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Genetic Control of Immune Response to Pseudorabies and Atrophic Rhinitis Vaccines: I. Heterosis, General Combining Ability and Relationship to Growth and Backfat

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Abstract

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Keywords

Immune Response, Heterosis, Aujeszky Virus, Rhinitis, Pigs

Disciplines

Agriculture | Animal Sciences | Biochemistry, Biophysics, and Structural Biology | Genetics | Veterinary Medicine

Comments

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GENETIC CONTROL OF IMMUNE RESPONSE TO PSEUDORABIES AND ATROPHIC RHINITIS VACCINES: I. HETEROSIS, GENERAL COMBINING ABILITY AND RELATIONSHIP TO GROWTH AND BACKFAT[1,2]

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ABSTRACT

Data from 988 pigs from 119 litters farrowed in two seasons of a three-breed diallel crossbreeding experiment were analyzed to estimate general combining abilities of breeds and heterosis for humoral immune response to pseudorabies virus and atrophic rhinitis vaccines. Twenty purebred boars and 85 sows of the Duroc, Landrace and Yorkshire breeds were mated to provide the nine breed-of-sire and breed-of-dam combinations. Immune response was measured after vaccination. A modified-live pseudorabies virus (PR) vaccine was administered to piglets at 28 d of age and response measured as $\log_2$ serum neutralization titers at 56 d. An inactivated B. bronchiseptica bacterin was administered at 28, 42 and 112 d. Antibody levels were measured relative to positive and negative controls at 28, 56 and 119 d by using an enzyme-linked immunosorbent assay. The results of this study showed that ranking by breed of sire and breed of dam did not differ for general combining ability, and no evidence of significant heterosis for any immune responses was observed. Higher immune response at 56 d to B. bronchiseptica vaccine was associated with lower weaning weight ($r = -.09$, $P<.01$). Correlations of days to 100 kg with 56-d and 119-d B. bronchiseptica antibody levels were .15 ($P<.01$) and .12 ($P<.01$). The relationship between humoral immune response to PR vaccine and growth traits was similar to that observed for B. bronchiseptica vaccine. Immune response to both antigens was not associated with backfat thickness. Further research using more specifically defined antigens and homogeneous populations of animals is needed to examine nonadditive gene action on the humoral immune response in swine.
(Key Words: Immune Response, Heterosis, Aujeszky Virus, Rhinitis, Pigs.)

Introduction

Selection for disease resistance in mammalian livestock has largely been ignored by quantitative animal breeders (Gavora and Spencer, 1983). A primary component of disease resistance is the humoral immune response (Gavora and Spencer, 1983; Buschmann et al., 1985; Warner et al., 1987). Genetic differences in the humoral immune response of pigs to antigens or vaccines have been reported for vaccination with sheep erythrocytes (Buschman et al., 1974), hen egg white lysozyme (Vaiman et al., 1978), E. coli vaccine (Edfors-Lilja et al., 1984, 1985), Bordetella bronchiseptica vaccine (Rothschild et al., 1984a) and pseudorabies vaccine (Rothschild et al., 1984b). Little is known about the relationships among immune response traits and production traits (Rothschild, 1985; Edfors-Lilja et al., 1986).

Selection for improved immune response leading to increased numbers of resistant animals could enhance vaccination and medication programs through synergistic interactions. However, this selection process is complicated by the fact that many diseases depend on a cellular, as well as a humoral, component to the immune response. Nevertheless, a study of the humoral immune response is at least a starting point for the study of breed differences and the association of production traits with the immune response.

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TABLE 1. NUMBER OF PIGS AND LITTERS BY BREED

Breed		Quantity	
Sire[a]	Dam	Pigs	Litters
Duroc	Duroc	127	18
Duroc	Landrace	114	13
Duroc	Yorkshire	122	13
Landrace	Duroc	132	17
Landrace	Landrace	103	11
Landrace	Yorkshire	88	9
Yorkshire	Duroc	140	17
Yorkshire	Landrace	93	11
Yorkshire	Yorkshire	69	10

[a]Numbers of sires used were seven Duroc, seven Landrace and six Yorkshire. All sires were bred to each breed of dam.

The objectives of this research were to 1) estimate general combining ability and heterosis for antibody production in response to Bordetella bronchiseptica and pseudorabies (Aujeszky) virus immunization and 2) examine the relationship between the humoral immune response to these vaccines and growth and backfat.

Experimental Procedure

Sows and boars of the Duroc, Yorkshire and Landrace breeds were mated in a complete three-breed diallel crossbreeding design. Twenty purebred boars and 85 purebred sows were used to produce 988 pigs from 119 litters in two seasons (table 1). These animals were part of a large research herd at Iowa State University's Bilsland Memorial Farm located near Madrid, Iowa. Introduction of semen and boars from outside sources is considered to have made this herd representative of the population of breeding herds in the United States. The sows in each of the three breeds represented at least six different pedigree lines within their respective breed. The sows ranged from first to fifth parity, and some were used in both seasons. Sows were randomly assigned and could produce different breed combinations each season. Sows were bred in each of three consecutive weeks. All the sows on this project tested negative to pseudorabies by a serum neutralization (SN) test. There were no clinical signs of atrophic rhinitis. All sows had low B. bronchiseptica antibody values when compared with the positive control. Sows were not immunized with either pseudorabies vaccine or B. bronchiseptica bacterin.

All pigs were immunized at 28 d of age with pseudorabies (PR) vaccine and B. bronchiseptica bacterin via separate intramuscular shots in separate hams. Bordetella bronchiseptica bacterin was again administered at 42 and 112 d of age. Immediately before the 28-d immunization, blood samples were taken from all pigs and dams, to serve as base levels. Blood samples were collected from all pigs again at 56 and 119 d. The pigs were weaned at 42 d, simultaneous with the second B. bronchiseptica immunization. Blood samples were collected with glass capillary tubes from the orbital venous sinus using the technique described by Huhn et al. (1969) for 28- and 56-d-old pigs. A modification of the technique was used for 119-d-old pigs and sows.

The two immunogens used were a chemically inactivated, adjuvanted culture of B. bronchiseptica[7], and a modified-live pseudorabies virus vaccine[8]. These immunogens were used according to the manufacturer's recommendations.

Ten milliliters of blood were collected from the pigs and dams at the designated time in the vaccination and bleeding schedule. The blood was allowed to clot at room temperature, and the clots were removed after contraction. The samples were then centrifuged twice to separate the serum. The sera were stored in aliquots at −20 C until assayed.

[7] Rhinobac, Norden Labs., Lincoln, NE.
[8] PR-Vac, Norden Labs., Lincoln, NE.

The assay used to determine PR vaccine titers was the microtitration serum neutralization (SN) test (Hill et al., 1977). This work was done at the Veterinary Diagnostics Laboratory, Iowa State University. In this test, fixed amounts of PR virus were added to serial dilutions of serum and incubated for 1 h at 37 C. Porcine kidney cells were added to the virus-serum mixture and incubated for 48 h at 37 C. Each microtiter well was examined for the absence or presence of cytopathic effect. The antibody titer of the test serum is reported as the highest dilution of serum causing 100% neutralization of the virus.

A modified enzyme-linked immunosorbent assay (ELISA; Venier et al., 1985) was used to determine antibody levels for B. bronchiseptica. In this assay, wells of microtiter plates were coated with B. bronchiseptica antigen[9]. Sample pig serum, rabbit antiswine IgG and Protein A β-galactosidase conjugate were added to each well in order, with the required washings and incubations after each step. Substrate, o-nitrophenol-β-D-galactopyranoside, was added and color intensities were measured spectrometrically. These color intensity measurements were recorded as the ELISA values. The 28-, 56- and 119-d samples from each pig were assayed in duplicate on the same plate, along with positive and negative controls, so that extraneous variation would be reduced.

The immune response traits measured in this experiment were response to PR vaccine at 56 d of age, response to B. bronchiseptica vaccine at 56 d of age (secondary response) and response to B. bronchiseptica vaccine at 119 d of age (memory response).

The SN titer data for response to PR vaccine were transformed to the $\log_2$ to normalize the distribution and to improve homogeneity of variance. The ELISA values for anti-B. bronchiseptica antibodies were standardized relative to positive and negative controls to adjust for differences between microtiter plates and test days.

Production traits measured were birth weight, 21-d weight, weaning weight at 42 d, days to 100 kg and backfat adjusted to 100 kg.

A mixed model analysis (Henderson, 1984) was used to analyze the immune responses for each of the nine breed combinations of the diallel design. The assumed model was as follows:

$Y = X\beta + Zu + e$,

where

Y = vector of immune response values,

X = a known incidence matrix,

β = an unknown vector of fixed season and breed effects,

Z = known incidence matrix,

u = a vector of random litter effects with mean 0 and variance σ^2_L and

e = an unobservable vector of random error with mean 0 and variances σ^2_e.

The mixed model equations are:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + Ik \end{bmatrix} \cdot \begin{bmatrix} \beta \\ u \end{bmatrix} = \begin{bmatrix} X'Y \\ Z'Y \end{bmatrix}$$

where k is a constant value σ^2_e/σ^2_L. The variances used in calculating k were estimated from the data by using Henderson's Method III (Henderson, 1953) with a model that included fixed effects for season and breed and random effects for litter and error.

Litters were absorbed into breeds and the solutions for the overall mean and second season mean were restricted to 0 because the resulting equations were not of full rank. Estimable functions for the nine breed combinations and for the difference between the two seasons were best linear unbiased estimates (Henderson, 1984).

Heterosis for each two-breed combination in the diallel was calculated by subtracting the mean of the two purebred values from the mean of the two reciprocal crossbred values. Overall heterosis estimates were calculated using all six crossbred combinations in the crossbred mean and all three purebred values in the purebred mean.

General combining abilities of breeds as sires were calculated by averaging the means of the three cells with the same breed of sire. General combining abilities of the breeds as dams were calculated in a similar manner. These estimates were pooled for each breed to get an estimate of general combining ability.

Correlations among the three immune response traits and the five production traits were calculated on a within-litter basis. Correlation values were then pooled across season and breed.

Results and Discussion

Generalized least-squares means of humoral immune responses for the nine breed combina-

[9] Prepared by Norden Lab., Lincoln, NE.

TABLE 2. GENERALIZED LEAST-SQUARES ESTIMATES AND STANDARD ERRORS OF HUMORAL IMMUNE RESPONSES FOR THE NINE BREED COMBINATIONS

Breed[a]	PR titer[b]		ELISA value[c]			
	56 d	SE	56 d	SE	119 d	SE
D X D	5.00	.52	.506	.047	.692	.056
D X L	3.22	.58	.517	.052	.796	.062
D X Y	4.52	.56	.526	.052	.596	.062
L X D	3.83	.52	.511	.047	.838	.057
L X L	3.43	.64	.408	.058	.864	.070
L X Y	5.51	.70	.515	.061	.808	.074
Y X D	3.77	.54	.518	.049	.623	.060
Y X L	5.62	.63	.544	.058	.886	.070
Y X Y	4.89	.69	.653	.060	.787	.073

[a]D = Duroc, L = Landrace, Y = Yorkshire; first breed is breed of sire.

[b]Log_2 SN titer for pseudorabies antibodies.

[c]B. bronchiseptica antibody levels.

tions are displayed in table 2. Differences among these breed combinations were not significant. For PR antibody titer the three purebreds ranked Duroc, Yorkshire and Landrace in order of highest titer. These results are similar to the study of Rothschild et al. (1984b), in which Yorkshire had the highest response and Landrace was lowest. Response to the B. bronchiseptica vaccine was highest for Yorkshire and lowest for Landrace at 56 d. These results differ for those of Rothschild et al. (1984a), who found Duroc to be significantly lower than both Yorkshire and Landrace. One possible explanation for this may be that Rothschild et al. (1984a) used an agglutination procedure to measure immune response which preferentially measures IgM as opposed to IgG antibody. The ELISA procedure used here measures only IgG. At 119 d, Landrace ranked highest and Duroc lowest. Thus, although the secondary response of Landrace is relatively low, the memory response of Landrace was the highest of the three breeds tested.

Heterosis estimates for immune response are in table 3. Heterosis was not significant for any of the breed combinations or overall. No previous estimates of heterosis for these immune responses have been reported. It is generally believed that there is heterosis for disease resistance. The lack of significant heterosis in the

TABLE 3. HETEROSIS[a] AND STANDARD ERRORS OF HUMORAL IMMUNE RESPONSES

Breed[b]	PR titer[c]		ELISA value[d]			
	56 d	SE	56 d	SE	119 d	SE
D X L	− .69	.62	.057	.049	.039	.059
D X Y	− .80	.56	−.058	.050	−.138	.060
L X Y	1.40	.64	−.001	.057	.022	.069
Overall	− .03	.61	.000	.052	−.023	.063
% heterosis	− .70		.0		− 3.7	

[a]Calculated as crossbred mean − purebred mean. None of these are different from 0 (P>.05).

[b]D = Duroc, L = Landrace, Y = Yorkshire.

[c]Log_2 SN titer for pseudorabies antibodies.

[d]B. bronchiseptica antibody levels.

study may indicate that epistatic loss did equal or exceed dominance gene effects that may have been present in purebred populations.

Failure to establish the existence of non-additive gene action in this study was not expected. However, the swine breeds studied in the experiments described in this paper are not genetically homogeneous for the many genes that control the humoral immune response. Each breed included several family lines, and were not inbred. Also, the antigens used in this experiment have compound determinants. It is possible that different animals had the necessary immune response genes for an immune response to different determinants of the polyvalent antigens. Research with geneticaly inbred animals and with more specifically defined antigens is needed to examine more fully non-additive gene action in the humoral immune response in swine.

The general combining abilities of the sire and the dam breeds (table 4) were similar for the humoral immune responses measured. This indicates that a breed's effect was the same whether it came from the sire or the dam side, so there was no apparent breed maternal effect on immune response to these vaccines. These values were pooled (table 5) to estimate the overall general combining ability of the breeds. Though the differences between these means were not great enough to be significant, the ranking of the breeds agrees reasonably well with previous work. Rothschild et al. (1984b) showed titers to PR vaccine ranking Yorkshire, Duroc, Landrace from highest to lowest, which is the same as in table 5. Rothschild et al. (1984a) showed titers to B. bronchiseptica vaccine at 56 d ranked Yorkshire, Landrace, Duroc from highest to lowest. General combining abilities are in table 5 for this response, and these rank Yorkshire, Duroc, Landrace from highest to lowest. However, as previously discussed, the assays used to detect the antibodies differed in the two studies because the ELISA assay measured IgG antibodies and that of Rothschild et al. (1984a) preferentially measured IgM. Ranking of breeds at 119 d placed Duroc as the lowest responder in this study.

Research on the relationships among disease resistance, immune response and production traits in swine has been limited. Huang (1977) found no evidence of an association between early growth in pigs and ability to develop humoral immune response to synthetic antigens. Higher gains and feed efficiency were found by Edfors-Lilja et al. (1986) to be related to susceptibility to K88 E. coli scours. Correlations of production traits and immune response to PR and B. bronchiseptica vaccines from this study are shown in table 6. Birth weight had little relationship to any of the immune response traits, but 21-d weight, 42-d weaning weight, and rate of gain had antagonistic relationships with most of the immune response traits. A positive correlation of immune response with days to 100 kg is actually a negative relationship with gain because more days to 100 kg indicates slower rate of gain. These data indicate that the faster growing, presumably

TABLE 4. GENERAL COMBINING ABILITIES[a] AND STANDARD ERRORS OF HUMORAL IMMUNE RESPONSES FOR BREED OR SIRE AND BREED OF DAM

	PR titer[b]		ELISA value[c]			
Breed	56 d	SE	56 d	SE	119 d	SE
Sire						
Duroc	4.25	.55	.516	.050	.695	.061
Landrace	4.25	.63	.478	.056	.837	.068
Yorkshire	4.76	.63	.572	.056	.765	.068
Dam						
Duroc	4.19	.53	.512	.048	.718	.058
Landrace	4.09	.62	.490	.053	.849	.068
Yorkshire	4.97	.65	.565	.058	.730	.070

[a]These are not different from each other (P>.05) within any one immune response.

[b]Log_2 SN titer for pseudorabies antibodies.

[c]B. bronchiseptica antibody levels.

TABLE 5. GENERAL COMBINING ABILITIES[a] AND STANDARD ERRORS OF HUMORAL IMMUNE RESPONSES FOR BREEDS

Breed	PR titer[b]		ELISA value[c]			
	56 d	SE	56 d	SE	119 d	SE
Duroc	4.22	.54	.514	.049	.706	.059
Landrace	4.16	.62	.484	.054	.843	.068
Yorkshire	4.87	.64	.568	.057	.748	.069

[a]These are not different from each other (P>.05) within any one immune response.

[b]Log_2 SN titer for pseudorabies antibodies.

[c]B. bronchiseptica antibody levels.

TABLE 6. CORRELATIONS[a] AMONG IMMUNE RESPONSE AND PRODUCTION TRAITS

	ELISA value[b]		PR titer[c]
	56 d	119 d	56 d
Birth wt	−.06	−.02	−.04
21-d wt	−.11**	.00	−.08*
Weaning wt	−.09**	−.04	−.06
Days to 100 kg	.15**	.12**	.08*
Adjusted backfat	−.03	−.05	.01
56-d ELISA		.29**	.12**
119-d ELISA			.02

[a]Calculated within litters and pooled across breed and season.

[b]B. bronchiseptica antibody levels.

[c]Log_2 SN titer for pseudorabies antibodies.

*P<.05.

**P<.01.

healthier pigs had a lower immune response. The slower growing pigs could have been at a higher level of immune system "readiness" because of stimulation from pathogens that caused slower growth. Another possibility is that pigs that grew more slowly may have ingested less colostrum and were less inhibited by maternal antibodies. Backfat had no significant correlation with any of the immune response traits.

Conclusions

Breed differences for the humoral immune response to PR and B. bronchiseptica vaccines were found to be similar to those reported previously. There was no evidence from this study to indicate significant non-additive gene action on the humoral immune response to these two vaccines. Thus, crossbred pigs appeared to have no advantage over purebreds in the generation of the humoral immune response. This was contrary to what was expected, because it is generally believed that heterosis exists for disease resistance and that immune response is associated with disease resistance.

The relationship of humoral immune response to growth traits was of interest. In general, a lower weight at 21 d and 42 d (weaning) indicated a higher immune response. Likewise, the more days to reach 100 kg, the higher was the immune response. There was no significant correlation of backfat thickness with the humoral immune response. It is clear that additional information is still needed before selection programs for immune response or disease resistance can begin.

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