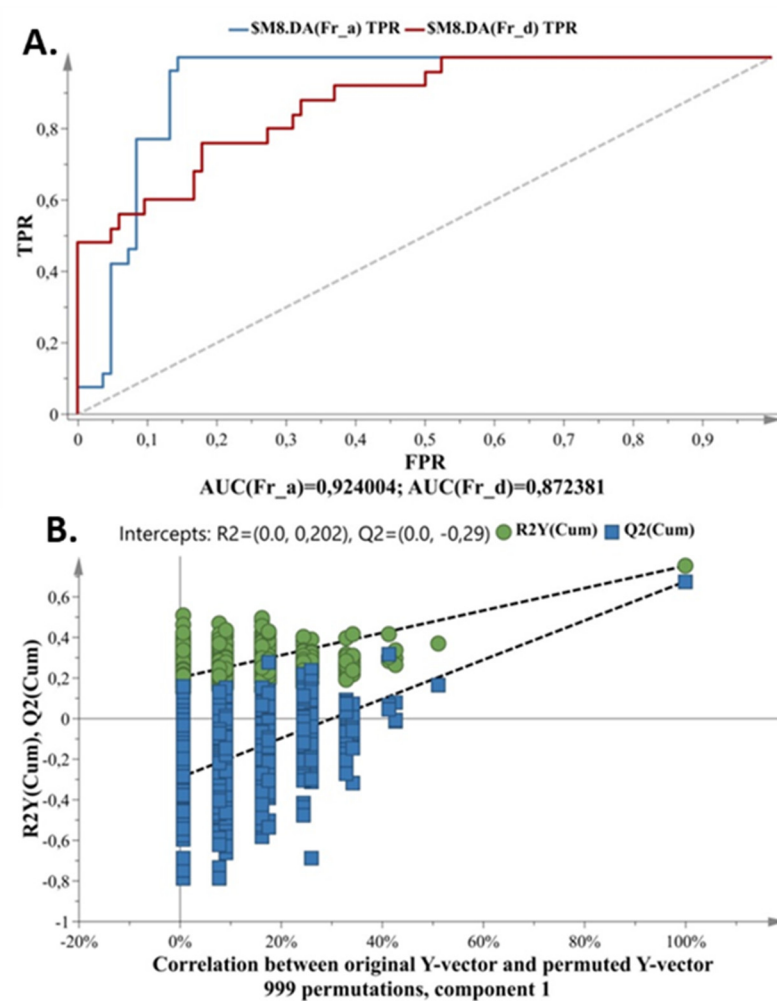
**Figure S9.** (a) OPLS-DA scores plot for formula-fed samples regarding day 15<sup>th</sup> and month 3, with  $A=1+1+0$ ,  $N=50$ ,  $R^2X(\text{cum})=0.59$ ,  $R^2Y(\text{cum})=0.49$ ,  $Q^2(\text{cum})=0.39$  for pareto scaling and 95% confidence level,  $p\text{-value}=8.3714\text{e-}005$ . (yellow squares: month 3, blue squares: day 15); (b) S-line plot demonstrates the metabolites responsible for discrimination. 1. methyl succinate, 2. citric acid, 3. dime-thylamine, 4. Taurine, (c) ROC curve AUC (15<sup>th</sup> day) = 0.689 and AUC (3<sup>rd</sup> month) = 0.820; (d) per-mutation testing for the OPLS-DA model formula-fed 15<sup>th</sup> day vs 3<sup>rd</sup> month.



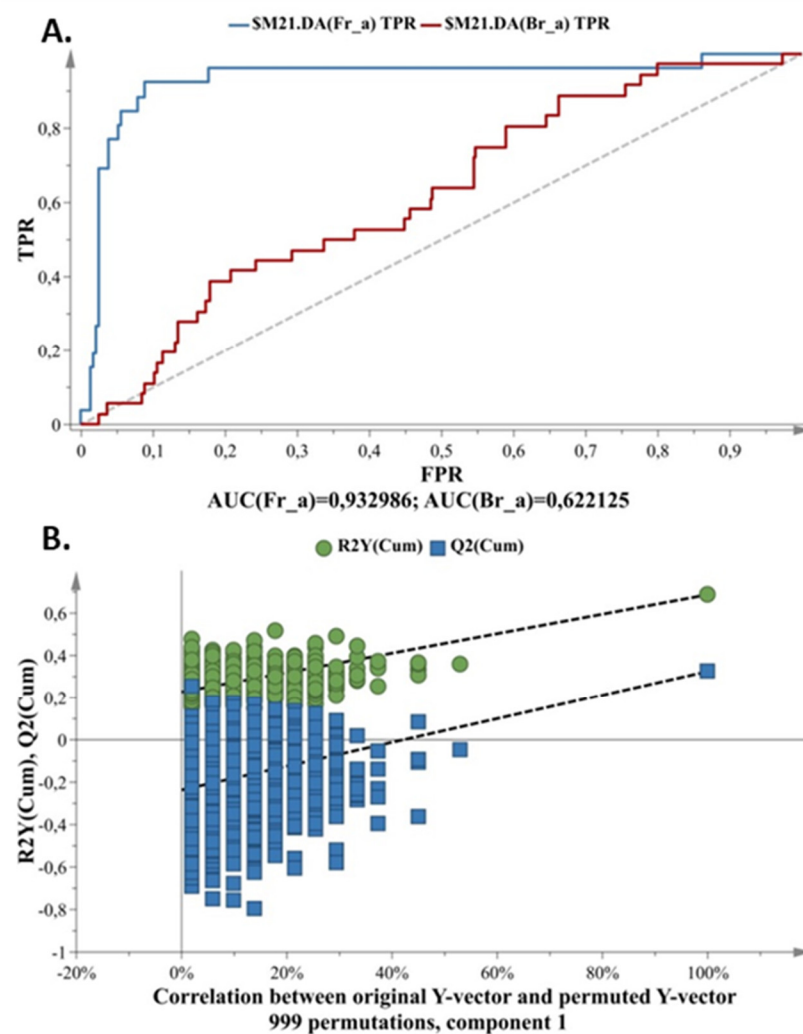
**Figure S10. (a)** ROC curve AUC 2<sup>nd</sup> month (Formula-fed) = 0.808 and AUC (Breast Fed) =0.757; **(b)** permutation testing for the OPLS-DA model for formula-fed 3rd day vs 15th day.



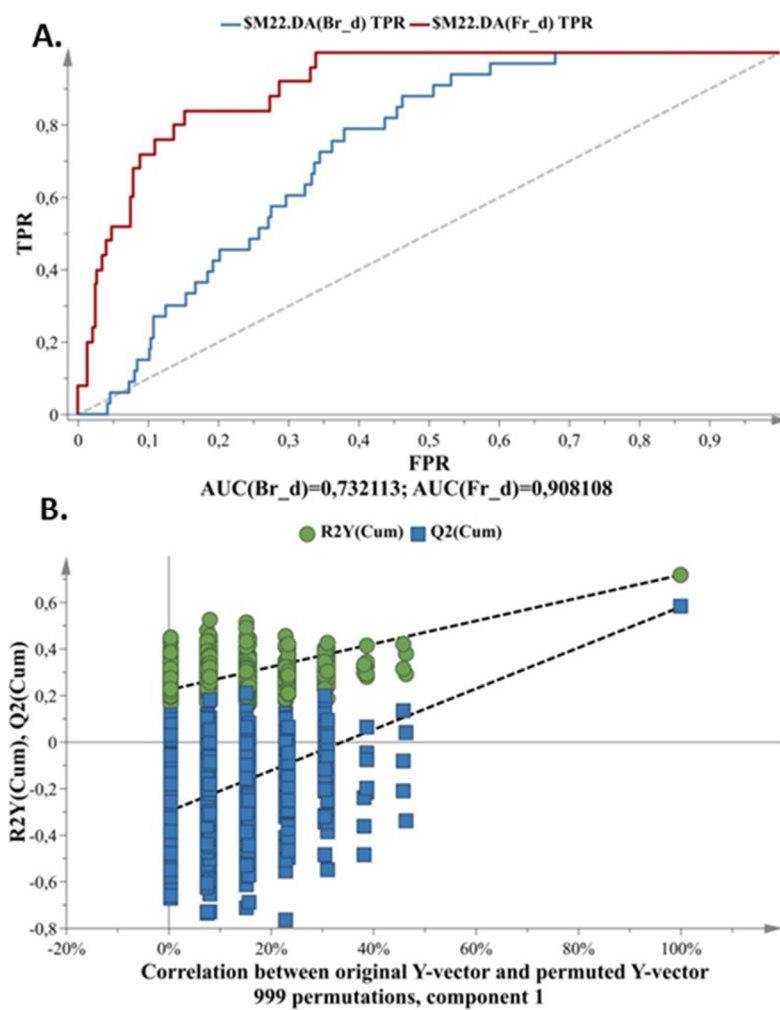
**Figure S11.** (a) ROC curve AUC (Breastfeeding day 3)=0.805 and AUC (Breastfeeding 3<sup>rd</sup> month)=0.778, (b) permutation testing for the OPLS-DA model in Figure 3.



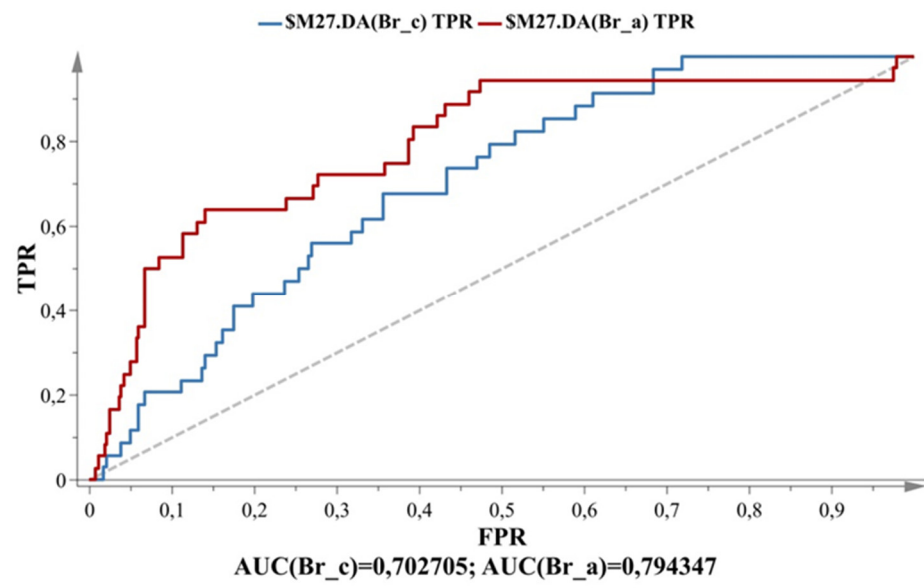
**Figure S12.** (a) ROC curve AUC (Synbiotic formula day 3)= 0.924 and AUC (Synbiotic formula 3<sup>rd</sup> month)=0.872, (b) permutation testing for the OPLS-DA model in Figure 4.



**Figure S13. (a)** ROC curve AUC (Synbiotic formula day 3)= 0.932 and AUC (Breastfeeding day 3)=0.622, **(b)** permutation testing for the OPLS-DA model of breastfeeding vs formula at 3<sup>rd</sup> day.



**Figure S14. (a)** ROC curve AUC (Breastfeeding 3<sup>rd</sup> month)= 0.732 and AUC (Synbiotic formula 3<sup>rd</sup> month)=0.908, **(b)** permutation testing for the OPLS-DA model of breastfeeding 3<sup>rd</sup> month vs synbiotic formula 3<sup>rd</sup> month.



**Figure S15.** ROC curve AUC (Breastfeeding month 2) = 0.702 and AUC (Breastfeeding day 3) = 0.794.

**Table S1.** Validation metrics for Statistical models of Breast-fed: Br vs Formula-fed: Fr at each time point of the study.

	PCA	PLS <sup>1</sup>	OPLS <sup>1</sup>	OPLS-DA (SIMCA <sup>2</sup> )
1st Sampling – Day 3		R <sup>2</sup> = 0.633	R <sup>2</sup> = 0.433,	R <sup>2</sup> Y (cum)=0.69,
Br= 36 vs Fr= 26	R <sup>2</sup> = 0.798	Q <sup>2</sup> = 0.344	Q <sup>2</sup> = 0.244	Q <sup>2</sup> (cum)=0.33
N=62		(3 predictors)	N=62	N=51
2nd Sampling – Day 15		R <sup>2</sup> = 0.573	R <sup>2</sup> = 0.406,	R <sup>2</sup> Y (cum)=0.59,
Br= 34 vs Fr= 31	R <sup>2</sup> = 0.858	Q <sup>2</sup> = 0.395	Q <sup>2</sup> = 0.276	Q <sup>2</sup> (cum)=0.42
N=65		(3 predictors)	N=65	N=57
3rd Sampling – Month 2		<b>R<sup>2</sup> = 0.834</b>	R <sup>2</sup> = 0.69,	<b>R<sup>2</sup>Y(cum)=0.75,</b>
Br= 34 vs Fr= 28	R <sup>2</sup> = 0.680	<b>Q<sup>2</sup> = 0.669</b>	Q <sup>2</sup> = 0.343	<b>Q<sup>2</sup>(cum)=0.58</b>
N=62		(4 predictors)	N=62	N=56
4th Sampling - Month 3		<b>R<sup>2</sup> = 0.922</b>	R <sup>2</sup> = 0.574,	<b>R<sup>2</sup>Y(cum)=0.72,</b>
Br= 34 vs Fr= 25	R <sup>2</sup> = 0.676	<b>Q<sup>2</sup> = 0.781</b>	Q <sup>2</sup> = 0.476	<b>Q<sup>2</sup>(cum)=0.58</b>
N=59		(8 predictors)	N=59	N=54

<sup>1</sup> *p* -value = 0.001 for all R<sup>2</sup> and Q<sup>2</sup> of the PLS and OPLS models. <sup>2</sup> Supervised SIMCA models were generated omitting outliers from the PCA models.



**Table S2.** Validation metrics for statistical models of Breast-fed: Br samples between different time points.

	PCA	PLS	OPLS	OPLS-DA (SIMCA <sup>1</sup> )
Day 3 (n= 36) vs Day 15 (n= 34)	R2 = 0.843	R2 = 0.245 p-value = 0.05 Q2 = 0.018 p-value = 0.281(2 predictors)	R2 = 0.283 p-value = 0.173 Q2 = 0.04 p-value = 0.067	OPLS: R2 =0.298, Q2 = 0.0338
Day 3 (n= 36) vs Month 2 (n= 34)	R2 = 0.813	R2 = 0.367 p-value = 0.003 Q2 = 0.267 p-value = 0.001 (2 predictors)	R2 = 0.367 p-value = 0.003 Q2 = 0.25 p-value = 0.001	R2X(cum)=0.61, R2Y(cum)=0.48, Q2(cum)=0.33 N=64
Day 3 (n= 36) vs Month 3 (n= 34)	R2 = 0.791	R2 = 0.391 p-value = 0.001 Q2 = 0.264 p-value = 0.001 (2 predictors)	R2 = 0.391 p-value = 0.001 Q2 = 0.201 p-value = 0.001	R2X(cum)=0.57, R2Y(cum)=0.55, Q2(cum)=0.41 N=54
Day 15 days (n=34) vs Month 2 (n= 34)	R2 = 0.858	R2 = 0.227 p-value = 0.05 Q2 = 0.104 p-value = 0.01 (2 predictors)	R2 = 0.227 p-value = 0.05 Q2 = 0.113 p-value = 0.003	OPLS: R2 = 0.362, Q2 = 0.183
Day 15 days (n=34) vs Month 3 (n= 34)	R2 = 0.838	R2 = 0.351 p-value = 0.002 Q2 = 0.187 p-value = 0.001 (2 predictors)	R2 = 0.267 p-value = 0.001 Q2 = 0.147 p-value = 0.001	OPLS: R2 = 0.489, Q2 = 0.34
Month 2 (n=34) vs Month 3 (n= 34)	R2 = 0.721	R2 = 0.335 p-value = 0.504 Q2 = 0.106 p-value = 0.104 (2 predictors)	R2 = 0.313 p-value = 0.07 Q2 = -0.201 p-value = 0.652	-

<sup>1</sup>Supervised SIMCA models were generated omitting outliers from the PCA models.

**Table S3.** Validation metrics for statistical models of formula-fed: Fr samples between different time points.

	PCA	PLS	OPLS	OPLS-DA (SIMCA <sup>1</sup> )
Day 3 (n= 26) vs Day 15 (n= 31)	R2 = 0.68	R2 = 0.516 p-value = 0.008 Q2 = 0.118 p-value = 0.046 (3 predictors)	R2 = 0.401 p-value = 0.007 Q2 = 0.246 p-value = 0.002	OPLS: R2 = 0.401, Q2 = 0.228
Day 3 (n= 26) vs Month 2 (n= 28)	R2 = 0.613	R2 = 0.732 p-value = 0.001 Q2 = 0.416 p-value = 0.001 (2 predictors)	R2 = 0.732 p-value = 0.001 Q2 = 0.591 p-value = 0.001	R2X(cum)=0.53, R2Y(cum)=0.75, Q2(cum)=0.55 n=53
Day 3 (n= 26) vs Month 3 (n= 25)	R2 = 0.644	R2 = 0.816 p-value = 0.001 Q2 = 0.681 p-value = 0.001 (4 predictors)	R2 = 0.698 p-value = 0.001 Q2 = 0.639 p-value = 0.001	R2X(cum)=0.51, R2Y(cum)=0.75, Q2(cum)=0.67 n=48
Day 15 (n= 31) vs Month 2 (n= 28)	R2 = 0.691	R2 = 0.36 p-value = 0.004 Q2 = 0.208 p-value = 0.002 (2 predictors)	R2 = 0.36 p-value = 0.003 Q2 = 0.158 p-value = 0.003	OPLS: R2 = 0.377, Q2 = 0.215
Day 15 (n= 31) vs Month 3 (n= 25)	R2 = 0.716	R2 = 0.518 p-value = 0.002 Q2 = 0.382 p-value = 0.001 (3 predictors)	R2 = 0.433 p-value = 0.001 Q2 = 0.405 p-value = 0.001	PCA: R2 = 0.805 OPLS: R2 = 0.492, Q2 = 0.386
Month 2 (n= 28) vs Month 3 (n= 25)	R2 = 0.674	R2 = 0.164 p-value = 0.466 Q2 = 0.033 p-value = 0.091 (2 predictors)	R2 = 0.162 p-value = 0.462 Q2 = 0.024 p-value = 0.122	PCA: R2 = 0.768 OPLS: R2 = 0.263, Q2 = 0.106

<sup>1</sup>Supervised SIMCA models were generated omitting outliers from the PCA models.

**Table S4. Average nutritional composition per 100 mL of human breast milk and formula (Ron-tamil® Complete 1) [1,2].**

	<b>Formula*</b>	<b>Breast<sup>1, 2</sup></b>
Energy (kcal)	68	63
Protein (g)	1.4	1.04
Carbohydrates (g)	7.8	7.84
Fibre (g)	0.34	1.2-1.4
Fat (g)	3.4	3.0
Saturated Fatty acids (g)	1.0	1.1
MUFA (g)	1.71	1.4
PUFA (g)	0.66	0.5
Linoleic acid (g)	0.52	0.4
$\alpha$ -linolenic acid (mg)	54	17.8
Arachidonic acid -AA (mg)	15.4	15.2
Docosahexaenoic acid – DHA (mg)	15.4	7.6
Minerals		
calcium (mg)	47	20-25
phosphorus (mg)	29	12-14
magnesium (mg)	5	3.0-3.5
iron (mg)	0.5	0.03-0.07
zinc ( $\mu$ g)	0.5	0.1-0.3
manganese ( $\mu$ g)	7.9	0.3-0.4
copper ( $\mu$ g)	50	0.01-0.03
iodine ( $\mu$ g)	12.6	14-15
sodium	23	15-25
potassium	74	40-55
chloride	54	40-45
selenium ( $\mu$ g) 2.71.0-2.5fluoride6.8-Other		

total nucleotides (mg)	2.2
Non-protein nitrogen (g)	0.18
Probiotics ( <i>Bifidobacterium animalis</i> ) (cfu/g)	1.00E+07

\*100mL formula milk is prepared by mixing 13.5 g powder with 90 ml water

**Table S5.** Data comparison between formula-fed and breast-fed groups.

	<b>Formula-fed</b>	<b>Breast-fed</b>
Birth weight Mean (g)	3,287	3,298
Birth weight Std Dev (g)	410	339
Sex Ratio (F/M)	0.78	0.59
Delivery Mode Ratio (Physical/Cesarean)	0.88	1.69

## References

1. Koletzko, B. Human Milk Lipids. *Ann Nutr Metab* **2016**, 69 Suppl 2, 28–40, doi:10.1159/000452819.
2. Kim, S.Y.; Yi, D.Y. Components of Human Breast Milk: From Macronutrient to Microbiome and MicroRNA. *Clin Exp Pediatr* **2020**, 63, 301–309, doi:10.3345/cep.2020.00059.