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Research Article

A Pilot Study: Gestating Sow Nutrition and Environment Impacts Piglet Immune Responsiveness to Weaning Stress

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Abstract

The primary objective was to determine the effects of feeding gestating sows different dietary fibers and housing them in pens with individual feeding places on their progeny's immune and endocrine responses to weaning stress at days 7 and 14 after weaning. Before weaning, forty-two piglets were selected based on body weight. The two heaviest and two lightest piglets per litter were used. These piglets were born to sows fed fiber diets modified with either 30% wheat middlings and 15% soybean hulls (MID-SOY) or 30% distillers dried grains with soluble and 30% corn germ meal (DDGS-GM) and housed in pens (9 sows/pen) equipped with feeding places that were made from barriers of either 58.4 cm (short) or 203.2 cm (long) in length. Piglets were weaned at 19 ± 2 d-of-age. Blood samples were obtained at 24-h before and 7 and 14 days after weaning, equivalent to 20, 28, and 35 days-of-age. Cortisol and aspects of both innate and adaptive immunity were measured. Pigs' immune status was affected by the dam's gestation diet, especially T-cell and B-cell proliferation, total IgG and cortisol. Pigs born to MID-SOY-fed sows had a greater T-cell response and were suppressed less by weaning stress, whereas the pigs born to DDGS-GM-fed sows had greater B-cell response, but this measure was dramatically suppressed at 7 and 14 days post-wean. Total IgG and cortisol were also differentially affected by the sow gestation diet. These data reveal that it is plausible that sows' gestation diet can modulate pigs' immune status and immune responsiveness to weaning stress 7 and 14 days post-wean. The type of fiber-fed to the sows can differentially skew the immune balance toward either a cell-mediated or humoral response.

Keywords: *Cortisol; Prenatal Experience; Sows; Stress; Weaning*

Abbreviations

ConA: Concanavalin-A; DDGS-GM: Distillers Dried Grains with Solubles and Germ Meal; IgG: Immunoglobulin-G; LPS: Lipopolysaccharide; MID-SOY: Wheat Middlings and Soyhulls; NRC: National Research Council; WBC: White Blood Cells; D 0: 24 Hours before Weaning; D 7: 7 Days Post-Weaning; D 14: 14 Days Post-Weaning

Introduction

Many studies with rodents, non-human primates, and humans have shown that maternal stress can program their offspring's physiological systems later in life [1]. Stress experienced during pregnancy, generally referred to as prenatal stress, can affect the fetal development in utero and have long-lasting consequences on the activity of the hypothalamic-pituitary-adrenal axis and immune function in the

offspring of many species [1,2]. Pregnant sows are frequently exposed to various stressors, especially social stress and competition during feeding when grouped-housed, which can negatively impact her welfare and impact her progeny when exposed to high levels of stress hormones in utero. High levels of glucocorticoids result in an unpredictable prenatal environment for the fetus [3], altering the offspring's endocrine and immune reactions. It has been hypothesized that maternal stress can negatively impact the neonate's immune system, affecting their immunological responsiveness to unavoidable stressors often confronted with, thus compromising their well-being [4-6]. In pigs, prenatal stress has been shown to modify offspring phenotype in stress reactivity, impaired growth, and modified immune function [1,5]. Data are limited on the long-term consequences of prenatal stress regarding the group-housing environment of gestating sows on their progeny's immune responsiveness to weaning stress. Therefore, this study assessed the effect of gestating sows dietary treatment and pen environment on the baseline immune status and immune and endocrine responsiveness to weaning stress at D 0, D 7 and D 14 post-wean.

Materials and Methods

All animal procedures used in this study were approved by the University of Illinois Institutional Animal Care and Use Committee.

Piglets selection and blood samples

Piglets (n = 42) used in this study were selected based on pre-weaning body weight and balanced across sow gestational treatments; thus, data reported are from the two heaviest and two lightest piglets per litter. Piglets were born to sows fed two different gestation diets and kept in group pens equipped with two different length feeding stall barriers. Blood samples were collected via jugular venipuncture 24-h before weaning (19 ± 2 days-of-age) and then at D 7 and D 14 post-wean. Piglets were penned together as littermates. All litters were processed using standard operating procedures of the University of Illinois Swine Research Center.

Sow gestation treatments

Piglets dams' were randomly assigned in a 2 x 2 factorial design to 1 of 2 fiber-modified gestation diets of either: (a) 30% wheat middlings and 15% soybean hulls (MID-SOY) or (b) 30% dried distiller grains and 30% corn germ meal (DDGS-GM), and to 1 of 2 feeding barriers of either: (c) 58.4 cm (short; width = 48.3 cm) or (d) 203.2 cm (long; width 57.2 cm) in length. All sows were housed in group-pens at a floor space allowance of 1.7m^2 *(*n = 9 sows/pen/treatment). Diet treatments were fed to sows for two days (day 35 post-breeding) before moving them to treatment pens to facilitate acceptance of the high-fiber diets without competition. The MID-SOY and DDGS-GM ingredients (Table 1) replaced a portion of the shelled corn and soybean meal and were formulated to meet or exceed NRC requirements for gestating sows.

	30% wheat middlings and 15% soybean hulls (MID-S0Y)	30%distillers dried grains w/solubles and 30% corn germ meal (DDGS-GM)
Ingredients, % (as-fed basis)		
Corn	38.90	33.65
Soybean meal, 48%	12.50	2.50
Soybean hulls	15.00	-
Wheat middlings	30.00	-
DDGS	-	30.00
Corn germ meal	-	30.00
Soybean oil	1.00	1.00
Limestone	1.30	1.60
Dicalcium phosphate	0.60	0.55

Salt	0.40	0.40
Vitamin mineral premix	0.30	0.30
Energy and nutrients		
Energy, Kcal ME/kg	2,999	3,177
CP, %	13.78	18.96
Ca, %	0.78	0.78
Total P, %	0.61	0.66
Available P, %	0.34	0.34
ADF, %	9.81	7.93
Nitrogen, %	23.97	25.75
SID Arginine, %	0.90	0.83
SID Histidine, %	0.35	0.52
SID Isoleucine, %	0.59	0.49
SID Leucine, %	1.05	1.34
SID Lysine, %	0.61	0.61
SID Methionine, %	0.21	0.45
SID Methionine + cysteine, %	0.46	0.66
SID Phenylalanine, %	0.60	0.58
SID Threonine, %	0.43	0.51
SID Tryptophan, %	0.15	0.23
SID Valine, %	0.59	0.59

Table 1: *Composition of the experimental diets fed to group-housed sows during gestation.*
[1]Diets were formulated by replacing a portion of the corn and soybean meal with either wheat middlings and soybean hulls (MID-SOY) or distiller's dried grains with solubles and germ meal (DDGS-GM).

Sample collection and cell isolation and counts

Pigs were placed in a V-trough in a supine position. Blood samples were obtained by anterior vena cava puncture using sodium heparin or EDTA vacutainers within 1 minute of initial restraint. Whole blood smears were made, fixed in methanol, stained with Hema-3 staining system (Fisher Scientific, Houston, TX), and then viewed under a light microscope to determine leukocyte differential counts. Total white blood cell numbers were counted electronically using a Coulter Z1 particle counter (Beckman Coulter, Miami, FL).

Whole Blood was diluted in RPMI medium (Gibco, Carlsbad, CA), layered over Histopauqe-1077 (density: 1.077 g/ml; Sigma) and -1119 (density: 1.119 g/ml; Sigma) and centrifuged at 700 × g for 30 min. Porcine lymphocytes were aspirated from the 1077 layer, washed twice in RPMI, resuspended, and counted. Neutrophils were isolated from the 1119 layer and washed in RMPI. Red blood cells were lysed using cold endotoxin-free water, and isotonicity was restored using 10× phosphate buffer saline. Neutrophils were centrifuged at 475 × g for 10 min, the supernatant decanted and the pellet was washed and then resuspended in RPMI.

Mitogen-induced lymphocyte proliferation

A mitogen-induced lymphocyte proliferation assay was performed as previously described [7]. Isolated porcine lymphocytes were used at a concentration of 5 x 10^6 cells/mL and run in triplicate. The mitogen concanavalin A (ConA; Sigma Aldrich) was added at 0, 2 and 20 µg/mL, and lipopolysaccharide (LPS; Sigma Aldrich) at 0, 5, and 50 µg/mL to stimulate T and B cells, respectively. Plates were

incubated, the reaction stopped, and then read at a wavelength of 550 nm with reference wavelength 690 nm using a microplate reader (Thermo Scientific Instruments). Results are expressed as proliferation percentage: Optical density$_{(550/690nm)}$ of stimulated cells ÷ Optical density$_{(550/690nm)}$ of nonstimulated cells x 100.

Total plasma immunoglobulin-G

Total porcine plasma IgG was measured using an indirect competitive Enzyme-Linked Immunosorbent Assay (ELISA) previously described by our lab [8]. First, plasma samples were diluted 1:3,000 in 0.05% Tween-PBS and then 120 µL of diluted sample or standard in duplicate was added to 96-well microtiter plates coated with porcine IgG (Jackson ImmunoResearch, West Grove, PA). Goat anti-swine IgG (120 µL; Sigma, St. Louis, MO) was added, and plates incubated for 2h at 25°C and then washed 3 times with 0.05% Tween-PBS. Two hundred microliters of enzyme-linked rabbit anti-goat IgG (Jackson Immunoresearch) was added at 1:7,500 dilution. After a 1-h incubation period, plates were washed 3 times. Then 200 µL of substrate solution (1 mg of p-nitrophenyl phosphate/mL; Sigma-Aldrich) was added, and plates were incubated for 30-min. The reaction was stopped by adding 100 µL of 2M NaOH, and plates were read at wavelength 405 nm using a microplate reader (BioTek Instruments, Winooski, VT). Total plasma IgG was estimated using a standard curve (0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50 µg of IgG/mL).

Total plasma cortisol

A validated radioimmunoassay kit (MP Biomedicals, Santa Ana, CA) was used to measure plasma cortisol concentrations. A standard curve of 0, 78, 156.25, 312.5, 625, 1250, and 2500 ng/mL was generated by adding 390 µL of pooled stripped pig plasma and 10 µL of cortisol standard (10 µg/mL of cortisol in phosphate-buffer-saline). In duplicates, 25 µL of pig plasma or standard and 1 mL of I^{125} cortisol were added to antibody-coated tubes and placed in a 37°C water bath for 45 minutes. The liquid was aspirated from the tubes and using a gamma counter, samples were counted. Inter- and intra-assay CVs were 7.4% and 5.2%, respectively, and sensitivity was 3 pg.

Statistical analysis

All data were analyzed using a linear mixed-effects model (Proc-Mixed, SAS version 9.4, SAS Institute, Cary, NC), with repeated measures utilizing a first-order autoaggressive structure. All traits were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. The model included fixed effects of sow diet (MID-SOY or DDGS-GM) and feeding barrier lengths (long or short), and sampling days (24 h before and 7 and 14 days post-wean) of piglets relevant to weaning as repeated measures. Least square means were generated and separated statistically with pairwise *t*-tests (PDIFF option). Significance was set at $P \leq 0.05$, whereas trends were discussed at $P \leq 0.10$.

Results and Discussion

The present study was designed to assess the prenatal impact of pregnant sow nutrition and pen environment on her offspring's immune status and stress responsiveness D 0 (24-h before) and D 7 and D 14 post-wean. These preliminary findings indicate that dietary fiber type sows consumed (MID-SOY vs. DDGS-GM) during gestation differentially affected baseline immune status and their piglets' immune responsiveness to weaning stress. The barrier length (long vs. short) used to create individual feeding places for group-housed gestating sows affected piglets' baseline cortisol ($P = 0.01$) and total IgG at D 7 post-wean ($P < 0.0001$). Pigs from sows kept in the pens with long barriers had higher cortisol at D 0 and higher IgG at D 7 post-wean (Table 2), and these pigs also had a higher mean neutrophil-to-lymphocyte ratio ($P = 0.01$; data not shown) than those from sows kept in pens with short barriers. Here we did not assess the acute effects of weaning stress. However, pigs born to sows that gestated in group-pens with long barriers had higher neutrophil-to-lymphocyte ratio, neutrophils, and cortisol, which are often elevated in response to weaning stress [9]. It is plausible that this greater stress response among these pigs from these sows reflects their dams' stress responsiveness during gestation to this pen environment. Sows that gestated in pens with long barriers also had higher neutrophils and the neutrophil-to-lymphocyte ratio at various days throughout gestation

compared to those sows in pens with short ones [10,11]. It is plausible that the sows' pen environment influences their piglets' stress responsiveness to wean stress, but diet influences immune status and immune responsiveness.

Measure	Long	Short	SEMp	P-value
Total WBC no./10 mL (10^7)			0.7	0.67
24h pre-wean	8.0	7.2		
D 7 post-wean	7.6	7.2		
D 21 post-wean	11.5	12.1		
Lymphocytes, %			2.1	0.20
24h pre-wean	65.1	67.1		
D 7 post-wean	65.7	59.7		
D 21 post-wean	60.2	59.5		
Neutrophils, %			1.4	0.11
24h pre-wean	31.2	28.6		
D 7 post-wean	29.7	35.8		
D 21 post-wean	34.9	34.5		
Monocytes, %			0.7	0.84
24h pre-wean	2.8	3.7		
D 7 post-wean	3.5	3.5		
D 21 post-wean	4.0	4.6		
Eosinophils, %			0.3	0.30
24h pre-wean	0.87	0.58		
D 7 post-wean	1.03	0.91		
D 21 post-wean	0.82	1.3		
ConA Proliferation, %			3.1	0.22
24 h pre-wean	115	112		
D 7 post-wean	115	105		
D 14 post-wean	106	107		
LPS-Proliferation, %			3.0	0.77
24 h pre-wean	133	139		
D 7 post-wean	99	100		
D 14 post-wean	102	101		
Total IgG, ug/ml			0.08	< 0.0001
24 h pre-wean	7.2	7.1		
D 7 post-wean	8.0^a	7.2^b		
D 14 post-wean	8.0	8.1		
Cortisol, ng/ml			0.6	0.01
24-h pre-wean	16.0^a	11.8^b		
D7 post-wean	11.3	10.5		
D 14 post-wean	10.3	10.3		

Table 2: *Immune measures at D 0 (24-h before) and D 7 and D 14 post-wean pigs from sows kept in group-pens with long or short feeding stall barriers during gestation.*

$^{a, b}$: Within a row, means without a common superscript letter differ (P < 0.05).

Interestingly, pigs' baseline immune status and immune responsiveness to weaning stress may have been differentially modulated by their dam's gestation diet. Pigs born to sows that were fed MID-SOY diet had a skewed T-cell (ConA; P = 0.05) proliferative response and pigs from DDGS-GM-fed sows had B-cell (LPS; P < 0.0001) proliferative responses at D 0 (Table 3). The pigs that had greater ConA response also had a higher number of total WBC (P = 0.06) and percent monocytes (P = 0.02), but less cortisol (P < 0.001) compared to those pigs whose dams were fed DDGS-GM diet (Table 3). The magnitude of suppression of T- and B-cell responses to weaning at D 7 and D14 was differentially affected by sow gestation diet; with a more profound suppressive effect occurring at D 7 on B-cell proliferation (LPS) among pigs from sows fed DDGS-GM diet and was still suppressed at D 14 post-wean compared to pigs from sows fed MID-SOY diet. The reduced B-cell proliferative response among these pigs did not directly affect IgG levels at D 7 since IgG levels increased among these pigs and their levels were higher than pigs from MID-SOY-fed sows (Table 3). Also, pigs from DDGS-GM-fed sows had greater cortisol D 0 and D 14 than those pigs from sows fed MID-SOY diet (P < 0.001; Table 3). Overall, pigs born to MID-SOY-fed sows had a more robust T-cell proliferative response (117 vs. 99 ± 2%; P < 0.0001), while those from DDGS-GM-fed sows had a more robust B-cell proliferative response (120 vs. 107 ± 2.2%; P < 0.005). Mean cortisol levels were also greater among pigs born to DDGS-GM-fed sows than those from MID-SOY-fed sows (14.5 vs. 10.0 ± 0.4 ng/ml; P < 0.0001). The lower cortisol levels and higher mitogen-induced T-cell response (ConA) may imply that pigs born to MID-SOYfed sows during gestation may have a more activated immune cell-mediated immune profile. In contrast, pigs from sows fed DDGS-GM diet were more skewed toward a T-helper 2 immune response (humoral), which may have been partly due to higher plasma cortisol. It has been shown that elevated glucocorticoids can suppress T-helper 1 immune response (cell-mediated), resulting in a shift toward humoral immunity in an attempt to achieve immune homeostasis [12]. Together, these results imply that maternal diet-fed and pen environment housed in may affect immune development and responsiveness of their offspring to weaning stress, as evident by differential immune phenotype and responsiveness.

Measure	MID-SOY	DDGS-GM	SEMp	P-value Diet x Day
Total WBC no./10 mL (10^7)			0.7	= 0.06
24h pre-wean	8.1	7.0		
D 7 post-wean	7.5	7.3		
D 21 post-wean	10.6	12.9		
Lymphocytes, %			2.2	=0.10
24h pre-wean	64.0	68.2		
D 7 post-wean	65.0	60.5		
D 21 post-wean	61.6	58.1		
Neutrophils, %			1.4	0.24
24h pre-wean	30.7	29.1		
D 7 post-wean	31.1	34.3		
D 21 post-wean	32.0	37.5		
Monocytes, %			0.7	0.02
24h pre-wean	4.7^a	1.8^b		
D 7 post-wean	2.9	4.0		
D 21 post-wean	5.2^a	3.2^b		
Eosinophils, %			0.2	0.62
24h pre-wean	0.6	0.9		
D 7 post-wean	0.8	1.1		
D 21 post-wean	1.1	1.0		

ConA Proliferation, %			3.0	= 0.05
24 h pre-wean	124[a]	100[b]		
D 7 post-wean	118[a]	102[b]		
D 14 post-wean	111[a]	100[b]		
LPS-Proliferation, %			2.8	< 0.0001
24 h pre-wean	110[a]	162[b]		
D 7 post-wean	101	98		
D 14 post-wean	113[a]	99[b]		
Total IgG, ug/ml			0.05	< 0.0001
24 h pre-wean	7.2	7.0		
D 7 post-wean	7.1[a]	8.1[b]		
D 14 post-wean	7.9	8.0		
Cortisol, ng/ml			0.4	< 0.001
24-h pre-wean	10.1[a]	18.6[b]		
D7 post-wean	11.1	10.8		
D 14 post-wean	8.9[a]	12.8[b]		

Table 3: *Immune measures at D 0 (24-h before) and D 7 and D 14 post-wean for born to sows fed either MID-SOY or DDGS-GM modified gestation diets.*

a, b: Within a row, means without a common superscript letter differ (P < 0.01).

Conclusion

These preliminary findings indicate that piglets' immune status may be affected by the type of dietary fiber their dams' are fed during gestation. In contrast, the stress sows experience during gestation can also impact her piglets' immune response to weaning for 14 days after weaning. These data also indicate that the type of fiber-fed to the w sows can differentially skew piglets' immune response. Pigs born to sows fed MID-SOY diet tended to be skewed toward a T-helper 1 cell-mediated immune response and pigs from sows fed DDGS-GM diet favored a T-helper 2 humoral immune response. It should be noted that those piglets born to sows on either diet but housed in group-pens with short length barriers exhibited a diminished immune response when collectively compared amongst other treatment combinations. Thus, indicating the sow's gestation environment may also influence her pigs' stress and immune responsiveness; however, more research should investigate the effects of other stressors and long-term consequences.

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Conflict of Interest

The authors have no conflict of interests.

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