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Swine Breed Differences in Agglutination Titers Following Vaccination with Sheep Red Blood Cells and Pasteurella Multocida (Serotype A)

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SWINE BREED DIFFERENCES IN AGGLUTINATION TITERS FOLLOWING VACCINATION WITH SHEEP RED BLOOD CELLS AND *PASTEURELLA MULTOCIDA* (SEROTYPE A)

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SWINE BREED DIFFERENCES IN AGGLUTINATION TITERS FOLLOWING VACCINATION WITH SHEEP RED BLOOD CELLS AND PASTEURELLA MULTOCIDA (SEROTYPE A)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SWINE BREED DIFFERENCES IN AGGLUTINATION TITERS FOLLOWING VACCINATION WITH SHEEP RED BLOOD CELLS AND PASTEURELLA MULTOCIDA (SEROTYPE A)

Kenneth J. Stalder May 1992 36 pages

Directed by: Gordon F. Jones, Elmer Gray, Cheryl D. Davis and James P. Worthington

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An investigation into the genetic differences in the humoral immune response of swine following vaccination with a sheep red blood cell solution (SRBC) and a commercially prepared Pasteurella multocida (serotype A) bacterin (PmA) was conducted on a total of 268 pigs from two individual trials. This study was also conducted to evaluate the humoral immune response of pigs to a non-pathogen (SRBC) and a known pathogen to swine (PmA). The pigs used in the first trial were from 22 litters born between January 1991 and July 1991. The pigs consisted of Hampshire x Yorkshire (n=114), purebred Yorkshire (n=70) and Hampshire (n=17). Individual pigs were vaccinated at five and eight weeks of age with 2 ml of a 5% SRBC solution and 1 ml of a killed PmA bacterin. At 11 weeks of age 8 ml of blood was collected from each animal and serum prepared to determine antibody titer levels against the two antigens by agglutination methods. Pigs utilized in the second study consisted of purebred Duroc (n=11), Hampshire (n= 10), Landrace (n=12) and Yorkshire (n=11) and crossbred Hampshire X Duroc (n= 12) and Yorkshire X Landrace (n=12). Results of trial 1 indicate that breed of pig affected the immune response against both PmA ($P<.01$) and SRBC ($P<.01$), with the Hampshire x Yorkshire crossbred pigs having higher titer levels against

the PmA than either Hampshire or Yorkshire purebred pigs. The purebred Hampshire were not statistically different from either the purebred Yorkshire or the Hampshire x Yorkshire crossbred pigs in their antibody response to SRBC; however, the Hampshire x Yorkshire crossbred pigs were statistically higher than the Yorkshire pigs. Results from trial 2 indicate highly significant ($P<.01$) breed differences in the humoral immune response to PmA. Purebred Landrace pigs were superior to both Duroc and Hampshire purebred pigs in their immune response to PmA. Purebred Yorkshire and crossbred Yorkshire X Landrace pigs were superior to purebred Durocs in their immune response to PmA. No other significant differences among breeds of pigs occurred in trial 2.

A low positive correlation of .22 was found between the pigs' antibody responses to PmA and SRBC in trial 1. Correlation differences among breeds were found between average daily gain while on test and the humoral immune response to both PmA and SRBC. Results suggest that further studies into breed differences of the immune response in swine are warranted. Results also suggest that further studies are needed to evaluate sheep red blood cells as a suitable antigen when conducting research to analyze the humoral immune response in swine.

CHAPTER 1

INTRODUCTION

Infectious diseases cost livestock producers millions of dollars each year. These costs include mortality, condemnation of products, veterinary services and antibiotic costs. Disease also costs producers in more hidden ways: increased days to market, poorer feed efficiency, decreased milk and/or egg production. The most hidden cost in seedstock herds may be a reduction in genetic progress resulting in an increase in phenotypic variance due to disease (Gavora and Spencer, 1983). It is estimated that disease costs U.S. swine producers in excess of 1.5 billion dollars annually (Rothschild, 1985). Swine producers have generally focused on vaccination, sanitation, medication, and eradication to produce healthy hogs. The identification and selection of pigs with superior immune systems has largely been ignored as a means to produce healthier swine.

There are various shortcomings of current methods to control the incidence of disease. Immunity obtained by the administration of a vaccine varies widely (Rothschild, 1985). Disinfectants used in sanitizing swine facilities also vary in the organisms controlled (Jensen and Kaeberle, 1975). In recent years more controls have been placed on the use of antibiotics in livestock production; therefore the cost of using antibiotics may become prohibitive. Eradication is both an expensive and impractical method of controlling a large number of

diseases in a swine herd. Other vectors of diseases that are infectious to swine also make eradication difficult. The use of identification and selection for improved immune response used in conjunction with more conventional methods of controlling disease might prove productive.

There has been limited research exploring the identification of animals with superior immune systems and the results of these limited studies vary with the species utilized. The level of disease resistance present in livestock herds is primarily due to natural selection and a correlated response with production traits (Gavora and Spencer, 1983). Research indicates there is a genetic component of the immune response which may be influenced by the environment (Warner et al., 1987). Correlated responses between production traits and immune response vary and will influence whether animal breeders will be successful in simultaneous improvement of both groups of traits.

The purpose of this study was two fold: to explore the differences in the immune response among breeds of swine, and to determine if the pigs' immune systems respond similarly to sheep red blood cells (a non-pathogen to swine) and Pasteurella multocida serotype A (a known pathogen to swine).

CHAPTER 2

LITERATURE REVIEW

Thoughts that the immune response, against a variety of antigens, may be under some genetic control are not new. As early as 1968 researchers had discovered that poultry resistant to Merek's disease was related to B alloalleles of the major histocompatibility complex (Cole, 1968). The major histocompatibility complex is intimately involved in the immune response of animals to foreign antigens (Biozzi et al., 1979). A study conducted with sheep also indicated the genetic influence on the quantitative immune response of sheep to chicken red blood cells (CRBC) (Nguyen, 1984).

In cattle, genetic controls are less defined. The major histocompatibility complex of cattle is known as the Bovine Leukocyte Antigen (BoLA). The exact control exerted by the BoLA over the immune response in cattle is not well understood (Lewin, 1989). It is known that BoLA differences do exist and that BoLA heterozygotes were significantly better than homozygotes in their immune response to a synthetic peptide antigen (Lewin, 1989).

The swine major histocompatibility complex is known as the swine Leukocyte Antigen (SLA). It is known that the SLA is related to production traits such as litter size, birth weight, weaning weight and piglet mortality (Hoganson et al., 1989). However, little is known concerning the effects of the SLA on disease resistance.

Efforts in determining immune response differences between lines or breeds of livestock have focused on exposing an animal to an antigen(s) and measuring differences. Current levels of disease resistance have likely resulted from natural selection gained when selecting farm animals for production traits only. Direct selection for disease resistance has largely been ignored (Gavora and Spencer, 1983).

Disease resistant lines within breeds have been developed in poultry. Two lines of White Leghorn chickens (High Avian, HA) and (Low Avian, LA) differed in their susceptibility to Mycoplasma gallisepticum, Escherichia coli, Staphylococcus aureus, New Castle disease and Merek's disease (Gross et al., 1980). Two chick lines were divergently selected fourteen generations for antibody response to sheep red blood cells (SRBC); high and low antibody response lines were produced and maintained (Martin et al., 1988).

Breed differences have also been explored in cattle. No differences in the humoral immune response were found among Angus, Hereford and Red Poll calves vaccinated with Infectious Bovine Rhinotracheitis Virus (IBRV) (Muggli et al., 1987).

Humoral immune response differences in swine have been found by a number of researchers. Differences between breeds have varied with the antigen utilized to stimulate the immune system. Buschmann et al. (1974) demonstrated significant breed differences when sheep red blood cells (SRBC) were utilized as the antigen. Purebreeds utilized were Duroc, Hampshire, Chester White, Large White, Pietrain, German Landrace and German Edelschwein. Crossbred pigs which were German Landrace X Hampshire and Hampshire X Pietrain were also utilized in this study.

Breed differences in the number of plaque forming cells (PFC) were measured (an indication of the magnitude of immune response). It was found that Duroc and Chester White had the highest yield of (PFC) while Pietrain had the lowest (Buschmann et al., 1974). Buschmann et al. (1974) concluded that differences in the immune response to SRBC among breeds should be interpreted as genetic variation.

Two studies conducted by Rothschild et al. (1984a and 1984b) found breed differences among pigs' humoral immune response. The first study utilized Bordetella bronchiseptica as the antigen. Chester White pigs had higher post-vaccination titers than any of the other breeds tested. Yorkshire and Landrace ranked intermediate in their response and were different ($P < .01$) from Hampshire and Duroc pigs (Rothschild et al., 1984a). The second study conducted by Rothschild et al. (1984) utilized a modified live Pseudorabies virus as the antigen. It was found that the Yorkshire and Chester White pigs had higher average titers ($P < .01$) than Duroc and Landrace pigs (Rothschild et al., 1984b). Rothschild (1984b) concluded that breed differences may effect the efficacy of vaccines among breeds.

Crossbreeding to improve immune response or disease resistance has not been researched extensively. A study conducted by Gailor (1989) demonstrated that F1 Hybrid mice resulting from crossing two highly inbred strains of mice, BALB/c and C57BL, exhibited a highly significant increase in the humoral immune response to SRBC over their contemporaries of parental strain breeds. A 177.1% degree of heterosis was found in the humoral immune response to SRBC in the F1 hybrid when compared to the contemporary inbred strains (Gailor, 1989).

Kennedy and Moxley (1980) concluded that pure breeds did not differ in their susceptibility to atrophic rhinitis; however, rhinitis incidence was lower and snout scores were improved 10% among two-breed crosses when compared to contemporary purebreeds (Kennedy and Moxley, 1980). It was concluded that the differences represent an estimate of heterosis under the assumption that the pure breeds did not differ in their susceptibility to atrophic rhinitis.

The study, previously mentioned, conducted by Buschmann (1974) revealed that the F1 crossbred Pietrain X Hampshire were superior to purebred Pietrain pigs in their immune response to SRBC. A positive deviation of 11.8 percent in the number of PFC was found for the Pietrain X Hampshire when compared to purebred Pietrain.

In order to capitalize on humoral immune response differences it must be determined if an improvement in the humoral immune response can be obtained by selection. Eide and co-workers (1991) selected Norwegian dairy goats for high and low antibody response to diphtheria toxoid for 12 years or approximately 5.5 generations. Mean phenotypic values from each line diverged slightly until the fourth year. After the fourth year no further divergence occurred. Realized heritability estimates were approximately .19 in the first selection trial, but approached zero in later cycles.

Selection in other species has also been successful. Martin et al. (1989) demonstrated that selection in poultry was successful in improving the immune response to SRBC. Warner et al. (1987) state that successful immune response selection trials have been conducted in sheep, guinea pigs and mice. Table 1, adapted from Gavora and Spenser

(1983) and Rothschild (1985) illustrates several approaches to breeding and selecting for genetic resistance to disease. The methods outlined indicate that selection can be utilized in a number of ways to improve the immune response of livestock. Unfortunately, a good response to one antigen does not necessarily predict a good response to another antigen (Warner et al., 1987). Thus selection for general disease resistance by the response to a single antigen may prove to be difficult.

Heritability estimates for the immune response in swine have varied. Warner et al. (1987) reported a study conducted by Li in which a heritability estimate of .62 was obtained for the immune response to Bordetella bronchiseptica in Chester White swine after one generation. Meeker et al. (1987) obtained a heritability estimate in swine of .18 $\pm$ .09 for Pseudorabies titers at 56 days of age and .52 $\pm$ .15 at 119 days. Rothschild et al. (1984) found a heritability estimate of .10 $\pm$ .12 to Bordetella bronchiseptica. Rothschild (1985) reported that European studies estimate the heritability of resistance to atrophic rhinitis to be from .12 to .42 in swine. The heritability of the immune response to IBRV vaccination ranged from -.06 $\pm$ .08 to .21 $\pm$ .12 in beef calves (Muggli et al., 1987). Nguyen et al. (1984) reported a heritability estimate of .82$\pm$ .32 for the immune response to chicken red blood cells (CRBC) in sheep. The heritabilities estimated further support the idea that selection for immune responsiveness is possible, but successful selection varies greatly depending on the antigens or the infectious agent involved.

Ideally, the immune response would be well associated with various production traits making it conducive to simultaneous progress when

selecting for a single trait. Researchers have found this not always to be the case. Meeker et al. (1987) found an antagonistic relationship between the immune response to a modified live pseudorabies vaccine and the production traits of 21-day weight, 42-day weaning weight and average daily gain in swine. Pigs resistant to the K88 strain of Escherichia coli grow slower than their susceptible littermates (Gibbons et al., 1987). Shook (1989) states that high producing dairy cows are more susceptible to mastitis resulting in the high producing cow having a shorter longevity than its low producing herdmates.

Identification and selection of animals with superior immune systems and the application to commercial swine production still remains a formidable problem. Suitable antigens, well associated with production traits, used to access the immune response remain elusive. Crossbreeding of pure lines or breeds appears to improve the immune response in various species of livestock. Breed differences in the humoral immune response in pigs exist; results vary depending on the antigen involved in the stimulation of the immune system.

CHAPTER 3

MATERIALS AND METHODS

Animals. Two trials were conducted to evaluate swine breed differences in the humoral immune response to sheep red blood cells and/or Pasteurella multocida (serotype A). The first trial was conducted at the Western Kentucky University farm. Twenty-two litters farrowed between January 1991 and July 1991 provided the pigs utilized in this trial. The pigs used in the study consisted of crossbred Hampshire x Yorkshire (n=114) and purebred Yorkshire (n=70) and Hampshire (n=17). All dams were primiparous with the exception of the last six litters farrowed which were from second litter sows. All sires and dams were obtained from specific pathogen free (SPF) purebred herds. Pigs were weaned at five weeks of age and given a minimum of three days adjustment period before beginning the trial. All pigs were fed a standard commercial 18% crude protein ration medicated with Furazolidone, Oxytetracycline and Arsanilic Acid at the rate of 200, 100, and 90 grams per ton, respectively. Pigs were housed in a nursery at 26° to 30° Celsius (C). Pigs were randomly assigned to 1.22 m x 1.22 m wire floor decks with approximately ten pigs per pen for the first three weeks of the trial. Pigs were then moved to 1.52 m x 3.65 m totally slotted floor pens for the duration of the trial. Pigs were fed ad-libitum and had access to fresh water at all times.

The second trial was conducted utilizing pigs from a commercial seedstock herd. Pigs used in this trial represented forty-four litters farrowed in June and July of 1991. The large number of litters represented was an attempt to sample pigs from a large number of sires. The pigs utilized to conduct the trial consisted of purebred Yorkshire (n=11), Landrace (n=12), Duroc (n=11) and Hampshire (n=10) females, and crossbred Hampshire X Duroc (n=12) and Yorkshire X Landrace (n=12) females. Pigs were housed in grow-finish total confinement buildings. A temperature of 21° to 26° C was maintained within the grow-finish buildings. Pigs were randomly assigned to 3.65 m x 4.87 m or 3.65 m x 3.05 m (a stocking density of .414 to .506 square meters per pig was used) totally slotted floor pens for the full six week trial. Pigs were provided ad-libitum access to a standard 16% crude protein meal ration medicated with BMD (Bacitracin Methylene Disalicylate 30g per ton) or ASP 250 (Aureomycin 100g per ton, Sulfamethazine 100g per ton and Penicillin 50g per ton). Pigs were provided access to fresh water at all times.

The number of sires represented within each breed of experimental pigs from trials 1 and 2 is noted in table 2.

Antigens and Immunizations. Sheep Red Blood Cells (SRBC) and Pasteurella multocida serotype A (PmA) were the antigens selected for use in this study. Sheep red blood cells were selected because they are non-pathogenic to swine and would not be normally found in the pigs' environment. Sheep red blood cells were also commercially available in a standard suspension (Cleveland Scientific, Bath, Ohio). A killed Pasteurella multocida serotype A bacterin was selected because it is a

known pathogen to swine and standardized procedures for measuring serum antibodies were commercially available (Ambico, Inc., Dallas Center, Iowa).

Sheep red blood cells were refrigerated in Alsever's solution at 7^{o} C until use. The SRBCs were washed two times in physiological saline prior to diluting to the desired concentration. A 5% SRBC solution was determined sufficient to elicit a measurable immune response when injected intramuscularly (IM) in pigs (Seymour, 1985). The SRBC antigen was made by mixing 5 ml of SRBCs with 95 ml of physiological saline resulting in the 5% SRBC concentration. The Pasteurella multocida serotype A bacterin was obtained prediluted to a known concentration of 1×10^{10} colony forming units (cfu) per ml. The diluent used was a 12% rehydrogel adjuvant (Ambico, Inc., Dallas Center, Iowa).

Pigs utilized in the first study were weighed at approximately five weeks of age and given the initial antigen injections to stimulate the primary immune response. It was determined that five week old pigs were of sufficient age to mount an immune response to an antigen(s) (Haye and Kornegay, 1979). Three weeks following primary injections, a second injection of each antigen was given in order to stimulate the secondary immune response. Pigs were immunized intramuscularlly (IM) utilizing separate neck injection sites for each antigen. Antigen injections consisted of 2 ml of the 5% SRBC and 1 ml of the PmA solution. Pigs were weighed at the time of final blood collection to determine average daily gain.

Pigs used in the second study were given an initial 1 ml injection of Pasteurella multocida (serotype A) at an average of twelve weeks of

age. The antigen was injected IM into the neck. A second 1 ml injection of the PmA antigen was given three weeks following the initial injection to stimulate the secondary immune response.

Blood Collection. A blood sample was taken from an initial group of 45 pigs at weaning to determine baseline antibody levels for the two antigens involved in trial 1. Ten random blood samples were taken from pigs in trial 2 to determine if any antibodies to PmA existed prior to the initiation of the trial. Blood samples were collected three weeks after the initial antigen injections to determine the primary immune response and again three weeks later to determine the secondary immune response. Previous research indicated that immunoglobulin G (IgG) does not appear in the sera of pigs for seven to fourteen days following the initial injection of antigens (Roth, 1992). Blood was collected via the anterior vena cava with a 16 gauge, 38.1 mm needle attached to a disposable 10 ml syringe. Each sample was placed in a Serum Separation Tube (SST) (Beckton Dickinson and Company, Rutherford, New Jersey) and allowed to clot at ambient temperature. Sera were harvested by centrifuging each blood sample for ten minutes at 1100 g. The sera were harvested and each sample was divided into two 10 mm x 75 mm borosilicate culture tubes. The serum samples were then frozen at approximately -10° C until laboratory analyses were conducted.

Serum Assays. The serum samples were taken to the Breathitt Veterinary Center Serology Laboratory, in Hopkinsville, KY, to determine antibody levels by microtitration. Serum samples were thawed in a 37° C water bath for thirty minutes. Antibody levels against PmA were determined by a direct Pasteurella agglutination assay (Ambico, Inc.,

Dallas Center, Iowa). Standard positive and negative as well as all the test sera were pre-diluted 1/10 in PAST diluent composed of 5% sodium chloride, 1/1000 ml formalin, 0.05% bovine serum albumin and distilled water. Twenty-five microliters (µl) of diluent was added to wells in columns B through L of each microtiter plate. Each sample was assayed in duplicate by placing 25 µl of the test sera in columns A and B in two rows of the microtiter plate. The samples were serially diluted from columns B through L. Bulk PmA antigen was diluted 1 to 400 in PAST diluent and 25 µl of diluted antigen was added to each well to give a final dilution scheme of 1:20 to 1:58,240. The plates were mechanically shaken for approximately one minute before being incubated at 37° C for two hours and refrigerated at approximately 4° C overnight. Antibody titer was recorded as the reciprocal of the highest dilution at which a visible agglutination occurred. The results were considered valid if the duplicated samples' agglutination end-points were within one well or one dilution of each other. Antibodies to SRBC from each sample were determined by a hemagglutination assay in 8 x 12 well U-bottom microtiter plate (DynaTech Laboratory Products, Alexandria, Virginia). Serum samples were placed in a 56° C water bath for thirty minutes to inactivate the complement prior to assaying for SRBC antibodies. Twenty-five µl of serum was serially diluted in duplicate with 25 µl of physiological saline to give a final dilution scheme of 1:2 to 4,096. Twenty-five µl of a 2% SRBC suspension was added to each well and plates were mechanically shaken for approximately one minute. The plates were incubated at approximately 25° C for thirty minutes and

then refrigerated for approximately two hours. Antibody titer was recorded as the reciprocal of the highest dilution at which visible agglutination had occurred. If the end-points of duplicated assays were not within one dilution of each other, the results were considered invalid.

Statistical Analysis. The reciprocal of the highest dilution at which a visible agglutination occurred was transformed using $\log_2$ (because the assay utilized a serial doubling dilution scheme) to normalize the distribution. The General Linear Models procedure (GLM) of the Statistical Analysis Services (SAS), (SAS, 1989), was used to analyze these data. Data from trials 1 and 2 were analyzed separately. An analysis of variance (ANOVA) was performed on these data to analyze for significant differences, and Duncan's Multiple Range (DMR) test was used to separate breed mean differences. The statistical model used to analyze these data is shown in figure 1. ANOVA tables are shown in tables 3 through 6.

CHAPTER 4

RESULTS AND DISCUSSION

None of the 45 serum samples from trial 1 that were collected prior to immunization contained detectable levels of antibodies to SRBC or PmA. The ten serum samples collected prior to the start of trial 2 had a preexisting titer range of negative at the 1:20 dilution to positive at the 1:80 dilution. The distribution of PmA and SRBC agglutination titers following immunizations is shown in figures 2 and 3 for trial 1 and figure 4 for trial 2. The titer values were transformed into $\log_2$, and utilizing the Shapiro-Wilk test, it was determined ($P<.0001$) that the values were normally distributed in both trials. Breed average of pigs' secondary humoral immune response to SRBC and PmA is illustrated in table 7. Results from trial 1 indicate that breed affected the humoral immune response against PmA ($P<.0001$) and SRBC ($P<.0029$) with the Hampshire x Yorkshire crossbred pigs having higher PmA titer levels than either Hampshire or Yorkshire purebred pigs. The two pure breeds were not statistically different in their humoral immune response against PmA. The purebred Hampshire pigs were not statistically different from either the Hampshire x Yorkshire crossbred pigs or the Yorkshire purebred pigs in their humoral immune response against SRBC; however, the Hampshire x Yorkshire crossbred pigs were statistically higher than the purebred Yorkshire ($P<.0029$).

Highly significant breed differences (P<.0001) for average daily gain were noted from trial 1. Breed average daily gain while on test is illustrated in table 7. The Hampshire X Yorkshire pigs had higher average daily weight gain during the test period than either Hampshire or Yorkshire purebred pigs. The purebred Yorkshire pigs also had higher average daily weight gain while on test than purebred Hampshire pigs.

Pigs utilized in trial 2 were given injections of Pasteurella multocida (serotype A) only. Breed average for pigs' humoral immune response to PmA is shown in table 8. Results indicate highly significant breed differences. Landrace pigs were statistically higher in their humoral immune response to PmA than either Duroc or Hampshire breeds of pigs. Yorkshire purebred and Yorkshire X Landrace crossbred pigs were higher than Duroc pigs in their humoral immune response to PmA. No other significant differences were observed in trial 2.

Trial 1 also revealed a low positive coefficient of correlation of .22 between the secondary immune responses to SRBC and PmA. Coefficients of correlation by breed and for the overall study are found in table 9. Substantial numerical differences in the correlations between the secondary immune response to both antigens and average daily gain were also found.

The results of this study suggest that differences do occur in the humoral immune response of pigs among breeds. This study also suggests that crossbred pigs may have superior immune systems to combat known pathogens to swine than their purebred counterparts.

The results of the two trials using PmA as the antigen, closely resemble the results of Rothschild et al. (1984a) where purebred

Yorkshire and Landrace pigs were superior to purebred Duroc and Hampshire pigs in their humoral immune response to Bordetella bronchiseptica. However, when Rothschild et al. (1984b) utilized a modified live Pseudorabies vaccine as the antigen the results differed. In that study purebred Yorkshire, Chester White and Hampshire pigs were statistically higher in their humoral immune response than purebred Landrace pigs, while purebred Durocs were intermediate in their response. These results conflict with the current demonstration where purebred Landrace pigs had the highest transformed titer average of any pure breed used in the trial. The different results support the idea of breed differences that are dependent on the antigen used to conduct the experiment.

The results from trial 1 utilizing sheep red blood cells (SRBC) as the antigen differ from those results obtained by Buschmann et al. (1974) who also used SRBC as the antigen. Buschmann et al. (1974) found that purebred Large White (considered similar to Yorkshire pigs in the United States) were superior to purebred Hampshire in the average number of plaque-forming cells (an indication of the intensity of immune response). The current study demonstrated that the purebred Hampshires were superior to purebred Yorkshire when measured by a hemagglutination method, but the limited number of purebred Hampshires utilized in the current study may not have given a true estimate of breed differences.

The superior humoral immune performance of crossbred pigs in this study also concurs with studies conducted by Gailor (1985), Buschmann et al. (1974), and Kennedy and Moxley (1980). Research using two highly inbred strains of mice were used to create F1 offspring. It was found

that the F1 mice had 10.1% higher titer values to SRBC than the parent strains of mice (Gailor, 1985). An experiment conducted by Buschmann et al. (1974) found that Pietrain X Hampshire pigs were 11.8% superior in their immune response to SRBC than contemporary purebred Pietrain. A study conducted by Kennedy and Moxley (1980) found that crossbred Yorkshire X Landrace, Yorkshire X Lacombe and Landrace X Lacombe pigs had 1.1% less rhinitis incidence and 10.1% better rhinitis snout scores than contemporary purebred Yorkshire, Landrace and Lacombe pigs. The results of all the trials to date suggest an advantage for the crossbred pig in building immunity to a variety of antigens.

The current study indicates than PmA could be a suitable antigen to detect differences in the humoral immune response among breeds and individual pigs should selection for immune response become advantageous. The results also support the conclusion of Warner et al. (1985) who stated that selection for a superior immune response to one antigen does not guarantee a superior immune response to all antigens. The results from this study indicate the coefficients of correlation between average daily gain and the humoral immune response to the antigens PmA and SRBC to be extremely variable as noted in table 8. The correlations suggest that selection only for disease resistance would yield very little or negative progress on average daily gain, an economically important trait in swine. Meeker et al. (1987) and Gibbons et al. (1977) found similar relationships between economically important traits and the immune response and/or disease resistance.

No previous articles reviewed utilized SRBC and an antigen known to be pathogenic in the same study. Variable correlations, noted in table

8, between the humoral immune response to SRBC and PmA raise questions concerning the suitability of SRBC as an antigen to evaluate the pigs' humoral immune response capabilities to known pathogens of swine. Errors in the accessment of the pigs' immune response are likely if the immune response to SRBC does not consistently and closely mimic the immune response to known pathogens of swine.

CHAPTER 5

IMPLICATIONS

This study suggests that breed differences exist in the immune response to PmA, a known pathogen to swine. This effort also suggests that crossbreeding improves the immune response to PmA. Commercial swine producers could exploit breed differences in a crossbreeding program to improve the immune response in pigs. A poor overall correlation was found between the immune response to PmA and ADG, indicating that simultaneous improvement in production traits and the immune response may be difficult. Comparing previous studies to the current, it appears that different results can be obtained when various antigens are utilized in the assessment of the immune response among pigs. Therefore, further work may be warranted to identify suitable antigens that could be used in identifying a general improvement in the immune response among pigs. Results also suggest that non-pathogenic organisms such as SRBCs are poor indicators of the pigs' humoral immune response to known pathogens such as PmA. Above all, this study indicates boundless research opportunities that could be conducted to learn more about the relationship between the immune system and economically important traits in swine.

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Table 1: Approaches to breeding for genetic resistance to disease [a].

Type of Selection	Method	Effects on production of Breeders	Expression of Disease Resistance	Costs
Direct	1. Observe Breeding Stock	0	Questionable	0
	2. Challenge Breeding Stock	Negative	Good	Low - High
	3. Challenge Sibs or Progeny Testing	0	Good	Low - High
Indirect	1. Vaccine Challenge	0	Good	Low
	2. Genetic Markers	0	Good	Low
Molecular Genetics	1. Construct Resistant Genotypes	0	Good	High

[a] Portions adapted from Gavora and Spenser, 1983 and Rothschild, 1985.

Table 2. Number of sires represented within each breed of experimental pigs from trials 1 and 2.

Breed of Experimental Pigs	number of sires	
	Trial 1	Trial 2
Duroc	0	6
Hampshire	2	7
Landrace	0	5
Yorkshire	4	5
Hampshire X Yorkshire	2	0
Hampshire X Duroc	0	4 Hampshires and 2 Durocs
Landrace X Yorkshire	0	3 Yorkshires and 1 Landrace

Table 3. Analysis of variance for pigs' secondary humoral immune response to sheep red blood cells from trial 1.

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F - Value	Probability > F Value
Total	198	827.08			
Breed	2	48.01	24.00	6.04	.0029
Error	196	779.07	3.97		

Table 4. Analysis of variance for pigs' secondary humoral immune response to Pasteurella multocida (serotype A) from trial 1.

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F - Value	Probability > F Value
Total	198	348.15			
Breed	2	42.42	21.21	13.6	.0001
Error	196	305.73	1.56		

Table 5. Analysis of variance for pigs' average weight gain while on test from trial 1.

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F - Value	Probability > F Value
Total	198	2.61			
Breed	2	.68	.34	34.52	.0001
Error	196	1.93	.009		

Table 6. Analysis of variance for pigs' secondary humoral immune response to Pasteurella multocida (serotype A) from trial 2.

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F - Value	Probability > F Value
Total	67	89.22			
Breed	5	19.45	3.89	3.46	.008
Error	62	69.77	1.13		

Table 7. Breed averages for pigs' humoral immune response to SRBC and PmA and average daily gain from trial 1.

Breed:	Hampshire x Yorkshire		Yorkshire		Hampshire	
	$\bar{X}$*	SE	$\bar{X}$*	SE	$\bar{X}$*	SE
Secondary SRBC titers	5.06 [a]	±1.94	4.00 [b]	±2.24	4.50 [a,b]	±1.09
Secondary PmA titers	10.65 [a]	±1.26	9.72 [b]	±1.27	9.71 [b]	±1.09
Average Daily	.518 [a]	±.11	.436 [b]	±.08	.33 [c]	± .06

[a,b,c] Means in rows with no common superscripts differ ($P<0.01$).

*Mean of agglutination titers in $\log_2$ units.

Table 8. Breed averages for pigs' humoral immune response to PmA from trial 2.

Breed	$\bar{x}^*$	SE
Hampshire	9.22 [b,c]	± .88
Yorkshire	9.78 [a,b]	± .82
Duroc	8.78 [c]	± 1.29
Landrace	10.41 [a]	± .67
Yorkshire X Hampshire	10.07 [a,b]	± 1.36
Hampshire X Duroc	9.57 [a,b,c]	± 1.14

[a,b,c] Means in column with no common superscript differ ($P < .01$)

* Means of agglutination titers in $\log_2$ units

Table 9. Coefficients of correlation between immune responses and average daily gain from trial 1.

		SRBC	Prob. > R	PmA	Prob. > R
PmA	O	.22	.007	1.0	
	X	.06	.523	1.0	
	Y	.31	.009	1.0	
	H	.07	.771	1.0	
ADG	O	.23	.005	.17	.001
	X	.25	.007	-.08	.398
	Y	.02	.845	.30	.012
	H	.18	.476	-.09	.727

O - Overall Coefficients of Correlations
X - Crossbred Coefficients of Correlations
Y - Yorkshire Coefficients of Correlations
H - Hampshire Coefficients of Correlations

Figure 1: Statistical model used in analyzing the experimental data.

$$y_{ij} = \mu + b_i + e_{ij}$$

where

y_{ij} = the agglutination titer of the pig,

μ = the overall constant,

b_i = fixed effect of the j^{th} breed and

e_{ij} = random residual.

Fig 2: Distribution of SRBC agglutination titers
following immunization from trial 1

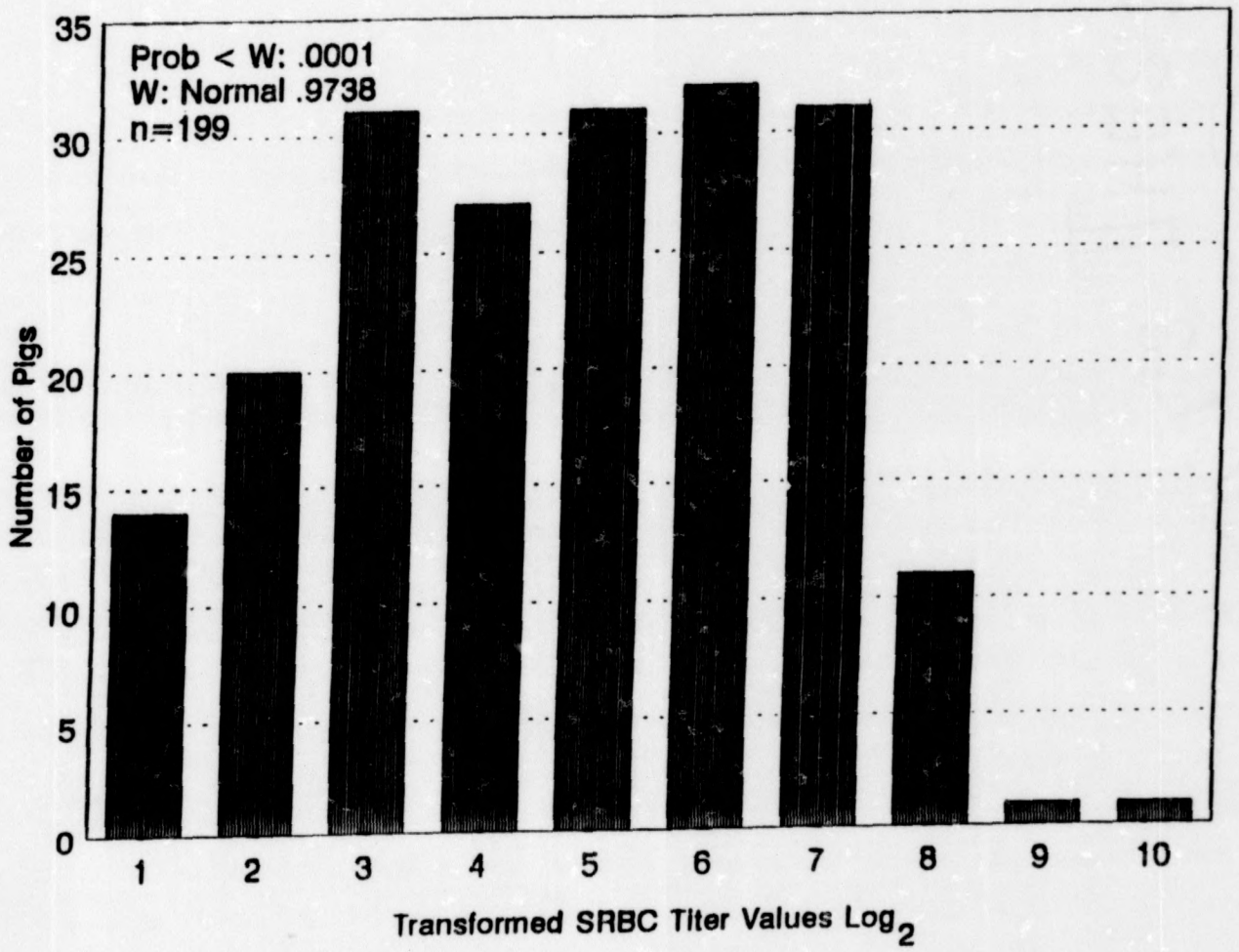

Fig 3: Distribution of PmA agglutination titers following immunization from trial 1

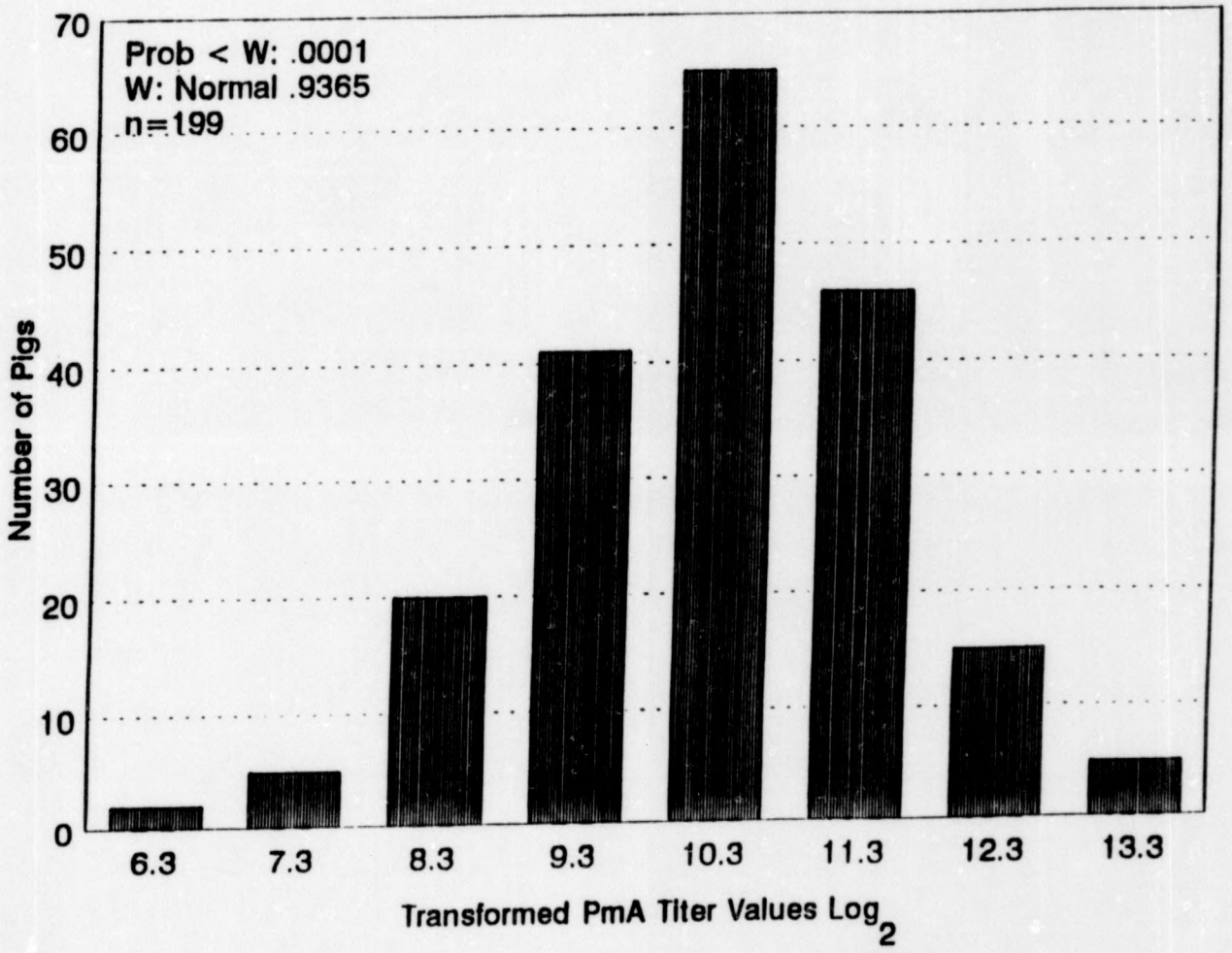

Fig 4: Distribution of PmA agglutination titers
following immunization from trial 2