IMUNNORESPONSIVENESS IN JAPANESE QUAIL AND CHICKENS

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Poultry Science

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June, 1991

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(ABSTRACT)

The association between selection for body weight and immunoresponsiveness was studied in Japanese quail and White Plymouth Rock chicken populations. Quail populations consisted of a randombred control (C) line and a line selected for high (HW) 28-day body weight. The chicken populations used were lines selected for high (HW) and low (LW) 56-day body weight, reciprocal F_1 crosses (HL and LH), and F_2 crosses of the F_1 (HLHL and LHLH).

Kinetics of primary and secondary antibody response to SRBC antigen was examined in Line C quail (Experiment 1). At most times post-primary inoculation (PPI), antibody titers were highest for antigen concentration 2.5%. Presence of MER antibodies was very low PPI, but increased following reinjection. Primary antibody response was then compared between C line and HW line of quail (Experiment 2). Antigen concentration 2.5% once again resulted in the highest titers. Line HW quail were less able to maintain high antibody titer levels to SRBC antigen than the randombred control line from which they originated.

Mode of inheritance for immunoresponsiveness in selected populations of chickens and crosses between them was examined (Experiment 3). Additive genetic variation was important in the inheritance of both

primary and secondary response to this antigen. Reciprocal differences and heterosis of the F_1 crosses were also factors in the inheritance of secondary response. Kinetics of primary and secondary responses were evaluated in the parental weight lines and in lines of White Leghorn chickens divergently selected for antibody response to SRBC antigen. At all times PPI, line HA chickens had the highest antibody titers, while those from line LA consistently had the lowest titers. Lines HW and LW reacted similarly to line LA early in response, but showed higher peak levels later on. In both primary and secondary responses, the weight lines peaked at similar levels. Thereafter, line LW maintained a hgih antibody titer level to SRBC antigen than line HW.

ACKNOWLEDGEMENTS

The author extends special appreciation to Dr. E. A. Dunnington for her support and understanding throughout my studies at Virginia Tech. I would also like to thank the other members of my graduate committee, Dr. P. B. Siegel, Dr. H. L. Marks, and Dr. J. H. Wolford for their support and friendship.

Additional appreciation goes to Neil O'Sullivan for computer consulting, advice, and overall help. I would also like to thank Amy Larsen and Don Noble for all their wonderful and cheerful assistance in data collection. Sincere appreciation to Dr. Gross, Dr. Larsen, and Opal Price for all their assistance with assays. Thanks also go to Charlie Orange and the whole farm crew for all their help and friendship during the past two years. My final thanks goes to my family for their unfailing support and encouragement.

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INTRODUCTION

Disease is an important factor in poultry production costs. Gavora and Spencer (1983) estimated economic losses from disease at 12% of the value of poultry products. Such economic losses result from mortality and overt sickness as well as subclinical problems which cause reduction in performance. Expenses associated with diseases would be lessened if the overall morbidity and mortality of the birds were reduced without adversely affecting production ability.

Methods of circumventing expenses incurred by poultry producers due to disease have been proposed, including selection for general disease resistance and for pathogen-specific disease resistance. Selection for general disease resistance, as championed by Hutt (1958), has been shown to be impractical since selection for resistance to one particular antigen type usually results in increased susceptibility to another type of antigen. Similarly, Gross et al. (1980) showed that chickens which have been selected for strong immune response to a foreign antigen, sheep erythrocytes (SRBC), had reduced defense against bacteria. Selection for resistance to a specific pathogen can be successful, as with Marek's disease (Gavora and Spencer, 1983), but generally does not confer resistance to other pathogens. Often the selection renders individuals more susceptible to other pathogens.

Selection for increased rate of growth is accompanied by many correlated responses, some of which are detrimental. A negative correlation between growth and immunoresponsiveness (SRBC antibody titer)

has been demonstrated in several experimental selected lines of chickens (Siegel and Gross, 1980; Siegel et al., 1982; Dunnington and Siegel, 1984) as well as in commercial broilers (Siegel et al., 1984; 1989).

The phenomena described above, such as general verses specific disease resistance, antibody verses bacterial defense, or growth verses immunoresponsiveness, indicate that a living organism must allocate its available resources to satisfy differing demands. Growth, maintenance, reproduction, and disease resistance all claim resources which must be provided by the organism throughout life. At any particular time, the necessity to accommodate one of these demands may render the organism vulnerable to other problems, due to preferential resource allocation.

The research presented in this thesis was designed to further the knowledge regarding immunoresponsiveness in Japanese quail and chickens.

LITERATURE REVIEW

RESOURCE ALLOCATION

A developing organism has a limited pool of resources which must be allocated to growth, maintenance, immune response, reproduction, and other demands (Siegel et al., 1982; Dunnington, 1990). Natural selection often favors individuals with intermediate values for traits, giving them optimum fitness for a changing environment (Haldane, 1954; Siegel and Dunnington, 1987; Dunnington et al., 1989). When selection is made in favor of any particular component of development, it reduces resources available for the other components. Resources will be redistributed according to the greatest pressures or demands being placed on the organism (Dunnington, 1990). In the broiler industry, chickens are selected with intense pressure for reaching market weight at an early age. Many effects are correlated with this particular characteristic. One negative correlation with selection for body weight in poultry is reduced immunoresponsiveness. High weight (HW) and low weight (LW) lines of chickens when injected with sheep red blood cell (SRBC) antigen showed antibody responses which were similar to those found in an unrelated line which had been selected for low antibody response to the antigen (Dunnington and Siegel, 1986). Martin et al. (1988) observed that when dosage concentration of SRBC antigen was at either lower or intermediate levels, LW chicks responded with higher antibody titers than HW chicks. This difference in response of the divergently selected weight line birds

is consistent with results previously recorded (Reddy et al., 1975; Mauldin et al., 1978; Marsteller et al., 1980).

Chickens selected for strength of antibody titer to SRBC antigen also show the negative correlation between immune response and growth. Martin et al. (1988) found that mean 56-day body weight was significantly higher for chicks selected for low antibody response to SRBC antigen (LA) than for chicks selected for high antibody response to SRBC antigen (HA). Thus, the increase in immune response results in a significant decrease in early growth. Siegel et al. (1982) and van der Zijpp et al. (1988) also discuss the negative correlation between the traits of immune response and body weight in chickens.

IMMUNORESPONSIVENESS

Antigens are substances that catalyze B and T lymphocytes into specific responses. The initial exposure to an antigen will prime an individual so that, when it is re-exposed to the antigen, it can produce a secondary response more quickly. The secondary response differs in both quantity and quality from the primary response. Sant'Anna et al. (1979) concluded that, in mice immunized with Salmonella antigens, a smaller number of genes regulated the secondary response compared to the primary response. Thus, the primary response may be genetically influenced to a greater degree than the secondary response. van der Zijpp et al. (1983) found that in chickens, the secondary response included a higher peak titer, earlier peak postinjection, and larger IgG production than the

primary response. The SRBC antigen triggered both IgG and IgM production when injected.

Mercaptoethanol-resistant (MER) antibodies are often used as a measure of IgG production response in chickens and ME-sensitive (MES) titers are made up primarily of IgM (Delhanty and Solomon, 1966). IgG which is the dominant antibody in most antisera differs from IgM in its ability to neutralize viral infectivity, in its bacterial immunity, in its interactions with other immune components, and in its serological reactions (Barrett, 1983). Genetic differences in MER antibody production response to SRBC antigen have been reported in chickens (Yamamoto and Glick, 1982; van der Zijpp, 1983b). Kreukniet and van der Zijpp (1990) found that, in lines of chickens selected for high or low antibody to SRBC, ME-resistant titers followed the dose level in the secondary response.

GENETIC VARIATION

Genetic variation in antibody-producing ability influences both resistance to infection and response to noninfectious antigens (Biozzi et al., 1979; Siegel and Gross, 1980; van der Zijpp, 1983a). With selection for high antibody response to a specific antigen, disease resistance to similar antigens can be improved (Gross et al., 1980). Improvement of resistance is limited to the class of antigen, rather than overall disease resistance. Biozzi et al. (1982) found that ability to produce antibody to SRBC antigen was controlled by multiple genes and

could be correlated with antibody response to several other antigens or to disease resistance.

Antibody titers to sheep erythrocytes have been utilized as a measure of immune response and general disease resistance (van der Zijpp, 1983a). Siegel and Gross (1980) and Gross et al. (1980) used the response to SRBC to develop lines of chickens which differ in the production and persistence of antibodies. In the lines exhibiting divergent response in antibody production, strong immune response to SRBC antigen was accompanied by reduced ability to protect against bacterial infection. Reduced growth was also associated with an increased immune defense against SRBC antigen. The non-persistent selected line generally had a poor response to all the antigens tested (Gross et al., 1980).

NEGATIVE CORRELATIONS

Reduced growth is associated with relatively high immune response to SRBC antigen (Siegel and Gross, 1980). It is thought that resources which would have been channeled into growth are used instead for immune response. Gross and Siegel (1988) reported that the high antibody (HA) chickens used in their experiment had lower body weight and poorer feed efficiency than the low antibody (LA) birds. Commercial broilers responded to SRBC antigen in much the same manner as the LA birds (Siegel et al., 1984; 1989). A negative genetic relationship exists between high antibody response and heavy 4-week body weight (Siegel and Gross, 1980). Chickens selected for high body weight have antibody responses to SRBC

which are similar to that of those which have been selected for low antibody response (Dunnington and Siegel, 1986).

EXTERNAL FACTORS

Many external factors may affect antibody titers. Dosage levels can have a considerable impact on subsequent immune response to the antigen. Martin et al. (1988) found that differences in SRBC response between the weight-selected lines were dose-dependent. The LW line had higher titers than the HW line at lower and intermediate doses. At higher doses of antigen there were no line differences. This finding agrees with the theory that genetic differences in antibody response are masked by either too high or too low a dose of antigen (van der Zijpp, 1983b; Ubosi et al., 1985b). Kreukniet and van der Zijpp (1990) stated that, in their line of chickens selected for high antibody response to SRBC, primary response followed level of SRBC dose. Secondary response in line H, however, showed an inverse response sequence. Their line selected for low antibody response exhibited no pattern in total antibody level. Route of administration also influences immune response to SRBC antigen (van der Zijpp et al., 1986) with intravenous injections resulting in higher titers than intramuscular injections at all days postimmunization.

Age of the bird at injection also influences immune response. Ubosi et al. (1985a) found that lines of chickens which were selected for antibody response to SRBC reached serological maturity by 14 days of age. Peak SRBC antibody response occurs between 3 and 6 months of age and declines thereafter in chickens (McCorkle and Glick, 1980). van der Zijpp

and Leenstra (1980) found sex differences and hatch effects in the humoral immune response which complicated their results in chicks at 40 days of age. It is thought that the chicks used in their study had not yet achieved immunological maturity at the age which they were tested. Other researchers have not noted significant sexual effects when examining humoral immune response (Siegel and Gross, 1980; Siegel et al., 1982; Martin et al., 1988).

Immunological responses can be affected by physical and behavioral stressors. Degree of immunodepression found in a stressful situation seems to be related to the perceived change to the environment (Gross and Siegel, 1981). Exposure to high environmental temperatures can cause suppressed antibody responses in chickens (Thaxton et al., 1968; Thaxton and Siegel, 1970; 1972). Gross and Siegel (1980) found that when physical stressors such as heat, chilling, or overheating are endured early in life, immune responses of HA and LA chickens are affected for at least 18 weeks. HA line chickens had reduced antibody titers, while those from the LA line showed an increase in titers after experiencing the stressor. However, in other lines studied, intermittent heat or cold stress had little or no effect on antibody production (Regnier et al., 1980; Donker et al., 1990). Treatment of chickens with adrenocorticotropic hormone (ACTH) also acts to reduce their ability to produce antibodies against SRBC antigen (Thaxton et al., 1968). Reduction was shown to be greatest when ACTH was injected 21 to 24 hours before the SRBC antigen was administered.

Social stress can have a large effect on the ability of chickens to mount an immunological response to infectious diseases. Chickens in a high stress environment show an increased resistance to bacterial infections and decreased antibody response to viral infections when compared to those maintained in less stressful situation (Gross and Colomano, 1969; Gross and Siegel, 1982). It is thought that the establishment of some degree of stress in the environment may act to increase a bird's ability to resist bacterial infection (Siegel, 1980; Gross et al., 1984; Siegel, 1984; Siegel, 1985; Katanbaf et al., 1987).

Socialization seems to reduce the amount of stress perceived by birds in the presence of human handlers. An improvement in environmental conditions seems to be followed by a reduction in the amount of resources needed for response (Gross and Siegel, 1982). These additional resources could be rerouted to other demands such as maintenance, growth, immune response, or reproduction. Gross and Siegel (1982) found adapted, or socialized, chickens had a greater antibody response to antigen than unadapted ones (Gross and Siegel, 1979). When fasting was added as an additional stressor, the adapted chickens had similar titers in both fasted and non-fasted groups. Unadapted fasted birds, however, had lower titers than unadapted fed ones. It is thought that the handling received by an individual may greatly affect subsequent antibody responses. Socialization increased antibody response to red blood cell antigens and resistance to E. coli infections in chickens, whereas the "hasseling" of them resulted in responses similar to those observed in stressed birds (Gross and Siegel, 1982).

QUAIL vs CHICKENS

One part of this thesis involves a population of Japanese quail that were selected for high (HW) 4-week body weight for 22 generations as well as a random-bred control (C) population (Darden and Marks, 1988). These lines of Japanese quail, because they have the genetic predisposition to grow rapidly (line H) or at an intermediate rate (line C), provide an animal model for the study of resource allocation in terms of growth and immunoresponsiveness.

Japanese quail are often used as an avian model for chickens. Selection can be performed on Japanese quail populations and the results observed relatively quickly because of their shorter reproductive cycle where individuals are sexually mature by 6 to 7 weeks of age. Three generations of selection can easily be observed in the same period of time needed for one generation of selection in chickens. Quail also reach a much smaller size at maturity than chickens which reduces the amount of space and feed necessary during experimentation (Porter and Terrill, 1986).

CHAPTER 1

IMMUNORESPONSIVENESS TO SHEEP ERYTHROCYTES IN SELECTED AND UNSELECTED LINES OF JAPANESE QUAIL

INTRODUCTION

Organisms are limited in resources available during development (Siegel et al., 1982; Dunnington, 1990). These resources must be divided among demands such as growth, immune response, reproduction, and maintenance. Not surprisingly, natural selection favors individuals with intermediate values for many traits. These individuals show greater adaptability in a changing environment (e.g., Haldane, 1954; Siegel and Dunnington, 1987; Dunnington et al., 1989). If selection favors any component of development, it may reduce resources available for other demands. This type of imbalance can result in a negative correlation between the selected trait and an unselected trait which may affect fitness (Rendel, 1963). For example, a negative correlation between growth and immunoresponsiveness as measured by antibody response to sheep erythrocytes has been demonstrated in several experimental lines of chickens (Marsteller et al., 1980; Siegel et al., 1982; van der Zijpp et al., 1988) as well as in commercial broilers (Siegel et al., 1984; 1989).

The objectives of this study were to examine the kinetics of immune response to sheep erythrocytes in a randombred population of Japanese quail and to examine the relationship between immunoresponsiveness and selection for high body weight in this species.

MATERIALS AND METHODS

Japanese quail used in this experiment were from a line selected (22 generations) for high 4-week body weight (HW) and the randombred control line (C) which was the base population for the selected line (Darden and Marks, 1988). At hatch chicks were toe clipped and placed in chick brooders with continuous lighting. At 3 weeks of age, quail were sexed according to breast plumage color, wingbanded, and transferred to colony cages where they remained as sex-separated flocks until the conclusion of each experiment. Feed and water were provided ad libitum throughout the experiment.

Ten cc of blood were collected from one sheep and transferred to a tube containing 20 drops of anticoagulant (Sequester-Sol). The blood was then centrifuged to allow separation of plasma and red blood cells (SRBC). Once the plasma was drawn off, physiological saline was used to wash the cells 3 times, saline was removed, and SRBC concentrations were made. Injections of antigen and bleedings of quail were via the jugular vein. During bleedings, a blood sample of 0.5 ml was drawn, transferred to a tube containing 2 drops of anticoagulant (Sequester-Sol), and refrigerated to allow the quail red blood cells (QRBC's) to settle. If sedimentation was not complete, samples were centrifuged to separate plasma and erythrocytes.

Antibody titers were determined by the microtiter method of Wegmann and Smithies (1966). Each well of a 96-well titer plate was filled with 25 ul of physiological saline. Plasma from each sample (25 ul) was added

to the first well of each row on the titer plate. Then, half (25 ul) of the solution from the first well, where the blood plasma was added, was transferred to the next well and mixed. The half dilution of the previous well was repeated for the entire row. Finally, 25 ul of sheep blood (0.75% suspension) was added to all wells. Titer plates were then incubated at 37 C for 3 to 5 hours. Antibody titers were the $\log_2$ of reciprocal of the last dilution in which there was agglutination (Wegmann and Smithies, 1966).

Antibodies resistant and sensitive to 2-mercaptoethanol (2-ME) were also determined by microtiter methods (Delhanty and Solomon, 1966). Twenty-five ul of plasma and an equal amount of 0.15 M 2-ME were mixed and diluted in plastic titer plates. Each well in all plates contained 25 ul of normal saline prior to the addition of plasma and 2-ME. Titer plates were then incubated at 37 C for 1 hour. After incubation, 25 ul of 0.75% suspension of SRBC was added to each well. Plates were then incubated for an additional 3 to 5 hours. Antibody titers were expressed as $\log_2$ of reciprocal of the last dilution in which there was agglutination (Wegmann and Smithies, 1966). These titers were recorded as 2-ME resistant antibody (IgG) and differences from the SRBC total titers were recorded as the 2-ME sensitive antibody (IgM).

Trial One.

A kinetics study was conducted to determine the immunological response to SRBC for the randombred control population. Quail were randomly assigned to one of three groups, each containing 84 individuals.

At 28 days of age, quail in each group were inoculated with 0.1 ml of either 0.025%, 0.25%, or 2.50% suspension of SRBC antigen. On the same day, prior to inoculation, 12 quail were bled to determine a base titer level for the line. Antibody determinations were made on 12 quail from each dosage on alternate days from day 3 to 13 following inoculation. No individual was bled more than once during this period.

On day 17 post-primary injection (PPI), half of the quail from each dosage group used in the previous section (n = 126) were injected with 0.1 ml of the 2.50% concentration of SRBC antigen. This concentration was chosen because of its tendency to result in a higher antibody titer during the measurement of the primary response. Blood was then collected from both the boostered and non-boostered quail on alternate days from day 19 to 27 and on day 31 PPI. The sample at each bleeding consisted of 6 quail per line and reinjection status with no individual bled more than once.

Trial Two.

Ninety-four HW and 64 C line quail were randomized within line into two groups and injected at 28 days of age with 0.1 ml of either 0.25% or 2.50% suspension of SRBC. Eight individuals from each line were bled prior to injection to obtain base titer levels for the populations. On days 4, 7, 10, and 13 PPI blood samples were obtained from 12 HW and 8 C line quail. No individual was bled more than once during this period.

Statistical Analyses.

All titers were transformed to the square root of the $\log_2$ before analyses were performed using the general linear model (GLM) procedure (SAS Institute, 1985). When significant differences were found for main effects having more than two means, Duncan's multiple range test was conducted for comparisons among means.

Analysis of variance for Trial 1 was performed within sampling day with dosage concentration (0.025, 0.25, and 2.5) as the main effect in the model. Because sexual dimorphism in antibody titers was observed for only one sampling, sexes were pooled. In Trial 2, analysis was performed within sampling day with line (HW and C) and dosage concentration (0.25 and 2.5) as main effects. Interactions between the main effects were also tested.

RESULTS

Trial One.

At most times PPI, total antibody titers to SRBC were higher for antigen concentration of 2.50% than 0.25% and 0.025% (Figure 1). Similarly, the titers for the 0.025% concentration were consistently lowest. The kinetics of the PPI response was similar for all 3 dosages with peak titers observed on day 7. Presence of MER antibodies PPI was very low throughout (Figure 2). Some MER was present at days 7 and 9 at the two higher concentrations (0.25% and 2.50%) and also at days 11 and 13 at the highest concentration (2.50%). Because of the low values for MER, results for MES were essentially the same as those for total antibody.

Antibody titers increased dramatically following reinjection of SRBC's on day 17 (Figure 1). Concentrations of SRBC used in the primary inoculation influenced secondary responses with separation among titer means for the primary injection concentrations present on day 25 (Figure 1). Comparisons among concentrations showed, once again, highest titers at the 2.50% dose. Levels of MER also increased after reinjection with peak PSI on day 6 (day 23 PPI). The percentage of MER antibodies contributing to total titer values was substantially more for the 0.25% and 2.50% dosages than the lower one (Figure 2).

Trial Two.

Although interactions between line and dose were not significant, line and dose effects are present by separation in Figure 3. Differences between lines at concentration of 2.50% SRBC were attributed to a lack of persistence in antibody titers in line HW with low values noted by 10 days after injection. Differences between dosages of antigen were present in line HW but not in line C. Also, a higher concentration of SRBC antigen was needed for the initiation of high levels of antibody response in HW than in C quail. Significant amounts of MER were not present, therefore, analysis of variance for MES antibodies yielded the same results as that for total SRBC titer.

DISCUSSION

Response of Japanese quail to an intravenous injection of SRBC differed in some respects from that observed in chickens. Dosage concentrations which mask genetic differences in chickens (van der Zijpp, 1983b; Ubosi et al., 1985b) are necessary to elicit an immune response in Japanese quail. Apparently quail are able to tolerate large amounts of foreign antigen (SRBC) without triggering a large humoral response.

The different pattern of MER observed between primary and secondary immune responses in quail is consistent with that reported for chickens (van der Zijpp et al., 1983; Ubosi et al., 1985b). Although large increases in MER proportions were seen after reinjection, results should be regarded with caution because the literature is inconsistent concerning the proportion of total titer which is due to the IgG antibodies (Yamamoto and Glick, 1982; van der Zijpp, 1983b; van der Zijpp, et al., 1983).

The inability of the HW line quail to maintain high antibody levels to SRBC antigen when compared to randombred control line from which they originated is consistent with the negative correlation between growth and immune response reported for chickens (Siegel et al., 1982; Martin et al., 1988; van der Zijpp et al., 1988). These results support the thesis that through selection, resources may be redirected to enhance responses for the selected trait (Dunnington, 1990). This redirection has a cost in that emphasis for other important functions is reduced.

SUMMARY

Kinetics of primary and secondary immune responses were evaluated in a randombred control (C) line of Japanese quail. Primary responses for line C were also compared with those from a line selected for high (HW) 4-week body weight which originated from line C. In the kinetics study, independent sampling of quail was made on alternate days from day 3 to 13, 19 to 27, and day 31 post-primary inoculation (PPI). Half of the quail sampled from day 19 on received an additional injection of SRBC antigen. Samplings for comparisons between line C and line HW were obtained on days 4, 7, 10, and 13 PPI. Plasma from each blood sample was examined for total, mercaptoethanol resistant (MER), and mercaptoethanol sensitive (MES) titers. At most times PPI, antibody titers were highest for antigen concentration 2.50%. Presence of MER antibodies was very low PPI, but increased following reinjection. Persistence of antibody to the 2.50% SRBC antigen was less in line HW than in line C from which line HW originated.

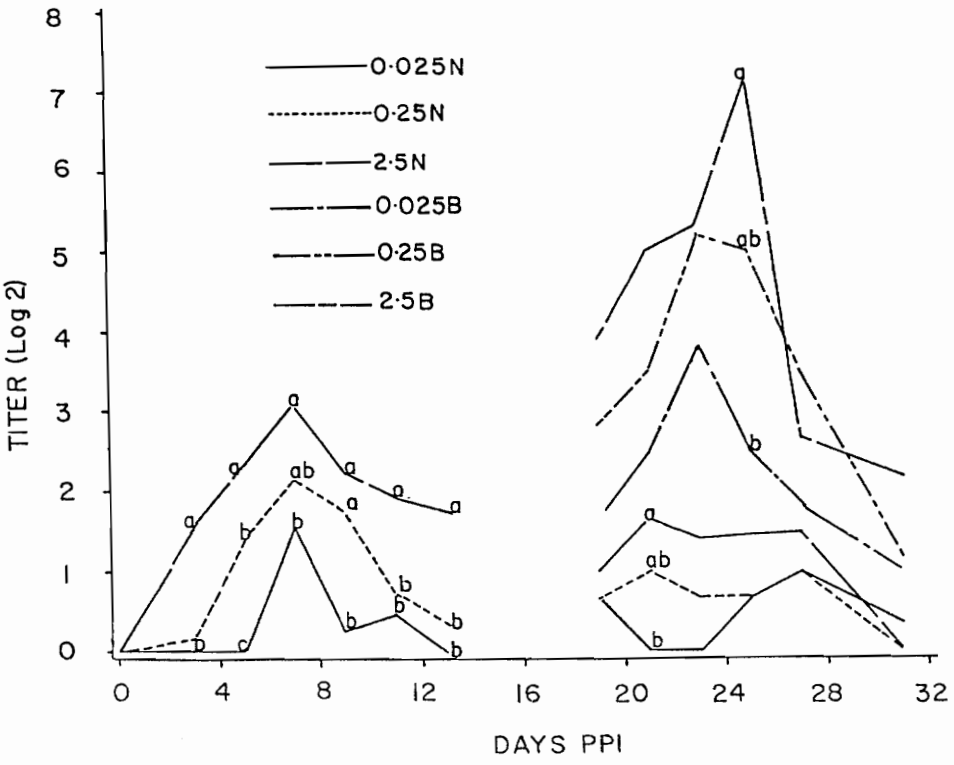

Figure 1. Primary and secondary antibody titer means with differing concentrations of SRBC antigen in control line quail (a b c, the different letters indicate differences among means within an age $P \leq .05$). For secondary responses, each group of birds was divided and half received a .1 ml booster of 2.5% SRBC (B) while the others were not injected (N).

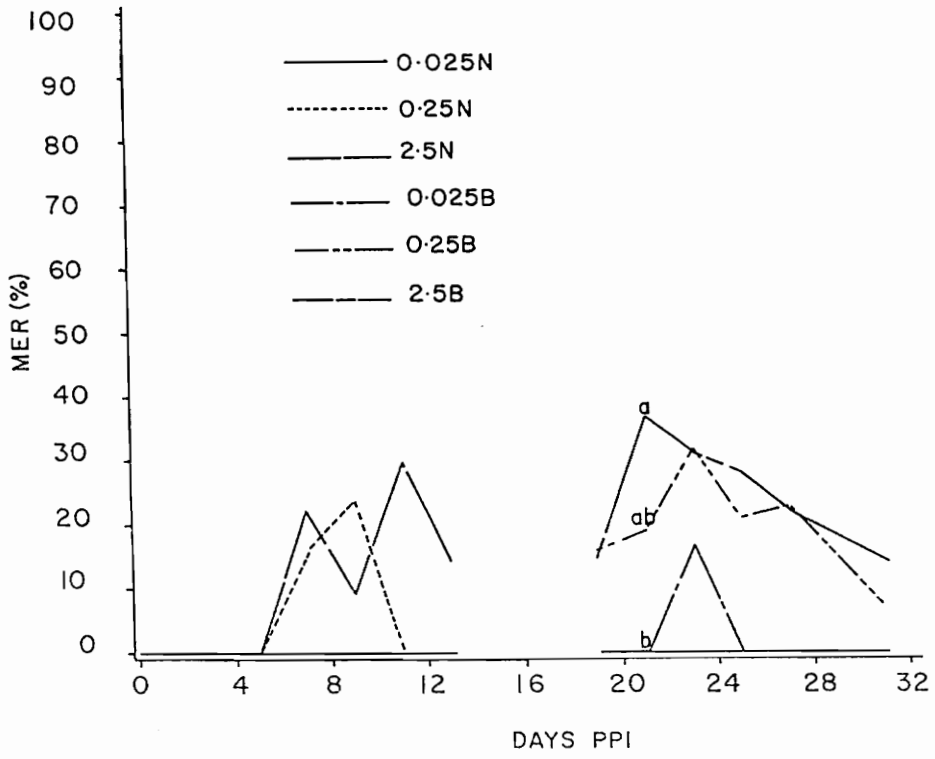

Figure 2. Primary and secondary MER resistant titers as a percentage of total antibody titer in boostered (B) and non-boostered (N) control line quail with differing primary concentrations of SRBC antigen (a b c, the different letters indicate differences among means within an age $P \leq .05$). For secondary responses, each group of birds was divided and half received a .1 ml booster of 2.5% SRBC (B) while the others were not injected (N).

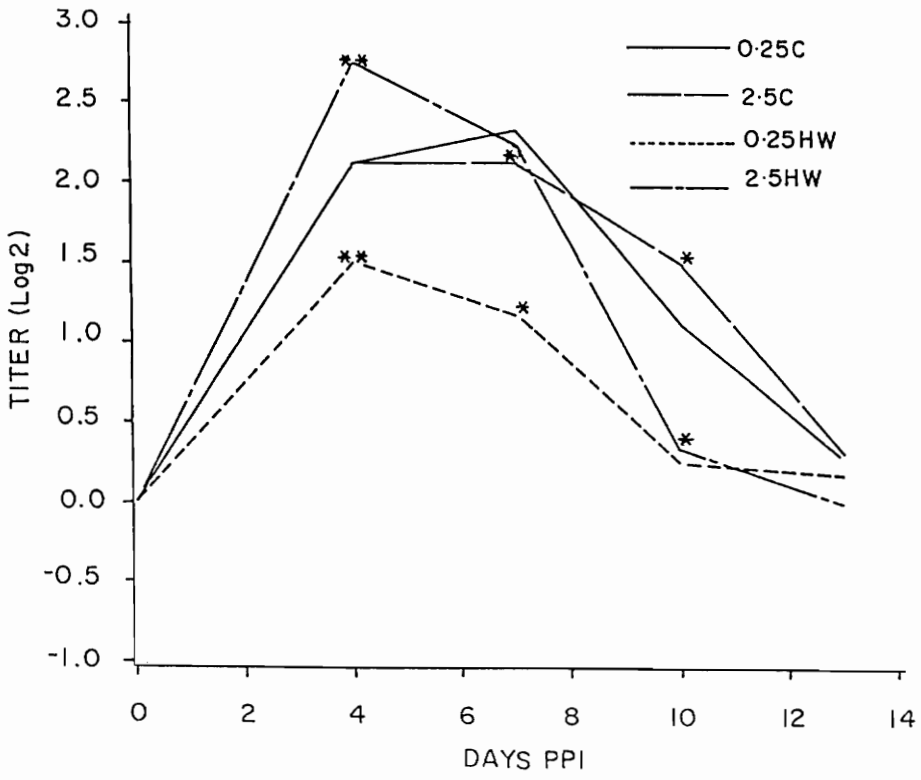

Figure 3. Primary antibody titer means in high (HW) and control (C) line quail in response to varying concentrations of SRBC antigen, ** and * denote significant differences among means within an age ($P \leq .01$) and ($P \leq .05$), respectively.

CHAPTER 2

INHERITANCE OF IMMUNORESPONSIVENESS TO SHEEP ERYTHROCYTES IN LINES OF CHICKENS DIVERGENTLY SELECTED FOR 56-DAY BODY WEIGHT AND THEIR CROSSES

INTRODUCTION

Throughout its life, an organism experiences allocation of its available resources. This finite pool of available resources must be divided among various demands which are made upon the individual. These demands include growth, immune response, reproduction, and maintenance (Siegel et al., 1982; Dunnington, 1990). Natural selection favors those individuals with intermediate values for many traits. Such individuals tend to exhibit a greater degree of adaptability in an unstable or changing environment (Haldane, 1954; Siegel and Dunnington, 1987; Dunnington et al., 1989; Falconer, 1989). Selection for a particular component of development may decrease the availability of resources to satisfy other demands. Redistribution of resources will occur according to the strongest demands or pressures experienced by the organism (Dunnington, 1990).

A negative correlation which may affect fitness can develop between the selected trait and a correlated trait due to a resource imbalance (Rendel, 1963). For example, the negative correlation between growth and immunoresponsiveness to sheep red blood cells (SRBC) has been demonstrated in several experimental lines of chickens (Marsteller et al., 1980; Siegel et al., 1982; van der Zijpp et al., 1988) as well as in commercial broilers (Siegel et al., 1984; 1989).

Selection and crossing are procedures which may be used in genetic analyses of quantitatively inherited traits. Comparisons of crosses with their parental lines result in clarification of the mode of inheritance

of a particular trait. The use of first (F_1) generation and second (F_2) generation crosses allows for a check on the presence of heterosis and recombination. The objective of this study was to determine the principal mode of inheritance for immunoresponsiveness in selected populations of chickens and their crosses as measured by antibody response to SRBC.

MATERIALS AND METHODS

Chickens from eight populations were used in this experiment. These populations consisted of lines which had undergone divergent selection for juvenile body weight (Siegel, 1962; Dunnington and Siegel, 1985) and lines which had been divergently selected for response to SRBC antigen (Siegel and Gross, 1980). White Plymouth Rocks which had undergone (33 generations) long-term selection for high (HW) and low (LW) 56-day body weight provided the pure parental lines. Crosses HL and LH were their reciprocal F_1 progeny (sire line designated first, dam line second), and crosses HLHL and LHLH were the F_2 progeny produced by mating the F_1 offspring. Lines HA and LA were White Leghorns which had undergone (17 generations) selection for high and for low antibody response to SRBC antigen. The antibody lines served as reference populations for the antibody responses of the weight populations. All the chickens used in the experiment were age contemporaries.

At hatch, chicks were wingbanded, vaccinated for Marek's disease, and housed in 3 electrically heated brooder batteries with continuous lighting. Each pen contained chicks of both sexes but only one line or cross. At 28 days of age, chicks were moved to developer batteries where they remained until the conclusion of the experiment. Feed and water were provided ad libitum throughout the experiment. A diet consisting of 20% crude protein and 2685 Kcal/kg was fed throughout.

Eight individuals from each population were bled at 34 days of age to determine base titer levels for their population. Then, chickens were

injected intravenously with 0.1 ml of a 0.25% suspension of SRBC antigen via the brachial vein. Blood samples were collected at 3, 4, 5, 6, 7, 10, and 14 days post-primary injection (PPI). Antibody determinations were made on 8 chickens from each line at each sampling. No individual was bled more than once during this period of time. During bleedings, a sample of 0.5 ml of blood was drawn, transferred to a tube containing 2 drops of anticoagulant (Sequester-Sol), and refrigerated to allow the erythrocytes to settle. If sedimentation was not complete, samples were centrifuged to separate plasma and erythrocytes. Antibody titers were determined by microtiter method (Wegmann and Smithies, 1966) and expressed as the $\log_2$ of reciprocal of the last dilution in which there was agglutination (Wegmann and Smithies, 1966).

Secondary intravenous inoculations were given 21 days PPI to half of the birds from each population. Chicks that received the concentration of 0.1 ml of 0.25% suspension of SRBC antigen were selected at random. For each population, 8 chicks from the boostered group and 6 from the non-boostered group were sampled on days 24, 26, 28, and 31 PPI. No individual was bled more than once during this period.

Antibodies resistant and sensitive to 2-mercaptoethanol (2-ME) were also determined by microtiter methods (Delhanty and Solomom, 1966). These methods have been described in Chapter 1.

Statistical Analyses.

Analyses of variance were conducted, within sampling day, among the parental antibody lines (HA, LA) and parental weight line (HW, LW) to compare the selected lines for total, MER, and MES titers, as well as % MER for both primary and secondary immune responses. All titers were transformed to the square root of the $\log_2$ before analyses were conducted. Titers were analyzed using the general linear model (GLM) procedure (SAS Institute, 1985). When the line effect was significant, Duncan's multiple range test was conducted to determine statistical differences among means.

Nonorthogonal contrasts (Table 1) were made among the parental, reciprocal F_1, and F_2 weight populations (SAS Institute, 1985) to determine the adequacy of an additive-dominance model of inheritance. The genetical inferences used were based upon Scheffe's nonorthogonal linear contrasts (Scheffe, 1970).

Data resulting from the assays performed were subjected to more than one statistical test. Each test was based on a 0.05 alpha rejection region. Reuse of the data increased probability of an occurrence of a Type 1 error to greater than the alpha level.

RESULTS

After day 3 PPI antibody responses of weight lines were bracketed by the responses recorded for those lines which had been selected for either high or for low antibody response to SRBC antigen (Figures 1 and 2). Because no sexual dimorphism was observed in antibody production, sexes were pooled. Kinetics of the PPI response differed among lines with peak titers on days 6 and 7 post-injection. Line LW chicks had higher antibody responses at day 5 PPI than those of line HW. Presence of MER antibodies during the primary response occurred only in the HA line. Because of this, results for analyses of MES titers were the same as those for total antibody titers.

Antibody titers increased following reinjection of SRBC antigen on day 21 PPI (Figure 3), except in Line HA where peaks for both primary and secondary response occurred at the same level. Persistence of high antibody levels was greatest in this line (Figure 3). In most lines peak titer levels occurred on day 5 post-secondary injection (PSI) i.e., day 26 PPI. Line HW exhibited low persistence of antibody levels following peak titers. These results were similar to those discussed previously for Japanese quail. MER percentages were also highest on day 5 PSI (Figure 4). Only line HA exhibited an ability to maintain a high MER proportion in subsequent antibody levels.

Differences were found, between the weight lines and their crosses, within sampling day (Table 2). These differences occurred mainly between lines HW and LW throughout the primary response. The reciprocal F_1 crosses were different at the end of the sampling period. Additive

genetic variation was important in the inheritance of primary response, while heterosis and recombination loss had little or no impact on it.

Comparisons among lines for secondary response showed a large additive component (Code 1) in the mode of inheritance (Table 3). Reciprocal differences (Code 2) and heterosis (Codes 3 and 4) of the F_1 crosses were additional factors which were important in the inheritance of total secondary response. Results for contrasts with MES antibody titers were similar to those found for total response. Parental differences were present on peak day for MER titers. Heterosis of HL was present only on day 3 PSI for MER analyses.

DISCUSSION

Data obtained during this experiment showed that the principal source of genetic variation for primary immune response to SRBC was additive. Previous studies have shown the same mode of inheritance for juvenile body weight (Siegel, 1962; Maloney et al., 1967; Pym and Nicholls, 1979; Barbato et al., 1983). This relationship increases the difficulty in improving immune response without decreasing growth.

During the primary response, titers were within the range of the populations selected for high and for low antibody response to SRBC antigen. This result was consistent with that which was previously reported (Martin et al., 1988). Peak occurrence was at days 6 and 7 PPI and 5 days PSI. Previous work by Ubosi et al. (1985b) reported antibody peaks at day 5 PPI and day 3 PSI. Although MER titers showed an increase after reinjection, results should be regarded with caution because the literature is inconsistent in the proportion of the total titer which is due to the IgG antibodies (Yamamato and Glick, 1982; van der Zijpp, 1983b; van der Zijpp, et al., 1983).

Sant' Anna et al. (1979) concluded that in mice, a smaller number of genes were involved in the regulation of the secondary response compared to the primary. Thus, primary response may be genetically influenced to a greater degree than secondary response. Comparisons among the lines during the secondary response showed the increased presence of non-additive effects that were absent during the primary response.

SUMMARY

Experiments were conducted to determine the principal mode of inheritance for immunoresponsiveness in selected populations of chickens and their crosses as measured by antibody response to sheep erythrocytes (SRBC). Additive genetic variation was important in the inheritance of both primary and secondary responses to this antigen. Reciprocal differences and heterosis were also factors in the inheritance of secondary response.

Kinetics of primary and secondary antibody responses were evaluated in pairs of parental lines divergently selected high (HW) or low (LW) juvenile body weight and high (HA) or low (LA) response to SRBC antigen. Independent sampling of birds occurred on days 3 to 7, 10, 14, 24, 26, 28, and 31 post-primary inoculation (PPI). Half of the chickens sampled from day 24 on received an additional injection of SRBC antigen. Plasma from each individual was examined for total, mercaptoethanol resistant (MER), and mercaptoethanol sensitive (MES) titers. Antibody titers PPI to SRBC were consistently highest in line HA and lowest in line LA. Lines HW and LW reacted similarly to line LA early in response, but showed higher levels later on. In both primary and secondary responses, the weight lines peaked at similar levels. Thereafter, line LW maintained a high antibody titer level to SRBC antigen than line HW.

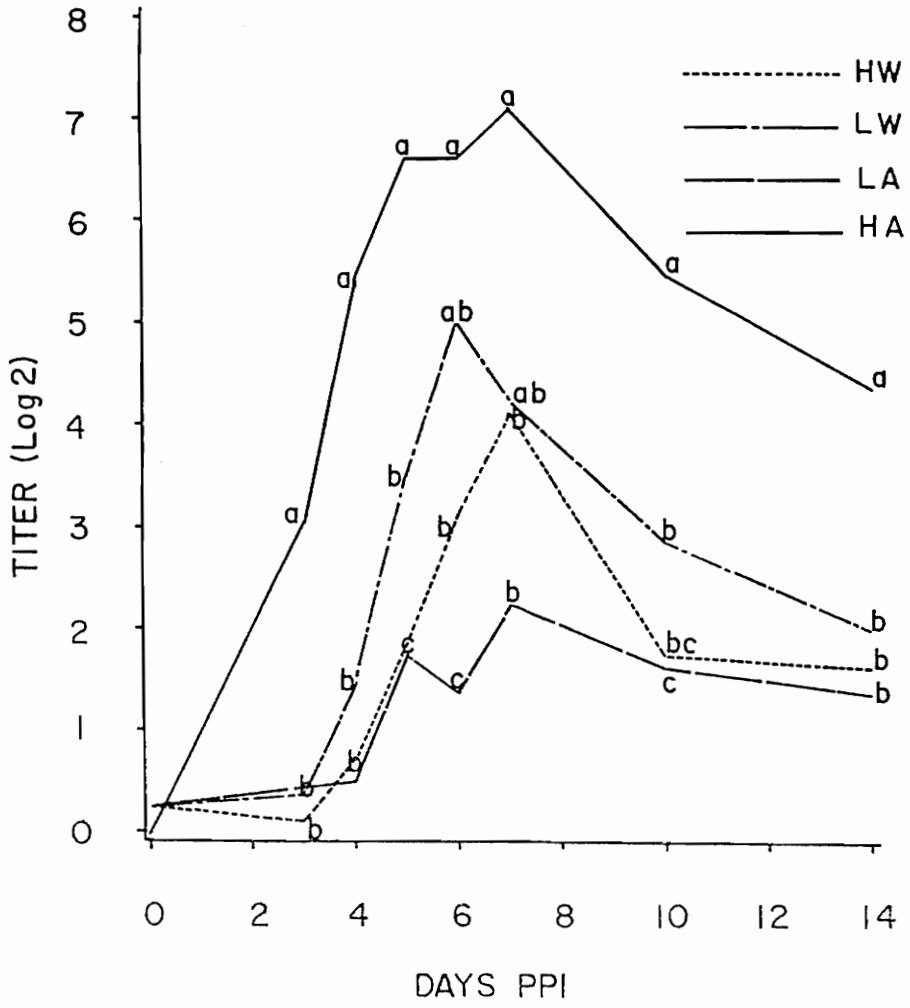

Figure 1. Primary antibody means to SRBC antigen in lines divergently selected for 56-day body weight (HW and LW) and lines divergently selected for response to SRBC antigen (HA and LA). a b c, denote significant differences among means within an age ($P \leq .05$).

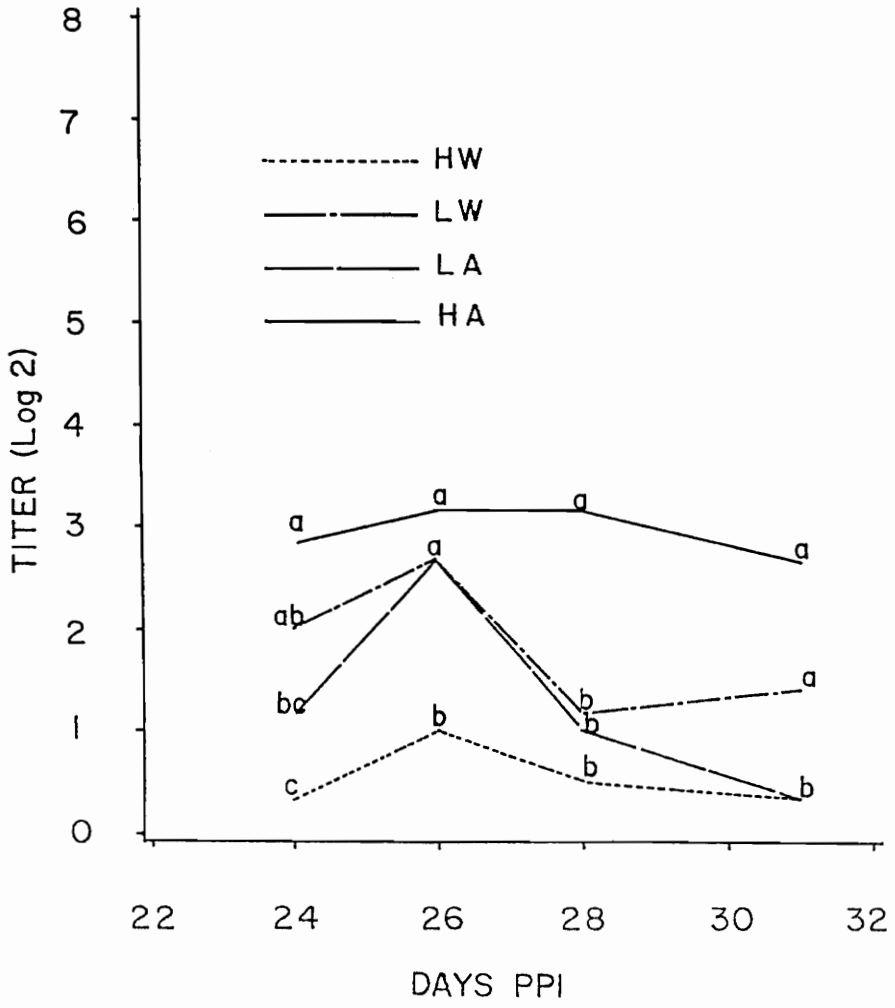

Figure 2. A continuation of the primary antibody means to SRBC antigen in lines divergently selected for 56-day body weight (HW and LW) and lines divergently selected for response to SRBC antigen (HA and LA). a b c, denote significant differences among means within an age ($P \leq .05$).

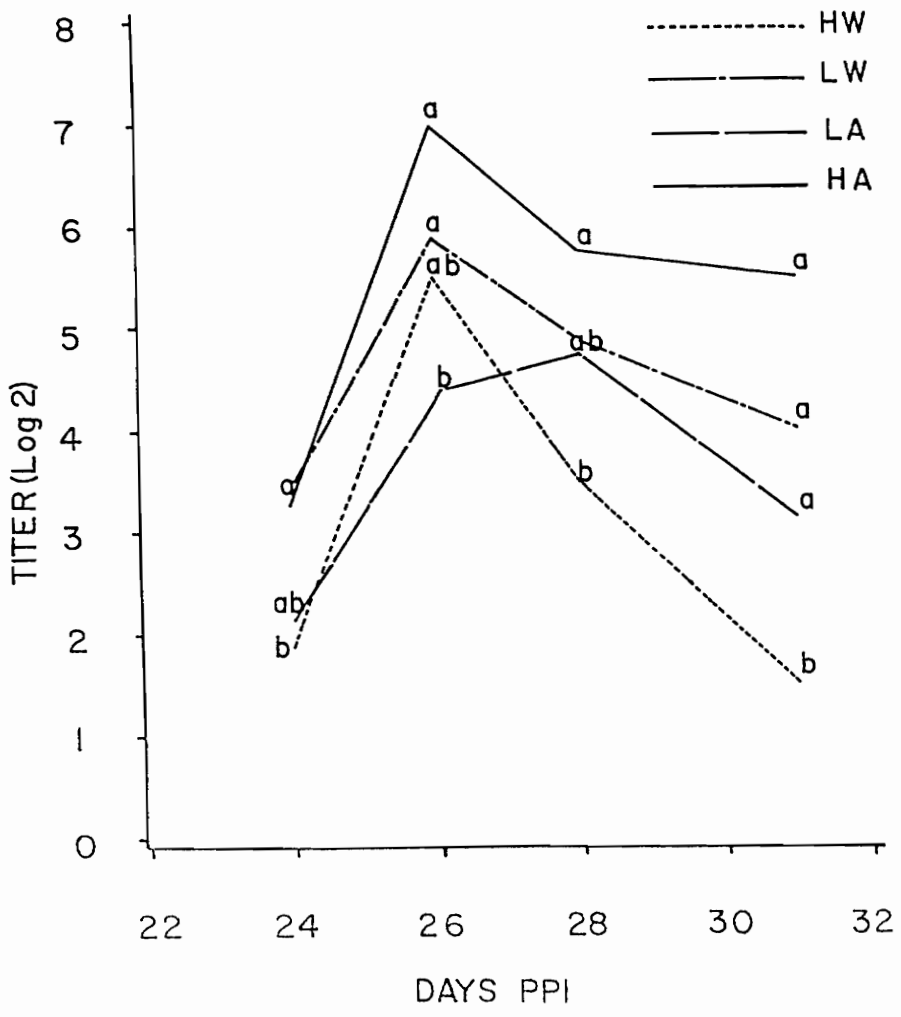

Figure 3. Secondary antibody means to SRBC antigen in lines divergently selected for 56-day body weight (HW and LW) and lines divergently selected for response to SRBC antigen (HA and LA). a b c, denote significant differences among means within an age ($P \leq .05$).

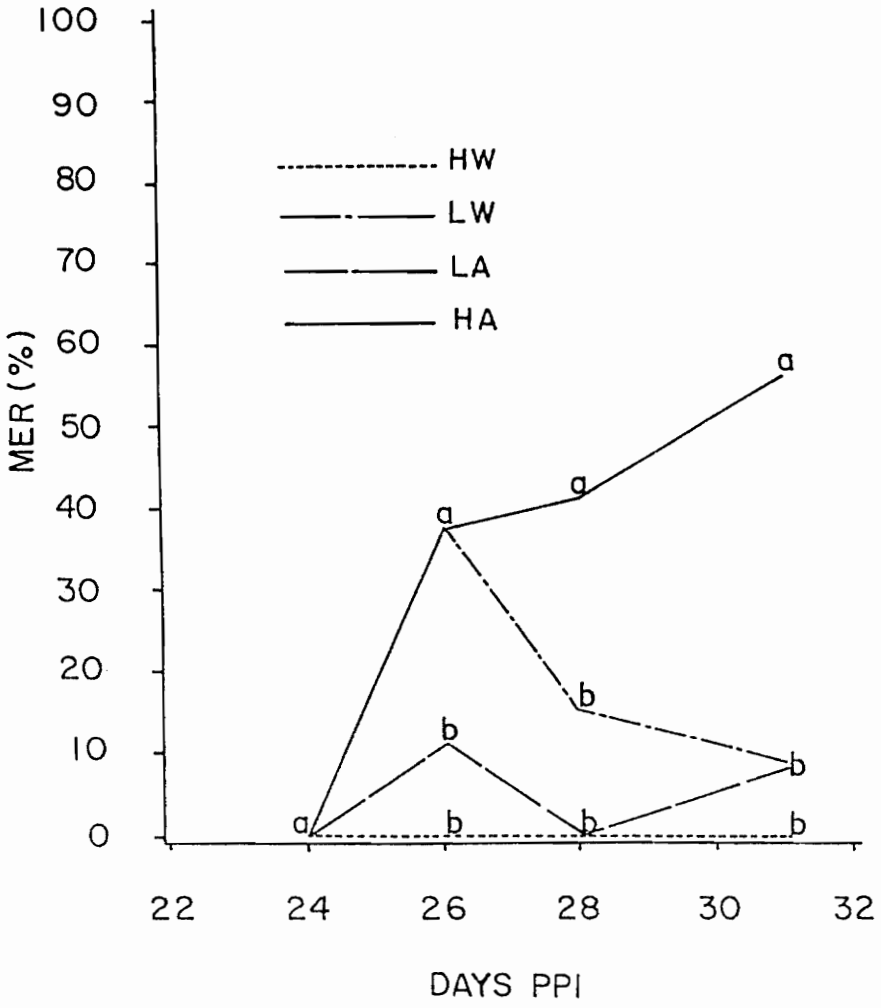

Figure 4. Secondary MER titers as a percentage of total antibody titer in lines divergently selected for 56-day body weight (HW and LW) and lines divergently selected for response to SRBC antigen (HA and LA). a b c, denote significant differences among means within an age ($P \leq .05$).

TABLE 1. *Nonorthogonal linear contrasts and genetical inferences for primary and secondary immune response.*

Contrast Code	Mating Comparison	Genetical Inference
1	HW vs LW	Parental
2	LH vs HL	Reciprocal F_1
3	HL vs (HW + LW)	Heterosis of HL
4	LH vs (HW + LW)	Heterosis of LH
5	(LHLH + HLHL) vs (LH + HL)	Recombination

TABLE 2. *Results of contrasts among lines for primary response (total and MES titers).*

	Contrast Code (see Table 1)				
Days (PPI)	1	2	3	4	5
3	ns	ns	*	ns	ns
4	ns	ns	ns	ns	ns
5	*	ns	ns	ns	ns
6	*	ns	ns	ns	ns
7	ns	ns	ns	ns	ns
10	*	ns	ns	ns	ns
14	ns	ns	ns	ns	ns
24	**	ns	ns	ns	ns
26	**	ns	ns	ns	ns
28	ns	ns	ns	ns	ns
31	*	**	*	ns	ns

** $p \leq .01$ * $p \leq .05$ ns $p > .05$

TABLE 3. *Results of contrasts among lines for secondary response.*

Titer	Days (PPI)	Contrast Code (see Table 1) 1	2	3	4	5
Total						
	24	**	ns	ns	ns	ns
	26	ns	*	*	ns	ns
	28	*	**	ns	*	ns
	31	**	ns	ns	ns	ns
MER						
	24	ns	ns	ns	*	ns
	26	**	ns	ns	ns	ns
	28	ns	ns	ns	ns	ns
	31	ns	ns	ns	ns	ns
MES						
	24	**	ns	ns	ns	ns
	26	ns	**	*	ns	ns
	28	ns	*	ns	*	ns
	31	**	ns	ns	ns	ns

** $p \leq .01$ * $p \leq .05$ ns $p > .05$

GENERAL SYNTHESIS

In the broiler breeding industry there is intense selection pressure for reaching market weight at an early age. Of the many traits correlated with this particular characteristic, immunocompetence appears to be one of those negatively correlated with body weight. Immune response involves various lines of defense such as phagocytic, cellular, and humoral immunity. With the use of sheep erythrocytes as a source of antigen the focus is on thymus-dependent humoral immune response.

An organism must allocate its available resources among various demands such as growth, maintenance, reproduction, and immune response (Siegel et al., 1982; Dunnington, 1990). When selection occurs in favor of any particular component of development, it reduces resources available to other components. Resources are redistributed according to the greatest pressures or demands being placed on the organism (Dunnington, 1990; Bacon and Dietert, 1991). The experiments reported in this dissertation were designed to explore the association found between selection for body weight and immunoresponsiveness in both Japanese quail and White Plymouth Rock chickens with emphasis on thymus dependent response. Levels of antibody observed in the blood of an animal may be dependent upon genetics, dose, and time. Accordingly, evaluation included measuring kinetics of antibody response in populations known to differ in growth potential.

Kinetics of primary and secondary immune responses were evaluated in a randombred control (C) line of Japanese quail using three dosages differing in concentration of SRBC antigen (0.025, 0.25, and 2.50%). At

most times PPI antibody titers were highest for antigen concentration 2.50%. Presence of MER antibodies was very low PPI, but increased following reinjection. Kinetics of primary immune responses were also compared between a control line (C) and a line selected for high (HW) 4-week body weight. Line HW quail were unable to maintain a high antibody titer level to SRBC antigen when compared to the randombred control line from which they originated.

Kinetics of primary and secondary responses of chickens were evaluated in lines divergently selected for body weight and for antibody response to SRBC antigen. The two antibody lines served as positive and negative control lines throughout the experiment. Weight lines HW and LW reacted at low levels similar to line LA early in response, but subsequently showed higher levels. In both primary and secondary responses, the weight lines peaked at similar levels. However, line HW was less able to maintain a high antibody titer level to SRBC antigen than line LW.

Experiments were conducted to determine the principal mode of inheritance for immunoresponsiveness in selected populations of chickens and their crosses as measured by antibody response to sheep erythrocytes. Additive genetic variance was important in the inheritance of both primary and secondary responses to this antigen. Reciprocal differences and heterosis of the F_1 crosses were important in the inheritance of secondary but not the primary response. Bacon and Dietert (1991) in their recent review article on genetic control of generalized immune responses pointed out that redistribution of resources may occur due to overemphasis or

selection of a particular trait. Data are presented here are consistent with this thesis because HW line birds were unable to maintain high levels of antibody titers when compared to an unselected randombred control line (quail) or a line selected for low body weight (chickens).

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APPENDIX

Appendix Table 1. *Primary antibody titer means and standard deviations with differing concentrations of SRBC antigen in control (C) line quail (Trial 1), Chapter 1.*

	Concentrations (%)		
Days PPI	0.025	0.25	2.5
3	0.0 ± 0.0b	0.2 ± 0.6b	1.6 ± 1.2a
5	0.0 ± 0.0c	1.4 ± 1.4b	2.3 ± 1.1a
7	1.5 ± 1.7b	2.2 ± 1.2ab	3.1 ± 1.4a
9	0.2 ± 0.9b	1.7 ± 1.4a	2.2 ± 1.1a
11	0.4 ± 0.8b	0.7 ± 1.0b	1.9 ± 1.2a
13	0.0 ± 0.0b	0.3 ± 0.8b	1.7 ± 1.2a
19	0.7 ± 1.0a	0.7 ± 1.0a	1.0 ± 1.1a
21	0.0 ± 0.0b	1.0 ± 1.1ab	1.7 ± 0.8a
23	0.0 ± 0.0a	0.7 ± 1.0a	1.4 ± 1.3a
25	0.7 ± 1.0a	0.7 ± 1.0a	1.4 ± 1.3a
27	1.0 ± 1.1a	1.0 ± 1.1a	1.5 ± 1.2a
31	0.3 ± 0.8a	0.0 ± 0.0a	0.0 ± 0.0a

PPI = post-primary inoculation
a,b indicate differences ($p \leq .05$) among titers within a row.

Appendix Table 2. *Primary mercaptoethanol resistant (MER) as a percentage of total antibody titer for non-boostered control (C) line quail with differing antigen concentrations (Trial 1), Chapter 1.*

	Concentrations (%)		
Days PPI	0.025	0.25	2.5
3	0	0	0
5	0	0	0
7	0a	15a	22a
9	0a	24a	9a
11	0a	0a	30a
13	0a	0a	14a
19	0	0	0
21	0	0	0
23	0	0	0
25	0	0	0
27	0	0	0
31	0	0	0

PPI = post-primary inoculation
a,b indicate differences ($p \leq .05$) among titers within a row.

Appendix Table 3. *Secondary antibody titer means and standard deviations with differing concentrations of SRBC antigen in control (C) line quail (Trial 1), Chapter 1.*

Titer	Days PPI	Concentrations (%) 0.025	0.25	2.5
Total				
	19	1.7 ± 0.8a	2.8 ± 2.0a	3.8 ± 1.9a
	21	2.8 ± 2.0a	3.5 ± 3.3a	5.0 ± 2.7a
	23	3.8 ± 1.5a	5.2 ± 2.2a	5.3 ± 1.4a
	25	2.5 ± 1.4b	5.0 ± 3.0ab	7.2 ± 3.0a
	27	1.8 ± 1.6a	3.5 ± 1.6a	2.7 ± 1.2a
	31	1.0 ± 1.1a	1.2 ± 1.8a	2.2 ± 2.2a
MES				
	19	1.7 ± 0.8a	2.2 ± 0.5a	3.2 ± 1.2a
	21	2.8 ± 2.0a	2.6 ± 1.1a	3.3 ± 0.2a
	23	3.3 ± 0.6a	3.8 ± 0.4a	3.6 ± 0.8a
	25	2.5 ± 1.4a	3.8 ± 0.3a	5.0 ± 0.2a
	27	1.8 ± 1.6a	2.8 ± 0.2a	2.2 ± 0.2a
	31	1.0 ± 1.1a	1.0 ± 1.2a	1.8 ± 1.2a

PPI = post-primary inoculation
a,b indicate differences ($p \leq .05$) between titers within a row.

Appendix Table 4. *Secondary mercaptoethanol resistant (MER) antibody means and standard deviations plus MER percentage of total antibody titer for non-boostered control (C) line quail with differing antigen concentrations (Trial 1), Chapter 1.*

	Concentrations (%)		
Days PPI	0.025	0.25	2.5
MER			
19	0.0 ± 0.0a	0.6 ± 1.5a	0.6 ± 1.5a
21	0.0 ± 0.0b	0.9 ± 2.2ab	1.7 ± 2.5a
23	0.6 ± 0.9a	1.4 ± 1.8a	1.7 ± 2.2a
25	0.0 ± 0.0b	1.2 ± 2.7ab	2.2 ± 3.0a
27	0.0 ± 0.0a	0.7 ± 1.4a	0.5 ± 1.0a
31	0.0 ± 0.0a	0.2 ± 0.6a	0.4 ± 1.0a
MER (%)			
19	0a	16a	15a
21	0b	19ab	37a
23	17a	32a	31a
25	0a	21a	28a
27	0a	23a	22a
31	0a	7a	14a

PPI = post-primary inoculation
a,b indicate differences ($p \leq .05$) between titers within a row.

Appendix Table 5. *Means and standard deviations for antibody titers in high (HW) and control (C) line quail in response to varying concentrations of SRBC antigen (Trial 2), Chapter 1.*

		Day (PPI)			
Line	Dose %	4	7	10	13
C	0.25	2.1 ± 2.0x	2.3 ± 1.8x	1.1 ± 1.2y	0.3 ± 0.7y
		NS	NS	NS	NS
	2.5	2.1 ± 1.1a	2.1 ± 1.4a	1.5 ± 0.9a	0.3 ± 0.8b
HW	0.25	1.5 ± 1.2x	1.2 ± 1.2x	0.2 ± 0.6y	0.2 ± 0.6y
		**	*	NS	NS
	2.5	2.7 ± 0.7a	2.2 ± 1.0a	0.3 ± 0.8b	0.0 ± 0.0b

PPI = post-primary inoculation
*, ** indicate differences (P≤ .05 and P≤ .01) between adjacent means.
NS no difference between adjacent means (P≥ .05).
a,b indicate differences (p≤ .01) between titers within a column and concentration of 2.5.
x,y indicate differences (p≤ .01) between titers within a column and concentration of 0.25.

Appendix Table 6. *Primary antibody titer means and standard deviations in chickens of different genetic stocks, Chapter 2.*

	Day (PPI)					
LINE	0	3	4	5	6	7
HA	0.0 ± 0.0a	3.0 ± 1.2a	5.4 ± 2.3a	6.6 ± 1.6a	6.6 ± 1.5a	7.1 ± 1.7a
LA	0.2 ± 0.7a	0.4 ± 1.0b	0.5 ± 0.9b	1.7 ± 1.6c	1.4 ± 1.2d	2.2 ± 1.7c
HW	0.2 ± 0.7a	0.1 ± 0.3b	0.7 ± 1.0b	1.9 ± 1.2bc	3.1 ± 1.0bc	4.1 ± 1.6abc
LW	0.2 ± 0.7a	0.4 ± 1.1b	1.5 ± 0.9b	3.5 ± 1.3b	5.0 ± 1.4ab	4.2 ± 2.8bc
HL	0.0 ± 0.0a	1.0 ± 1.1b	1.5 ± 1.2b	2.0 ± 2.1c	4.0 ± 0.9bc	2.7 ± 1.7bc
LH	0.0 ± 0.0a	0.2 ± 0.7b	1.5 ± 1.4b	3.2 ± 1.6bc	4.0 ± 1.2bc	3.5 ± 2.3bc
HLHL	0.0 ± 0.0a	0.2 ± 0.7b	1.4 ± 1.2b	2.5 ± 1.4bc	3.0 ± 1.4c	4.7 ± 1.5ab
LHLH	0.2 ± 0.7a	0.4 ± 1.1b	0.9 ± 1.2b	3.7 ± 1.2ab	4.0 ± 1.5bc	3.5 ± 1.1bc

a,b,c,d indicate differences ($p \leq .05$) between titers within a column.

Appendix Table 7. *Primary antibody titer means and standard deviations in chickens of different genetic stocks (non-boostered), Chapter 2.*

	Day (PPI)					
LINE	10	14	24	26	28	31
HA	5.5 ± 0.9a	4.4 ± 1.1a	2.8 ± 0.7a	3.2 ± 0.7a	3.2 ± 1.2a	2.7 ± 1.2a
LA	1.6 ± 1.1c	1.4 ± 1.2b	1.2 ± 1.0abcd	2.7 ± 0.8a	1.0 ± 1.1b	0.3 ± 0.8bc
HW	1.7 ± 0.7bc	1.6 ± 1.1b	0.3 ± 0.8d	1.0 ± 0.9b	0.5 ± 0.8b	0.3 ± 0.8bc
LW	2.9 ± 0.8b	2.0 ± 1.3b	2.0 ± 1.3ab	2.7 ± 0.8a	1.2 ± 1.3b	1.4 ± 0.9a
HL	2.5 ± 0.7bc	2.1 ± 1.0b	1.0 ± 1.0bcd	2.3 ± 0.5a	1.2 ± 1.0b	0.0 ± 0.0c
LH	2.6 ± 0.5b	2.4 ± 0.5ab	0.5 ± 0.8cd	2.0 ± 1.0a	0.8 ± 1.0b	1.3 ± 1.0ab
HLHL	2.6 ± 0.8b	1.7 ± 0.7b	1.6 ± 1.3abc	2.0 ± 0.6a	0.2 ± 0.5b	0.0 ± 0.0c
LHLH	1.6 ± 1.1c	2.3 ± 1.0b	0.8 ± 1.1bcd	2.3 ± 1.2a	1.1 ± 1.1b	0.7 ± 1.6bc

a,b,c,d indicate differences ($p \leq .05$) between titers within a column.

Appendix Table 8. *Secondary antibody titer means and standard deviations in chickens of different genetic stocks (boostered), Chapter2.*

	Day (PPI)			
LINE	24	26	28	31
HA	3.2 ± 0.5a	7.0 ± 1.3a	5.7 ± 1.4a	5.5 ± 1.1a
LA	2.1 ± 0.3ab	4.4 ± 1.6ab	4.7 ± 1.3abc	3.1 ± 1.0b
HW	1.9 ± 1.6b	5.5 ± 1.3ab	3.5 ± 1.5d	1.5 ± 1.5c
LW	3.4 ± 0.9a	5.9 ± 1.3ab	4.9 ± 1.4abc	4.0 ± 1.8ab
HL	2.7 ± 0.7a	4.4 ± 3.1b	3.7 ± 1.0cd	2.7 ± 1.6b
LH	2.5 ± 1.3ab	6.2 ± 1.8a	5.4 ± 0.5ab	3.4 ± 1.3ab
HLHL	2.9 ± 0.9a	6.1 ± 0.8a	5.1 ± 1.1ab	4.4 ± 1.7ab
LHLH	2.4 ± 0.5ab	5.7 ± 1.6ab	4.0 ± 0.7bcd	3.7 ± 1.0acb

a,b,c,d indicate differences ($p \leq .05$) between titers within a column.

Appendix Table 9. *Secondary MES titer means and standard deviations in chickens of different genetic stocks (boostered), Chapter 2.*

	Day (PPI)			
LINE	24	26	28	31
HA	3.2 ± 0.5a	4.4 ± 2.0abc	3.4 ± 1.7b	2.4 ± 1.3a
LA	2.1 ± 0.3ab	3.9 ± 1.6abc	4.7 ± 1.3ab	2.9 ± 1.2a
HW	1.9 ± 1.6b	5.5 ± 1.3ab	3.5 ± 1.5b	1.5 ± 1.5b
LW	3.4 ± 0.9a	3.6 ± 2.1bc	4.1 ± 1.7ab	3.6 ± 1.9a
HL	2.7 ± 0.7a	3.4 ± 2.5c	3.7 ± 1.0ab	2.7 ± 1.6a
LH	2.4 ± 1.4ab	6.2 ± 1.8a	5.4 ± 0.5a	3.4 ± 1.3a
HLHL	2.9 ± 0.9a	6.1 ± 0.8a	4.7 ± 1.8ab	3.7 ± 0.9a
LHLH	2.4 ± 0.5ab	5.6 ± 1.6ab	3.6 ± 1.3ab	3.5 ± 1.2a

a,b,c,d indicate differences ($p \leq .05$) between titers within a column.

Appendix Table 10. *Secondary MER percentages in chickens of different genetic stocks (boostered), Chapter 2.*

	Day (PPI)			
LINE	24	26	28	31
HA	0	37.5	41.3	56.8
LA	0	11.4	0	8
HW	0	0	0	0
LW	0	38.3	15.4	9.4
HL	0	22.5	0	0
LH	5	0	0	0
HLHL	0	0	7.3	14.3
LHLH	0	2.5	9.4	6.7

VITA

Libbie Leann Miller, the daughter of Sue Miller and Arthur Miller, was born on June 16, 1967 in Lebanon, Indiana. She attended Western Boone High School, Dover, Indiana, and graduated in May, 1985. Libbie was awarded the Bachelor of Art in Biology from Randolph-Macon Woman's College in Lynchburg, Virginia, in May of 1989. Her studies in Poultry Genetics with Dr. E. A. Dunnington at Virginia Polytechnic Institute and State University began two weeks later.

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