

Table S1. Nutrition and Lipid Delivery Protocol

Protocol Day/Pig Age (in days)¹	0	1	2	3	4	5	6	7	8
C-section 7-8 days prior to due date	✓								
<u>Nutrition</u>									
<u>Enteral feedings</u> - Litterlife (ml/kg/day)	100	120	150	180	240	240	240	240	240
Number of feedings per day	24	24	24	12	12	12	8	8	8
Interval feeding schedule	q1	q1	q1	q2	q2	q2	q3	q3	q3
% of energy needs as Enteral	42	50	63	75	100	100	100	100	100
<u>Lipid Supplement Delivery</u>									
Lipid supplement		✓	✓	✓	✓	✓	✓	✓	✓
Lipid supplement dosing		q6	q6	q6	q6	q6	q6	q6	q6

q, every (e.g. q1 = every 1 hour)

¹Day 0 = Date of birth

Table S2. Piglet Cohort Characteristics

Groups	Litter	n	Birth Weight, grams, Median (IQR)	Growth Velocity, g/k/d, Median (IQR)	Mortality, n (%)	Mortality Cause
Intralipid		7			2 (29)	intestinal perforation, diarrhea
	Litter 1	2	1135 (55)	37.8 (18.9)	0	
	Litter 2	3	840 (80)	40.3 (5.9)	2	
	Litter 3	2	1225 (305)	17.7 (13.6)	0	
BW and GV Median (IQR), all litters			920 (290)	31.4 (16.7)		
CLS1		10			1 (10)	diarrhea
	Litter 1	2	1130 (90)	23.4 (1.9)	0	
	Litter 2	4	840 (40)	38.0 (11.0)	1	
	Litter 3	4	1135 (201)	18.6 (6.3)	0	
BW and GV Median (IQR), all litters			1068 (428)	23.4 (15.1)		
CLS2		4			0 (0)	
	Litter 1					
	Litter 2					
	Litter 3	4	1402 (79)	15.9 (12.8)	0	

BW, birth weight; CLS1, complex lipid supplement 1; CLS2, complex lipid supplement 2; GV, growth velocity; IQR, interquartile range

Table S3. Median plasma levels of select fatty acids in the n-3 pathway, expressed as mol% (\pm IQR)

Group	Day	ALA, 18:3,n-3	EPA, 20:5,n-3	DHA, 22:6,n-3
Intralipid	0	0.07 \pm 0.03	0.2 \pm 0.04	2.4 \pm 0.6
	2	0.9 \pm 0.1	0.1 \pm 0.04	1.2 \pm 0.2
	4	1.0 \pm 0.9	0.1 \pm 0.03	1.5 \pm 0.03
	6	0.8 \pm 0.1	0.1 \pm 0.04	1.2 \pm 0.5
	8	0.7 \pm 0.1	0.1 \pm 0.09	1.3 \pm 0.7
CLS1	0	0.08 \pm 0.04	0.2 \pm 0.03	2.4 \pm 0.7
	2	0.3 \pm 0.06*	0.6 \pm 0.08*	3.7 \pm 0.9*
	4	0.4 \pm 0.03*	1.0 \pm 0.05*	4.7 \pm 0.3*
	6	0.5 \pm 0.02	0.8 \pm 0.2*	4.1 \pm 0.6*
	8	0.5 \pm 0.06	0.8 \pm 0.1*	4.0 \pm 0.8*
CLS2	0	0.1 \pm 0.01	0.2 \pm 0.05	2.3 \pm 0.06
	2	0.4 \pm 0.04*	0.6 \pm 0.1	2.9 \pm 0.3*
	8	0.5 \pm 0.06	0.6 \pm 0.03	4.1 \pm 0.4*

Column values for each fatty acid with an asterisk are different from the Intralipid® control group at that time point, $P < 0.05$.

ALA, α -linolenic acid; CLS1, complex lipid supplement 1; CLS2, complex lipid supplement 2; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IQR, interquartile range

Table S4. Median plasma levels of select fatty acids in the n-6 pathway, expressed as mol% (\pm IQR)

Group	Day	LA, 18:2,n-6	GLA, 18:3,n-6	DGLA, 20:3, n-6	AA, 20:4, n-6
Intralipid	0	5.1 \pm 0.9	0.3 \pm 0.2	0.4 \pm 0.1	14.3 \pm 1.7
	2	17.4 \pm 1.8	0.7 \pm 0.4	0.4 \pm 0.0	8.6 \pm 0.7
	4	20.2 \pm 1.3	0.4 \pm 0.3	0.3 \pm 0.0	8.4 \pm 0.2
	6	24.1 \pm 0.9	0.3 \pm 0.1	0.4 \pm 0.1	7.6 \pm 1.4
	8	23.8 \pm 5.8	0.4 \pm 0.2	0.4 \pm 0.2	7.0 \pm 1.4
CLS1	0	4.9 \pm 0.7	0.3 \pm 0.2	0.4 \pm 0.1	13.6 \pm 2.2
	2	11.9 \pm 0.7*	1.5 \pm 0.8*	0.5 \pm 0.1*	9.9 \pm 2.0
	4	14.5 \pm 1.2*	2.1 \pm 0.4*	0.6 \pm 0.1*	9.4 \pm 0.6
	6	17.3 \pm 0.6*	1.6 \pm 0.0*	0.7 \pm 0.1*	8.4 \pm 0.1
	8	19.9 \pm 1.1*	1.5 \pm 0.4*	0.7 \pm 0.1*	7.3 \pm 1.1
CLS2	0	5.7 \pm 0.3	0.2 \pm 0.1	0.5 \pm 0.0	13.1 \pm 0.5
	2	12.5 \pm 0.6	1.1 \pm 0.3	0.7 \pm 0.1*	11.2 \pm 0.6
	8	17.8 \pm 1.0*	1.5 \pm 0.1	0.6 \pm 0.0	11.3 \pm 0.6* [†]

Column values for each fatty acid with an asterisk are different from the Intralipid® control

group at that time point, $P < 0.05$. Column values for each fatty acid in the CLS2 group with a superscript dagger are different from the CLS1 group at that time point, $P < 0.05$.

AA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; GLA, gamma-linolenic acid; IQR, interquartile range; LA, linoleic acid

Table S5. Median ileum tissue levels of select fatty acids in the n-3 and n-6 pathways, expressed as mol% (\pm IQR)

Group	n-3 Fatty Acids			n-6 Fatty Acids			
	ALA, 18:3,n-3	EPA, 20:5,n-3	DHA, 22:6,n-3	LA, 18:2,n-6	GLA, 18:3,n-6	DGLA, 20:3,n-6	AA, 20:4,n-6
Birth	0.08 \pm 0.04 ^a	0.2 \pm 0.2 ^{ab}	2.0 \pm 0.7 ^{ab}	5.6 \pm 1.7 ^a	0 \pm 0.0 ^{ab}	1.3 \pm 0.1 ^a	17.7 \pm 1.1 ^a
Intralipid	0.7 \pm 0.6 ^b	0.1 \pm 0.03 ^a	1.1 \pm 0.3 ^a	14.0 \pm 6.1 ^b	0.07 \pm 0.04 ^a	0.9 \pm 0.1 ^b	10.9 \pm 4.6 ^b
CLS1	0.2 \pm 0.2 ^c	0.7 \pm 0.3 ^c	5.7 \pm 1.2 ^b	11.0 \pm 1.3 ^c	1 \pm 0.7 ^c	1.7 \pm 0.06 ^c	12.0 \pm 2.7 ^b
CLS2	0.1 \pm 0.01 ^a	0.3 \pm 0.05 ^b	5.8 \pm 0.9 ^b	7.8 \pm 0.9 ^a	0.6 \pm 0.3 ^{bc}	1.2 \pm 0.2 ^a	17.2 \pm 0.6 ^a

Medians in a column for each group without a common letter differ, $P < 0.05$.

AA, arachidonic acid; ALA, α -linolenic acid; CLS1, complex lipid supplement 1; CLS2, complex lipid supplement 2; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; IQR, interquartile range; LA, linoleic acid

Table S6. Median brain tissue levels of select fatty acids in the n-3 and n-6 pathways, expressed as mol% (\pm IQR)

Group	n-3 Fatty Acids			n-6 Fatty Acids			
	ALA, 18:3,n-3	EPA, 20:5,n-3	DHA, 22:6,n-3	LA, 18:2,n-6	GLA, 18:3,n-6	DGLA, 20:3,n-6	AA, 20:4,n-6
Birth	0 \pm 0	0.1 \pm 0.03 ^a	7.2 \pm 0.8 ^a	0.3 \pm 0.04 ^a	0.02 \pm 0.01	0.3 \pm 0.08	11.6 \pm 0.2
Intralipid	0.02 \pm 0.01	0.06 \pm 0.05 ^b	8.1 \pm 0.5 ^a	0.9 \pm 0.3 ^b	0.03 \pm 0.01	0.5 \pm 0.3	12.0 \pm 0.3
CLS1	0.02 \pm 0	0.05 \pm 0.01 ^b	9.7 \pm 0.5 ^b	0.7 \pm 0.08 ^c	0.03 \pm 0.01	0.5 \pm 0.05	11.9 \pm 0.3
CLS2	0.02 \pm 0	0.06 \pm 0.01 ^{ab}	9.7 \pm 0.2 ^b	0.6 \pm 0.1 ^{ac}	0.03 \pm 0.01	0.4 \pm 0.03	12.0 \pm 0.0

Medians in a column for each group without a common letter differ, $P < 0.05$.

AA, arachidonic acid; ALA, α -linolenic acid; CLS1, complex lipid supplement 1; CLS2, complex lipid supplement 2; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; IQR, interquartile range; LA, linoleic acid

Table S7. Lutein, Tocopherol, Vitamin D Body Compartment Levels

Compartment	Group	n	Lutein, ng/g	Tocopherol, ug/g	25 Hydroxyvitamin D3, ng/g	Vitamin D, ng/g
Plasma	Baseline ¹	5	BDL ^{ab}	0.2 ± 0.0 ^a	BDL ^a	0.5 ± 0.3 ^a
	Intralipid	8	BDL ^a	2.7 ± 2.4 ^{ab}	2.6 ± 3.4 ^{ab}	16.2 ± 12.6 ^b
	CLS1	11	BDL ^{ab}	7.5 ± 4.1 ^{bc}	6.9 ± 6.9 ^{bc}	21.2 ± 10.3 ^{bc}
	CLS2	4	5.0 ± 1.0 ^b	8.9 ± 1.3 ^c	8.9 ± 1.3 ^c	33.6 ± 3.4 ^c
Liver	Baseline	5	BDL ^{ab}	0.3 ± 0.0 ^a	BDL ^a	0.5 ± 0.3
	Intralipid	8	2.8 ± 2.9 ^a	40.9 ± 36.6 ^b	34.7 ± 38.2 ^b	15.8 ± 23.1
	CLS1	11	35.5 ± 36.7 ^b	85.3 ± 24.1 ^{bc}	83.6 ± 35.5 ^{bc}	38.6 ± 25.3
	CLS2	4	60.3 ± 22.9 ^b	101.7 ± 44.5 ^c	101.7 ± 44.5 ^c	54.7 ± 32.3
Eye	Baseline	5	BDL ^a	BDL	BDL	BDL
	Intralipid	8	BDL ^a	BDL	BDL	BDL
	CLS1	11	0.7 ± 0.7 ^b	BDL	BDL	BDL
	CLS2	4	0.6 ± 0.1 ^b	BDL	BDL	BDL
Frontal cortex	Baseline	5	BDL	0.4 ± 0.1 ^a	BDL ^a	BDL
	Intralipid	8	BDL	2.0 ± 0.9 ^a	1.7 ± 1.5 ^a	BDL
	CLS1	11	BDL	3.9 ± 0.7 ^b	3.9 ± 1.4 ^b	BDL
	CLS2	4	BDL	5.3 ± 0.8 ^c	5.3 ± 0.8 ^c	BDL
Occipital cortex	Baseline	5	BDL	0.3 ± 0.1 ^a	BDL ^a	BDL
	Intralipid	8	BDL	2.6 ± 1.4 ^{ab}	2.0 ± 1.7 ^{ab}	BDL
	CLS1	11	BDL	3.3 ± 1.7 ^b	3.2 ± 1.7 ^b	BDL
	CLS2	4	BDL	4.6 ± 0.9 ^{ab}	1.9 ± 3.8 ^{ab}	BDL
Cerebellum	Baseline	5	BDL	0.4 ± 0.1 ^{ab}	BDL ^{ab}	BDL
	Intralipid	8	BDL	4.1 ± 0.3 ^a	3.6 ± 3.8 ^a	BDL
	CLS1	11	BDL	5.1 ± 2.5 ^{ab}	4.9 ± 3.6 ^{ab}	BDL
	CLS2	4	BDL	6.5 0± 0.7 ^b	6.5 0± 0.7 ^a	BDL

¹Baseline = birth samples

BDL, below detection limit

Labeled points without a common letter represents a statistically significant difference, $P < 0.05$.

Table S8. Sources of Fatty Acids and Lipophilic Nutrients

Nutrient	Source
Docosahexaenoic acid	Structured lipid; Nippon Kaisha, Ltd.
Gamma-linolenic acid	Borage oil; Bioriginal Food and Science Corp.
Arachidonic acid	Mortierella alpine oil; DSM Nutritionals
Mixed tocopherols	BASF Corp.
Vitamin D3	DSM Nutritionals
Lutein	DSM Nutritionals
Vitamin E	Prinova Solutions

Figure S1. Western blot (A) and quantification (B) of pancreatic lipase in immature, preterm piglets (n=6) versus mature pigs (n=2).

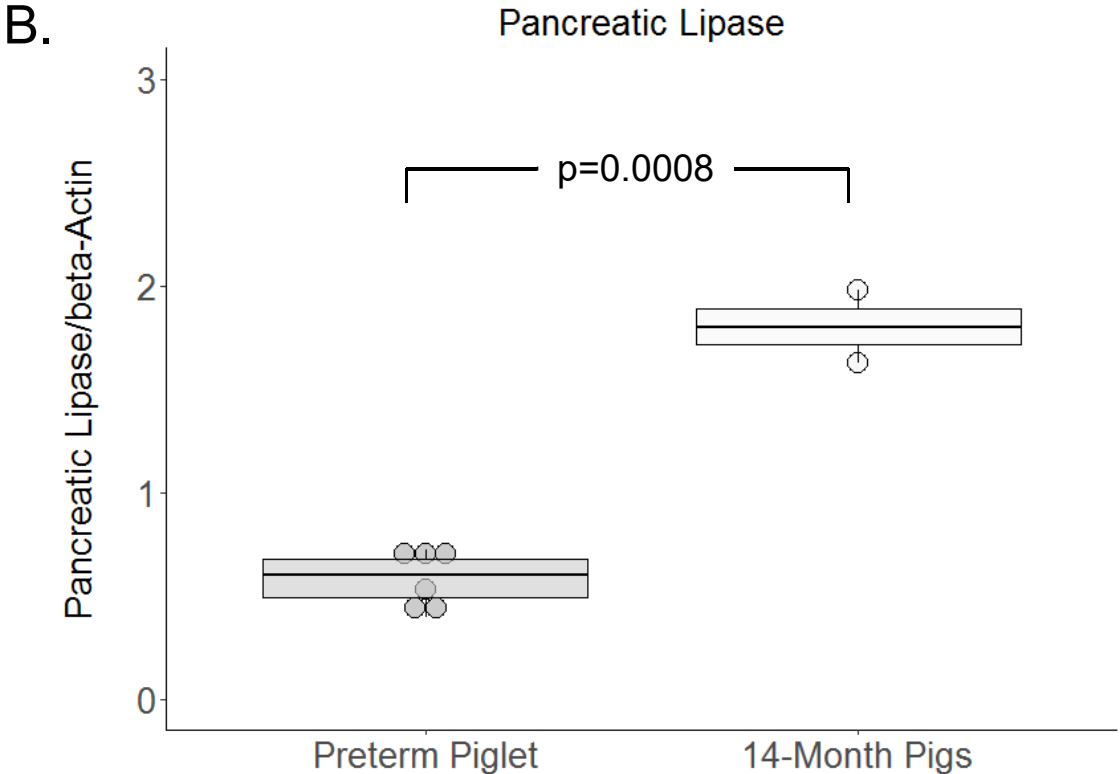
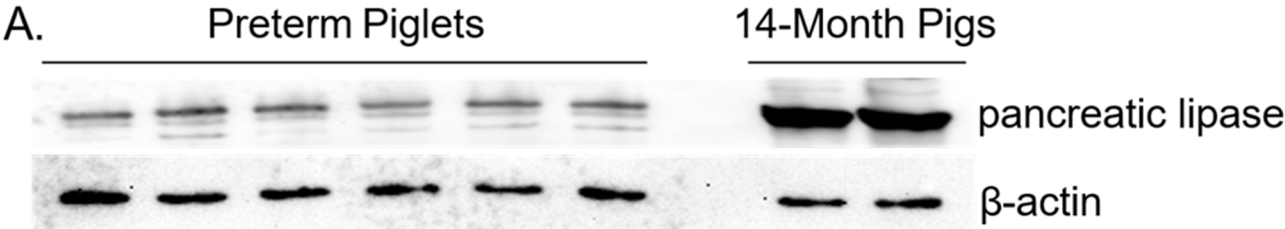


Figure S2. Quantification of TUNEL staining in the distal ileum tissue represented as dot plots. Staining intensity score of 0 for none, 1 for weak, 2 for moderate, and 3 for strong. All samples scored a 0 or 1. Open circles represent a single sample. Black circles represent the median value for each group. CLS, concentrated lipid supplement; IL, Intralipid®-control.

