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Effects of energy restriction during gilt development on milk nutrient profile, milk oligosaccharides, and progeny biomarkers[1]

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ABSTRACT: An ongoing study at the University of Nebraska-Lincoln (which included 14 batches of gilts; $n = 90$ gilts/batch) demonstrated that energy restriction during the developmental period of a gilt increases longevity and may also have beneficial effects on progeny health and growth, particularly, parity 1 progeny. Therefore, we hypothesized that energy restriction during gilt development may affect milk nutrient profile, milk oligosaccharides (**OS**), and postnatal progeny biomarkers. During the development period, batch 14 gilts ($n = 128$, 8 gilts/pen) were fed 3 dietary treatments including the following: 1) Control diet formulated to NRC (2012) specifications (CTL); 2) Restricted (20% energy restriction via addition of 40% soy hulls; RESTR); and 3) CTL diet plus addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet (CTL+). All diets were fed ad libitum and applied in a 3-phase feeding regimen during gilt development (days 123 to 230 of age). Average daily feed intake was used to estimate daily metabolizable energy intake (Mcal/d) during each phase (Phase 1: 10.13, 6.97, 9.95; Phase 2: 11.25, 8.05, 10.94; and Phase 3: 9.47, 7.95, 11.07) for CTL, RESTR, and CTL+, respectively. After 230 d of age, gilts were bred and fed a common diet. Milk samples were collected from batch 14 gilts ($n = 7$ per treatment) on days 0 and 14 postfarrowing for compositional analysis of N, CP, dry matter (DM), GE, insulin, and OS. Piglet blood samples ($n = 6$ piglets/gilt) were obtained on days 1 and 15 postfarrowing for quantification of glucagon-like peptide-2 (**GLP-2**) and insulin. No effects of developmental diet were observed for milk N, CP, DM, or GE; however, N, CP, DM, and insulin were increased ($P < 0.05$) on day 1 compared with day 14. A total of 61 different milk OS were identified. Milk OS profile was significantly different for neutral and acidic OS ($P < 0.05$) on day 0, but there were no significant differences on day 14. For piglet GLP-2, a treatment by day interaction was observed ($P < 0.009$); specifically, on day 1 GLP concentrations were greater ($P < 0.001$) in CTL+ compared with RESTR (6.73 vs. 1.21 ng/mL). For serum insulin, a treatment by day interaction was observed ($P < 0.01$); specifically, insulin in RESTR progeny was greater ($P < 0.03$) than CTL on day 1. In conclusion, nutritional management of the developing gilt may affect milk nutrient composition, milk OS profile, and piglet serum biomarkers.

Key words: energy restriction, gilts, milk, oligosaccharides

INTRODUCTION

Ongoing research at the University of Nebraska investigating the effects of energy restriction on gilt development (which included 14 batches of gilts, n = 90 gilts/batch) led to the observation that energy restriction increases sow longevity and may also provide beneficial effects to first-parity progeny with respect to health and growth (Barnett et al., 2017). Developing gilts fed an energy-restricted diet that is adequate in all other nutrients will cause a restriction in fat deposition, but should not have a significant effect on gilt muscle development (Miller et al., 2011). Although most studies focus on diet alteration during gestation/lactation rather than prior to breeding, there is evidence that backfat (**BF**) and body weight (BW) will affect milk nutrient profile and offspring development (Chen et al., 2009).

In addition, growth biomarkers such as glucagon-like peptide-2 (**GLP-2**) and insulin are maintained through enteral food intake and nutrients ingested (Sangild et al., 2000). Insulin and GLP-2 have been shown to help increase protein synthesis of the neonate. Although insulin has its greatest impact on fast-twitch muscles, GLP-2 is related to intestinal adaptation (Davis et al., 2001; Burrin et al., 2007).

Over the last few years, research on milk oligosaccharides (**OS**) has received a lot of attention due to their impact on postnatal infant intestinal development. Specifically, milk OS are resistant to digestion in the upper gastrointestinal tract; therefore, OS may reach the large intestine where they serve as a carbon-source for commensal bacteria and contribute to shaping a health-promoting microbiota (Boehm et al., 2005). The diversity and complexity of OS functions may be attributed to the many different linkages by which a few monosaccharides can be attached to the core lactose molecule (Wu et al., 2011). As milk OS play key roles in the young mammal's development and health, there is much interest in characterizing and quantifying OS structures in milk across stages of lactation and investigating the potential effect diet may have on milk OS composition.

We hypothesized that energy restriction during gilt development may affect milk nutrient profile, milk OS profile, and postnatal progeny biomarkers. Specifically, this experiment was designed to take advantage of a unique resource population that has been used to investigate the effects of energy restriction on gilt development to further explore potential effects on progeny related to health and growth. Therefore, the objective of this experiment was to evaluate how management strategies (i.e., energy restriction) in gilt development may affect milk nutrient composition, milk OS profile, and piglet serum biomarkers.

MATERIALS AND METHODS

The University of Nebraska-Lincoln Animal Care and Use Committee approved all animal care and handling procedures used in this experiment. The experiment was carried out at the University of Nebraska Swine Research Center.

Animals and Experimental Design

Batch 14, parity 1 gilts (n = 128) were randomly allotted to a dietary treatment (3 treatments, 8 gilts/pen) during their developmental period (days 120 to 230 of age). Gilts were housed in a temperature-controlled room and were given ad libitum access to water. Gilts were fed in a 3-phase feeding regimen in which phases 1 and 2 were 42 d, and phase 3 was 28 d. After 230 d of age, gilts were bred and moved to into gestation where they were all fed a common diet to meet the requirements of a gestating sow (NRC, 2012). At day 109 of gestation, the gilts were moved to farrowing crates.

Dietary Treatments

Diet ingredients and nutrient composition are presented in Tables 1 and 2. Diets were fed ad libitum and varied based on energy content. Dietary treatments included the following: 1) Control (CTL; formulated to 2012 NRC requirements); 2) Restricted (RESTR; containing 40% soy hulls and 20% energy restricted); and 3) Control Plus (CTL+; containing an addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet).

Data and Sample Collection

Feed disappearance was measured by pen every 2 wk from days 120 to 230 during the gilt development period to calculate ADFI and estimate metabolizable energy (ME) intake per gilt. During lactation, sows were allowed free access to feed and individual feed disappearance was recorded to calculate ADFI. When the gilts were moved to farrowing crates (day 109 of gestation), BF (pre-BF) was measured using an Aloka 500V real-time ultrasound instrument equipped with a 3.5-MHz, 17-cm linear transducer (Corometrics Medical System,

Table 1. Ingredient composition and nutrient analysis of diets (as-fed basis) fed to developing gilts days 123 to 230

Item	Phase 1			Phase 2			Phase 3		
	CTL[a]	RESTR[b]	CTL+[c]	CTL	RESTR	CTL+	CTL	RESTR	CTL+
Ingredient, %									
Corn	72.52	39.59	70.38	76.32	43.17	74.66	80.13	47.16	78.60
Soybean Meal	21.53	17.79	23.35	17.66	14.13	19.00	13.79	10.05	15.00
Soybean Hulls	–	40.00	–		40.00	–		40.00	–
Beef Tallow	3.00	–	3.00	3.00	–	3.00	3.00	–	3.00
Dicalcium phosphate	1.37	1.72	1.37	1.46	1.80	1.46	1.54	1.89	1.54
Limestone	0.68		0.68	0.66		0.66	0.64		0.64
Sodium Chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin Premix[d,e]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[f]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	–	–	0.15	–	–	0.15	–	–	0.15
Methionine	–	–	0.05	–	–	0.05	–	–	0.05
Threonine	–	–	0.09	–	–	0.09	–	–	0.09
Tryptophan	–	–	0.03	–	–	0.03	–	–	0.03
Calculated composition									
ME, kcal/kg	3406	2705	3408	3408	2706	3410	3410	2707	3412
Lys, g/kg	0.7	0.7	0.86	0.61	0.61	0.76	0.51	0.51	0.66
CP, %	13.72	12.68	14.34	12.36	14.41	12.81	11.01	12.79	11.41
P, %	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Ca, %	0.67	0.71	0.68	0.67	0.72	0.68	0.68	0.73	0.68
Lys/ME (g/Mcal)	2.06	2.57	2.53	1.78	2.24	2.22	1.50	1.87	1.93

Diets consisted of a control (CTL), restricted (RESTR), or control plus (CTL+). Phases 1 and 2 are each 6 wk in duration and phase 3 is 4 wk in duration.

[a]Control diet (CTL) is formulated to meet 2012 NRC requirements for developing gilts.

[b]Energy restricted diet (RESTR) is 20% restricted in energy with increased fiber.

[c]Control Plus (CTL+) contains an addition of crystalline amino acids equivalent to the SID Lys:Met of the RESTR diet.

[d]Provided per kilogram of diet for phases 1 and 2: 5,500 IU of Vitamin A, 550 IU of Vitamin D_3, 30 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 33.00 μg of Vitamin B_{12}.

[e]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 22.05 μg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin.

[f]Provided per kilogram of diet: 10.50 mg of Copper Sulfate Pentahydrate, 0.26 mg of Calcium Iodate, 127.50 mg of Ferrous Sulfate, 30.00 mg of Manganese Oxide, 0.30 mg of Sodium Selenite, 127.50 mg of Zinc Sulfate, 226.03 mg of Calcium Carbonate.

Inc., Wallingford, CT) and BW (pre-BW) was recorded. After farrowing, at the time progeny were weaned (day 21 postfarrowing), gilt BF (post-BF) and BW (post-BW) were observed and recorded as described previously. Piglets that were cross-fostered were moved to a farrowing crate with a gilt on the same treatment as that from which it was derived. All piglets' birth weight (**BiW**) and weaning weight (**WW**) were recorded to measure progeny performance based on developmental diet. Milk samples were collected on days 0 and 14 postfarrowing from 21 gilts (7 gilts/treatment). Oxytocin (1 to 2 mL) was administered in the neck via intramuscular injection to facilitate milk letdown. Piglets (*n* = 6 per litter) from the gilts selected for milk sampling were randomly selected, and blood samples were collected on days 1 and 15 postfarrowing. All blood samples were collected via the jugular vein. Serum was harvested following centrifugation (20 min at 2,500 × *g*). Serum and milk samples were frozen at −20 °C for subsequent analyses.

Milk Composition Analysis

Milk samples were analyzed for dry matter (DM; procedure 930.15; AOAC, 1995) and N (TruSpec N Determinator; Leco Corporation, St. Joseph, MI; procedure 984.13; AOAC, 1995). The energy concentration in milk samples was determined by bomb calorimetry (Parr 1241 Calorimeter; Parr Instrument Co., Moline, IL). A porcine-specific enzyme-linked immunosorbent assay (ELISA) was used to quantify insulin concentration in milk samples (Mercodia; Uppsala, Sweden). The intra- and inter-assay CV for milk insulin were 3.47% and 3.23%, respectively. All samples were analyzed in duplicates.

Table 2. Ingredient composition and nutrient analysis of diets (as-fed basis) fed during gestation and lactation

Item	Gestation[a]	Lactation[b]
Ingredient, %		
Corn	77.25	65.68
Soybean Meal, 47.5 % CP	16.00	27.50
Tallow	3.00	3.00
Dicalcium Phosphate	1.90	2.33
Limestone	0.93	0.60
Salt	0.50	0.50
Vitamin Premix[c]	0.25	0.25
Trace Mineral	0.15	0.15
Phytase	0.02	−
Calculated composition		
ME (kcal/kg)	2605	2536
CP, %	11.74	15.75
Lys	0.56	0.85
Total P, %	0.67	0.80
Ca, %	0.87	0.90

[a]Gestation diet is fed from the day of breeding until farrowing.

[b]Lactation diet is fed beginning at farrowing through day 21 postfarrowing, sows are put immediately back on gestation diet at day 21 postfarrowing.

[c]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 22.05 µg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin.

OS Analysis

For milk OS analyses, only milk samples from gilts in the RESTR and CTL groups were analyzed (n = 7 samples per treatment on days 0 and 14 postfarrowing).

Chemicals and reagents. Acetonitrile (ACN), chloroform, formic acid (FA), methanol (MeOH), ethanol (EtOH), trifluoroacetic acid (TFA), and sodium hydroxide (NaOH) were obtained from Thermo Fisher Scientific (Waltham, MA); sodium acetate (NaAc) was from Sigma-Aldrich (St Louis, MO). OS standards Lacto-N-difucohexaose (LNDFH), Lacto-N-fucopentaose I (LNFP-I), Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LNnT), Lacto-N-hexaose (LNH), Lacto-N-neohexaose(LNnH), N-acetylgalactosaminyllactose, α1–3,β1-4-D-galactotriose (3-Hex), 3'-Sialyllactose (3′SL), 6′-Sialyllactose (6′SL), 3′-Sialyl-N-acetyllactosamine (3′SLN), and 6′-Sialyl-N-acetyllactosamine (6′SLN) were purchased from V-Labs Inc. (Covington, LA), whereas LNH and LDFT standards were purchased from Prozyme Inc. (Hayward, Ca). All solvents were mass spectrometry (**MS**) grade, and the water used was nanopure (18.2 ohms).

OS isolation and purification. Milk OS were isolated and purified as previously described, with minor modifications (Barile et al., 2010). Briefly, frozen milk samples were completely thawed, and a 0.5-mL aliquot of each sample was mixed with an equal volume of nanopure water and centrifuged at 14,000 × g in a microfuge for 30 min at 4 °C to remove lipids. The top fat layer was removed, and 4 volumes of chloroform/methanol (2:1, vol/vol) were added, vigorously mixed, and the resulting emulsion was centrifuged at 4,000 × g for 30 min at 4 °C. The upper methanol layer containing OS was transferred to a tube, 2 volumes of cold ethanol were added, and the solution was frozen for 1 h at –30 °C, followed by centrifugation for 30 min at 4,000 × g and 4 °C to precipitate the denatured protein. The supernatant (OS-rich fraction) was collected and freeze-dried using a speed vacuum centrifuge.

For OS characterization by nanoliquid chromatography on chip quadrupole time-of-flight (**QToF**)-MS (Agilent Technologies; Santa Clara, CA), extracts were purified from the mixture by solid-phase extraction using nonporous graphitized carbon cartridges. Prior to use, each cartridge was activated with 3 column volumes of 80% acetonitrile, 0.1% TFA (vol/vol) and equilibrated with 3 column volumes of nanopure water. The carbohydrate-rich solution was loaded onto the cartridge, and salts and mono/disaccharides were removed by washing with 6 column volumes of nanopure water. The OS were eluted with a solution of 40% ACN with 0.1% TFA (vol/vol) in water and dried in speed vacuum centrifuge at 35 °C overnight.

Characterization by nano liquid chromatography chip QTOF-MS. Prior to MS analysis, dried OS samples were reconstituted in 100 µL of nanopure water. MS analysis was performed with an Agilent 6520 accurate-mass QToF liquid chromatography/MS (**LC/MS**) with a microfluidic nanoelectrospray chip (Agilent Technologies) as described previously (Wu et al., 2011). The acquisition rate was 0.63 spectra/s for both MS and MS/MS modes. Automated precursor selection was employed based on ion abundance, performing up to 6 MS/MS spectra per individual MS when precursor was above ion-abundance threshold. The precursor isolation window was selected to be narrow (1.3 m/z) to improve accuracy. Fragmentation energy was set at 1.8 V/100 Da with an offset of −2.4 V. Internal calibration was continuously performed by infusing 2 reference masses: m/z 922.009 and 1221.991 (ESI-TOF Tuning Mix G1969-85000, Agilent Technologies).

QToF data analysis. The Molecular Feature Extraction function of Mass Hunter Qualitative Analysis Version B.06.00 (Agilent Technologies) was used to generate a list of deconvoluted masses selected to be in a range of 450 to 1500 m/z with a ≥1000 height count and a typical isotopic distribution of small biological molecules. Charge states allowed were restricted to single and double species. OS compositions were determined from the deconvoluted mass list with in-house software, and all OS compositions were confirmed by tandem MS (MS/MS) analysis. Following MS/MS identity validation and assessment of reproducible retention times (**RT**), individual peaks for each OS were automatically integrated using the Targeted Feature Extractor from MassHunter Profinder Version B.06.00 (Agilent Technologies). The RT window allowed for compound matching was restricted to ± 0.5 min and ± 0.25% of the RT at each time point. Each sample was analyzed in triplicate, 2-fucosyllactose (2′-FL) added as internal standard to minimize instrumental variation.

OS quantification by high-performance anion exchange chromatography—pulsed amperometric detection (*HPAEC-PAD*). The quantification of 9 neutral OS standards (LNDFH, LDFT, LNFP-I, LNT, LNnT, N-acetylgalactosaminyllactose, 3-Hex, LNH, and LNnH) and 4 acidic OS standards (6′-SLN, 3′-SLN, 6′-SL, and 3′-SL) was carried out with a HPAEC-PAD(Thermo Scientific HPAE-PAD ICS-5000), equipped with a detector/chromatography module including a pulsed amperometry electrochemical detector, an electrochemical cell with a disposable gold working electrode, a pH-Ag/AgCl reference electrode, an auto-sampler, and a single pump. Samples were diluted and filtered through a 0.22-μm membrane (Pall, Port Washington, NY) before analysis. A 25-μL sample was injected into the CarboPacPA200 analytical column (3 × 250 mm, Dionex, Sunnyvale, CA) and a CarboPacPA200 Guard Column (3 × 50 mm, Dionex) for OS analysis, eluting with 0.5 mL/min and a nonisocratic gradient: 0 to 10 min 50% B, 10 to 50 min 45% B to 10% C. The column was equilibrated for 5 min with 10% B followed by 10 min with 50% B. Solvent A was deionized water, solvent B 200 mM NaOH, and solvent C was 100 mM NaAc in 100mM NaOH.

Quantification was assessed by external calibration using a mixture of all OS standards ranging from 0.0001 to 0.03 g/liter (coefficient of correlation > 0.999).

Serum Biomarker Analyses

A porcine-specific ELISA was used to quantify circulating insulin (Mercodia; Uppsala, Sweden) using manufacturers' instructions with an intra-assay and interassay CV percent of 3.47 and 3.23, respectively. GLP-2 concentrations were measured by ELISA (AssayPro, St. Charles, MO, USA) using manufacturers' instructions and an intra-assay and interassay CV percent of 5.03 and 9.1, respectively.

Statistical Analysis

Data were analyzed in JMP 12 and used LSMEANS Differences with Tukey-HSD adjustment. $P < 0.05$ was considered significant; nonsignificant factors were dropped and the model was run again. During the developmental period, feed intake was analyzed on a pen basis. For BiW, gilt pre-BW, gilt pre-BF, diet, and total number born were included in the model as fixed effects, sire and litter nested in sire were random effects. When analyzing WW, gilt pre-BF, gilt post-BF, number nursed, number weaned, average BiW of litter, and diet were included in the model as fixed effects, sire and litter nested within sire were random effects. When analyzing preweaning mortality and sow longevity, diet was used as a fixed effect. For the analysis of milk, CP, N, DM, GE, and insulin were analyzed separately as the response variables with diet as a fixed effect. Serum analysis for GLP-2 and insulin was analyzed separately as the response variable with diet as a fixed effect. The model for milk and biomarker analysis included diet, day, and diet x day as fixed effects. All means are presented as least-squares means (SEM). For the OS analysis, the normal distribution of the data was evaluated using the Kolmogorov–Smirnov test ($P < 0.05$), whereas homoscedasticity was checked using Levene's test. A two-way analysis of variance (ANOVA) was carried out to evaluate the effect of diet and/or time of lactation on OS abundances and concentrations. In all cases, the Tukey test was also used to assess differences between groups. R package "stats" (version 2.15.3) was used for all the analyses.

RESULTS

Gilt and Litter Performance

Growth performance data are presented in Table 3. Gilts fed the RESTR diet had decreased ($P < 0.05$) ME intake at all phases of development, decreased ($P < 0.003$) BW on day 109 of gestation, and decreased BF ($P < 0.041$; Pre-BF) upon entering the farrowing crate (day 109 of gestation) when compared with gilts on the CTL or CTL+ diets. However, RESTR gilts tended to have greater ($P < 0.053$; post-BF) BF at weaning and had greater

Table 3. Effects of feeding gilts control (CTL; $n = 34$), restricted (RESTR; $n = 27$), or control plus (CTL+; $n = 27$) diets on gilt and litter performance

	CTL	RESTR	CTL+	SEM	*P*-value
ME intake (Mcal/kg)*					
Phase 1 (120 to 162 d of age)	10.13[a]	6.97[b]	9.95[a]	0.045	0.004
Phase 2 (162 to 204 d of age)	11.25[a]	8.05[b]	10.94[a]	0.045	0.016
Phase 3 (204 to 230 d of age)	9.47[a]	7.95[b]	11.07[a]	0.045	< 0.0001
BW[†] of gilts at d 109 gestation, kg	232.06[a]	216.33[b]	226.70[a]	0.102	0.003
BW loss of gilts, kg	-38.89[a]	-38.30[a]	-42.89[a]	0.213	0.072
Pre-BF of gilts, mm[‡]	2.40[a]	1.83[b]	2.10[a]	0.040	0.041
Post-BF of gilts, mm[§]	1.54	1.59	1.54	0.256	0.053
Gilt ADFI[‖] during lactation, kg	8.12[a]	9.24[b]	8.00[a]	0.110	< 0.0001
Progeny BiW, kg[¶]	1.30	1.35	1.378	0.036	0.390
Progeny WW, kg**	5.62	5.62	5.70	0.109	0.840
Pre-weaning mortality[††]	3.48	3.48	2.67	0.588	0.296

Means in the same row not connected by the same letter differ ($P < 0.05$).

*Metabolizable energy (ME) intake during the gilt development period by phase beginning at 120 d of age.

[†]Body weight (BW).

[‡]Gilt backfat at day 109 of gestation (pre-BF).

[§]Gilt backfat at weaning (post-BF; 21 d post-farrowing).

[‖]Average daily feed intake (ADFI).

[¶]Progeny average birth weight (BiW).

**Progeny average weaning weight (WW).

[††]Piglet mortality per litter before weaning.

($P < 0.0001$) average daily feed intake during lactation compared with CTL and CTL+ gilts. Dietary treatment had no effect on progeny average BiW ($P = 0.39$; BiW), piglet mortality before weaning ($P = 0.296$), or progeny average weaning weight ($P = 0.84$; WW).

Milk Nutrient Composition

There were no effects of dietary treatment or time on the average GE of milk (data not shown). DM of milk was not affected by diet; however, milk DM decreased over time ($P = 0.003$; data not shown). Milk percent N (Figure 1A) and percent CP (Figure 1B) decreased over time ($P < 0.0001$). Lastly, when milk insulin was analyzed, there was a diet × day interaction ($P = 0.035$) where the milk from RESTR gilts had the highest insulin at day 0, but the lowest insulin concentration at day 14.

OS Profile

Across the 2 diets (RESTR and CTL), 61 different milk OS were identified. Of the OS identified, 58.73% were neutral, 15.87% were fucosylated, and 25.40% were acidic (Table 4). At day 0, CTL had more neutral OS and less acidic OS ($P < 0.05$) when compared with RESTR (Figure 2). Of the neutral OS quantified, RESTR had more LNnT than CTL ($P < 0.05$). Also, both RESTR and CTL
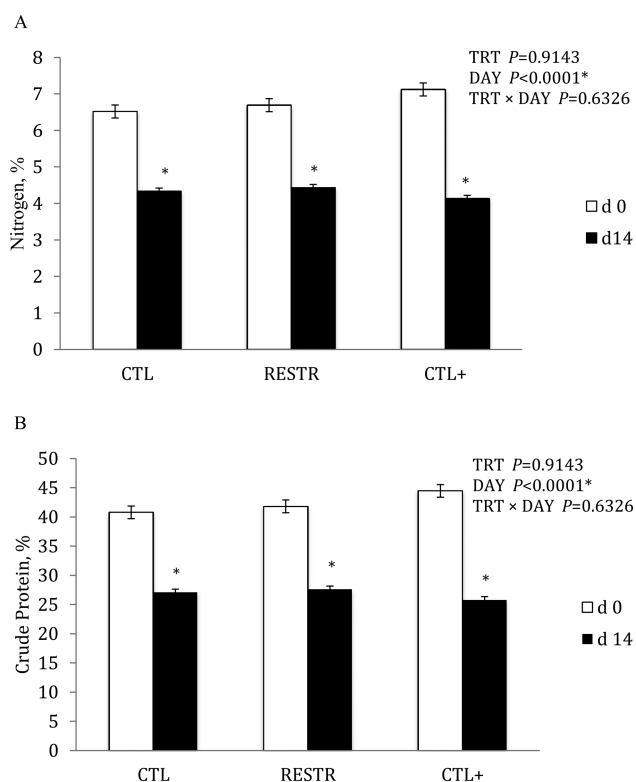
Figure 1. Effects of feeding gilts a control (CTL), restricted (RESTR), or control plus (CTL+) diet on milk composition. Each bar represents the LSM for % N (A) or % CP of 7 sows/diet on days 0 and 14. Bars of the same diet group with * differ based on day with * representing a difference of $P < 0.05$.

had an increase in fucosylated OS and decrease in acidic OS from days 0 to 14 ($P < 0.05$; Figure 3). Of the fucosylated OS quantified, samples from

Table 4. Qualitative profile of porcine milk oligosaccharides (OS) identified in all porcine milk samples organized by molecular mass (least to greatest)

No.	Compound[a]	Mass[b]	RT[c]	No.	Compound	Mass	RT	No.	Compound	Mass	RT
1	2_0_1_0_0	488.1723	14.84	21	3_1_0_0_0	707.2477	15.82	41	3_2_0_0_0	910.3267	22.66
2	3_0_0_0_0	504.1685	12.74	22	3_1_0_0_0	707.2492	17.86	42	4_0_0_1_0	957.3281	26.59
3	3_0_0_0_0	504.1683	13.38	23	3_1_0_0_0	707.2478	20.44	43	6_0_0_0_0	990.3279	16.59
4	3_0_0_0_0	504.1684	13.97	24	3_1_0_0_0	707.2477	22.63	44	3_1_0_1_0	998.3431	25.58
5	3_0_0_0_0	504.1685	15.02	25	3_1_0_0_0	707.2481	28.36	45	3_1_0_1_0	998.3446	29.05
6	3_0_0_0_0	504.1683	16.22	26	2_2_0_0_0	748.2741	17.54	46	4_1_1_0_0	1015.358	15.87
7	2_1_0_0_0	545.1948	13.44	27	2_2_0_0_0	748.2744	15.96	47	4_1_1_0_0	1015.359	26.8
8	2_1_0_0_0	545.1949	15.77	28	2_2_0_0_0	748.2747	19.45	48	4_1_1_0_0	1015.355	17.33
9	2_1_0_0_0	545.1948	19.14	29	3_0_0_1_0	795.2644	26.07	49	4_2_0_0_0	1072.381	22.64
10	1_2_0_0_0	586.2208	13.42	30	3_0_0_1_0	795.2648	27.28	50	4_2_0_0_0	1072.378	25.03
11	1_2_0_0_0	568.2211	15.08	31	3_0_0_1_0	795.2643	24.91	51	4_2_0_0_0	1072.379	30.62
12	2_0_0_1_0	633.2116	19.03	32	5_0_0_0_0	828.2748	15.4	52	3_3_0_0_0	1113.406	20.77
13	2_0_0_1_0	633.2116	23.92	33	2_1_2_0_0	837.3014	24.38	53	3_3_0_0_0	1113.404	23.44
14	4_0_0_0_0	666.2209	15.69	34	4_1_0_0_0	869.3016	20.43	54	4_1_0_1_0	1160.397	28.27
15	4_0_0_0_0	666.2229	20.55	35	4_1_0_0_0	869.3015	21.6	55	3_2_0_1_0	1201.42	27.13
16	4_0_0_0_0	666.2217	21.47	36	4_1_0_0_0	869.3007	28.28	56	4_2_1_0_0	1218.437	18.95
17	4_0_0_0_0	666.2215	13.55	37	2_2_1_0_0	894.3348	12.08	57	4_2_1_0_0	1218.436	20.56
18	1_1_0_1_0	674.2345	17.2	38	3_2_0_0_0	910.3268	18.43	58	4_3_0_0_0	1275.456	22.28
19	1_1_0_1_0	674.2374	18.96	39	3_2_0_0_0	910.3271	19.37	59	4_2_0_1_0	1363.481	27.09
20	1_1_0_1_0	647.2378	23.48	40	3_2_0_0_0	910.3271	20.19	60	4_2_0_1_0	1363.478	30.63
								61	4_3_0_1_0	1566.55	26.22

[a]The compound name, or composition of the OS, is shown as a set of 5 monomers. The following order is as stated with their abbreviations: hex = glucose or galactose; HexNAc = N-acetylhexososamine; Fuc = Fucose; Neu5Ac = N-acetylneuramic acid; and Neu5Gc = N-glycolylneuramic acid (e.g., 3_1_0_0_0 is equal to 3 Hex, 1 HexNAc).

[b]Mass is the molecular mass measured by the mass spectrometer.

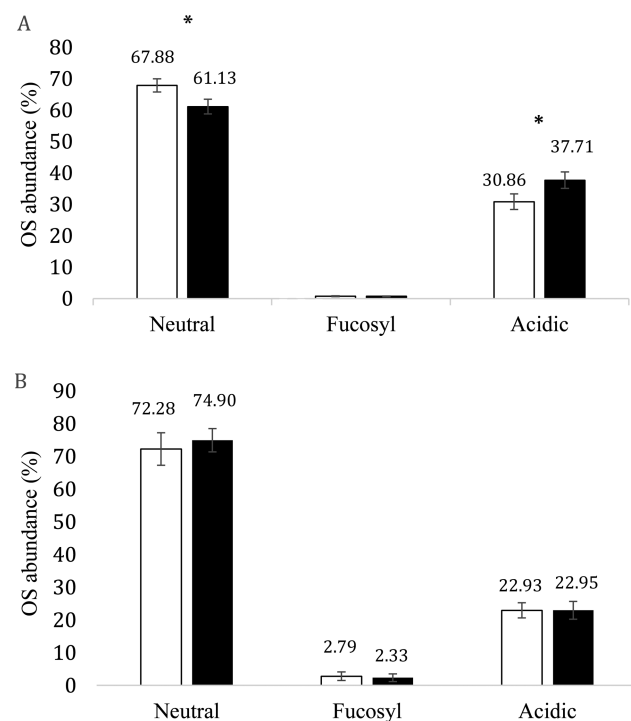[c]Retention time (RT) is the specific elution time from the LC analytical column for each compound.

Figure 2. Effects of feeding gilts a control (CTL; open bars) or restricted (RESTR; closed bars) diet on milk oligosaccharide (OS) profile. Each bar represents the LSM for OS abundance of 7 sows/diet on days 0 (panel A) and 14 (panel B). Bars of the same OS group with * differ based on abundance. * represents a significant difference of $P < 0.05$.

CTL had more LNDFH-I than RESTR ($P < 0.05$) at day 0. Lastly, only the RESTR showed an increase in neutral OS over time (Figure 3). Total OS quantification was greater in the RESTR when compared with CTL ($P < 0.05$; Figure 4). Total OS also decreased in both dietary treatments over time ($P < 0.0001$; Figure 4).

Growth Biomarkers

A diet × day interaction ($P = 0.0149$; Figure 5A) was observed in which progeny derived from RESTR gilts had the highest (0.044 mlU/liter) and CTL had the lowest (0.019 mIU/liter; $P = 0.032$) circulating concentrations of insulin on day 1, whereas no differences between dietary treatments were observed on day 15. For GLP-2, main effects of day ($P < 0.0001$) and diet ($P = 0.0008$), as well as a day × diet interaction ($P = 0.0087$; Figure 5B) were observed. Across all treatments, concentrations of GLP-2 decreased ($P = 0.0087$) with time. Across all time points, CTL+ had the greatest concentration and RESTR had the lowest concentration when compared with the other treatments. Control had concentrations of 3.97 on day 1 and 0.21 on day 15,
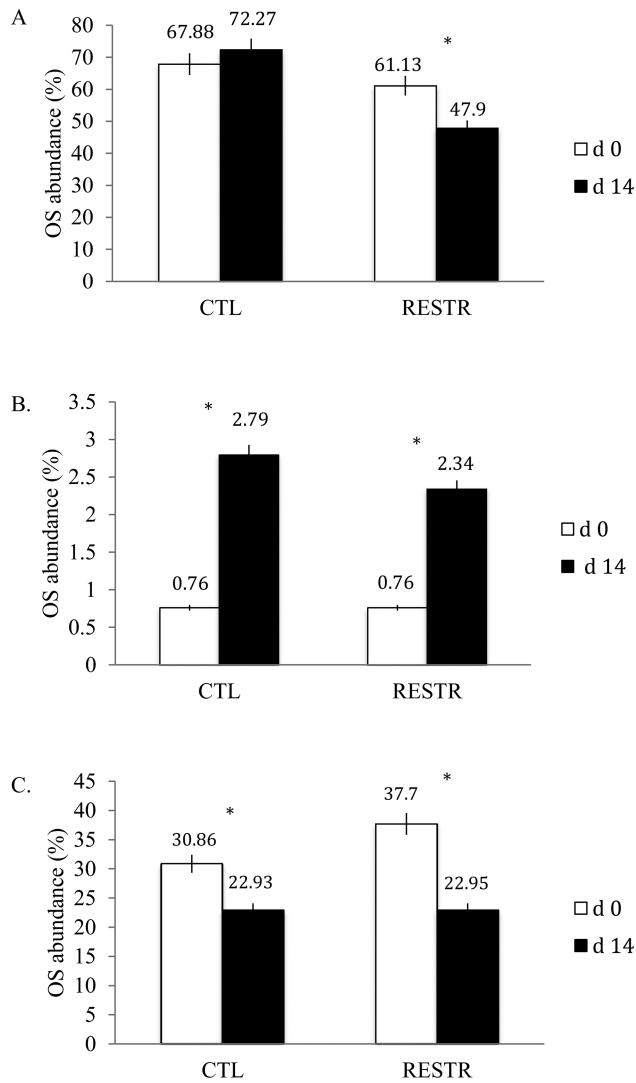
Figure 3. Effects of feeding gilts a control (CTL) or restricted (RESTR) diet on Neutral (panel A), Fucosylated (panel B), or Acidic (panel C) milk oligosaccharides (OS) based on time. Each bar represents the LSM for OS abundance of 7 sows/diet on days 0 and 14. Bars of the same diet group with * differ based on abundance. * represents a significant difference of $P < 0.05$.
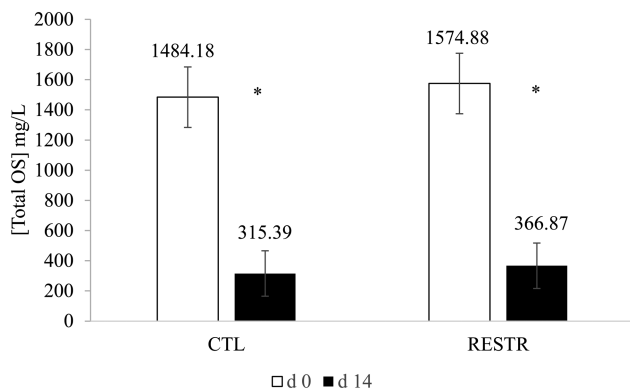

Figure 4. Effects of feeding gilts a control (CTL) or restricted (RESTR) diet on milk oligosaccharide (OS) abundance. Each bar represents the LSM for OS abundance of 7 sows/diet on days 0 (open bars) and 14 (closed bars). Bars of the same diet group with * differ based on abundance. * represents a significant difference of $P < 0.05$. Oligosaccharide abundance decreased significantly over time ($P < 0.0001$).
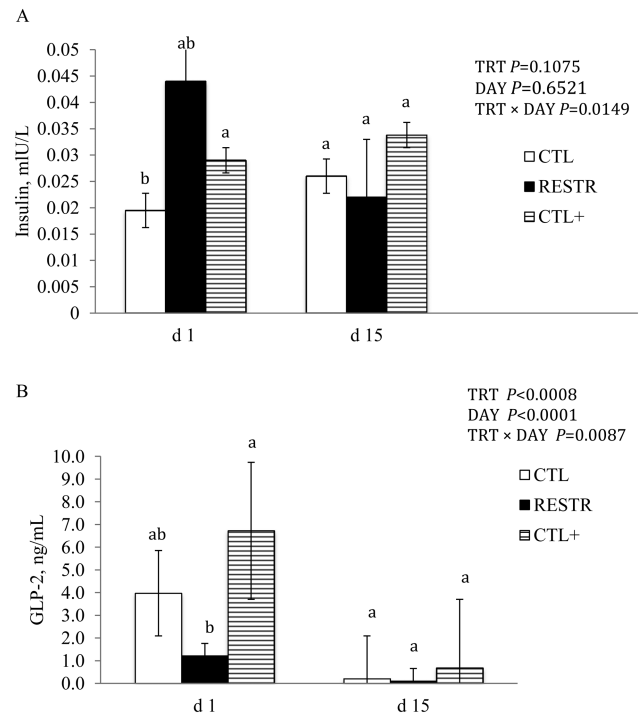

Figure 5. Effects of feeding gilts a control (CTL), restricted (RESTR), or control plus (CTL+) diet on circulating insulin (A) and GLP-2 (B), in piglets on days 1 and 15. Bars at same time point with different letters differ based on diet of the mother ($P < 0.05$).

RESTR had concentrations of 1.21 on day 1 and 0.107 on day 15, and lastly, CTL+ had concentrations of 6.73 on day 1 and 0.681 on day 15.

DISCUSSION

Today there is a greater need for research focusing on increased longevity of the sow, while maintaining or improving offspring health and growth performance. In a study conducted by Miller et al. (2011), it was observed that gilts on an energy-restricted diet have greater longevity and may result in offspring with a greater WW (Barnett et al., 2017). The idea of restricting energy during gilt development is based on the premise that restricting metabolizable energy intake should result in decreased fat deposition, but muscle accumulation may not be affected (Miller et al., 2011) Although results from the batch of gilts utilized in the current experiment do not show significant differences in piglet WW, there is a numerical difference, and with more statistical power, a greater weight difference may be seen. Furthermore, it was concluded that restricting energy during gilt development increased feed intake during lactation; thus, energy intake of restricted gilts is greater than the gilts fed a control diet. Also, restricted gilts lost less BF than control gilts. In a study by Amdi et al. (2013), it was observed that gestation feeding level affects the number of offspring born alive per

litter and offspring BiW, whereas sow body condition score affects WW and growth of offspring. A sow that loses excessive weight during lactation has higher cortisol levels. Cortisol is known to be a stress hormone that has the capability of crossing the placental barrier, and excess exposure to it can cause a fetus to have reduced BiW (Seckl, 2004; Kranendonk et al., 2006).

Our results indicate that developmental diet had no effect on milk nutrient (N, CP, DM, and GE) composition during lactation. Even though this was contrary to our hypothesis, this may not be surprising given that all gilts were fed a common diet after breeding and through gestation and lactation. To the best of our knowledge, no previous studies have analyzed milk samples based on diets fed during the developmental stage of gilts. However, many studies demonstrate that diet does affect milk composition when fed during late gestation and lactation. In a study conducted by Amdi et al. (2013), it was concluded that fatty acid and fat composition change based on dietary interventions during the gestation and lactation period. Although the gilts in this study were not restricted during gestation/lactation, they did weigh significantly less than control gilts during gestation and lactation due to previous diets. According to Amdi et al. (2013), restricted sows pull from fat reserves and use these components to add nutrients to the secreted milk. Sows are able to produce adequate quality and quantity of milk in spite of the nutrient deficit that may be associated with an inadequate diet. However, in order to compensate, they must mobilize more tissues to meet lactation requirements resulting in lower litter weight (O'Grady et al., 1973). Additionally, energy from the diet is the primary contributor of fat for milk synthesis (Amdi et al., 2013). In other previous research, diets targeting limiting amino acids showed an increase in mammary milk protein through increased amino acid absorption to the mammary gland, as well as having increased nitrogen retention and utilization in the milk protein during peak lactation periods (Huber et al., 2015). The current experiment was not in agreement with the study by Huber et al. (2015). The development diet with added crystalline amino acids (i.e., CTL+) showed no differences in milk nitrogen when compared with the other dietary treatments; however, a main cause of these varying results may be due to the time period difference of when the treatment diets were fed.

Insulin regulates energy metabolism and milk production. Furthermore, the energy source in feed will have an effect on insulin secretion. In this experiment, we observed a day by diet interaction for milk insulin concentration. Interestingly, milk from gilts developed on the restricted diet had the highest insulin concentration in early lactation (day 0), but the lowest concentration during mid to late lactation (day 14). Sows that have greater weight loss during lactation tend to have lower insulin levels (Spinka et al., 1999). Due to the fact that control gilts lost significantly more BF than restricted gilts, this could explain the higher day-0 insulin concentrations in milk from restricted gilts. Additionally, the more a neonate suckles, the lower the insulin levels become according to Spinka et al. (1999). Due to the fact that restricted gilts may wean heavier piglets, lower insulin levels could correlate with increased suckling frequency in restricted gilts.

OS are carbohydrates that are made up of simple sugars known as monosaccharides that are resistant to gastric digestion (Engfer et al., 2000). Furthermore, OS contribute to the health benefits of milk, by adding prebiotic and anti-infective factors to it (Bode, 2006). Due to great similarities between swine and human brain development and physiology, there is an increased interest in characterizing porcine milk with OS being one of the main compounds of interest.

Previous studies by Mudd et al. (2016) resulted in the characterization of 60 unique OS structures; however, in the current study, 61 types of OS were quantified, including isomers and anomers. Currently, there are well over 100 human milk OS quantified (Ninonuevo et al., 2008). OS composition observed in this study showed a slight difference based on gilt-developmental diet. Total OS abundance decreased over time, which has also been observed in human and other mammalian milks. Restricted gilts produced milk with significantly less neutral OS and significantly more acidic OS on day 0. Of the 3 subgroups of neutral OS identified (2 Hex-1 HexNAc, Lacto-N-neotetraose, and Lacto-neotetraose), colostrum (day-0 samples) obtained from restricted gilts had greater amounts of Lacto-N-neotetraose (LNnt). Lacto-N-neotetraose belongs to the "bifidum factor" group of OS that have been shown to have a prebiotic effect and stimulate growth of bifidobacterium (LoCascio et al., 2007). According to Tao et al. (2010), LNnt is one of the few OS that increases in mammals throughout lactation; however, our results showed the opposite effect in porcine milk. In general, total neutral OS had a slight increase in abundance through lactation, unlike acidic and fucosylated OS, in agreement with the results

reported by Mudd et al. (2016). Additionally, there were no overall significant differences in fucosylated OS, except for LNDFH-I (a type of Fucosylated OS) which was significantly lower in restricted gilts on day 0. Fucosylated OS normally remain constant or decrease over lactation; however, this was not seen in our results. Fucosylated OS can inhibit diarrhea caused by *Escherichia coli,* campylobacter, and caliciviruses; furthermore, higher levels of fucosylated OS correlate with improved *E. coli* protection (Newburg et al., 2004). Simple fucosylated OS are beginning to make their way into infant formula and are said to make formula more closely related to human breast milk and the microbial profile of infant fecal samples more similar to fecal samples obtained from infants that consumed breast milk (Steenhout et al., 2016). However, more complex fucosylated OS, larger than 2-fucosyllactose, are hypothesized to have more beneficial effects. Fucosylated OS are found at very low levels in porcine milk (Tao et al., 2008; Salcedo et al., 2016) as were they in this experiment. Tao et al. (2010) observed that fucosylated OS make up 1% to 4% of OS in porcine milk; however, in humans, concentrations of fucosylated OS can reach levels as high as 70%. In the current experiment, fucosylated OS ranged between 0.76% and 2.8%, thus, even lower than previous studies reported.

Lastly, there was a significant difference in overall acidic OS abundance among diets on day 0, but no particular acidic OS was different between gilts fed the different developmental diets. Acidic OS, also known as sialylated OS, contain sialic acid in its structure, which plays an important role in neural development and neural protection (Tao et al., 2008). Through competing for the adhesion sites on epithelial surfaces, sialylated OS are able to inhibit certain pathogens and possibly even help with postweaning diarrhea (Newburg et al., 2004).

Growth Biomarkers

Both insulin and GLP-2 concentration were measured in piglets for insight on growth biomarkers. Although both play a role in protein synthesis, insulin plays a key role in the development of fast-twitch muscles, and GLP-2 stimulates intestinal growth (Drucker 1998, Davis et al., 2001). In this experiment, a main effect of diet and a day by diet interaction was observed in blood samples obtained from progeny for both insulin and GLP-2. Progeny from restricted gilts had the lowest GLP-2 concentrations at both time points, but was only significantly different from CTL+ at day 1. Interestingly,

the restricted diet was the only treatment that did not have a great decrease in GLP-2 concentration from days 1 to 15. Increased GLP-2 can help reduce weaning diarrhea and stimulate intestinal adaptation to new diets (Thyman et al., 2014). GLP-2 is stimulated by enteral intake of nutrients and piglet total parenteral nutrition (**TPN**) fed will have significant decreases in circulating GLP-2 (Petersen et al., 2001).

GLP-2 has growth-related effects on a neonate through epithelial cell proliferation resulting in increased intestine mucosal mass, colon mass, villus height, and crypt depth. However, low levels of GLP-2 do not correlate with increased weight. Our results did agree with Petersen et al. (2001) where there was a decrease in plasma GLP-2 through the postnatal period. Furthermore, Petersen et al. (2001) reported no difference in BW based on a control group of neonate pigs compared with pigs infused with GLP-2 same as our results. However, Petersen et al. (2001) studied the piglet's intestines where there was a difference in small intestine and colon weight.

Insulin concentrations were significantly higher in progeny from restricted gilts when compared with control on day 1. The circulating insulin concentration coincided with milk insulin of restricted because, as previously stated, gilts fed restricted diets had numerically increased milk insulin concentration at day 0. The growth rate of a mammal is greatest at its neonate stage (Young, 1970) and the insulin receptor protein is 2-fold higher in a newborn piglet than that of a weanling (Suryawan et al., 2001). The results of the current experiment conflict with those of Suryawan et al. (2001). Although the insulin concentration decreased with age in restricted progeny, there was an increase in insulin concentration for both CTL and CTL+ treatments. The results of varying insulin concentration may relate back to nursing frequency and maternal stress. Insulin and the efficiency of it in its signaling pathways are essential determinants of efficient growth during development periods and will decrease with age as seen in energy-restricted piglets (Davis et al., 2001). Furthermore, insulin regulates stimulation of protein synthesis in peripheral tissues, as well as whole body amino acid disposal (Davis et al., 2000). Increased insulin may play a role in the increased BiW and WW of piglets from restricted gilts.

In conclusion, nutritional management of the developing gilt may affect milk nutrient composition, milk OS profile, and piglet serum biomarkers. It is important to note that dietary treatments were

only fed to gilts during the developmental period (days 120 to 230 of age); thereafter, all gilts were fed a common diet during gestation and lactation. Therefore, the developmental period may have long-lasting effects on gilt and litter performance and health. In addition, the results described herein warrant future research examining the interactive effects of nutrition, gut health, and gut microbiota.

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