

FINAL REPORT

North Carolina Pork Council Research Proposal, 2009-2010

EFFECTS OF STRESS ON IMMUNE CELL POPULATIONS IN PIGS

PI: Glen W. Almond, Col: Andrew R. Kick, College of Veterinary Medicine

OBJECTIVES

- a) Develop reliable methods to assess immune cell populations in pigs,
- b) Evaluate the effects of acute and chronic stress on immune cell populations and adaptive immunity in nursery pigs.

INTRODUCTION

Presumably, stress has negative effects on pig productivity. Various studies endeavored to assess the effects of stress on pigs from gestation through finishing. Stressors included social interaction (crowding/mixing), temperature variation, restraint, transportation, food deprivation, and light/dark cycles. In general, previous studies used rather rudimentary methods to assess immunity. Examples include neutrophil to lymphocyte ratios, B-lymphocyte development and immunoglobulin levels, and lymphocyte proliferation. These studies reported a negative effect of stress on short-term immunity; however, they failed to identify the precise cell type(s) affected. The immune response is more complex than described in these early studies. Immune cells possess “clusters of differentiation” or CD molecules. The reactions of monoclonal antibodies with different cell types, which have different CD molecules, are measured by the technique of flow cytometry. One of the cell types of greatest interest is the “T” lymphocyte or T cell. Therefore, the proposed studies evaluated effect of stress on short- and long-term adaptive immunity, and identify the specific cell type(s) affected.

(The primary objectives were completed within the proposed budget. To use the small quantity of remaining funds, we conducted preliminary studies to determine immune cell types in sow colostrum and milk)

EXPERIMENTAL PROCEDURES

Animals, Facilities and Blood Collection:

Phase 1: Summer Stress

Twenty-four piglets were selected from nine crossbred sows ($\geq 4^{\text{th}}$ parity) at the NC State University Swine Educational Unit (SEU). Experiments were conducted from June through August. In the nursery, pigs were randomly assigned to two treatment groups: stress (n=12) and control (n=12). Barrows (n=12) and gilts (n=12) were equally allocated between treatments. Piglets were assigned to pen based upon weight and treatment. There were two pens (6 pigs / pen) for stress treatment and two pens (6 pigs / pen) for control. All pens were in the same nursery room. At approximately seven weeks of age, pigs in the stress group were mixed in one pen with a

reduction in space allowance per pig from 0.46 m² to 0.174 m². Additionally, the stress group had access to one nipple drinker and one feeder.

For the weaning phase of the study, all pigs were weaned at 21±7 days of age. Blood samples were collected when pigs were 14, 20, 22, 23, 24 and 28 days of age. For the stress phase, which began when pigs were 50 days of age, pigs were bled before mixing, at the conclusion of stress treatment on day 55, and then on days 56, 57, 58 and 65. All blood sampling occurred between 6:30 – 8:00 am, and bleeding normally lasted less than one minute per pig. Pigs were weighed on the following days: 14, 22, 50, 55, and 58.

Phase II: Effects of Weaning Age

Twenty-four piglets were selected from four crossbred sows (≥2nd parity) at the Swine Educational Unit (SEU). Experiments were conducted from October through November. Farrowing dates for all piglets were within one day (day 0). On day 6, pigs were selected by sex in order to ensure equal number of barrow and gilts per treatment and were randomly assigned to treatment group with six pigs selected per sow with two pigs per treatment. Pigs were assigned to three treatment groups consisting of being weaned on day 14, 21 or 28 of age. Pigs from the same litter not in the study were also weaned on the same day but were not placed in the same nursery room. Upon weaning, pigs were placed in adjacent pens in the same nursery room. Blood samples were collected from all pigs on days 13, 14, 15, 20, 21, 22, 27, 28, 29, and 35. Complete blood counts and flow cytometry were conducted on all samples. Lymphocyte stimulation procedures were conducted on samples collected on days 15, 22, 29 and 35. Pigs were weighed on days 6, 14, 21, 28, and 35 (prior to blood sample collection).

Differential Blood Leukocyte Concentrations:

All blood samples were submitted to Antech Diagnostics (Antech Diagnostics, Lake Success, NY) for Complete Blood Counts (CBCs). Differential blood leukocyte concentrations were obtained for porcine blood and reported as absolute numbers of neutrophils and lymphocytes.

Flow Cytometry:

One of our primary objectives was to establish reliable flow cytometric procedures to quantify porcine immune cells, namely, T cell and B cell composition of the peripheral blood. Using one, two or three color flow cytometry, the following cells were quantified in each blood sample: $\gamma\delta$ T cells, CD8 α^+ T cells, CD4⁺ T cells, CD4⁺CD8⁺ T cells, CD3⁺ T cells, and mature B cells (CD21⁺). Samples were analyzed by FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) and analyzed using CELLQuest Pro software with 20,000 events and lymphocyte gating based upon forward and side scattering.

Cortisol Assays:

Whole blood was centrifuged for 10 min at 400 x g. Plasma was removed, frozen and stored at -20° C. Plasma samples were tested using a Coat-A-Count cortisol kits in accordance with the manufacturer's protocol (Siemens, Los Angeles, CA). Tubes were counted on a Cobra II Auto Gamma counter (Packard Instrument Company, Meriden, CT).

Phase III: Preliminary Studies of Immune Cells in Sow Colostrum and Milk.

Approximately 15ml of colostrum was collected from 5 sows (3 first parity and 2 second parity). These samples were collected at time 0, 24 or 48 hours post-partum in 50ml conical tubes. Each sample was taken after the third piglet had been born at time 0 or other time points and placed immediately in a cooler with ice packs for transport to the lab. At the lab, the colostrum was diluted to twice its original volume with non-sterile phosphate buffered saline (PBS), pH 7.4 at 4^oC. The samples were then centrifuged at 4^oC and 1500 rpm for 10 minutes. After centrifugation, the fat/lipid layer was removed with cotton-tipped applicator sticks. The remaining supernatant was removed and the pellet reconstituted with 10mls of non-sterile PBS and again centrifuged at 1500 rpm for 10 minutes. Again the fat and supernatant were removed.

The pellet was reconstituted with 2 ml of Gibco (Carlsbad, CA) Advanced Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% Cell Generation Fetal Bovine Serum (FBS). The pH was checked and adjusted to a pH of 7.4 if needed. Cell viability was then determined using the Hansen Method A. After the cells were found viable, 100µl of the cell suspension was pipetted into BD Falcon polystyrene round-bottomed tubes containing 100µl antibody. The samples were prepared for flow cytometry as previously described.

RESULTS & DISCUSSION

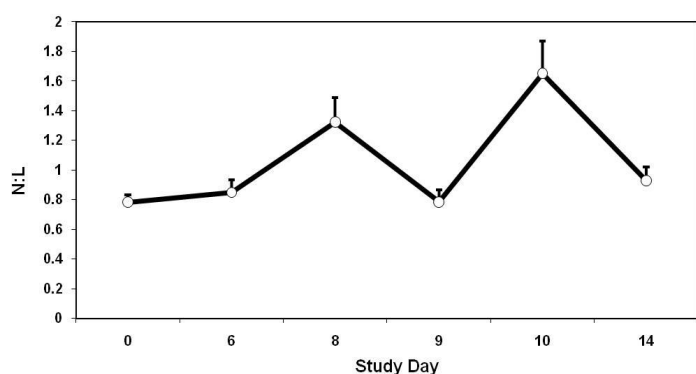
Phase I – Summer Stress:

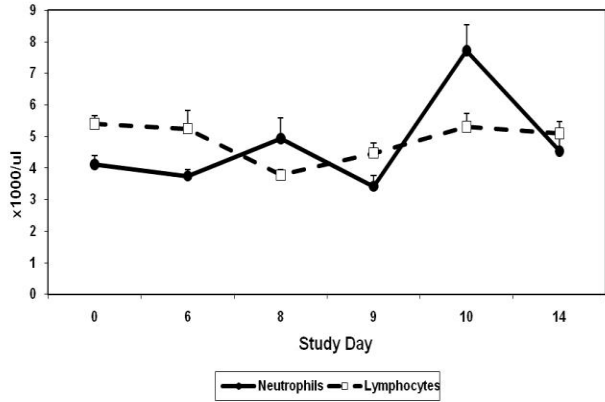
This study was conducted in two parts: namely, an assessment of changes in immune cell populations at weaning and then following a 5-day stress period in the nursery phase. For the sake of brevity, figures are limited to cell types, which illustrate changes associated with weaning and subsequent stress in the nursery.

As shown in Figure 1, there was a modest increase in the number of neutrophils on the day after weaning. Conversely, total lymphocytes decreased after weaning. This phenomenon is commonly referred to as “lymphocytic trapping”. The lymphocytes are trapped in lymph nodes, spleen and liver and thus, a transient decrease in cell number is evident in the peripheral blood. The precise function of lymphocytic trapping is not clear; however, it may play a critical role in response to vaccinations and the immune response. This was the basis of our Phase II study, in which we examined the influence of weaning age on immune cell populations. Many of our current vaccines are approved for use at the time of weaning. There are clear implications for the interactions between vaccination and lymphocytic trapping.

Numerous previous studies used the neutrophil to lymphocyte ratio (N:L; Figure 1, right graph) to assess the influence of stress on immune function. Of the 19 studies, only 11 noted an effect of stress on the N:L. This raises some doubt as to the usefulness of this test to monitor the effects of stress. In the present study (Figure 1), the ratio increased after weaning and this increase in N:L appears to be typical for stressful events. Our interpretation is that this simply reflects the changes in neutrophil counts and the lymphocyte trapping.

Figure 1. Changes in lymphocyte and neutrophil concentrations (left) in the peripheral blood and the neutrophil:lymphocyte ratio (right figure). Pigs were 14 days of age at study day 0. Pigs were weaned at 21-days of age (Study Day 7).





To further examine the influence of weaning on immune cells, we assessed several types of immune cells. As shown in Figure 2, the number of both CD4+ and CD8+ lymphocytes (CTL's) decreased on the day after weaning and then slowly increased to pre-weaning values. Similar changes in CD4+CD8+, CD21+ and gamma-delta T cells were noted (data not shown).

Figure 2. Changes in CD4+ cells (number and % of lymphocytes; left) in the peripheral blood and the CD8+ cells (cytotoxic T cells; right figure). Pigs were 14 days of age at study day 0. Pigs were weaned at 21-days of age (Study Day 7).

As described in the materials and methods, we used a five-day stress period to examine the influence of crowding and limited access to water on immune cell populations. It was evident that minimal differences were observed between the stress group and the control group (Figure 3). For example, the CD4+ and CTL cells were similar between groups prior to the stress period, and then decreased after the stress. The lack of differences in immune cell numbers also was noted for other immune cell types. The ambient temperature in the nursery room typically exceeded 90 degrees F for all 5 days of the stress period. Evidently, heat stress affected both groups of pigs and the influence of crowding did not provide an additive effect on the stress pigs.

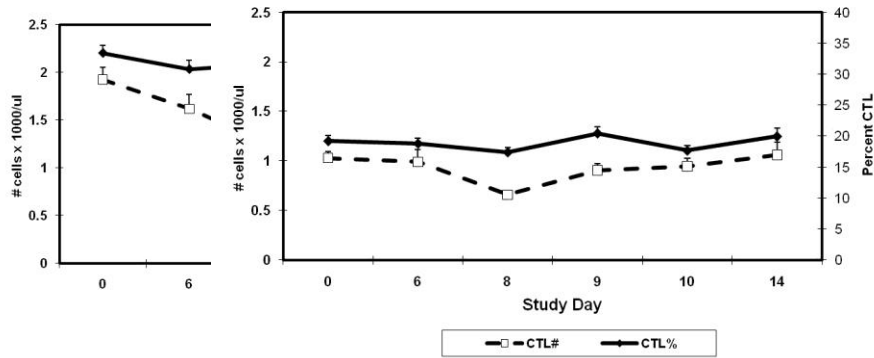
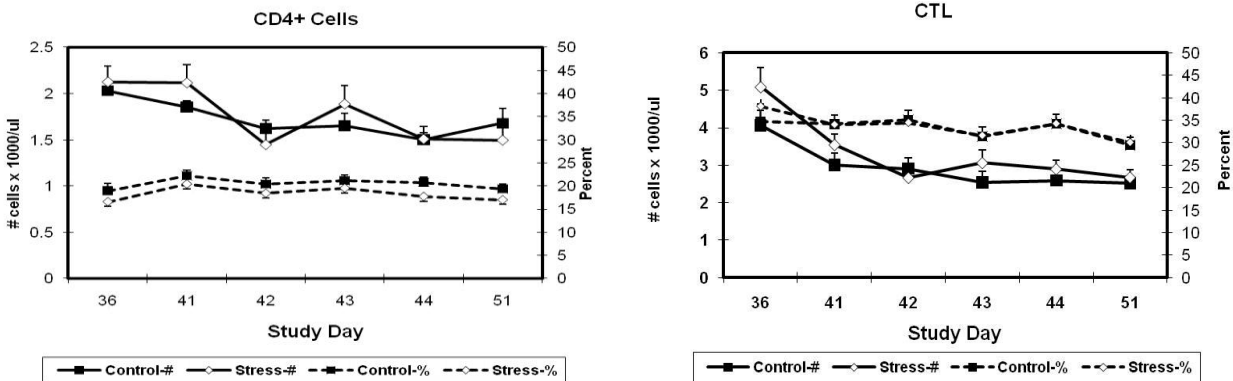


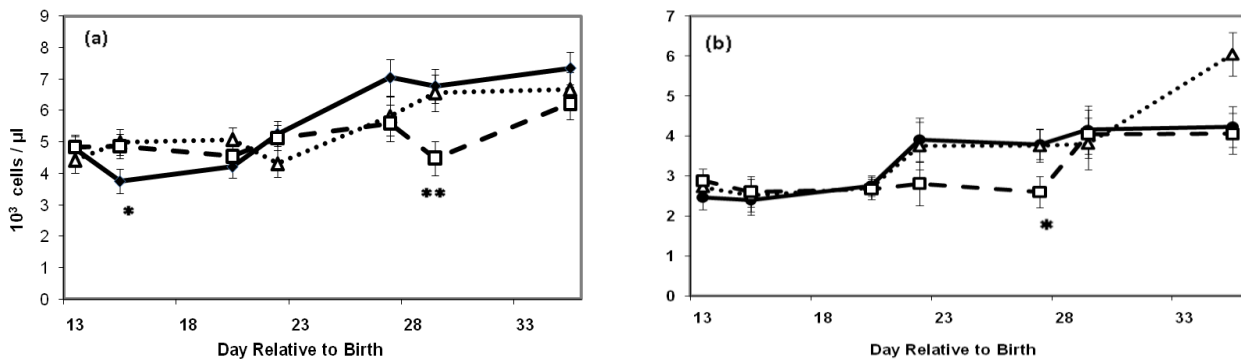
Figure 3. Changes in CD4+ cells (number and % of lymphocytes; left) in the peripheral blood and the CD8+ cells (cytotoxic T cells; right figure). Pigs were 14 days of age at study day 0. Blood samples were collected prior to (Day 36) a 5-day stress period in the stress group. Five additional blood samples were collected from Day 41 to Day 51. Pigs were weaned at 21-days of age (Study Day 7).



Phase II - Effects of Weaning Age

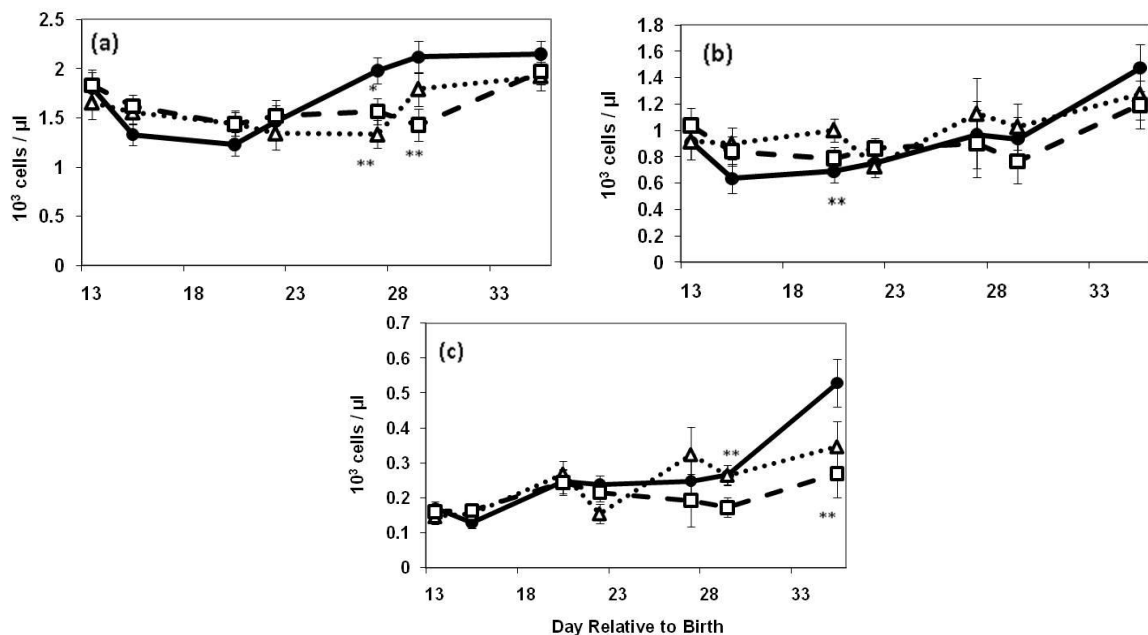
The main effects of neutrophil and lymphocyte concentrations did not differ ($P > 0.1$) among treatments (Table 1; Figure 4). Minor differences in neutrophil and lymphocyte concentrations were evident during the study.

Figure 4. Lymphocyte (a) and neutrophil (b) peripheral blood concentrations for different weaning ages: 14D (n=8 pigs; ●), 21D (n=7 pigs; △) and 28D (n=8 pigs; □). Day relative to birth. Blood samples were collected from all pigs on each day. Values are least squares means \pm SEM. Within day, differences among treatments designated: *, $P < 0.1$; **, $P < 0.05$



The main effects for the number of $CD4^+$ T cells and CTLs did not differ ($P > 0.1$) among treatments, while there was a treatment effect ($P < 0.05$) for the number of $CD4^+CD8^+$ T cells (Figure 5). The only differences in the number of $CD4^+$ T cells were apparent on d 27 and d 29. Similarly, few differences were evident in the number of CTL, with the notable exception of d 20. On d 29, the number of $CD4^+CD8^+$ T cells tended to be higher ($P = 0.07$) in the 14D pigs and the 21D pigs ($P = 0.08$) than in the 28D pigs. On d 35, the percentage ($P = 0.05$) and number ($P = 0.0351$) of $CD4^+CD8^+$ T cells was higher for the 14D pigs compared to the 28D pigs (Table 1).

Figure 5. Number (Least square means \pm SEM) of $CD4^+$ T cells (a), CTLs (b) and $CD4^+CD8^+$ T cells (c) in the peripheral blood for different weaning ages: 14D (n=8 pigs; ●), 21D (n=7 pigs; △) and 28D (n=8 pigs; □). Day relative to birth. Within day, differences among treatments designated: *, $P < 0.1$; **, $P < 0.05$.



The percentage of CD21⁺ cells of the PBL differed ($P = 0.02$) among treatments (Figure 6). On d 27, the number of CD21⁺ cells was higher ($P < 0.01$) for the 14D pigs compared to the 21D pigs and the 28D pigs. On day 29, the number of CD21⁺ cells was lower for the 28D pigs compared to the 14D pigs ($P < 0.01$) and the number tended to be lower than the 21D pigs ($P = 0.08$).

There were no by day differences ($\alpha = .05$) for the following immunological measures: percentages of CD4⁺ T cells, CTLs and CD4⁺CD8⁺ T cells of the PBL; percentage and concentration of $\gamma\delta^+$ -T cells in the peripheral blood; N:L ratio; and the CD4:CD8 (actually CD4:CTL) ratio (Table 1).

Cortisol

There was a treatment x time interaction for cortisol concentration ($P < 0.001$). Thus, the main effect of time was not considered. On d 15, d 22 and d 29, the weaned group had higher ($P < 0.01$) cortisol concentrations than the other groups (Figure 7).

Figure 6. The percentages (a) of CD21⁺ cells of the PBL and numbers (b) of CD21⁺ cells in the peripheral blood for different weaning ages: 14D (n=8 pigs; ●), 21D (n=7 pigs; △) and 28D (n=8 pigs; □). Blood samples were collected from all pigs on each day. Values are least squares means \pm SEM. Within day, differences among treatments designated: *, $P < 0.1$; ***, $P < 0.01$.

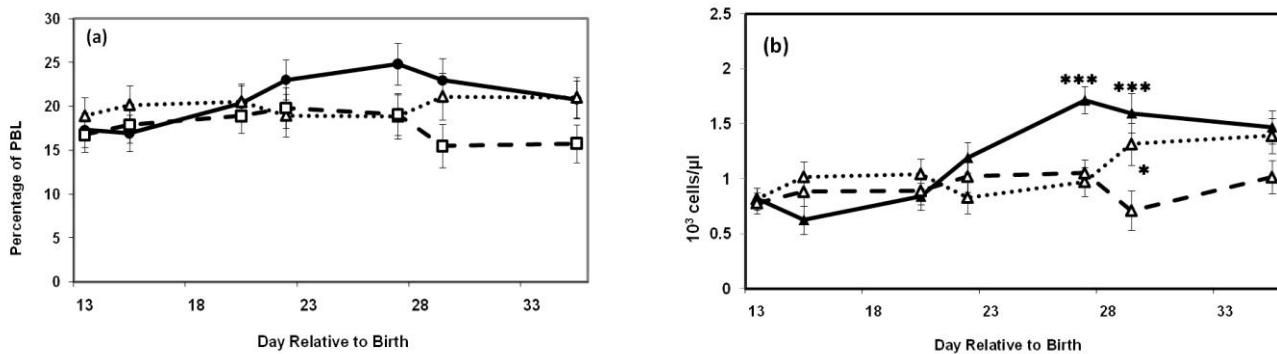
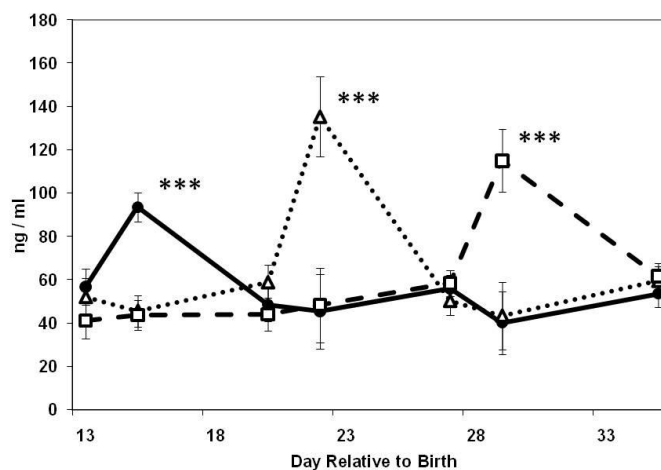


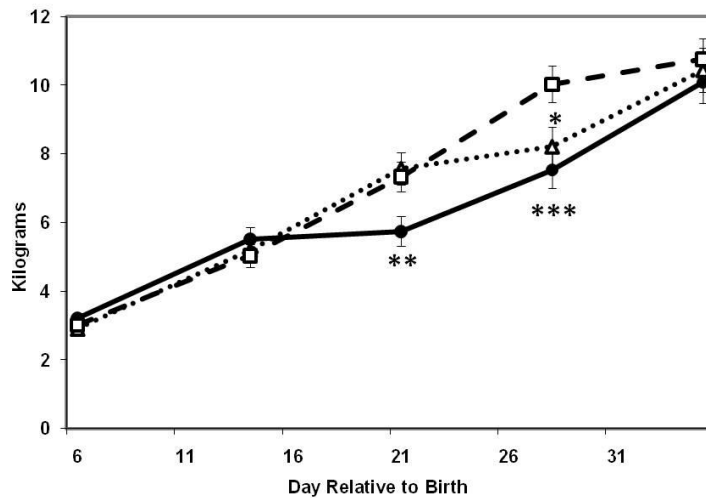
Figure 7. Plasma cortisol concentrations in pigs weaned at 14 d (n=8 pigs; ●), 21 d (n=7 pigs; △) or 28 d (n=8 pigs; □) of age. Blood samples were collected from all pigs each day. Values are least squares means \pm SEM. Within day, differences among treatments designated: ***, $P < 0.01$.



Body Weight

There was a treatment x time interaction for BW ($P = 0.015$). For all three treatments following weaning, the percentage change in BW over the next seven days was lower than for those pigs not weaned (14D, $P < 0.0001$; 21D, $P < 0.017$; 28D, $P < 0.0001$). Before weaning on d 6 and d14, and on d 35, there was no difference in BW among treatments. On d 21 ($P = 0.0159$) and d 28 ($P = .0103$), BW differed among treatments (Figure 8).

Figure 8. BW of pigs weaned at 14 d (n=8 pigs; ●), 21 d (n=7 pigs; △) or 28 d (n=8 pigs; □) of age. Values are least squares means \pm SEM. By day, differences among treatments designated: *, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.01$.



Phase III: Preliminary Studies of Immune Cells in Sow Colostrum and Milk.

This phase of the study was more challenging than expected. Sow colostrum (and milk) is laden with lipids and fats, which required special attention in the preparation of samples for flow cytometry. The conventional method of using histopaque actually killed the cells. Despite these problems, colostrum and milk were evaluated for 5 sows. There were approximately 2.6×10^3 immune cells/ μL ; however, epithelial cells also were present (2-3x more than immune cells). The results of the flow cytometry are shown in the Table 2.

OVERALL DISCUSSION AND CONCLUSIONS

Percentages and numbers of T cells subsets and B cells in the peripheral blood at different ages were consistent with previous studies (Solano-Aguilar et al., 2001; Borghetti et al., 2006). Cortisol concentrations also were comparable to previously reported values for pigs of this age and following stress (Niekamp et al., 2007; Kojima et al., 2008; Sutherland et al., 2009; Tuchscherer et al., 2009).

The comparison of cortisol concentrations of the pigs one day after weaning to other days demonstrated that weaning was a stressful event, regardless of age at weaning. Serum cortisol concentrations were expected to be higher 24 hours after weaning (Kojima et al., 2008). Blood

samples were not collected daily following weaning; however, by 6 days after weaning serum cortisol concentrations returned to normal levels for all treatments affirming the limited duration of weaning stress at each weaning age.

Body weight changes also demonstrated that weaning was a stressful event with significant decreases in the BW by seven days for the pigs weaned at d 14 and d 21. Notably, by d 35, there were no differences in BW among treatments; however, it is unknown what the long term effects of weaning age would have had on BW since the present study was completed on day 35. In a previous study, pigs weaned at 21 days of age gained weight faster than those weaned at 14 days of age (Davis et al., 2006). At 10 weeks of age, BW of pigs weaned at 28 days of age was higher than pigs weaned at 14 days of age. In contrast, body weight of pigs weaned at 21 days of age did not differ from weights of pigs weaned at 14 or 28 days (Niekamp et al., 2007).

A common method to determine stress-induced changes in T lymphocytes was to measure proliferation following mitogenic stimulation. Early weaning and combinations of stresses, such as heat, cold and social rank decreased lymphocyte proliferation or had no effect (Blecha et al., 1983; Davis et al., 2006; Morrow-Tesch et al., 1994; Niekamp et al., 2007; Sutherland et al., 2006 & 2007; Rudine et al., 2007; Tuchscherer et al., 2009). Also, isolation of weaning age pigs resulted in lower *in-vitro* T cell cytokine production and a lower CD4:CD8 ratio as measured with flow cytometry (Tuchscherer et al., 2009). In addition, four hours of transportation decreased the percentage of B cells in the PBL; however, no effect was observed for CD4⁺ or CD8⁺ T cells (McGlone et al., 1993). Based on these inconsistent observations, the influence of stress on immunity of young pigs was poorly defined in previous studies.

In one study, N:L ratios differed among weaning ages; however, age related differences in leukocyte concentrations were not accounted for (Niekamp et al., 2007). In general, few differences in N:L ratios were evident in the present study. Perhaps the N:L ratio is not a suitable indicator of stress-induced changes in immune cell populations.

As previously mentioned, lymphocytic trapping, albeit not reported for pigs, is an observable effect of stress on immunity in mammals. This response presumably is due to increased corticosteroid levels in the peripheral blood (Lundin and Hedman, 1978). Acute stress and lymphocytic trapping results in a greater percentage of lymphocytes in sites of potential pathogen invasion. In the present study, weaning increased plasma cortisol concentrations in the three treatment groups and thus, the appropriate endocrine events were in place for lymphocytic trapping. Although an animal responds to acute stress with lymphocytic trapping, repeated stress will degrade the animal's immunological response (McEwen, 1998). In the present study, lymphocytic trapping was most pronounced in pigs weaned at 28 days of age.

Significant differences were observed among treatments for the number of T cell subsets (CD4⁺ T cells, CTLs and CD4⁺CD8⁺ T cells) and the percentage of CD21⁺ cells in the peripheral blood; however, these differences were not consistently associated with weaning. The percentages of and numbers of $\gamma\delta^+$ -T cells on days 15, 22, 29 and 35 were lower than the respective total on days 13, 20, 27 because the blood on those days (15, 22, 29 and 35) was refrigerated for 24 hours before flow cytometry was conducted. The observable trend that the $\gamma\delta^+$ -T cells begin to die in refrigeration was not known until after all flow cytometry was completed.

In this study, physiological and immunological changes were observed with weaning for each treatment; however, those differences were eliminated by day 35, one week after all pigs were weaned. There was not a definitive immunological or physiological benefit among weaning ages, which conflicts with previous studies. It is apparent that available laboratory tests remain ill-

equipped to definitively decide on whether weaning age affects the immunological competence of pigs.

The preliminary study of immune cells in colostrum and milk revealed that the quantity and concentration of many immune cells are similar to values in whole blood. The notable exceptions are the CD⁺25 cells (T regulatory cells) and $\gamma\delta^+$ T cells, which were in far greater concentrations in milk and colostrum than in blood. The precise roles of the latter cells are poorly understood for milk. Overall, it was evident that sufficient immune cells are present to provide some protection to the mammary glands; however, their fate in piglets is unknown.

In summary, weaning at 14, 21 or 28 days of age was a stressful event and immunological changes were observed, particularly lymphocytic trapping, at 14 and 28 days of age. Differences in the percentage of T cell subsets and B cells were minimal. By day 35, pigs from 14D, 21D and 28D appeared to be equally physiologically and immunologically competent. **Based upon this data, it can be concluded that weaning at 14, 21 or 28 days of age does not compromise the welfare of pigs.**

Table 1. Immune measures in the peripheral blood for different weaning ages of pigs.

Variable	D13			D15			D20			D22		
	14D	21D	28D	14D	21D	28D	14D	21D	28D	14D	21D	28D
CD4 ⁺ T cells, %	37.8 ± 1.8	37.3 ± 1.9	38.4 ± 1.8	35.6 ± 1.6	31.7 ± 1.7	33.9 ± 1.6	29.8 ± 1.5	28.6 ± 1.6	31.3 ± 1.5	27.8 ± 2.0	31.2 ± 2.2	29.5 ± 2.0
CTL, %	19.0 ± 1.7	20.7 ± 1.9	21.1 ± 1.7	16.8 ± 1.5	18.0 ± 1.6	17.2 ± 1.5	16.0 ± 1.2	19.8 ± 1.3	17.7 ± 1.2	14.2 ± 1.2	17.5 ± 1.3	16.9 ± 1.2
CD4 ⁺ CD8 ⁺ T cells, %	3.6 ± 0.36	3.3 ± 0.38	3.3 ± 0.36	3.4 ± 0.22	3.1 ± 0.24	3.4 ± 0.22	5.7 ± 0.74	5.2 ± 0.79	5.6 ± 0.74	4.5 ± 0.4	3.8 ± 0.43	4.2 ± 0.4
γδ ⁺ T cells, %	16.5 ± 1.8	17.5 ± 1.9	18.4 ± 1.8	12.9 ± 1.6	13.6 ± 1.8	14.1 ± 1.6	20.9 ± 1.8	18.2 ± 2.0	19.7 ± 1.8	16.7 ± 1.7	13.7 ± 1.8	15.8 ± 1.7
γδ ⁺ T cells, No. 10 ⁶ /ml	0.78 ± 0.11	0.76 ± 0.12	0.94 ± 0.11	0.49 ± 0.09	0.66 ± 0.10	0.71 ± 0.09	0.89 ± 0.12	0.92 ± 0.13	0.9 ± 0.12	0.90 ± 0.12	0.61 ± 0.13	0.83 ± 0.12
N:L Ratio	0.54 ± 0.11	0.61 ± 0.12	0.7 ± 0.11	0.65 ± 0.11	0.52 ± 0.11	0.59 ± 0.11	0.66 ± 0.04 ^a	0.53 ± 0.04 ^b	0.59 ± 0.04 ^{ab}	0.75 ± 0.1	0.86 ± 0.11	0.55 ± .10
CD4:CD8 Ratio	2.1 ± 0.22	1.9 ± 0.23	1.9 ± 0.22	2.2 ± 0.23	1.8 ± 0.25	2.2 ± 0.23	1.9 ± 0.17	1.5 ± 0.19	1.8 ± 0.17	2.0 ± 0.2	1.9 ± 0.21	1.8 ± 0.2

Variable	D27			D29			D35			Overall Effect	
	14D	21D	28D	14D	21D	28D	14D	21D	28D	Treatment	Treatment x Day
CD4 ⁺ T cells, %	27.7 ± 1.8	24.6 ± 1.9	28.4 ± 1.8	31.3 ± 1.9	28.1 ± 2.0	32.2 ± 1.9	29.8 ± 1.8	28.9 ± 1.9	32.5 ± 1.8	0.0673	0.8412
CTL, %	13.4 ± 1.9	16.5 ± 2.0	16.2 ± 1.9	13.9 ± 1.5	14.9 ± 1.6	16.4 ± 1.5	19.6 ± 1.9	19.4 ± 2.1	19.2 ± 1.9	0.0501	0.9870
CD4 ⁺ CD8 ⁺ T cells, %	3.4 ± 0.57	4.7 ± 0.61	3.4 ± 0.57	4.0 ± 0.46	4.1 ± 0.49	3.9 ± 0.46	6.9 ± 0.70 ^a	5.3 ± 0.74 ^{ab}	4.4 ± 0.70 ^b	0.2356	0.2825
γδ ⁺ T cells, %	22.7 ± 2.5	23.2 ± 2.7	24.3 ± 2.5	17.1 ± 1.8	18.4 ± 2.0	15.8 ± 1.8	16.1 ± 2.1	21.8 ± 2.3	20.9 ± 2.1	0.6834	0.7852
γδ ⁺ T cells, No. 10 ⁶ /ml	1.6 ± 0.17	1.2 ± 0.18	1.4 ± 0.17	1.1 ± 0.15 ^{ab}	1.2 ± 0.16 ^a	0.72 ± 0.15 ^b	1.2 ± 0.2	1.5 ± 0.22	1.3 ± 0.2	0.9731	0.1999
N:L Ratio	0.54 ± 0.08	0.71 ± 0.08	0.48 ± 0.08	0.63 ± 0.16	0.62 ± 0.17	1.0 ± 0.16	0.59 ± 0.09 ^a	0.91 ± 0.09 ^b	0.7 ± 0.09 ^{ab}	0.6060	0.0480
CD4:CD8 Ratio	2.2 ± 0.19	1.7 ± 0.2	1.8 ± 0.19	2.3 ± 0.22	2.1 ± 0.24	2.1 ± 0.22	1.6 ± 0.17	1.6 ± 0.18	1.8 ± 0.17	0.0921	0.9627

^{ab} Within day and within row, least squares means without a common superscript differ ($P < 0.1$).

¹ The farrowing day was considered d 0. Blood samples were collected from all pigs on days 13, 15, 20, 22, 27, 29 and 35.

² Pigs were weaned at 14 (14D; n=8 pigs), 21 (21D; n=7 pigs) or 28 (28D; n=8 pigs) days of age.

Table 2. Immune cell percentages in sow milk and colostrum (n=5 sows).

Cell type	CD3 ⁺ T cells	CD4 ⁺ T cells	CTL	CD21 ⁺	CD25 ⁺	CD4 ⁺ CD8 ⁺	γδ ⁺ T cells
Mean	21.576	16.898	6.4	10.544	26.893	3.838	38.846
Std	10.189	8.2656	2.1891	4.540047	21.084	1.04339	33.5782

References

- Blecha, F., D. S. Pollmann, and D. A. Nichols. 1983. Weaning pigs at an early age decreases cellular immunity. *J. Anim. Sci.* 56: 396-400.
- Borghetti, P., E. De Angelis, R. Saleri, V. Cavalli, A. Cacchioli, A. Corradi, E. Mocchegiani, and P. Martelli. 2006. Peripheral T lymphocyte changes in neonatal piglets: Relationship with growth hormone (GH), prolactin (PRL) and cortisol changes. *Vet. Immunol. Immunopathol.* 110: 17-25.
- Davis, M. E., S. C. Sears, J. K. Apple, C. V. Maxwell, and Z. B. Johnson. 2006. Effect of weaning age and commingling after the nursery phase of pigs in a wean-to-finish facility on growth, and humoral and behavioral indicators of well-being. *J. Anim. Sci.* 84: 743-756.
- Kojima, C. J., H. G. Kattesh, M. P. Roberts, and T. Sun. 2008. Physiological and immunological responses to weaning and transport in the young pig: Modulation by administration of porcine somatotropin. *J. Anim. Sci.* 86: 2913-2919.
- Lundin, P., and L. Hedman. 1978. Influence of corticosteroids on lymphocyte recirculation. *Lymphology.* 11: 216.
- McEwen, B. S. 1998. Stress, adaptation, and disease. allostasis and allostatic load. *Ann. N. Y. Acad. Sci.* 840: 33-44.
- McGlone, J. J., J. L. Salak, E. A. Lumpkin, R. I. Nicholson, M. Gibson, and R. L. Norman. 1993. Shipping stress and social status effects on pig performance, plasma cortisol, natural killer cell activity, and leukocyte numbers. *J. Anim. Sci.* 71: 888-896.
- Morrow-Tesch, J. L., J. J. McGlone, and J. L. Salak-Johnson. 1994. Heat and social stress effects on pig immune measures. *J. Anim. Sci.* 72: 2599-2609.
- National Research Council . Subcommittee on Swine Nutrition. 1998. Nutrient requirements of swine. 10th ed. Washington, D.C., National Academy Press.
- Niekamp, S. R., M. A. Sutherland, G. E. Dahl, and J. L. Salak-Johnson. 2007. Immune responses of piglets to weaning stress: Impacts of photoperiod. *J. Anim. Sci.* 85: 93-100.
- Rudine, A. C., M. A. Sutherland, L. Hulbert, J. L. Morrow, and J. J. McGlone. 2007. Diverse production system and social status effects on pig immunity and behavior. *Livestock Science.* 111: 86-95.
- Solano-Aguilar, G. I., K. G. Vengroski, E. Beshah, L. W. Douglass, and J. K. Lunney. 2001. Characterization of lymphocyte subsets from mucosal tissues in neonatal swine. *Dev. Comp. Immunol.* 25: 245-263.

- Sutherland, M. A., P. J. Bryer, B. L. Davis, and J. J. McGlone. 2009. Space requirements of weaned pigs during a sixty-minute transport in summer. *J. Anim. Sci.* 87: 363-370.
- Sutherland, M. A., S. R. Niekamp, R. W. Johnson, W. G. Van Alstine, and J. L. Salak-Johnson. 2007. Heat and social rank impact behavior and physiology of PRRS-virus-infected pigs. *Physiol. Behav.* 90: 73-81.
- Sutherland, M. A., S. R. Niekamp, S. L. Rodriguez-Zas, and J. L. Salak-Johnson. 2006. Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds. *J. Anim. Sci.* 84: 588-596.
- Tuchscherer, M., E. Kanitz, B. Puppe, A. Tuchscherer, and T. Viergutz. 2009. Changes in endocrine and immune responses of neonatal pigs exposed to a psychosocial stressor. *Res. Vet. Sci.* 87:380-388.