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Immune status of PIC Camborough-15 sows, 25% Meishan sows, and their offspring kept indoors and outdoors¹

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ABSTRACT: Newer genetic lines of pigs are being used in indoor and outdoor production systems. The objectives of Exp. 1 were to describe the effects of the maternal sow line genotype, environment (indoor vs outdoor), and the genotype × environment interactions on blood hemoglobin (Hb), immunoglobulin G (IgG) concentrations, white blood cell (WBC) numbers, lymphocyte transformation/blastogenesis (LTA), natural killer (NK) cell activity, neutrophil chemotaxis, cortisol concentrations, and leukocyte differentials. Studies were performed using two genotypes: PIC Experimental-94 (Exp-94, an experimental line containing 25% Meishan) and PIC Camborough-15 (C-15). The Exp-94 sows had lower LTA at 0.2 µg/mL mitogen than the C-15 sows, whereas Exp-94 sows had higher NK cytotoxicity than the C-15 sows. When indoors, the two genotypes showed

similar neutrophil chemotaxis. When outdoors, the C-15 genotype had higher ($P < .01$) neutrophil chemotaxis than the Exp-94 sows. The other immune measures were statistically similar for the two genotypes for each environment and for the genotype × environment interaction of sows. Experiment 2 sought to determine the effects of genotype on the immune system of nursery-age offspring of the experimental lines. Each sow line was bred to a common PIC 405 boar line. The Exp-94 × 405 pigs had elevated WBC numbers than C-15 × 405 pigs. The social status of the Exp-94 × 405 or the C-15 × 405 pigs showed no effect on any of the immune measures studied. The other immune measures were statistically similar for the two lines of pigs. The Exp-94 line had marginally increased NK activity but reduced lymphocyte blastogenesis and neutrophil chemotaxis compared with the C-15 line.

Key Words: Environment, Genotype, Immunity, Pigs

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Introduction

Very little is known about the immune system of common and newer genetic lines of pigs. Genetic differences in different breeds of pigs have been reported in the immune responses to antigens or vaccines for sheep red blood cells, *E. coli*, and others (Meeker et al., 1987). King (1971) suggested that differences in performance between different breeds of pigs may be altered in the presence or absence of a particular disease. There is little research about the health or immune status of pigs raised in indoor or outdoor environments. Barnett et al. (1988) concluded that physiological differences (corticosteroid concentrations) were

seen in two genotypes of pigs, but genotypes showed similar responses to tether stalls compared to group housing.

The objectives of this study were to determine the effects of maternal sow line genotype and their offspring, environment (indoor vs outdoor), and the genotype × environment interactions on blood hemoglobin (Hb) and various immune measures. The initial hypothesis was that the immune systems of indoor and outdoor sows and their offspring would be similar. Second, the diverse genotypes examined had not had their immune systems evaluated and thus, if genotypes were to differ in immune measures, examination of the interactions may indicate whether one genotype may be better suited to a given environment.

Materials and Methods

Experiment 1: Immune Status of Exp-94 and C-15 Sows in Outdoor and Indoor Environments

This study was conducted at the Texas Tech University swine research unit. The unit, which comprised indoor and outdoor production systems, had been pop-

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ulated in a single step that produced contemporary gilts and sows in each environment. For this study, gilts were obtained from a common source farm and were kept in isolation, as a group, for 30 to 60 d before being randomly assigned to the indoor or outdoor systems. Thus, the animals were presumed to have been exposed to similar pathogens prior to initiation of treatments. During the entire study gilts and sows were fed identical diets composed of sorghum-soybean meal and vitamins and minerals. The outdoor sows were fed in pellet form (to avoid loss of nutrients in the wind) and the indoor sows were fed in meal form. The breeding herd management and care and the experimental protocol were consistent with the *Guide for the Care and Use of Agricultural Animals in Agricultural Animal Research and Teaching* (Consortium, 1988).

The indoor unit was mechanically ventilated and contained pens equipped with modified gestation crates that served as feeding stalls. The crates measured .6 × 2.1 m but the back of each crate was open to allow each group of 10 sows to interact socially. The open area behind each crate was the same size as the crate (.6 × 2.1 m). The flooring was solid concrete under the gestation crate and concrete slats behind the crates. Outdoor sows experienced a social group of 6 to 12 sows per pen. The pen was surrounded by electric fence. Each social group had an average of 6 sows per acre, or 15 sows per ha.

To evaluate the effect of environment and genotype on immune function, a total of 16 second-parity PIC Camborough-15 (**C-15**) and 16 25% Meishan (**Exp-94**) sows that had been housed either indoors (8 from each genotype) or outdoors (8 from each genotype) were evaluated. Genetic lines were produced by PIC (Franklyn, KY). All sows were in mid to late gestation when they were bled (d 60 to 90 of gestation). Approximately 20 mL of blood was taken by venipuncture over sodium heparin. Both indoor and outdoor sows were sampled simultaneously to control variation. Sow blood samples were collected in the cool morning hours of the summer.

Assays performed on blood samples were natural killer (**NK**) cytotoxicity, neutrophil chemotaxis, leukocyte differentials, white blood cell (**WBC**) numbers, Hb concentrations, immunoglobulin G (**IgG**) concentrations, lymphocyte transformation/blastogenesis (**LTA**), and cortisol levels. Assays were selected to attempt to sample from several components of the immune system.

The NK assay was performed as previously described for porcine NK cell activity (Lumpkin and McGlone, 1992; McGlone et al., 1993). Briefly, nonadherent splenic lymphocytes were isolated and used at effector:target (**E:T**) ratios of 100, 50, 25, and 12.5:1. K562 cells (American Type Culture Collection, Rockville, MD) were used as the target cell. Target cells were labeled with inorganic ⁵¹Cr (Na₂⁵¹CrO₄). A constant 10⁴ target cells were used in each culture well.

Maximum ⁵¹Cr release was determined by adding 7.5% Triton-X detergent (Sigma Chemical Co., St. Louis, MO) to lyse all targets. Spontaneous ⁵¹Cr release was determined by adding culture media to target cells and counting radioactive label in the supernatant. Effector and target cells were incubated in a 5% CO₂ humidified chamber for 18 h. Supernates were collected by pipette and were counted for 1 min on a gamma counter. Percentage cytotoxicity was calculated using the following formula:

$$\% \text{ NK cytotoxicity} =$$

$$\frac{\text{experimental release cpm} - \text{spontaneous release cpm}}{\text{maximum release cpm} - \text{spontaneous release cpm}}$$

The neutrophil chemotaxis was performed as previously described (Salak et al., 1993). Eight milliliters of heparinized whole pig blood was mixed with 5 mL of RPMI. Ten milliliters of the whole blood-RPMI mixture was layered onto 3 mL of Histopaque 1077 and 3 mL of Histopaque 1119 (Sigma Chemical Co.) and centrifuged at 700 × g for 30 min at 25°C. The opaque interface containing the mononuclear cells was aspirated and discarded. The polymorphonuclear cells (**PMN**) interface including some red blood cells (**RBC**) was aspirated and transferred to a clean, sterile conical centrifuge tube and washed one time in RPMI (Sigma). Red blood cells were lysed using distilled water and .10% phosphate buffered saline (PBS). Supernates were discarded and the PMN pellet was washed one time in RPMI and centrifuged at 600 × g for 10 min. The PMN preparations were more than 90% neutrophils, as verified by differential counts. The PMN were counted using an electronic Coulter counter (Coulter Electronics, Hialeah, FL) and cell concentration was adjusted in PBS to 5 × 10⁴ cells per 50 μL.

A modified Boyden chamber (Neuro Probe, Cabin John, MD) was used to measure the migration of neutrophils toward RPMI (control; chemokinesis) or toward 10⁻⁸ M of recombinant human complement C5a (rhC5a, Sigma; chemotaxis). The RPMI (30 μL) or rhC5a was pipetted into the wells of the bottom chamber. The chamber was placed in an incubator for thermal equilibration of the bottom chamber. In the wells of the top chamber, 50 μL of PMN was incubated. Samples were assayed in duplicate. The chamber was incubated for 1 h at 37°C in a humidified chamber (5% CO₂). The polycarbonate filter (pore size 5 μm; Neuroprobe) was fixed and stained using methanol and Hemo 3 (Biochemical Sciences, Bridgeport, NJ). The cells that migrated to the underside of the filter were counted in a blind fashion. Five fields per well were counted at 1,000× with a light microscope and duplicates were averaged.

Blood smears were made using whole blood. The smears were fixed in methanol and stained with Hemo-3 for differential leukocyte counts. One hundred cells were counted per slide.

The WBC numbers and Hb concentrations were determined using a Coulter cell counter at a 1:500 dilution of blood in Isolyse (Beckman, Fullerton, CA). Plasma IgG concentrations were determined by ELISA. Full descriptions and validations of this assay were presented by Morrow-Tesch et al. (1994).

The lymphocyte blastogenesis assay used 15 mL of whole blood centrifuged at $850 \times g$ for 20 min. The buffy coat was removed and mixed with 9 mL of RPMI 1640 medium. Buffy coat-RPMI mixture was layered over 3 mL of histopaque 1077 (Sigma) and centrifuged at $400 \times g$ for 30 min. Mononuclear cells were collected and washed once in RPMI. The cells were counted and adjusted to a cell concentration of 5×10^6 cells/mL in RPMI-FBS.

One hundred microliters of diluted cells was added in triplicate to a 96-well, flat-bottomed sterile plate. Phytohemagglutinin (PHA) at various doses (0, .2, 2.0 and 20 $\mu\text{g}/\text{mL}$) was added to each well in triplicate. Plates were incubated for 48 h at 37°C in 5% CO_2 incubator. Plates were pulsed with 1 μCi of ^3H per well for 24 h. After 72 h, the cells were harvested on glass fiber filters. Each filter disk was placed in a scintillation vial. Five milliliters of scintillation fluid was added to each vial and samples were counted on a scintillation counter.

The cortisol assay was performed by using cortisol antibody coated tubes (Coat-A-Count Cortisol, Diagnostic Products, Los Angeles, CA) for maximum binding and in duplicate for samples and standards. Twenty-five microliters of the zero calibrator was placed into the nonspecific binding tube and in the first group of tubes 25 μL of standards with increasing cortisol concentration was placed into the standard curve tubes (1, 5, 10, 20, and 50 μg). Twenty-five microliters of each sample was placed in duplicate into each of the sample tubes. One milliliter of [^{125}I]cortisol was added to each tube and then vortexed. The samples were incubated for 45 min in a waterbath at 37°C . Tubes were decanted thoroughly and then counted for 1 min on the gamma counter. Results were calculated by linear regression and expressed in average nanograms/milliliter.

Statistical analyses were performed using SAS software (SAS, 1988). The experimental design was a randomized complete block with a 2×2 factorial arrangement of treatments. Sows within each block were the experimental units. A complete block included each of the two genotypes, at a common stage of gestation, in each housing system (indoor and outdoor). Eight complete blocks were evaluated, and thus 32 sows were used. The statistical model included effects of genotype, housing system, interaction between genotype and housing system, and block. Least squares means were calculated and separated by the predicted difference test when protected by a significant overall F -value from the ANOVA.

Experiment 2: Immune Status of Offspring From Two Genetic Lines

A total of 48 female nursery pigs were obtained from the two genetic lines of sows crossed with a common sire line (PIC 405). The pigs were housed in 1.82- \times 1.82-m pens with two pigs per pen. Pigs were placed randomly in one of three treatment pens: Exp-94 \times 405 + Exp-94 \times 405; Exp-94 \times 405 + C-15 \times 405; C-15 \times 405 + C-15 \times 405. The methods resulted in four treatments: 1) Exp-94 \times 405 housed in a pair, 2) Exp-94 \times 405 sampled while they were housed with C-15 \times 405, 3) C-15 \times 405 sampled while housed with Exp-94 \times 405, and 4) C-15 \times 405 housed in a pair. Six pigs represented one complete block. Eight complete blocks were evaluated.

Pigs were randomly selected based on litter and body weight. They were weaned at approximately 29 d of age, weighed, and placed in cleaned nursery pens. Pigs were weighed again 20 d later. Feed consumption was recorded and feed:gain ratio was calculated. Weanling pigs were fed a 19% crude protein sorghum-soybean meal diet for ad libitum consumption. Water was available from a single nipple waterer per pen.

Approximately 3 mL of blood was taken by venipuncture on d 42 of age for control antibody titer and pigs were injected with 40% sheep red blood cells (SRBC). Approximately 20 mL of blood was taken on d 49 for assays.

During the first 24 h after weaning, pig behavior was video recorded by time-lapse (72-h) speed with a Panasonic AG 1040 video recorder. Behavior was recorded at .8 frames/s and tapes were reviewed at 4.8 frames/s. Dominance status was determined by dyad with two pigs per pen. From the video records, each pig was labeled as either dominant, submissive, or non-aggressive (if no agonistic interactions were observed) based on the outcome of social interactions.

Blood was evaluated for NK activity, neutrophil chemotaxis, leukocyte differentials, WBC numbers, Hb concentrations, IgG concentrations, LTA, plasma cortisol concentrations, and antibody titer to SRBC. The antibody titer was determined by plating plasma sample from control of d 42 and from d 49 onto microtiter plates. The samples were serially diluted and 1% SRBC was added. These were incubated for 48 h and the antibody titer was determined by observation of agglutination as in Morrow-Tesch et al. (1994).

The experimental design was a randomized complete block with four treatments. The assumption could not be made ahead of time that one genotype would have a similar immune status if housed with a like genotype or a different genotype. Thus, for the mixed-genotype pen (Exp-94 \times 405 plus C-15 \times 405), the individual pigs were considered as having a different experience. That is, an Exp-94 \times 405 pig might have a different immune status when housed with another Exp-94 \times 405 than when housed with a C-15 \times 405 genotype. In addition, each pig in each pen of two pigs was determined to be either dominant or submissive.

Table 1. Selected least squares means and standard errors for immune measures for sows housed either indoors or outdoors (Exp. 1)^a

| Measure | Indoors | | Outdoors | | SE | <i>P</i> -values | | |
|--|---------------------|---------------------|---------------------|---------------------|--------|------------------|-----|-------|
| | C-15 | Exp-94 | C-15 | Exp-94 | | G | E | G × E |
| Number of pigs | 8 | 8 | 8 | | | | | |
| Hb, g/dL | 12.94 | 11.49 | 13.33 | 12.4 | .69 | .10 | .35 | .71 |
| WBC, cells/ μ L $\times 10^3$ | 13.16 | 12.63 | 10.95 | 12.76 | .76 | .41 | .19 | .14 |
| NK cytotoxicity E:T = 1:100 ^b | 20.39 | 23.90 | 21.47 | 25.11 | 1.72 | .05 | .51 | .97 |
| Neutrophil, % | 43.88 | 44.75 | 40.63 | 47.75 | 3.76 | .30 | .97 | .42 |
| Lymphocyte, % | 47.00 | 47.88 | 52.13 | 48.00 | 4.02 | .69 | .52 | .54 |
| Monocyte, % | 1.75 | 1.63 | 2.63 | 1.63 | .42 | .20 | .31 | .31 |
| Other cells, % | 6.25 | 5.63 | 4.63 | 2.63 | 1.37 | .35 | .11 | .62 |
| Neutrophil:lymphocyte | 1.06 | 1.08 | .80 | 1.07 | .19 | .43 | .47 | .52 |
| LTA (20 μ g/mL), cpm | 52,435 | 24,916 | 50,043 | 56,294 | 12,343 | .40 | .25 | .19 |
| LTA (2 μ g/mL), cpm | 76,020 | 52,871 | 49,930 | 51,937 | 8,856 | .25 | .15 | .17 |
| LTA (.2 μ g/mL), cpm | 49,551 ^c | 21,634 ^d | 24,643 ^d | 24,636 ^d | 6,864 | .05 | .13 | .05 |
| LTA (0 μ g/mL), cpm | 34,181 | 19,295 | 27,849 | 29,594 | 10,114 | .52 | .85 | .42 |
| IgG, mg/mL | 19.06 | 21.85 | 33.28 | 10.72 | 5.65 | .14 | .81 | .06 |
| Cortisol, ng/mL | 92.9 ^c | 21.8 ^d | 54.9 ^{cd} | 55.3 ^{cd} | 18.3 | .08 | .91 | .06 |

^aWBC, white blood cell; Hb, hemoglobin; NK, natural killer cell; LTA, lymphocyte transformation assay; IgG, immunoglobulin G; G, genotype; E, environment; and G × E, genotype × environment interaction.

^bOther effector:target ratios were not significantly affected by treatments.

^{c,d}Least squares means with different superscripts differ ($P < 0.05$).

The statistical model included effects of genotype, social status (dominant vs submissive), and block. Three pens containing two pigs each represented a block. Eight blocks were evaluated.

Results

Experiment 1: Immune Status of Exp-94 and C-15 Sows in Outdoor and Indoor Environments

There was a significant genotype effect ($P < .05$) for NK cell cytotoxicity. The Exp-94 sows had 17% higher NK cytotoxicity than the C-15 sows (24.51 ± 1.21 vs 20.93 ± 1.21 ; Table 1).

A significant genotype effect ($P \leq .05$) was observed for the low mitogen dose (.2 μ g/mL) in lymphocyte blastogenesis. C-15 sows had greater lymphocyte blastogenesis at lower mitogen doses than the Exp-94 sows ($37,097 \pm 4,854$ vs. $23,135 \pm 4,854$ cpm, respectively). A significant genotype × environment interaction was found for the low mitogen dose in lymphocyte blastogenesis (Table 1). The C-15 genotype indoors had elevated blastogenic response compared with the other three treatment groups.

A genotype effect ($P < .01$) was observed for chemotaxis. The C-15 sows had significantly higher neutrophil chemotaxis than the Exp-94 sows (184.2 ± 7.49 vs 150.8 ± 8.09 cells/five fields, respectively). The sows kept indoors had significantly ($P < .02$) higher neutrophil chemotaxis than the outdoor sows (182.4 ± 8.09 vs 152.6 ± 7.49 cells/five fields, respectively). There was a significant genotype × environment interaction ($P \leq .05$) for neutrophil chemotaxis (Figure 1). When indoors, the two genotypes showed similar neutrophil chemotaxis. When outdoors, the C-15 genotype had

higher ($P < .01$) neutrophil chemotaxis than the Exp-94 sows.

A genotype × environment interaction was observed for cortisol concentrations ($P = .06$). The C-15 sows housed indoors had significantly higher concentrations of cortisol than the Exp-94 sows housed indoors (Table 2).

There were no significant differences for effects of genotype, environment, or genotype × environment for hemoglobin concentrations, WBC numbers, neutrophil, lymphocyte, or monocyte percentages, or neutrophil:lymphocyte ratios. No significant differences were observed in IgG concentrations among treatments.

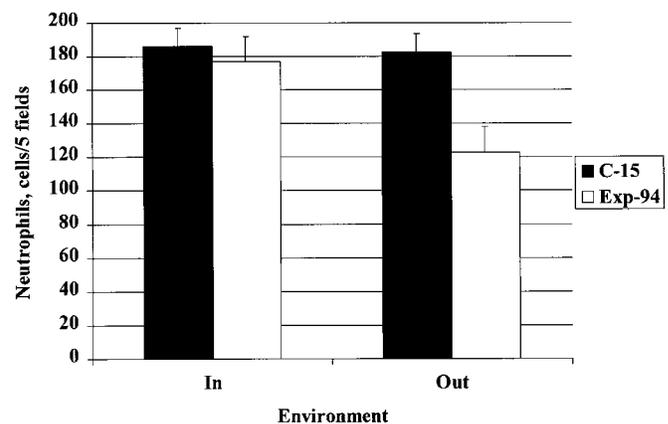


Figure 1. Genotype × environment intersection ($P < .05$) for neutrophil chemotaxis for sows (Exp. 1). N = 8 for each bar.

Table 2. Selected least squares means and standard errors for immune measures for nursery-age pigs housed two pigs per pen (Exp. 2)^a

| Measure | Genotypes | | | | P-values | |
|-----------------------------|----------------------------|------------------------------|------------------------------|--------------------------------|----------|-----|
| | C-15 × 405 with C-15 × 405 | C-15 × 405 with Exp-94 × 405 | Exp-94 × 405 with C-15 × 405 | Exp-94 × 405 with Exp-94 × 405 | G | Trt |
| Number of piglets | 16 | 8 | 8 | 16 | | |
| Hb, g/dL | 8.03 ± .334 | 7.19 ± .472 | 8.26 ± .472 | 8.05 ± .334 | .19 | .33 |
| NK cytotoxicity E:T = 1:100 | 18.36 ± 1.78 | 19.71 ± 2.52 | 16.30 ± 2.52 | 21.49 ± 1.78 | .95 | .23 |
| Neutrophil, % | 36.81 ± 3.35 | 35.88 ± 4.73 | 38.00 ± 4.73 | 35.00 ± 3.35 | .97 | .86 |
| Lymphocyte, % | 58.38 ± 3.27 | 61.50 ± 4.62 | 57.00 ± 4.62 | 60.81 ± 3.27 | .80 | .69 |
| Monocyte, % | 2.69 ± .374 | 2.38 ± .528 | 2.38 ± .528 | 2.69 ± .374 | 1.0 | .79 |
| Other cells, % | 1.50 ± .320 | .250 ± .452 | 1.38 ± .452 | 1.19 ± .320 | .31 | .09 |
| Neutrophil:lymphocyte | .724 ± .104 | .728 ± .147 | .698 ± .147 | .635 ± .104 | .64 | .94 |
| LTA (20 µg/mL) | 54,878 ± 12,752 | 54,743 ± 18,034 | 69,754 ± 18,034 | 34,029 ± 12,752 | .85 | .28 |
| LTA (2 µg/mL) | 76,642 ± 10,751 | 77,363 ± 15,204 | 81,109 ± 15,204 | 62,609 ± 10,751 | .70 | .61 |
| LTA (.2 µg/mL) | 46,861 ± 9,149 | 41,599 ± 12,939 | 65,396 ± 12,939 | 48,621 ± 9,149 | .26 | .55 |
| LTA (0 µg/mL) | 51,385 ± 10,719 | 41,653 ± 15,159 | 74,358 ± 15,159 | 45,208 ± 10,719 | .32 | .27 |
| Antibody titer (d 0) | 3.19 ± .217 | 2.50 ± .307 | 3.00 ± .307 | 2.59 ± .217 | .86 | .12 |
| Antibody titer (d 7) | 4.28 ± .247 | 4.19 ± .350 | 4.19 ± .350 | 3.88 ± .261 | .52 | .77 |
| IgG (d 0), mg/mL | 25.2 ± 3.42 | 16.8 ± 5.57 | 17.0 ± 5.57 | 16.0 ± 3.69 | .32 | .42 |
| IgG (d 7), mg/mL | 18.7 ± 4.99 | 20.8 ± 6.47 | 22.4 ± 5.94 | 14.1 ± 4.00 | .78 | .50 |
| Chemotaxis | 157.0 ± 7.9 | 185.0 ± 11.2 | 165.8 ± 11.2 | 158.9 ± 7.9 | .38 | .13 |
| Cortisol, ng/mL | 44.8 ± 9.7 | 69.5 ± 11.7 | 64.6 ± 13.9 | 65.5 ± 9.3 | .49 | .28 |

^aHb, hemoglobin; NK, natural killer cell; LTA, lymphocyte transformation assay; IgG, immunoglobulin G; G, genotype; and Trt, treatment.

Experiment 2: Immune Status of Offspring from Two Genetic Lines

White blood cell numbers were significantly higher ($P < .05$) among the Exp-94 × 405 pigs in the treatments in which they were mixed with the C-15 × 405 pigs (Figure 2). The WBC numbers were higher ($P < .02$) among the C-15 × 405 in the treatment in which they were not mixed with the C-15 ± 405 pigs (Figure 2).

There were no significant differences in cortisol concentrations, lymphocyte blastogenesis, Hb concentra-

tions, NK cytotoxicity, neutrophil, lymphocyte, or monocyte numbers for C-15 offspring or the Exp-94 offspring when mixed or not mixed (Table 2). There were also no significant differences in neutrophil:lymphocyte ratios, antibody titers, IgG concentrations, or neutrophil chemotaxis for the C-15 offspring or the Exp-94 offspring when mixed or not mixed (Table 2).

No significant differences were seen in immune measures between dominant, submissive, and non-fighting pigs (Table 3). No significant differences were seen for average daily gain, feed intake, or feed efficiency (Table 4) among lines.

Discussion

Indoor and outdoor Exp-94 sows had higher NK cytotoxicity than C-15 sows. Each genotype was exposed to similar microbes and thus had an opportunity to express a similar degree of immune system activation. Animals and humans with suppressed NK activity have increased likelihood of illness associated with viral infection (Glaser and Kiecolt-Glaser, 1998). The enhanced NK cytotoxicity among Exp-94 sows suggested that they may have some increased immunity to viral or malignant challenges compared to the C-15 sows; however, actual viral challenge studies would be required to test this hypothesis.

One recent report by Halbur et al. (1998) demonstrated an improvement of lung lesions due to the Porcine Respiratory and Reproductive virus (PRRS) in Meishan and Duroc breeds compared with Hampshire pigs. The finding that lesions associated with PRRS virus infection might be reduced among Meishan pigs is consistent with our finding of enhanced NK activity among Meishan-cross pigs.

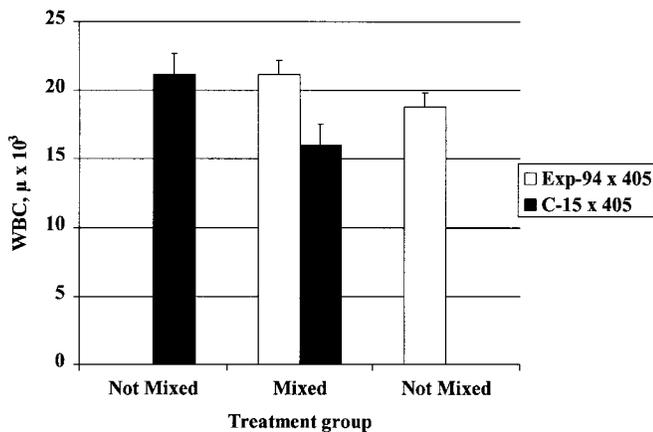


Figure 2. Treatment effect ($P < .02$) for white blood cell (WBC) numbers for nursery piglets (Exp. 2). N = 16, 8, 8, and 16 for each bar from left to right. Three treatment pens were: 1) single genotype pair of Exp-94 × 405, 2) a pair of pigs with one Exp-94 × 405 plus one C-15 × 405 pig, or 3) single pair of C-15 × 405 genotype.

Table 3. Selected least squares means and standard errors for immune measures for dominant and submissive nursery pigs housed two pigs per pen (Exp. 2)^a

| Measure | Dominant | Submissive | Neither | P-value |
|---------------------------------------|---------------------|---------------------|---------------------|---------|
| Number of pigs | 15 | 16 | 4 | |
| WBC, cells/ $\mu\text{L} \times 10^3$ | 18.6 \pm 1.8 | 18.0 \pm 1.8 | 18.0 \pm 3.6 | .97 |
| Hb, g/dL | 7.6 \pm .5 | 7.2 \pm .49 | 6.8 \pm .97 | .68 |
| NK cytotoxicity E:T = 1:100 | 18.2 \pm 3.8 | 19.1 \pm 3.7 | 13.9 \pm 7.4 | .82 |
| Neutrophil, % | 37.9 \pm 3.5 | 33.6 \pm 3.4 | 29.5 \pm 6.8 | .48 |
| Lymphocyte, % | 58.1 \pm 3.4 | 61.6 \pm 3.3 | 67.0 \pm 6.5 | .45 |
| Monocyte, % | 3.0 \pm .36 | 2.3 \pm .35 | 2.3 \pm .69 | .30 |
| Other cells, % | 1.0 \pm .38 | 1.3 \pm .36 | 1.3 \pm .73 | .88 |
| Neutrophil:lymphocyte | .75 \pm .11 | .62 \pm .10 | .46 \pm .20 | .39 |
| LTA (20 $\mu\text{g/mL}$) | 57,923 \pm 10,845 | 47,356 \pm 10,500 | 26,266 \pm 21,001 | .40 |
| LTA (2 $\mu\text{g/mL}$) | 84,527 \pm 13,324 | 84,396 \pm 12,901 | 80,083 \pm 25,802 | .99 |
| LTA (.2 $\mu\text{g/mL}$) | 58,660 \pm 13,895 | 58,984 \pm 13,453 | 42,067 \pm 26,907 | .84 |
| LTA (0 $\mu\text{g/mL}$) | 59,952 \pm 14,457 | 56,564 \pm 13,998 | 36,762 \pm 27,996 | .76 |
| Antibody titer (d 0) | 2.7 \pm .33 | 3.2 \pm .32 | 3.4 \pm .64 | .48 |
| Antibody titer (d 7) | 3.7 \pm .26 | 3.5 \pm .26 | 4.4 \pm .51 | .29 |
| IgG (d 0), mg/mL | 21.0 \pm 4.1 | 13.0 \pm 3.6 | 14.2 \pm 8.1 | .34 |
| IgG (d 7), mg/mL | 27.0 \pm 5.0 | 15.5 \pm 4.8 | 21.4 \pm 10.0 | .27 |
| Chemotaxis | 175.3 \pm 16.0 | 151.9 \pm 15.5 | 182.3 \pm 30.9 | .50 |
| Cortisol, ng/mL | 68.4 \pm 12.4 | 53.3 \pm 10.6 | 50.3 \pm 23.8 | .61 |

^aWBC, white blood cell; Hb, hemoglobin; NK, natural killer cell; LTA, lymphocyte transformation assay; and IgG, immunoglobulin G.

Indoors, Exp-94 sows had lower lymphocyte blastogenesis at lower mitogen doses (.2 $\mu\text{g/mL}$) than the C-15 sows. This finding indicates that there may be a slight reduction of cellular immunity among Exp-94 sows when they are housed indoors. Any speculation that differences in cellular immune responses might be associated with protection against specific disease challenges would need to be tested in challenge studies.

The C-15 sows housed indoors had higher cortisol concentrations than Exp-94 sows housed indoors. This finding could suggest a sign of stress in the C-15 sows that was not observed among the Exp-94 sows. The C-15 sows were more vocal and resisted handling more so than the calmer Meishan-cross sows. The relative calmness of the Meishan-cross sow, especially indoors, may have contributed to the lower plasma cortisol concentrations.

The C-15 sows housed both indoors and outdoors had higher neutrophil chemotaxis than the Exp-94 sows housed outdoors (Figure 1). Neutrophil recruitment to bacterial sites of infection plays an important role in counteracting bacterial infections. For example, weak recruitment of neutrophils contributes to increased severity of coliform mastitis (Shuster et al., 1996). The Exp-94 sows kept outdoors had lower neutrophil chemotaxis than C-15 sows outdoors. Although the lower neutrophil chemotaxis is predictive of lower resistance to bacterial infection, this relationship would have to be demonstrated in actual bacterial challenge studies.

One possible explanation for lower neutrophil chemotaxis might be elevated glucocorticoids in response to a perceived stressful environment. Neutrophil chemotaxis is very sensitive to the effects of circulating cortisol. Elevated plasma glucocorticoids resulted in a

Table 4. Nursery pig performance from 4 to 7 wk of age for two genotypes housed alone or in pens containing one of each genotype

| Measure | Genetic lines in each pen | | | SE | P-value ^b |
|---|---|---|--|-----|----------------------|
| | Exp-94 \times 405 with Exp-94 \times 405 | C-15 \times 405 with C-15 \times 405 | Exp-94 \times 405 with C-15 \times 405 ^a | | |
| Number of pigs | 16 | 16 | 16 | — | — |
| Number of pens | 8 | 8 | 8 | — | — |
| Weight (0 d), kg | 6.96 | 6.49 | 6.75 | .54 | .83 |
| ADG (0 to 20 d), kg | .16 | .18 | .16 | .02 | .78 |
| Feed intake (0 to 20 d), kg $\cdot\text{d}^{-1}\cdot\text{pig}^{-1}$ | .54 | .64 | .57 | .04 | .27 |
| Feed:gain | 3.77 | 3.77 | 3.47 | .37 | .80 |

^aPen containing one Exp-94 \times 405 and one C-15 \times 405 per replicate. Pen was the experimental unit.

^bP-value for the effect of three treatment pen types.

proportional decline in neutrophil chemotaxis (Salak-Johnson, et al., 1997). However, in this data set, suppressed neutrophil chemotaxis was not associated with elevated plasma cortisol concentrations. Even though Exp-94 sows had lower neutrophil chemotaxis outdoors (Figure 1), they did not have elevated glucocorticoid concentrations (Table 1). Thus, the mechanism for the observed effect on neutrophil chemotaxis is not likely to be elevated glucocorticoid concentrations.

The WBC numbers were significantly ($P < .02$) higher in C-15 \times 405 piglets when not mixed than when they were mixed with Exp-94 piglets. The Exp-94 piglets had significantly lower WBC numbers when mixed with the C-15 \times 405 piglets and had higher WBC numbers when not mixed. Thus, when crossbred lines containing the Meishan breed are released, they may be best housed alone (not in mixed genotype groups). However, the differences among genotypes were small and we cannot be sure what the impact of the reported immune differences would have in the face of a pathogenic challenge of viral or bacterial origin.

Implications

The Exp-94 sow line had marginally increased immunity in terms of increased natural killer activity and reduced lymphocyte blastogenesis and neutrophil chemotaxis when housed outdoors compared with the C-15 line. In low-pathogen environments, each line (Exp-94 or C-15) is expected to remain healthy, but their response to pathogenic challenge may differ. Weanling pigs did not differ in immune measures collected. Social status showed no significant effects on any of the immune measures taken. Although genotypic and environmental differences in immune measures were documented, actual disease challenge studies need to be conducted to confirm differences in susceptibility to viral and bacterial pathogens.

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