

IMMUNE RESPONSIVENESS IN SWINE: EIGHT GENERATIONS OF SELECTION FOR HIGH AND LOW IMMUNE RESPONSE IN YORKSHIRE PIGS

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SUMMARY

Pigs were bred for 8 generations for high (H), low (L) and control (C) immune responsiveness using estimated breeding values (EBV) of antibody (Ab) and cell-mediated immune responses (CMIR). Response to selection was determined by differences in least squares means and average EBV between the H and L lines. Lines diverged steadily between generations 1 to 3 (G1-G3); however, there was little or no response to selection following G4. Antibody responses to various antigens were significantly higher in the H line. Following infection with *Mycoplasma hyorhinis* in G4 and G8 H line pigs had significantly less peritonitis and pleuritis, but had more severe arthritis. Increased arthritis may be due to the formation of immune complexes or more severe inflammatory responses based on antigen-specific CMIR. From G0 to G7 H line pigs had higher rate of gain compared to L or C. If enhanced rate of gain is due to selection for H immune response and not due to founder, or other effects, then increased productivity may be the greatest benefit of this selection.

Keywords: Immune Response, Estimated Breeding Values, Genetic Selection, Disease Resistance

INTRODUCTION

The concept of breeding for disease resistance was discussed in the 1940's by J. L. Lush (1948) and later by others (Legates and Grinnells 1952; Hutt 1958) as a prophylactic approach to animal health. Original studies focused on identifying resistant livestock during disease outbreaks, recognizing that these animals were often related, and multiplying these lines through within-herd selection. This approach gave way to selection based on breed or line differences and established heritability estimates of disease resistance. These methods were successful in specific instances, but the principal disadvantages were slow response and high cost. Consequently, the consensus among animal breeders was that selection for disease resistance should only be considered when the disease had significant economic impact and less costly methods of disease control were not effective (Kennedy 1980; McDaniel 1984). However, reliance on exogenous methods, such as antibiotics, chemicals, elaborate management schemes, and vaccination, has caused animal and human welfare concerns. Thus contemporary concepts of genetic selection to enhance disease resistance have been well received given their potential to reduce the use of chemicals and antibiotics in food producing animals.

Genetic approaches to improved health may be most useful when disease susceptibility is based on

a single gene effect; however, resistance to infectious disease is more often controlled by multiple host resistance genes making selection complex. There is also concern that selection for resistance to one disease might increase susceptibility to other diseases. Furthermore, the agents of disease are genetically complex, and express and vary several virulence factors, necessitating different host response attributes. This information prompted genetic selection for enhanced host resistance as a method to improve broad-based disease resistance. Attempts to breed mice for H or L Ab response proved feasible (Biozzi *et al.* 1979). However, due to negative genetic relationships between the various mechanisms of host immunity, high Ab responders were more resistant to extracellular pathogens, but were susceptible to intracellular pathogens, such as *Salmonella typhimurium*, which are better controlled by enhanced phagocytic cell function and cell mediated immunity (CMI).

In several species, including pigs, MHC genes were reported to control some of the variation in immune response and to have relevance to the outcome of infection (Biozzi *et al.* 1979; Mallard *et al.* 1989). Indirect selection for improved resistance to Marek's disease was initially applied to chickens based on expression of particular MHC genes with no adverse effects on production and enhanced response to vaccination (Simonsen 1987). However, the MHC is only one set of many groups of genes mediating host resistance, and with the possible appearance of more virulent pathogenic strains it may prove necessary to modify the selection criteria. Furthermore, this selection could result in the loss of valuable genes required to combat the ever changing set of pathogens.

In 1988, with the described constraints of genetic selection for disease resistance in mind, we set out to devise a multi-trait selection index using EBVs of immune responses to improve broad-based disease resistance. It was necessary to identify relevant, easily measured immunological markers, determine their heritability, and the genetic covariances between them. It was also necessary to determine the marker relationships to production and disease traits. In order to efficiently test the technology we chose a livestock species in which genetic progress could be made relatively rapidly. The remainder of this paper describes our experience through 8 generations of breeding of pigs for high and low immune response in attempt to attain broad-based resistance to infectious disease.

RESULTS AND DISCUSSION

Details of the experimental design and selection index have been described elsewhere (Mallard *et al.* 1992). Briefly, a random bred population of 65 female and 33 male Yorkshire pigs (GO) were characterized using 14 indicators of immune and innate host resistance. Based on initial heritability estimates and genetic correlations between the traits, two measures each of Ab and CMI, one specific (secondary antibody response to HEWL, delayed-type hypersensitivity (DTH) response to purified protein derivative {PPD} of Bacillus Calmette Guerin) and the other general (serum IgG, lymphocyte blastogenesis to ConA mitogen), and one indicator of innate monocyte function (uptake and killing of *S. typhimurium*) were chosen as breeding criteria. Individual trait EBVs were calculated for each pig and animals were assigned to High (H), Low (L) or Control (C) breeding groups on the basis of a combined index which was the sum of the single trait EBVs each expressed in standard deviation units. Approximately 120 piglets were similarly evaluated at each of 8 subsequent generations of selection. Approximately 20 females were bred using 5 boars for each line at each generation. Monocyte function

was eliminated from the selection index after one generation of selection due to negligible heritability. Response to selection was determined by differences in least squares means and average combined EBVs between the H and L lines. The impact of selection on health and performance was determined.

Response to Selection. Estimates of heritability (h^2) calculated using the cumulative data from the parents and subsequent generations are summarized in Table 1. Heritability of the four selected traits remained relatively constant from G0 to G8 ($n > 1200$) when h^2 was 0.268 for Ab response, 0.163 for DTH, 0.160 for blastogenic response, and 0.070 for serum IgG. Differences between H, L, and C lines based on individual and combined EBVs are summarized in Table 2. Responses between lines diverged up to G3 when H and L were separated by 2.0 EBV (or approximately one phenotypic standard deviation). After G4 there was little or no response to selection, particularly in the H line, possibly reflecting rising inbreeding (Table 1).

Table 1. Heritability estimates, litter variances (c^2), and inbreeding coefficients of pigs selected for high and low immune response									
Traits	Generation of Selection								
	0	1	2	3	4	5	6	7	8
Heritability									
Antibody	0.75	0.25	0.30	0.32	0.34	0.32	0.36	0.24	0.27
DTH	0.27	0.10	0.21	0.26	0.23	0.18	-	0.19	0.16
IgG	0.15	0.08	0.16	0.13	0.18	0.14	0.09	0.04	0.07
ConA	0.15	0.23	0.26	0.19	0.11	0.12	0.14	0.17	0.16
Inbreeding									
All Lines	0	0.01	0.06	0.08	0.11	0.12	0.15	0.18	0.20
High Line	-	-	-	-	-	0.13	0.16	0.20	0.23
Control Line	-	-	-	-	-	0.11	0.13	0.17	0.16
Low Line	-	-	-	-	-	0.12	0.15	0.20	0.20
Standard errors for all traits vary between 0.03 and 0.08. Traits measured include antibody response to HEWL (Antibody), cutaneous DTH to PPD (DTH), serum IgG concentration (IgG), and lymphocyte blastogenesis to ConA (ConA). Data not available is shown as (-).									

Table 2. Combined and individual trait estimated breeding values (EBV) of Yorkshire pigs selected for high and low immune responses. The values represent all pigs tested at each generation (40-60 pigs/line/year). G4 was relaxed selection.

EBV by Line	Generation of Selection								
	0	1	2	3	4	5	6	7	8
Combined EBV									
High	0.66	0.65	0.77	0.93	-	0.59	0.60	-	0.47
Control	-0.13	-0.08	0.01	0.05	-	-0.04	0.01	-	0.16
Low	-0.64	-0.66	-0.89	-1.07	-	-0.98	-0.88	-	-0.76
HEWL EBV									
High	0.35	0.24	0.26	0.17	-	0.06	0.09	0.02	0.04
Control	-0.12	-0.09	-0.01	-0.01	-	-0.03	-0.02	0.07	0.08
Low	-0.39	-0.33	-0.49	-0.61	-	-0.61	-0.62	-0.55	-0.53
DTH EBV									
High	0.18	0.29	0.47	0.68	-	0.46	0.54	-	0.45
Control	-0.01	-0.10	-0.17	-0.17	-	-0.24	-0.23	-	-0.13
Low	-0.10	-0.16	-0.25	-0.29	-	-0.20	-0.14	-	-0.10
IgG EBV									
High	-0.02	-0.03	-0.08	-0.07	-	-0.08	-0.11	-0.10	-0.09
Control	0.02	0.07	0.09	0.18	-	0.17	0.17	0.17	0.15
Low	-0.01	-0.02	-0.03	-0.06	-	-0.03	-0.03	-0.02	-0.02
ConA EBV									
High	0.15	0.15	0.11	0.15	-	0.12	0.09	0.08	0.06
Control	-0.02	0.04	0.09	0.06	-	0.06	0.09	0.08	0.07
Low	-0.15	-0.15	-0.13	-0.11	-	-0.14	-0.10	-0.12	-0.11

Single trait EBVs varied, with Ab and DTH having the largest line-related differences. Genetic correlation between Ab and DTH remained negligible to G8 ($r=0.09$), although EBV rank correlations revealed some negative relationships. As anticipated, phenotypic differences between the lines varied widely (Wilkie *et al.* 1997). Problems with reagents made it **difficult** to measure DTH in G6 to G8, although h^2 remained stable.

Correlated Traits. Although selection for altered monocyte function was **practised** for one generation, no differences were observed between the lines for uptake and killing of *S. typhimurium*, O_2 production, or SLA-DR and DQ expression on blood monocytes in the subsequent two generations (Groves *et al.* 1993). In contrast, mice differed in phagocytic function following selection for high and low primary Ab response to heterologous erythrocytes (Biozzi *et al.* 1984). Increased respiratory burst, tumoricidal activity, and altered antigen presenting capacity of macrophages from the L line mice were proposed to contribute to their increased resistance to intracellular pathogens and their low Ab response (Adorine and Doria 1981; Dockrell *et al.* 1985). This inverse relationship between Ab responsiveness and **macrophage** function has also been noted in cattle resistant or susceptible to *Brucella abortus* (Harmon *et al.* 1985). However, Ab responses do not inversely correlate with phagocytic cell function in all species, including chickens selected for H or L Ab to sheep RBC (Van der Zijpp *et al.* 1988).

Antibody responses of H line pigs were higher to a wide range of antigens, including TGAL (Mallard *et al.* 1992); carbohydrate 1 and 5, and lps 1 of *Actinobacillus pleuropneumonia* (Magnusson *et al.* 1997a); and *Mycoplasma hyorhinis* (Magnusson *et al.* 1997b). In addition, mean antibody avidity of H line pigs is higher ($p<0.05$) than that of L line pigs (Appleyard *et al.* 1992). The ability of individuals to produce Ab of increased avidity is known to be under genetic control and relates, at least in part, to somatic mutation events in the immunoglobulin V-region genes. Individuals producing more Ab with higher binding strength may have a survival advantage when antibody-mediated protection is required. Alternatively, they may be more susceptible to antibody-mediated disease.

Recent studies of NK cell number and **function** at G6 and G8 show that the H line pigs have a significantly ($p<0.05$) higher proportion of NK cells in blood with greater **lytic** activity ($p<0.10$) than L or C lines (Raymond *et al.* 1995). Alterations in NK activity have been reported to influence health, and reproductive performance (Croy *et al.* 1994).

Resistance to Infectious Disease. To examine the effects of selection for resistance-mediating traits on ability to resist a complex infectious disease, 22 pairs of H and L litter-mates from G4 were challenged (intra peritoneal) with 2×10^9 cfu of *M. hyorhinis* in PBS (Magnusson *et al.* 1997b). Serum antibody increased more rapidly and was of higher ($p<0.05$) titre in the H line pigs. No differences were detected in synovial fluid antibody. Protection from infection and disease due to mycoplasmas has been reported to associate with Ab, although exceptions may apply (Lai *et al.* 1991).

Postmortem (PM) signs of peritonitis and pleuritis were more ($p<0.01$) severe in L line pigs, but arthritis scores were more ($p<0.005$) severe in the H line. Given that the objective of this selection

was to achieve general improvement in disease resistance, this fundamental goal may not have been attained. Since both Ab and DTH are enhanced in the H line the increased arthritis following challenge may relate to the **formation** of detrimental immune complexes or more severe inflammation based on antigen-specific CMIR. Although these experiments confirm that selection for H and L immune response can alter resistance to infectious disease, further study is required to precisely determine disease resistance to a **variety** of pathogens. Previously, mice selected for H and L Ab or CMI showed changes in disease resistance which were consistent with the expected roles of either Ab or cellular immunity, but not both (Biozzi *et al.* 1984. In experiments which successfully selected quails for neutralizing Ab against Newcastle disease virus (Takahashi *et al.* 1993) and chickens for Ab response to *Escherichia coli* (Leitner *et al.* 1989), animals also exhibited increased susceptibility to *Salmonella sp.* or NDV, respectively. Given the dual protective and inflammatory functions of Ab and CMI, uniform enhancement of disease resistance in high immune response lines may be **difficult** to achieve.

Since porcine cytokine production has shown genetic variation (Edfors-Lilja *et al.* 1991; Jordan *et al.* 1995) and because the increased arthritis in the H line pigs may have resulted from the pattern of cytokines released, ongoing investigations are focused on evaluation of cytokines and cytokine binding proteins. In one study chemokine binding proteins were detected using ¹²⁵I-labelled human recombinant C-C chemokines (RANTES and MIP-1B) and a C-X-C chemokine (IL-8) incubated with sera from H and L pigs pre and post mycoplasma challenge. These chemokines preferentially bound to a 130 kDa protein. Substantially higher amounts of this chemokine binding protein were detected in H line pigs after infection with *M. hyorhinitis* (Banga 1997). This increase corresponded with an increased induction of haptoglobin in the H line. It is possible that the chemokine binding **differences** are the result of an increased acute phase response in the H line pigs and that the 130 kDa protein is an inducible regulator of certain cytokines. Following *M. hyorhinitis* infection, cells from arthritic joints of H line pigs expressed more IFN-gamma and IL-6 than L line pigs, as determined using quantitative RT-PCR (Reddy *et al.* 1996). This may contribute to the line-related differences in arthritis.

Table 3. Lsmeans of days to 100 kg of Yorkshire pigs selected for high and low immune response							
Line	Generation of Selection						
Guelph SPF Facility	1	2	3	4	5	6	7
High	144.00	163.02	156.16	-	155.55	147.87	161.87
Control	147.43	-	168.18	-	176.85	160.70	172.92
Low	153.43	159.83	170.48	-	166.11	159.13	178.66

Production parameters. From G0 to G7 of selection the H line pigs showed consistently higher rate of gain than L or C pigs. In fact, H line pigs reach market weight at least 10 days sooner than L and C line pigs (Table 3). Following *M. hyorhinis* infection pigs from H and L lines had comparable weight loss (Magnusson et al. 1997b). When pigs from G5 and G6 were moved to satellite herds with high incidence of disease, some pigs from all lines suffered morbidity and mortality, but H line pigs maintained increased rate of growth. On commercial site I growth rate was monitored between 18-24 weeks of age and H line pigs had higher ($p < 0.001$) weight gain based on linear regression of weight values over time. On commercial site II, growth rate was determined from weekly weight recording and age to 100 kg was predicted using a standardized quadratic formula to be 238 \pm 11 days, 275 \pm 14 days, and 244 \pm 12 days for the H, C, and L lines respectively. Theoretically, the growth advantage of the H line may reflect a lower incidence of subclinical disease, thereby helping to maintain appetite and preventing energy diversion to other body systems. It is also possible that selection for H and L immune response had indirect effects on gene(s) which regulate growth. Alternatively, founder gene(s) may have played a role. No differences in carcass traits, including backfat, were noted. Although H line sows had some advantages in terms of live piglets/litter, % of litters with 3 or less piglets, and number of deformed piglets these line differences were inconsistent (Wilkie *et al.* 1997).

CONCLUSIONS

Data from 8 generations of selection for H and L immune response using EBVs in a multi-trait selection index indicate that it is possible to separate pigs into breeding lines based on immune response and that this can have effects on infectious disease outcomes. Whether or not H line pigs exhibit broad-based resistance to disease is still not clear. The fact that an unweighted additive index of Ab and CMI, with no individual threshold values, was utilized through out this selection, likely had effects on response to selection and disease resistance. For instance, a pig could be classified as H and utilized for further selection based on approximately four equal EBV measurements or because one or two of the individual traits were of extreme values. This may have resulted in inconsistent immune responses of pigs within each line. When selection is based on an unweighted index of standardized EBVs, response is optimum if the traits are under additive genetic control, population parameters are known without error and, in this case, if the index weights are those that would maximize overall immune responsiveness. In practice, none of these assumptions may hold. Phenotypic variances of the different index traits fluctuated across generations and EBVs in the index were standardized to the average standard deviation across generations. Although superior to unstandardized EBVs, this undoubtedly led to substantial variation in relative weighting on the four traits across generations. If such fluctuations in variances remain a feature of the tests for immune response, it may be preferable to practice a form of independent culling across the four traits, rather than an unweighted selection index.

Whether the four traits chosen for use in the selection are optimal to obtain maximum benefits for swine health, and whether all four traits are actually required to make these changes in Ab and CMI, is not known. Traits were chosen based on moderate to high heritabilities, correlation coefficients, and a general understanding of the roles of Ab and CMI in host resistance. High line pigs consistently grew faster than L and C line pigs. The economic benefit from this increased productivity has been

estimated at Cdn \$3-10 per slaughter pig. In a national market of 10 million pigs this would translate to a minimum increase of \$30 million. Lines of pigs genetically selected for H and L immune response also provide a tool to investigate mechanisms of host resistance and a means to identify genes or QTL involved in disease resistance and immune response.

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