

**Feeding sows milk biofortified with n-6 and n-3 modulates immune status of sows and drives positive transgenerational effects**

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Short Title: Biofortified Milk's Immune Modulation in Sows and Transgenerational Benefits with n-6 and n-3

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Table 1. Gene names and primer sequences of liver

Gene abbreviation <sup>a</sup>	Gene name	Primer sequences (5'-3')	GenBank sequence	References
ACC	Acetyl-CoA Carboxylase	F: ATGTTTCGGCAGTCCCTGAT R: TGTGGACCAAGCTGACCTTG	NM_001114269.1	Chen et al. (2017)
SREBP-1	Sterol Regulatory Element-Binding Protein 1	F: CTACCACAAGCTGCACCGAG R: GGGAGACTGGTCTGACTCG	NM_214157.1	Li et al. (2016)
SCD	Stearoyl-CoA Desaturase	F: TTGATCCCCACCTGCAAGAT R: CGTGTGGCAATGATCAGGA	NM_213781.1	Cirera et al. (2020)
PPAR- $\alpha$	Peroxisome Proliferator Activated Receptor Alpha	F: TTTCCCTCTTGTTGGCTGCT R: GGGGTGGTTGGTCTGCAAG	NM_001044526.1	Mentzel et al. (2018)
FADS2	Fatty acid desaturase 2	F: ACATGAACCTGTTGAGAGC R: TGATGAACCTGTGGACGATG	NM_001171750.1	Meadus, et al. (2011)
FAS	Fatty Acid Synthase	F: GTGGGCTACAGCATGATAAG R: GAATTGCAGCGAGGAGTTAG	NM_001099930.1	Meadus, et al. (2011)
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	F: AAGGACCCCTCGAAGTGTG R: CACAAACATGATTCAAGTCCCTG	NM_001032376.2	Svobodová, Bílek and Knoll (2008)
ACTB	$\beta$ -Actin	F: TCTGGCACCAACACCTTCT R: TGATCTGGTCATCTTCTCAC	NM_021086047.1	Jiao, et al. (2020)

3 <sup>a</sup>Housekeeping genes: HPRT1 and ACTB.

Table 2. Gene names and primer sequences of ARH

Gene abbreviation <sup>a</sup>	Gene name	Primer sequences (5'-3')	GenBank sequence	References
CART	Cocaine and Amphetamine-Related Transcript	F: CCGCCCTGCTGCTGCTGCTAC R: AGGGACTTGGCCATACTTCTTCTC	NM_001099925.1	Óvilo et al. (2010)
INSR	Insulin Receptor	F: AAACGCCAGGGACATCGTCAAGG R: CCGCAGGGAACCGCAGGTAACCT	XM_021083940.1	Óvilo et al. (2014)
LEPR	Leptin Receptor	F: GAAAAAACACCGGAATGATGC R: AAAAGAAAGAGGGCAAATGTC	NM_001024587.1	Óvilo et al. (2014)
NPY	Neuropeptide Y	F: TCGGCCTTGAGACATTACATCA R: GTCTCGGGACTAGATCGTTTCC	NM_001256367.1	Óvilo et al. (2010)
HPRT1	Hypoxanthine Phosphoribosyltransferase 1	F: AAGGACCCCTCGAAGTGTG R: CACAAACATGATTCAAGTCCCTG	NM_001032376.2	Svobodová, Bílek and Knoll (2008)
ACTB	$\beta$ -Actin	F: TCTGGCACCAACACCTTCT R: TGATCTGGTCATCTTCTCAC	NM_021086047.1	Jiao, et al. (2020)

5 <sup>a</sup>Housekeeping genes: HPRT1 and ACTB.

6 Table 3. Concentration of elaidic acid ( $\mu\text{g}/\text{mL}$ ) at 40 and 107 days of gestation and at 1 and 21 days of lactation of  
 7 sows

<b>Treatment</b>	<b>Gestation</b>		<b>Lactation</b>	
	<b>D40G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D107G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D1L<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D21L<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>
CON	2.430 $\pm$ 0.19 <sup>a,B</sup>	1.875 $\pm$ 0.18 <sup>a,B</sup>	1.725 $\pm$ 0.09 <sup>a,B</sup>	8.482 $\pm$ 0.16 <sup>a,A</sup>
n-6	3.593 $\pm$ 0.20 <sup>a,A</sup>	2.237 $\pm$ 0.18 <sup>a,B</sup>	0.903 $\pm$ 0.08 <sup>b,C</sup>	5.514 $\pm$ 0.17 <sup>a,A</sup>
n-3	3.922 $\pm$ 0.20 <sup>a,B</sup>	2.279 $\pm$ 0.18 <sup>a,B</sup>	1.782 $\pm$ 0.08 <sup>a,C</sup>	8.105 $\pm$ 0.16 <sup>a,A</sup>

8 Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by  
 9 the same lowercase letter, do not differ.

10 <sup>a</sup>D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.

11 <sup>b</sup>SEM, standard error of the mean.

12 Table 4. Concentration of  $\gamma$ -linolenic acid ( $\mu\text{g}/\text{mL}$ ) at 40 and 107 days of gestation and at 1 and 21 days of lactation of  
 13 sows

<b>Treatment</b>	<b>Gestation</b>		<b>Lactation</b>	
	<b>D40G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D107G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D1L<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D21L<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>
CON	3.835 $\pm$ 0.08 <sup>a,B</sup>	4.979 $\pm$ 0.10 <sup>a,B</sup>	2.686 $\pm$ 0.09 <sup>b,C</sup>	7.365 $\pm$ 0.08 <sup>a,A</sup>
n-6	4.228 $\pm$ 0.08 <sup>a,B</sup>	4.658 $\pm$ 0.11 <sup>a,A</sup>	4.425 $\pm$ 0.09 <sup>a,B</sup>	6.162 $\pm$ 0.08 <sup>a,A</sup>
n-3	3.754 $\pm$ 0.09 <sup>a,B</sup>	6.358 $\pm$ 0.10 <sup>a,A</sup>	3.216 $\pm$ 0.09 <sup>b,B</sup>	5.329 $\pm$ 0.08 <sup>b,A</sup>

14 Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by  
 15 the same lowercase letter, do not differ.

16 <sup>a</sup>D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.

17 <sup>b</sup>SEM, standard error of the mean.

18 Table 5. ARA/EPA ratio at 40 and 107 days of gestation and at 1 and 21 days of lactation of sows

<b>Treatment</b>	<b>Gestation</b>		<b>Lactation</b>	
	<b>D40G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D107G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D1L<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D21L<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>
CON	63.264 $\pm$ 7.16 <sup>a,A</sup>	59.917 $\pm$ 7.59 <sup>a,A</sup>	41.825 $\pm$ 3.04 <sup>a,B</sup>	25.339 $\pm$ 4.26 <sup>a,C</sup>
n-6	70.146 $\pm$ 7.67 <sup>a,A</sup>	47.843 $\pm$ 7.40 <sup>a,B</sup>	31.797 $\pm$ 2.69 <sup>b,B</sup>	31.222 $\pm$ 4.39 <sup>a,B</sup>
n-3	64.069 $\pm$ 7.40 <sup>a,A</sup>	29.090 $\pm$ 7.59 <sup>b,B</sup>	33.114 $\pm$ 2.86 <sup>b,B</sup>	23.872 $\pm$ 4.26 <sup>a,B</sup>

19 Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by  
 20 the same lowercase letter, do not differ.

21 <sup>a</sup>D40G: 40 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.

22 <sup>b</sup>SEM, standard error of the mean.

23 Table 6. Backfat thickness average (mm) from 40 to 107 days of gestation of gilts

<b>Treatment</b>	<b>Day</b>	
	<b>D40G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>	<b>D107G<sup>a</sup> (<math>\pm \text{SEM}^b</math>)</b>
CON	15.17 $\pm$ 0.66 <sup>b,A</sup>	15.29 $\pm$ 0.45 <sup>a,A</sup>
n-6	15.76 $\pm$ 0.66 <sup>b,B</sup>	16.41 $\pm$ 0.44 <sup>a,A</sup>

n-3	$17.29 \pm 0.56^{\text{a,A}}$	$15.44 \pm 0.46^{\text{a,B}}$
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24 Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by  
25 the same lowercase letter, do not differ.

26 <sup>a</sup>D40G: day 40 of gestation; D107G: day 107 of gestation.

27 <sup>b</sup>SEM, standard error of the mean.

28 Table 7. Triglyceride's concentration (mg.dL<sup>-1</sup>) at 40 and 107 days of gestation and at 1 and 21 days of lactation of  
29 sows

Treatment	Gestation		Lactation	
	D40G <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D107G <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D1L <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D21L <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )
CON	$129.00 \pm 13.92^{\text{a,A}}$	$100.00 \pm 13.92^{\text{a,A}}$	$24.10 \pm 9.84^{\text{a,B}}$	$100.40 \pm 13.92^{\text{a,A}}$
n-6	$95.80 \pm 10.81^{\text{a,A}}$	$81.40 \pm 10.81^{\text{a,A}}$	$35.33 \pm 8.06^{\text{a,B}}$	$67.00 \pm 10.81^{\text{b,A}}$
n-3	$80.40 \pm 9.69^{\text{b,A}}$	$105.80 \pm 9.69^{\text{a,A}}$	$31.11 \pm 7.22^{\text{a,B}}$	$54.80 \pm 9.69^{\text{b,B}}$

30 Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed  
31 by the same lowercase letter, do not differ.

32 <sup>a</sup>D40G: day 40 of gestation; D107G: day 107 of gestation; D1L: day 1 of lactation; D21L: day 21 of lactation.

33 <sup>b</sup>SEM, standard error of the mean.

34 Table 8. Effects of supplementing biofortified milk in estimated colostrum production of sows and intake of their  
35 piglets in the first 24 hours

Item <sup>a</sup>	Treatment <sup>b</sup>			P-value <sup>d</sup>		
	CON ( $\pm$ SEM <sup>c</sup> )	n-6 ( $\pm$ SEM <sup>c</sup> )	n-3 ( $\pm$ SEM <sup>c</sup> )	Treatment	C1	C2
Production, kg	$4.867 \pm 0.364$	$4.835 \pm 0.393$	$4.864 \pm 0.556$	0.998	0.984	0.954
Intake, g	$422.943 \pm 0.066$	$409.797 \pm 0.070$	$397.298 \pm 0.072$	0.827	0.581	0.756

36 <sup>a</sup>Colostrum intake of piglets alive was estimated by the model to predict of Theil et al. (2014) 24 h after the first-born  
37 piglet. The sum of the individual colostrum intake of each piglet within the litter was used to calculate colostrum  
38 production of the sow.

39 <sup>b</sup>Sows fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

40 <sup>c</sup>SEM: standard error of the mean.

41 <sup>d</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

42 Table 9. Histological characteristics of the *Longissimus dorsi* muscle from gilts at 28 days of gestation – batch B

Item <sup>a</sup>	Treatment <sup>b</sup>			P- value <sup>d</sup>		
	CON ( $\pm$ SEM <sup>c</sup> )	n-6 ( $\pm$ SEM <sup>c</sup> )	n-3 ( $\pm$ SEM <sup>c</sup> )	Treatment	C1	C2
<b>Adipose cells</b>						
Area, $\mu\text{m}$	$1806.480 \pm 226.140$	$2250.370 \pm 225.620$	$2195.670 \pm 214.750$	0.321	0.135	0.862
Maximum diameter, $\mu\text{m}$	$63.266 \pm 2.897$	$67.845 \pm 2.909$	$67.819 \pm 2.753$	0.436	0.200	0.995

Minimum diameter, $\mu\text{m}$	$35.097 \pm 2.238$	$38.107 \pm 2.247$	$38.648 \pm 2.127$	0.477	0.233	0.863
Medium diameter, $\mu\text{m}$	$47.816 \pm 2.475$	$51.605 \pm 2.486$	$51.763 \pm 2.352$	0.441	0.204	0.964
Per-area, %	$0.220 \pm 0.024$	$0.270 \pm 0.024$	$0.255 \pm 0.023$	0.327	0.152	0.648
<b>Muscle fiber</b>						
Area, $\mu\text{m}^2$	$4396.880 \pm 737.560$	$5265.260 \pm 755.280$	$4542.460 \pm 780.220$	0.413	0.364	0.329
Maximum diameter, $\mu\text{m}$	$88.693 \pm 6.062$	$104.200 \pm 6.124$	$92.680 \pm 6.169$	0.187	0.197	0.197
Minimum diameter, $\mu\text{m}$	$57.750 \pm 4.732$	$62.934 \pm 4.842$	$58.452 \pm 5.001$	0.466	0.418	0.352
Medium diameter, $\mu\text{m}$	$72.201 \pm 6.200$	$80.194 \pm 6.342$	$74.028 \pm 6.559$	0.358	0.297	0.324
Per-area, %	$0.345 \pm 0.028$	$0.381 \pm 0.028$	$0.396 \pm 0.028$	0.429	0.217	0.698

43 <sup>a</sup>Per-area: percentage of the area filled by the object delimited within the total image, as a way of quantifying the

44 relative area.

45 <sup>b</sup>Gilts fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

46 <sup>c</sup>SEM: standard error of the mean.

47 <sup>d</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

48

Table 10. Histological characteristics of the *Longissimus dorsi* muscle from 14 day-old piglets – batch A

Item <sup>a</sup>	Treatment <sup>b</sup>			P-value <sup>d</sup>				
	CON ( $\pm$ SEM <sup>c</sup> )	n-6 ( $\pm$ SEM <sup>c</sup> )	n-3 ( $\pm$ SEM <sup>c</sup> )	Sex	Weight	Treatment	C1	C2
<b>Adipose cells</b>								
Area, $\mu\text{m}$	841.240 $\pm$ 2.760	883.790 $\pm$ 2.760	1076.050 $\pm$ 2.770	0.006	0.006	0.210	0.245	0.182
Maximum diameter, $\mu\text{m}$	43.633 $\pm$ 2.087	44.638 $\pm$ 2.222	48.222 $\pm$ 2.494	0.123	0.004	0.265	0.260	0.227
Minimum diameter, $\mu\text{m}$	25.108 $\pm$ 1.432	25.797 $\pm$ 1.510	28.841 $\pm$ 1.717	0.013	0.004	0.152	0.196	0.134
Medium diameter, $\mu\text{m}$	33.701 $\pm$ 1.722	34.555 $\pm$ 1.823	37.808 $\pm$ 2.054	0.035	0.003	0.206	0.225	0.182
Per-area, %	0.098 $\pm$ 0.029	0.098 $\pm$ 0.029	0.114 $\pm$ 0.029	0.065 <0.001		0.522	0.580	0.318
<b>Muscle fiber</b>								
Area, $\mu\text{m}$	250.61 $\pm$ 0.10	240.51 $\pm$ 0.10	284.72 $\pm$ 0.12	0.830	0.002	0.405	0.688	0.192
Maximum diameter, $\mu\text{m}$	21.606 $\pm$ 0.048	21.276 $\pm$ 0.049	23.257 $\pm$ 0.060	0.980	0.002	0.414	0.637	0.208
Minimum diameter, $\mu\text{m}$	13.980 $\pm$ 0.038	13.776 $\pm$ 0.040	14.765 $\pm$ 0.048	0.576	0.003	0.448	0.679	0.221
Medium diameter, $\mu\text{m}$	17.653 $\pm$ 0.045	17.262 $\pm$ 0.048	18.769 $\pm$ 0.056	0.848	0.001	0.427	0.722	0.203
Per-area, %	0.030 $\pm$ 0.001	0.029 $\pm$ 0.001	0.034 $\pm$ 0.001	0.949	0.003	0.444	0.626	0.232

49 <sup>a</sup>Per-area: percentage of the area filled by the object delimited within the total image, as a way of quantifying the relative area.50 <sup>b</sup>Sows fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.51 <sup>c</sup>SEM: standard error of the mean.52 <sup>d</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

53      Table 11. Liver expression levels of genes involved in lipid metabolism in gilts from control, n-6 and n-3 groups –  
 54      batch B

Item	Treatment <sup>a</sup>			P-value <sup>c</sup>		
	TC ( $\pm$ SEM <sup>b</sup> )	n-6 ( $\pm$ SEM <sup>b</sup> )	n-3 ( $\pm$ SEM <sup>b</sup> )	Treat	C1	C2
ACC	1.084 $\pm$ 0.212	1.049 $\pm$ 0.268	1.143 $\pm$ 0.212	0.959	0.963	0.786
D6D	1.325 $\pm$ 0.326	1.674 $\pm$ 0.412	0.966 $\pm$ 0.326	0.414	0.991	0.195
FAS	1.308 $\pm$ 0.454	1.994 $\pm$ 0.525	1.786 $\pm$ 0.428	0.589	0.317	0.762
SCD	1.487 $\pm$ 0.461	1.114 $\pm$ 0.532	1.170 $\pm$ 0.435	0.475	0.495	0.358
PPAR- $\alpha$	1.028 $\pm$ 0.123	0.933 $\pm$ 0.155	0.889 $\pm$ 0.116	0.710	0.462	0.822
SREBP-1	1.131 $\pm$ 0.681	2.594 $\pm$ 0.786	0.893 $\pm$ 0.681	0.235	0.868	0.093

55      <sup>a</sup>Gilts fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

56      <sup>b</sup>SEM: standard error of the mean.

57      <sup>c</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

58      Table 12. Relative expression of genes in the gilt hypothalamus supplemented with milk biofortified with PUFA n-6  
 59      and n-3

Item	Treatment <sup>a</sup>			P-value <sup>c</sup>		
	TC ( $\pm$ SEM <sup>b</sup> )	n-6 ( $\pm$ SEM <sup>b</sup> )	n-3 ( $\pm$ SEM <sup>b</sup> )	Treat	C1	C2
CART	6.198 $\pm$ 2.445	3.439 $\pm$ 2.445	3.010 $\pm$ 2.305	0.986	0.895	0.926
INSR	1.134 $\pm$ 0.181	0.637 $\pm$ 0.169	1.008 $\pm$ 0.159	0.130	0.163	0.125
LEPR	1.842 $\pm$ 0.787	1.188 $\pm$ 0.736	1.316 $\pm$ 0.787	0.794	0.631	0.652
NPY	2.409 $\pm$ 0.873	2.211 $\pm$ 0.873	2.025 $\pm$ 0.823	0.920	0.739	0.812

60      <sup>a</sup>Gilts fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

61      <sup>b</sup>SEM: standard error of the mean.

62      <sup>c</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

63      Table 13. Hemolytic activity of the alternative pathway of the complement system for swine females and piglets

Item	Treatment <sup>a</sup>			P-value <sup>c</sup>		
	CON ( $\pm$ SEM <sup>b</sup> )	n-6 ( $\pm$ SEM <sup>b</sup> )	n-3 ( $\pm$ SEM <sup>b</sup> )	Treat	C1	C2
T1/2 Sow batch. A	6.942 $\pm$ 0.156	5.966 $\pm$ 0.140	4.880 $\pm$ 0.156	<0.001	<0.001	<0.001
$\Delta$ OD Piglet batch. A	0.088 $\pm$ 0.028	0.088 $\pm$ 0.028	0.050 $\pm$ 0.037	0.680	0.613	0.4343
T1/2 Sow batch. B	6.841 $\pm$ 0.31	5.752 $\pm$ 0.306	5.774 $\pm$ 0.288	0.028	0.008	0.959

64      <sup>a</sup>Sows fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

65      <sup>b</sup>SEM: standard error of the mean.

66      <sup>c</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

67      Table 14. Concentration of IgM (mg.mL<sup>-1</sup>) at 40, 75 and 107 days of gestation and at 1 and 21 days of lactation of  
 68      sows

Treatment	Gestation			Lactation	
	D40G <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D75G <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D107G <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D1L <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )	D21L <sup>a</sup> ( $\pm$ SEM <sup>b</sup> )

CON	15.793 ± 4.123 a,B	14.332 ± 4.123 a,B	16.373 ± 4.123 a,B	29.405 ± 4.123 a,A	20.038 ± 4.123 a,AB
n-6	18.711 ± 3.934 a,A	20.083 ± 3.987 a,A	20.942 ± 4.225 a,A	19.838 ± 3.987 a,A	23.351 ± 3.987 a,A
n-3	15.952 ± 4.123 a,B	14.863 ± 4.123 a,B	17.271 ± 4.123 a,B	17.529 ± 4.123 a,B	27.032 ± 4.123 a,A

Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.

<sup>a</sup>D40G: 40 days of gestation; D75G: 75 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.

<sup>b</sup>SEM, standard error of the mean.

Table 15. Concentration of TNF- $\alpha$  (pg.mL $^{-1}$ ) at 40, 75 and 107 days of gestation and at 1 and 21 days of lactation of sows

<b>Treatment</b>	<b>Gestation</b>			<b>Lactation</b>	
	<b>D40G<sup>a</sup> (± SEM<sup>b</sup>)</b>	<b>D75G<sup>a</sup> (± SEM<sup>b</sup>)</b>	<b>D107G<sup>a</sup> (± SEM<sup>b</sup>)</b>	<b>D1L<sup>a</sup> (± SEM<sup>b</sup>)</b>	<b>D21L<sup>a</sup> (± SEM<sup>b</sup>)</b>
CON	32.927 ± 5.376 a,A	30.057 ± 5.376 a,A	37.482 ± 5.376 a,A	22.580 ± 6.191 b,A	22.102 ± 5.376 a,A
n-6	38.544 ± 4.802 a,A	35.048 ± 4.806 a,A	37.403 ± 5.363 a,A	23.122 ± 4.806 b,A	24.078 ± 4.806 a,A
n-3	26.040 ± 5.376 a,B	40.827 ± 5.376 a,AB	29.369 ± 6.191 a,AB	56.531 ± 6.191 a,A	33.560 ± 5.376 a,AB

Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.

<sup>a</sup>D40G: 40 days of gestation; D75G: 75 days of gestation; D107G: 107 days of gestation; D1L: first day of lactation; D21L: 21 days of lactation.

<sup>b</sup>SEM, standard error of the mean.

Table 16. Concentration of IgG (mg.mL $^{-1}$ ) at 1 and 14 days of lactation in plasma of piglets

<b>Treatment</b>	<b>Day</b>	
	<b>D1L<sup>a</sup> (± SEM<sup>b</sup>)</b>	<b>D14L<sup>a</sup> (± SEM<sup>b</sup>)</b>
CON	27.375 ± 17.831 a,B	160.69 ± 18.813 b,A
n-6	25.441 ± 17.831 a,B	262.69 ± 18.813 a,A
n-3	21.867 ± 17.831 a,B	113.65 ± 17.831 c,A

Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by the same lowercase letter, do not differ.

<sup>a</sup>D1L: 1 day of lactation; D14L: 14 days of lactation.

<sup>b</sup>SEM, standard error of the mean.

Table 17. Concentration of IgM (mg.mL $^{-1}$ ) at 1 and 14 days of lactation in plasma of piglets

<b>Treatment</b>	<b>Day</b>	
	<b>D1L<sup>a</sup> (± SEM<sup>b</sup>)</b>	<b>D14L<sup>a</sup> (± SEM<sup>b</sup>)</b>
CON	2.988 ± 0.940 a,B	6.358 ± 0.992 b,A
n-6	0.812 ± 0.940 b,B	10.295 ± 0.992 a,A
n-3	0.989 ± 0.940 b,B	7.395 ± 0.992 ab,A

87 Average on the same line followed by the same capital letter, do not differ. Averages in the same column followed by  
88 the same lowercase letter, do not differ.

89 <sup>a</sup>D1L: 1 day of lactation; D14L: 14 days of lactation.

90 <sup>b</sup>SEM, standard error of the mean

Table 18. Plasma concentrations of LTB4, PGF2 $\alpha$  and TXB2 in sows at 13 ± 4 days of lactation

Item <sup>a</sup>	Treatment <sup>b</sup>					P-value <sup>d</sup>				
	CON ( $\pm$ SEM <sup>c</sup> )	n-6 ( $\pm$ SEM <sup>c</sup> )	n-3 ( $\pm$ SEM <sup>c</sup> )	TAP	DMSO	Treat	Stimulus	Treat*Sti	C1	C2
LTB4, pg.mL <sup>-1</sup>	580.281 ± 213.378	647.393 ± 197.550	448.515 ± 261.334	558.729 ± 130.321	n.e.	0.879	n.e.	n.e.	0.715	0.723
PGF2 $\alpha$ , pg.mL <sup>-1</sup>	46.911 ± 36.291	57.158 ± 28.111	41.504 ± 38.936	72.363 ± 19.754	n.e.	0.390	0.063	0.302	n.e.	0.463
TXB2, pg.mL <sup>-1</sup>	20.266 ± 24.119	38.421 ± 26.554	58.128 ± 43.023	74.793 ± 18.777	3.084 ± 32.280	0.847	0.0001	0.922	0.908	0.580

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Table 19. Plasma concentrations of LTB4, PGF2 $\alpha$  and TXB2 in piglets at 13 ± 4 days of age

Item <sup>a</sup>	Treatment <sup>b</sup>					P-value <sup>d</sup>				
	CON ( $\pm$ SEM <sup>c</sup> )	n-6 ( $\pm$ SEM <sup>c</sup> )	n-3 ( $\pm$ SEM <sup>c</sup> )	TAP	DMSO	Treat	Stimulus	Treat*Sti	C1	C2
LTB4, pg.mL <sup>-1</sup>	257.400 ± 205.240	498.349 ± 229.466	568.989 ± 458.931	441.579 ± 184.209	n.e.	0.625	n.e.	n.e.	0.472	0.551
PGF2 $\alpha$ , pg.mL <sup>-1</sup>	48.433 ± 126.229	278.644 ± 105.937	75.158 ± 180.405	147.565 ± 86.231	n.e.	0.913	0.176	n.e.	n.e.	n.e.
TXB2, pg.mL <sup>-1</sup>	45.245 ± 6.095	31.884 ± 5.220	76.663 ± 7.869	88.2901 ± 5.502	14.238 ± 5.086	0.608	<0.0001	0.094	0.347	0.796

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aAnalytes present in greater evidence in the sample

bSows fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.

cSEM: standard error of the mean.

dC1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.

n.e: not estimable. The values were not estimable due to missing, as a consequence of the limitations of the method to measure the concentration of analytes in the sample.

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Table 20. Plasma concentrations of LTB4, PGF2 $\alpha$  and TXB2 in gilts at 24  $\pm$  6 days of gestation

Item <sup>a</sup>	Treatment <sup>b</sup>						P-value <sup>d</sup>			
	CON ( $\pm$ SEM <sup>c</sup> )	n-6 ( $\pm$ SEM <sup>c</sup> )	n-3 ( $\pm$ SEM <sup>c</sup> )	TAP	DMSO	Trat	Stimulus	Treat*Sti	C1	C2
LTB4, pg.mL <sup>-1</sup>	240.085 $\pm$ 324.069	330.581 $\pm$ 330.016	129.152 $\pm$ 322.062	403.904 $\pm$ 129.271	62.641 $\pm$ 352.802	0.465	0.037	0.784	0.359	0.414
PGF2 $\alpha$ , pg.mL <sup>-1</sup>	26.425 $\pm$ 18.191	72.973 $\pm$ 18.191	22.171 $\pm$ 24.255	31.180 $\pm$ 9.902	49.866 $\pm$ 21.391	0.403	0.890	0.110	0.960	0.183
TXB2, pg.mL <sup>-1</sup>	11.924 $\pm$ 4.755	7.042 $\pm$ 4.842	16.615 $\pm$ 3.381	19.852 $\pm$ 2.606	3.8685 $\pm$ 4.331	0.139	<0.0001	0.550	0.114	0.212

105 <sup>a</sup>Analytes present in greater evidence in the sample.106 <sup>b</sup>Gilts fed with a Control milk (CON), supplemented with cow's milk biofortified with n-6 or n-3.107 <sup>c</sup>SEM: standard error of the mean.108 <sup>d</sup>C1, contrast Control vs n-6+n-3; C2, contrast n-6 vs n-3.