Lipidome Profiles of Postnatal day 2 vaginal swabs reflect fat composition of gilt's postnatal diet

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Project:

Project Title	Lipidome profiles of postnatal day 2 vaginal swabs reflect fat composition of gilt's postnatal diet.
Project Type	MRM-profiling
Project Summary	In this study, we further investigated the efficacy of using MRM-profiling of vaginal lipids to differentiate PND 2 vaginal swabs between gilts suckled by sow or fed milk replacer. Secondly, we tested the effect of a lard based supplement on vaginal lipid profiles of gilts.
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Department	Animal Sciences
Laboratory	Metabolite Profiling Facility - Purdue University
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Funding Source	AgSEED Crossroads funding to support Indiana's Agriculture and Rural Development
Contributors	KaLynn Harlow, Christina R. Ferreira, Tiago J.P. Sobreira, Theresa Casey, and Kara Stewart

Study:

Study Title	Vaginal swab lipidome profiles at 48 h reflect the fat composition of neonatal diet during first two days postnatal
Study Type	MRM-profiling
Study Summary	In this study, we further investigated the efficacy of using MRM-profiling of vaginal lipids to differentiate PND 2 vaginal swabs between gilts suckled by sow or fed milk replacer. Secondly, we tested the effect of a lard based supplement on vaginal lipid profiles of gilts.
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Address	1203 W. State St, West Lafayette, IN, 47906, USA
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Phone	7654095924
Num Groups	colostrum suckled (S, n=8); S plus fat supplement (SF, n=5); bottle-fed milk replacer (B, n=8); or B plus fat supplement (BF, n=7
Total Subjects	28
Num Females	28

Subject:

Subject Type	Mammal
Subject Species	Sus scrofa
Taxonomy Id	9823
Age Or Age Range	2 days after birth
Weight Or Weight Range	above 1.3Kg at birth
Gender	Female
Animal Animal Supplier	Purdue University Animal Sciences Research and Education Swine facility
Animal Housing	Education Swine facility
Animal Water	ad libitum
Animal Inclusion Criteria	Three to four gilts above 1.3Kg at birth were selected per litter from eight different sows which were monitored during parturition

Samples and experimental variables (factors): (Factor headings shown in green)

Subject	Sample	Treatment	Sample Type	SampleData
-	1-R18	B	serum	
-	1-R18b	B	serum	
-	10-Y24	S	serum	
-	11-Y27	S	serum	
-	12-Y29	S	serum	
-	12-Y29b	S	serum	
-	13-Y31	S	serum	
-	14-Y32	S	serum	
-	14-Y32b	S	serum	
-	15-Y34	S	serum	
-	15-Y34b	S	serum	
-	16-Y39	S	serum	
-	19-4N-H24	M	milk	
-	2-R20	B	serum	
-	20-MR	MR	milk replacer	
-	24-14A-H24	M	milk	
-	28-11Z-H24	M	milk	
-	3-R21	B	serum	
-	32-4U-H24	M	milk	
-	36-1T-H24	M	milk	
-	4-R25	B	serum	
-	4-R25b	B	serum	
-	40-4M-H24	M	milk	
-	44-14S-H24	M	milk	
-	47-L14-H24	M	milk	
-	47-L14-H24b	M	milk	
-	5-R26	B	serum	
-	6-R27	B	serum	
-	7-R29	B	serum	
-	7-R29b	B	serum	
-	8-R30	B	serum	
-	8-R30b	B	serum	
-	9-Y23	S	serum	
-	R18_M1	B	swab	
-	R20_M1	B	swab	
-	R21_M1	B	swab	
-	R25_M1	B	swab	
-	R26_M1	B	swab	
-	R27_M1	B	swab	
-	R29_M1	B	swab	
-	R30_M1	B	swab	
-	Y23_M1	S	swab	
-	Y24_M1	S	swab	
-	Y27_M1	S	swab	
-	Y29_M1	S	swab	
-	Y31_M1	S	swab	
-	Y32_M1	S	swab	
-	Y34_M1	S	swab	
-	Y39_M1	S	swab	

Treatment:

Treatment Summary	Three to four gilts were selected per litter from eight different sows which were monitored during parturition. Immediately after delivery, all gilts were towel-dried, weighed, and placed in a holding cart until at least three gilts above 1.3 kg were delivered. Within litter, each gilt was randomly assigned to one of four treatment groups and ear tagged for identification. The four treatment groups were: suckled (S; n=8); suckled plus fat-supplement (SF; n=5); bottle-fed with milk-replacer (B; n=8), and; bottle-fed milk replacer plus fat-supplement (BF; n=7). Body weights were recorded at birth and 48 h. All gilts were administered a 2 ml dose of Camas experimental antibody product (Camas Incorporated; Le Center, MN) using Pump It™ Automatic Delivery System (Genesis Industries, Inc.) at birth, and at 3 h and 9 h after the first dose.

Collection:

Collection Summary	Blood samples were collected from gilts at 48 h postnatal. Gilts were euthanized approximately 48 h after birth using CO2 inhalation. Following euthanasia, skin of the abdominal and genital regions was cleaned thoroughly using 70% ethanol, and vaginal swabs were taken using a cytology brush (Puritan 2196 Removable Stiff Bristle Tip Brush; QuickMedical; Issaquah, WA) by inserting the tip of the brush into the vulva angled dorsally at 45˚. Once inserted to the base of the bristles, the brush was rotated 360˚ against the vaginal surface. Two consecutive swabs were collected from each animal, and swabs were placed in separate 15 ml sterile polypropylene conical tubes (Falcon™, Fisher Scientific, San Jose, California) and immediately placed on dry ice for transport. Samples were stored in a -80°C freezer until lipid extraction and analysis. Sows were milked during farrowing, and at 24 h after delivery of first piglet. For milk collection piglets were removed from the sow for approximately an hour and then 1 ml oxytocin (VetOne; Boise, ID; 20 USP/ml) was administered IM using a 20g x 1.5-inch needle into the vulva to stimulate milk letdown. Colostrum samples were collected manually from all teats and combined to create a uniform sample. Samples were stored until further analysis at -20°C
Sample Type	Vaginal epithelium
Collection Method	swab
Collection Location	West Lafayette
Collection Frequency	once
Collection Duration	1min
Storage Conditions	-80°C
Collection Vials	Puritan 2196 Removable Stiff Bristle Tip Brush; QuickMedical; Issaquah, WA
Storage Vials	15 ml sterile polypropylene conical tubes (Falcon™, Fisher Scientific, San Jose, California)
Additives	none

Sample Preparation:

Sampleprep Summary	The Bligh & Dyer lipid extraction technique was slightly modified to extract lipids from the vaginal swab samples. A volume of 500 µl distilled water was added to the conical tube containing the swab brushes and vortexed to remove biological material from the brush. The brushes were removed, the sample homogenate was transferred to a new tube, and phase separation was performed by mixing with chloroform/methanol/distilled water (1:2:0.8). Samples were centrifuged, the organic phase (bottom phase) was separated from aqueous phase, divided into four aliquots, and dried in a centrifugal evaporator (Savant SpeedVac AES2010, ThermoFisher Scientific, San Jose, CA). Dried lipid extracts were stored at -20˚C until mass spectrometry analysis. The Bligh & Dyer lipid extraction technique was also used to extract lipids from 48 h serum samples from suckled and bottle-fed piglets; colostrum samples taken from all eight sows (during parturition, 6 h after first piglet born, 12 h after, and 24 h after); and milk replacer used for bottle-feeding. The procedure for these samples began at the phase separation step (i.e. no water was added to the samples).
Processing Storage Conditions	Room temperature
Extraction Method	Bligh & Dyer
Extract Storage	-80°C
Sample Resuspension	acetonitrile/methanol/ammonium acetate 300 mM at 3:6.65:0.35 (v/v)
Sample Derivatization	none
Sample Spiking	none

Chromatography:

Chromatography Summary	Direct infusion
Chromatography Type	None (Direct infusion)
Instrument Name	Agilent 6410
Column Name	none

Analysis:

Analysis Type	MS
Laboratory Name	Metabolite Profiling Facility
Operator Name	Christina Ferreira

MS:

Instrument Name	Agilent 6410 QQQ
Instrument Type	Triple quadrupole
MS Type	ESI
Ion Mode	UNSPECIFIED
MS Comments	Pooled sample injections (8 µl) were delivered to the micro-autosampler (G1377A) in a QQQ6410 triple quadrupole mass spectrometer (Agilent Technologies, San Jose, CA) equipped with an ESI ion source. The solvent pumped between injections (CapPump G1376A, Agilent Technologies, San Jose, CA) was acetonitrile with 1% formic acid at 10µL/min. Between sample injections, methanol/chloroform was injected to remove any remaining lipids before the next sample injection
Laboratory Name	Metabolite Profiling Facility
Operator Name	Christina Ferreira